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ÍNDICE

CONFERENCIAS PLENARIAS

Use of genomic technologies for de prediction of risk in common diseases 12

Study of translation regulation for the identification of key proteins involved in plant adaptation to environmental stresses 13

Biomolecules from biosludge 14

Marine Microbiome as a Source of Anticancer Compounds. The experience of PharmaMar 15

PRESENTACIONES KEYNOTE

Plants as a source of tools for the development of molecular farming and nanobiotechnology. 17

MTBVAC, a new freeze dried vaccine of M. *tuberculosis* against TB. 18

Yarrowia lipolytica as a microbial host for flavors and fragrances production. 19

Sustainable chemical processes in ionic liquids and supercritical carbon dioxide. 20

Sustainable integrated Algae Biorefinery for the production of bioactive compounds for Agriculture and Aquaculture (SABANA). 21

Exploiting bacterial metabolism for removal of aromatic pollutants and biowaste revalorization. 22

The impact of nanobiotechnology in the design of new medicines 23

Modification of regulatory systems in *Streptomyces* to increase antibiotics production. 24

PREMIO SEBIOT JÓVENES INVESTIGADORES

In vitro metabolic engineering at the interface between solid materials and enzymes 26

COMUNICACIONES ORALES

Biotecnología vegetal

Establishment of plant cell suspension cultures for the production of bioactive compounds from Kalanchoe. 28

Microbial biopesticides to control fungal diseases in grapevine. Production, field monitoring and analysis of the plant response. 29

Phenolic compounds profiles of different parts of the plant *Cynara cardunculus* L. var. altilis. 30

Biotecnología de la salud

Preparation and validation of a DLC (Drug-in-Liposome-in-Cyclodextrin) system in cancer cell lines 31

Nanoencapsulation of controlled size superparamagnetic nanoparticles for biomedical applications. 32

Minicircle Gene Delivery to Transiently modulate Mesenchymal Stromal Cell function in the context of angiogenesis-based regenerative medicine. 34

Biotecnología alimentaria

Synthesis of potential prebiotic sugars by enzymatic extracts from the nectaries yeast *Metschnikowia reukafii.* 36

Sequential three-step biocatalytic process for the transformation of milk whey into the novel sweetener D-tagatose. 37

Production and characterization of a functional beverage obtained by fermentation of kiwifruit juice with kefir grains. 38

Innovative functional yogurt incorporating microencapsulated calcium lactobionate. 40

Nutritional value of different microalgae isolated in the south of Spain and Portugal on the basis of their amino acidic profile. 41

Antioxidant potential of Kalanchoe extracts for the preservation of water-in-oil emulsions. 42

Surfactants of microbial origin extracted from agro-industrial wastes: Agrifood applications. 44

Analysing the transferase activity of α-glucosidase GAM1 from *Schwanniomyces occidentalis.* 45

Biocatálisis

Production of furandicarboxylic building block from renewable methoxymethylfurfural through a self-sustained enzymatic cascade. 46

Travelling back and forth: Directed evolution of an ancestral high-redox potential laccase. 47

Towards the search of powerful enzymes by applying metagenomics and computational techniques. 48

Production of neo-fructooligosaccharides by the b-fructofuranosidase from *Xanthophyllomyces dendrorhous* immobilized by different methodologies. 49

Characterizacion of a novel fungal laccase synthesized de novo and expressed in yeast by using directed evolution. 50

Immobilization of chitinase chit42 on chitosan beads and magnetic nanoparticles for chitooligosaccharides production. 51

The generation of thermostable fungal laccase chimeras by SCHEMA-RASPP structure-guided recombination in vivo. 52

Design of sequential chemoenzymatic strategies towards optically active allylic alcohols and amines. Use of laccases in combination with alcohol dehydrogenases and amine transaminases. 53

Biotecnología industrial

Modelling the production of VFA from proteins by mixed culture fermentations. 54

Scaling-up continuous lignocellulosic biobutanol production. 55

Dihydroxyacetone production from glycerol using *Gluconobacter oxydans*: a kinetic model. 56

A microfluidic platform for downstream process optimization. 57

Evaluation of batch ABE extractive fermentation with solvents of different nature. 58

Bioprocessing of recombinant proteins using alternative purification platforms. 59

Microorganisms for use as a biocatalyst for green chemistry processes. 61

Gene dosage and specific growth rate impacts on recombinant protein production in *Komagataella phaffi* regulated by the AOX1 promoter. 62

Biotecnología ambiental

Extraction and Quantification of Antibiotics using Aqueous Two-Phase Systems based on Citrate Salts and Acetonitrile. 63

Cheese whey, a Galician dairy industry by-product, as potential feedstock for VFA production using a mixed culture. 64

Hydrolysis of butcher´s waste by Alcalase. 65

Anaerobic populations and enzymes involved in the removal of organic micropollutants. 66

Pilot-scale composting of organic fraction of municipal solid waste for agricultural use. 67

Valorization of methane emissions into polyhydroxyalkanoates by methanotrophs enriched from Sphagnum mosses: Assessing the effect of temperature and phosphorus limitation. 68

Power to gas technology by biological methanation. 69

Gene expression study of *E. coli* growing in glucose and acetate as carbon sources. 70

Nuevos retos en biotecnología

Synthesis, use and modelling of a gel containing Levan-capped silver nanoparticles for bactericidal purposes. 71

Controlled size nanocolloids production for bioapplications. 72

ProBone - New tools for prospecting the marine bone-degrading microbiome for novel enzymes. 73

Biotecnología microbiana

Isolation and characterization of xylanolytic bacteria from Cachiyacu of *Lupuna salterns* in Peru. 74

Identification of target genes to reduce acetate yield by *Saccharomyces cerevisiae* under aerobic conditions. 75

Random mutagenesis of *Rhodospirillum rubrum* to improve growth in syngas. 76

PÓSTERS

Biotecnología vegetal

Induction of resistence in cell suspensions of pepper (Capsicum annuum L.annuum) by elicitation with extracts of Moringa oleifera leaves. 78

New molecular tools for the expression of foreign genes in microalgal nucleus. 79

Antimicrobial and defence elicitor peptides in plant protection. From nature to the biotechnological product. 80

Influence of culture media composition and solvent aqueous ratio on phenolic content and antioxidant activity of Kalanchoe cultured in vitro. 81

Study of ectomycorrhizae communities in young chestnut trees using ITS sequences. 82

Identification of key factors to optimize plant tissue culture using neurofuzzy logic. 83

Biotecnología de la salud

Polydopamine nanoparticles: a study of the conditions that determine their size and loading-capacity of different metals. 84

Evaluation of salicylic acid permeation through silicone membrane in presence of biosurfactant produced from corn steep wáter. 85

A biosurfactant extract obtained from corn as L-ascorbic acid stabilizer. 86

Engineering high copy number plasmids for pharmaceutical-grade pDNA production in Lactococcus lactis. 87

Biotecnología alimentaria

Peptide biomarkers for biogenic amine-producing bacterial characterization by LC-ESI-MS/MS. 88

Peptide biomarkers for mastitis producing Streptococcus spp. identification and characterization by LC-ESI-MS/MS. 89

Fish Parasite Biobank: supplier of sample and data for studying the genetic structure of zoonotic Anisakis spp. from European hake Merluccius merluccius. 90

Analysis of the composition of commercial fish feed by NGS: implications for parasite risk exposure assessment. 91

Putative probiotic lactic acid bacteria isolated from non-conventional sources. 92

Potential of surplus harvest vegetables as new sources of protein for human consumption. 93

Dairy hydrolysates obtained using an enzymatic extract from Aspergillus niger. 94

Polyunsaturated fatty acid production by microalgae: optimisation and scale-up. 95

Amelioration of Omega-3 docosahexaenoic acid production by microalgae. 96

Effectiveness of dietary supplementation of microalgae as aquafeed additives for the juvenile white prawn, Litopenaeus vannamei. 97

A new procedure to prepare edible films from porcine and bovine blood proteins. 98

Influence of egg yolk proteins on the physical properties of gum arabic microparticles loaded with polyphenols. 99

Antioxidant capacity of macro and micro algae extracts of fresh and marine water with potential application in aquaculture. 100

Improving the nutraceutical potential of Eryngium viviparum J. Gay through in vitro culture elicitation. 101

Valorisation of a threatened plant species through in vitro culture elicitation of phenolic compounds. 102

Elaboration of films-based antibacterial plant extracts to preserve liquid food products. 103

Biofunctional characterization of protein hydrolysates obtained from cod (gadus morhua) byproducts. 104

Effect of the stages of the deodorization process of cochayuyo (Durvillaea antarctica) on the yield and minerals and dietary fiber content, of deodorized algae flour. 105

Phytomelatonin as a natural alternative to synthetic melatonin in food supplements and others applications. 107

Rheological properties and development of Staphylococcus sp. in structured foods. 108

Is the correct identification of chimaera a chimera? 109

Implementation of the traceability process in products of autochthonous Portuguese poultry breeds. 110

Implementation of a traceability system in the rooster production controlled by the "Confraria Gastronómica O Galo de Barcelos". 111

Study of the adsorption procedure of phenolics from Kalanchoe aqueous extracts onto activated carbón. 112

Biocatálisis

Re-using laccase for the production of oligorutin in a reaction media free of organic solvents. 113

Changes in the substrate preference of aryl-alcohol oxidase allows a stereoselective oxidation of secondary alcohols. 114

A designer natural deep eutectic solvent to recycle the nicotinamide cofactor in alcohol dehydrogenase-catalysed bioreductions. 115

Functional expression and characterization on a new chitinase from Pseudoalteromonas tunicata with nematicidal activity. 116

Copper and polymerized lignin by laccase as wood treatment for marine uses. 117

Sustainable enzymatic synthesis of panthenyl monoesters in ionic liquids. 118

Sustainable approach for the synthesis of sorbitol monoesters in Ionic Liquids 119

Cell-free immobilized multi-enzyme systems; from the design to the application. 120

Enzymatic alcoholysis of dimethyl adipate by 2-ethylbutanol. Clean synthesis of biolubricants in solvent-free medium. 121

Lipase comparison in solvent-free neopentyl glycol dilaurate synthesis. 122

Combined use of cyclodextrin glucanotransferase from Geobacillus sp. and amyloglucosidase from Saccharomyces cerevisiae to produce glucosylated derivatives of rutin. 123

Nicotinate phosphoribosyltransferase Escherichia coli characterization. 125

Characterization of a novel thermostable lypolitic hydrolase found by functional metagenomics from a hot spring. 126

In-situ and post-synthesis Immobilization of lipase CALB on MOF NH2-MIL-53(Al). 127

Esterification reactions in free solvent systems with cutinase covalently immobilized or only by adsorption. 128

Chemoenzymatic synthesis of muramic and glucuronic acids derivatives and preparation of multivalent dendrimers as Dengue virus antagonists. 129

Chemoenzymatic strategies for the preparation of glycoproteins of interest and molecular modelling studies of the enzyme-substrate recognition. 130

Stereoselective reduction of halogenated ketones with an alcohol dehydrogenase from Pichia Glucozyma overexpressed in E.coli. 131

Selective production and characterization of acidic xylooligosaccharides from beechwood xylan by an enzymatic cocktail. 132

Biocatalytic synthesis of oxygenated biofuels through the technologies of ionic liquids and supercritical carbon dioxide. 133

Preparation of SBA-15 in different conditions to obtain optimal supports for laccase immobilization. 134

Laccase-catalyzed oxidations of propargylic alcohols. Application to deracemization experiments by combination with alcohol dehydrogenases. 135

Biotecnología industrial

Autohydrolysis followed by ionic liquid treatment for Eucalyptus globulus wood fractionation. 136

Preparative chromatography of recombinant nucleic acids using ionic liquids as multimodal ligands. 137

Medium chain length polyhydroxyalkanoates production from Camelina oil. 139

MEP pathway optimization by directed enzyme evolution to produce highly valuable chemicals in microbes. 140

Growth rate and final protein production can be dramatically increased by using a secretion signal that drives the proteins through co-translational translocation in Pichia pastoris. 141

The new derepressible promoter P2: a promising expression system for methanol-free recombinant protein production in Pichia pastoris. 142

Oxygen-limiting conditions to improve recombinant protein production in Pichia pastoris regulated by the GAP promoter. 143

Evaluation of the bioreactor configuration in the production of microalgae Crypthecodinium cohnii. 144

Optimization of the culture medium for growth and DHA production of microalgae Crypthecodinium cohnii. 145

Simulation of biobutanol production at industrial scale. 146

Acetogenic bioconversion of greenhouse gases (CO_2, CO) and syngas to fuel-alcohols. 147

Citrus processing waste valorisation for the production of pigments by fungi fermentation. 148

Steering volatile fatty acid production during protein fermentation by adjusting substrate composition and pH. 150

Proteins involved in the acquisition of the active structure of the secreted proteins in Streptomyces lividans. 151

Succinic acid production from xylose by Actinobacillus succinogenes: influence of inoculum built up. 152

Biotecnología ambiental

Scaling-up Ionic Liquid biodegradation: sequencing batch for fixed-bed reactor. 153

Hugging a green approach to remove antibiotics from swine wastewater. 154

Wastes from hydrocolloid and fibre industry as low-cost adsorbents: adsorption and regeneration. 155

Study of the sorption process and removal of diuron by Trametes versicolor immobilised in wood chips in a biodisc reactor operating in continuous mode 156

Challenges in analysis and remediation of pharmaceutical pollution in water: A human concern. 157

Evaluation of the adsorption potential of biochar prepared from vine shoots for the removal of pirimicarb. 158

Scaling-up Ionic Liquid biodegradation: immobilization approach for fixed-bed reactor. 159

Bioelectro-Fenton process, a promising system for the removal of ionic liquids? 160

Valorization of sewage sludge to produce volatile fatty acids through acidogenic fermentation: batch and continuous reactor experiments. 161

Vermicomposting increases bacterial diversity and changes composition and function yielding a high quality plant biostimulant. 162

Biological treatment of anaerobic digestion liquor by white-rot fungi. 164

Effects of different enzymes on the enzymatic hydrolysis of proteins from butcher's waste. 165

Low cost biogas upgrading using ashes. 166

Anaerobic degradation of sulfamethoxazole using anthraquinone-2-sulfonate as redox mediator. 167

Ibuprofen, diclofenac and triclosan dissipation and associated microbial ecotoxicity in biopurification systems. 168

Removal of pharmaceutical compounds in wastewater using biochar from rice bran as adsorption matrix. 169

Water remediation by means of chestnut shell wastes. 170

Nuevos retos en biotecnología

Application of the invasive macroalgae Undaria pinnatifida in the synthesis of gold and silver nanoparticles. 171

Food waste application in the synthesis of gold nanoparticles. The case of Galician Brassica oleracea var. Acephala. 172

Biotecnología microbiana

A Bacillus sp. strain for biosurfactants production as biofungicides of pathogenic cocoa fungi Moniliophtora perniciosa and Moniliphtora roreri. 173

Cross-talks between two paralogous cascades contribute to the regulation of the General Stress Response in Sphingopyxis granuli TFA. 174

Characterization of an ibuprofen-eater bacteria. 175

SfbR2, a pseudo-gamma-butyrolactone receptor from Streptomyces filipinensis represses filipin production. 176

Streptomyces tsukubaensis production of the immunosuppressant tacrolimus: negative effect of adpA regulator as revealed by the use of a microcultivation system. 177

Deciphering the Regulation of safracin cluster in Pseudomonas poae strain PMA22. 178

Active and thermostable E. coli large-oligomer protein. 179

Brad-new ability of acclimated Pseudomonas strain in the presence of neoteric pollutans. 180

Evaluation of the extraction process of biotechnologically produced biosurfactant from Lactobacillus pentosus. 181

Study of the changes induced in the organoleptic properties of beer after genetic modification of yeast. 182

Characterization of extracellular vesicles produced by wine yeast strains under winemaking conditions. 183

Biotecnología azul

Study of the antimicrobial activity of different species of microalgae extracts against pathogenic bacteria in aquaculture. 184

Simultaneous extraction and fractionation of lipids from the marine microalga Nannochloropsis sp. for the production of EPA rich polar lipid concentrates. 185

Biotecnología y sociedad

NAFEI – Un proyecto de movilización de estudiantes graduados para la promoción y valorización de la industria agroalimentaria y agroambiental. 186

Newfood Project - Promoting the transfer of knowledge and innovation to the scientific community and society about traditional food products. 187

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10 - 13 JUNIO VIGO

CONFERENCIAS PLENARIAS

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Use of genomic technologies for de prediction of risk in common diseases

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Study of translation regulation for the identification of key proteins involved in plant adaptation to environmental stresses

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Thematic Areas: Biotecnología vegetal.

Abstract: One of the main challenges for agriculture is to optimize crop production to meet the food needs for the increasing world's population. In this sense, it is essential to face the drastic reduction on crop yield caused by environmental stresses

Most plants complete their life cycle in a single location and, therefore, their development and reproduction depend on the environmental conditions they are exposed to. Environmental stresses such as drought and high temperatures seriously affect plant development and growth. To cope with these challenges, plants have evolved molecular strategies that allow them to survive under such adverse conditions.

One of the earliest plant responses to environmental stresses is the regulation of translation. This regulation usually involves a general inhibition of protein synthesis. Under such conditions, some mRNAs able to bypass the global inhibition are selectively translated. These mRNAs are usually involved in the establishment of the stress response and are crucial regulators of plant adaptation to environmental cues.

Our lab aims to contribute to understand how plants respond to stress and, from this knowledge, to provide molecular tools to improve crop security and production under stress. To achieve this goal, our lab focuses on the study of translational regulation to identify new key regulators of plant adaptation to stress, especially to the increase of temperatures associated to the current context of climate change. Following this approach, our lab has identified a new family of cochaperones that play a main role in plant adaptation to multiple stresses. In addition, our group is currently analysing the biotechnological use of these proteins to enhance plant performance under environmental stresses.

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Biomolecules from biosludge

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Abstract: Operation of municipal and industrial wastewater treatment plants generate a large amount of sludge with a high biological content. Its management is an important environmental problem with serious costs associated with treatment and disposal. Many proposals have been studied, in particular those related with anaerobic digestion to produce biogas and energy, either directly or previously hydrolyzed to have more fermentable molecules. Nevertheless, at the end, this results in the generation of a new liquid phase that must be treated before being discharged into the environment, and a solid one which cannot be easily applicable as a fertilizer. Additionally, the legal normative is also introducing more restrictions, and other solutions should be conveniently tested.

In this context, the introduction in 2012 of the Europe´s Bioeconomy Strategy addresses the production of renewable bioresources for the future sustainable society. Thus, biological sludge in particular, is one of the best options to develop new products beyond energy uses. The centralized availability, reasonable uniformity and potentiality of the materials involved, are some of the advantages. But for many uses, the break or hydrolysis of the raw material results essential. Afterwards, some separation processes, depending on its use, are necessary, which led to different applications, like new materials, or substrates for other reactions or transformations. In this way, many different options appear to be evaluated in the different steps of the sludge “exploitation”.

One approach to the challenge in which we are contributing, begins with the use of thermal hydrolysis or wet oxidation to break the cell components, regulating the process to obtain desirable intermediate compounds. In this step, the conditions of temperature, pressure, time and catalysts, are crucial. Besides, it should also be considered the impact of possible pretreatments, and the differences in the products depending on the raw material used for hydrolysis. Proteins, carbohydrates, humic and uronic acids among others, together with a large number of different acids, are some of the interesting products that can be obtained. The physical, chemical and biological characterization of the resource and the products are important for the technical process. The first steps of separation should be economical, thus precipitation, centrifugation and membranes separation presenting good forecasts. More specific and new operations should also be analysed, especially in the following separation steps. Various applications, such as films or solid materials, present opportunities for uses, but also their utilization for transformations like in fermentation processes. Many other aspects will also have a definitive interest, in particular the presence of impurities for a given application. Finally, it is evident that a lot of work should be made in most of the options suggested for each step, and also, studies related to the economy and sustainability of the process will be necessary.

Marine Microbiome as a Source of Anticancer Compounds. The experience of PharmaMar

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Thematic Areas: Plenary Conference 4.

Abstract: The marine microbiome contains many billions of genes with the ability to express an unimaginable arsenal of small molecules, as well as proteins, lipids and others classes of products with ecological functions that can be “humanized” for the discovery of new pharmaceuticals, improved enzymes and novel biomaterials, as well as new resources for food and feed stocks, nutraceuticals, diagnostic devices, personal care and cosmetic products and an ever increasing list of marine natural products.

Early studies of the marine environment focused on natural products from invertebrates and tunicates have led to the isolation of several classes of bioactive natural products mainly small molecules such as polyketides and non-ribosomal peptides. However, there is an emerging a rational suspicion, based on marine sponge/tunicate metagenomics that these compounds originally isolated from metazoan organisms are in fact of bacterial origin. The cases of pederin, ecteinascidins (trabectedin, Yondelis©) or didemnins are some examples of the role of marine microbiome as producers of bioactive metabolites with application in oncology. This metabolites are being developed by PharmaMar.

Currently, genomic mining for polyketide synthases (PKS) and non ribosomal peptide synthetases (NRPS) improves the probability of success in drug discovery using marine microorganisms, both in isolated genomes and metagenomes.

The current state of the art in marine biotechnology applied to drug discovery will be discussed under the experience of PharmaMar, a spanish company focused in research, development and commercialization of marine derived drugs for the treatment of cancer.

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PRESENTACIONES KEYNOTE

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Plants as a source of tools for the development of molecular farming and nanobiotechnology

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Thematic Areas: Plant Biotechnology.

Abstract: Plants are a preferred object for the development of biotechnology. The central role that plants play in human society as long-recognized 5F-providers (Food, Feed, Fibres, Fun and *Ph*armacy) have favoured the vision that application of biotechnologies to plant breeding (transgenesis, molecular-assisted breeding, genome editing, *in vitro* culture, etc), or to plant deep characterization (molecular markers, genome sequencing, automated phenotyping) are the only aspects of plant biotechnology. However, although biotechnology applied to plant material is key to modern agriculture, plants are also an important source of biotechnological tools for other applications, often not so close to classical agricultural interests, albeit these are not excluded.

Molecular farming is normally the term used for the exploitation of plants as biofactories of recombinant proteins, peptides, or metabolites. These plant-made products have found their place in very different areas such as biomedicine, cosmetics, enzymes, and others. The field has kept growing and there are already a good number of companies which totally or partially exploit plants in their biological production systems. Even though transgenesis was the first technological approach to these purposes, most present systems are based on transient expression in plants, where plant virus-derived biotechnological constructs have become the most popular approach. Most of these companies were born as start-ups, spinned-off the academic world. This is also the case of our own work on plant viruses.

More recently, plant viruses have become the objects of a new rapidly expanding biotechnology. Viruses promote their own assembly into natural nanoparticles (virions, virus-like particles), mostly or exclusively made out of proteins, collectively named as VNPs (viral nanoparticles). The application of recombinant technology and/or chemical derivatization of these VNPs has revealed them as a versatile and potent approach to the generation of functionalized nanoparticles, with potential of application in many academic and economic areas. Often, the functionalizing molecules are of plant origin, too.

We will be presenting and discussing our own work and developments in these two broad fields of plant biotechnology as nearby examples of their potential.

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MTBVAC, a new freeze dried vaccine of *M. tuberculosis* against TB

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Thematic Areas: Health Biotechnology

Abstract: Tuberculosis remains the leading cause of death from a single infectious agent worldwide. The current TB vaccine, Bacille Calmette-Guérin (BCG), a live attenuated strain of *Mycobacterium bovis*, is the only licensed vaccine against this disease. In infants, BCG is considered to afford protection against disseminated (miliary and meningeal) forms of TB, but appears to be of limited efficacy against adult pulmonary disease, the most common form of the disease responsible for transmission. WHO End TB Strategy has vowed to end the TB epidemic by 2035. To achieve this ambitious goal, among others tools, new effective vaccines capable to prevent pulmonary TB are urgently needed.

MTBVAC is a live freeze dried vaccine based on a genetically attenuated strain of a clinical isolate of *M. tuberculosis*. MTBVAC was constructed to contain two independents non-reverting deletion mutations, without antibiotic markers, in the virulence genes phoP and fadD26 (1). Many genetic regions encoding important immunodominant antigens absents in BCG are still present in MTBVAC. MTBVAC is expected to stimulate specific long-lasting immune responses in the host mimicking those evoked during natural tuberculosis infection without causing disease.

Rigorous preclinical studies have demonstrated adequate attenuation, robust safety and improved immunogenicity and protective efficacy as compared to BCG, providing proof-of-concept to progress to first-in-human Phase 1 evaluation. In 2012, MTBVAC was approved to enter first in human Phase 1 clinical evaluation in healthy adults at Lausanne University Hospital in Switzerland. The study objective was to evaluate safety, local tolerance and immunogenicity of MTBVAC in comparison with BCG. Following the successful Phase 1(2), a Phase 1b trial in the target population, heathy newborns in an endemic country (South Africa) was initiated in 2015. The study goal was to evaluate the safety and immunogenicity of MTBVAC in comparison with BCG in newborns living in a high-burden TB-endemic region. In both studies, MTBVAC was well tolerated and as safe as BCG and induced a dose dependent and stronger Th1 response. This year two new clinical trials have started, a dose-defining Phase 2a in newborns infants and a Phase2a in BCG-vaccinated adolescents/adults in South Africa, to select the final dose formulation of MTBVAC to be evaluated in efficacy studies.

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Yarrowia lipolytica as a microbial host for flavors and fragrances production

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Thematic Areas: Food Biotechnology.

Abstract: Flavors and fragrances constitute a large part of the market of natural products and they represent a wide variety of chemical groups including ketones, aldehydes, alcohols, carboxylic acids, esters, lactones and terpenoids[1]. This class of compounds is widely used in the food, cosmetic, pharmaceutical and chemical sectors, and constitutes platforms to manufacture everyday life items. More than a quarter of the world market for food additives is represented by the flavor market[1] and it is expected that in 2020 its market will be close to $ 15.1 billion[2]. However, the demand of consumers for flavors and fragrances produced by natural means has led to a decrease of natural resources used for this purpose, being of great importance the development of biotechnological processes that meet consumer needs. Biotechnological production arises as an interesting alternative for its production, since the products obtained are labelled as “natural”.
Yarrowia lipolytica is a non-conventional and strictly aerobic yeast, with GRAS status, with many biotechnological applications due to the wide range of substrates that can use as carbon source and the ability to produce a large variety of metabolites with industrial interest. This yeast has been proved to be a robust cell for the biotechnological production of compounds that can be used as additives in food industry, such as organic acids, enzymes, biosurfactants, sweeteners and aroma and fragrances compounds[3].
γ-Decalactone (GDL) is a peach-like aroma widely used in many industrial applications and *Y. lipolytica* is able to produce it by biotransformation of ricinoleic acid, the main constituent of castor oil. The production of GDL has been intensively studied in order to better understand all process and optimize it. The role of lipases in substrate hydrolysis, the effect of substrate concentration, dissolved oxygen concentration and different fermentation strategies - batch and step-wise fed-batch – and bioreactor designs (STR and airlift) in the GDL production was investigated[4]. The characterization of GDL production by genetic modified strains at lab-scale bioreactor was also performed.
The potential of *Y. lipolytica* to produce other flavors and fragrances were also explored. 2-Phenylethanol (2-PE) is an aromatic alcohol with a fresh rose scent, and can be synthesized in yeast through shikimate and Ehrlich pathways. Either way, the main bottleneck for 2-PE production is its toxicity due to the fact that concentrations between 2 and 3 g L^{-1} inhibit the cellular growth[5]. Among several microorganisms able to produce 2-PE, *Y. lipolytica* appears to be promising due to its interesting characteristics, such as the Crabtree negative trait. Based on this, different strategies have been explored in order to improve 2-PE productivity combining strain selection, medium composition, culture condition optimization and application of *in situ* product removal techniques (ISPR).
New insights on the biotechnological production of GDL and 2-PE were achieved contributing with some different strategies to increase the flavors production, and a maximum GDL and 2-PE concentrations of 7 g L^{-1} and 2 g L^{-1}, respectively, were achieved.

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Sustainable chemical processes in ionic liquids and supercritical carbon dioxide

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Thematic Areas: Biocatálisis – Key Note.

Abstract: The development of sustainable chemical processes turns around two main axes: the selectivity of catalytic transformations, and the easy and clean separation of pure products. The use of enzymes in ionic liquids (ILs) was reported great advantages (*i.e.* improved activity and enantioselectivity, enhanced stability, etc.),[1] and its combination with supercritical technologies was resulted in synergetic and outstanding platforms for developing (multi)catalytic green chemical processes under flow conditions able to directly provide pure products.[2] This has been demonstrated by several examples of flow synthetic processes in IL/scCO_2 biphasic systems at 100 bar and 40-100ºC (*e.g.* DKR of *rac*-1-phenylethanol,[3] biodiesel,[4] monoglycerides,[5] etc.
Straightforward protocols for carrying out biotransformations, product recovering and biocatalysts/ILs reusing without any loss in the overall greenness character of the process, have been developed in recent years. Sponge-Like Ionic liquids (SLILs) are hydrophobic ILs based on cations with long alkyl side-chains, *e.g.* octadecyltrimethyl-ammonium bis(trifluoromethylsulfonyl)imide, etc.), which behaves as sponge-like systems by switching from liquid to solid phase with temperature.[6] Based on this new property, the SLILs have been applied for developing straightforward and clean approaches for biocatalytic synthesis and extraction of nearly pure compounds of added value (*e.g.* terpene esters,[7] biodiesel,[8] etc.).As can be seen in Figure 1, the SLILs are able for dissolving (soaking) hydrophobic compounds at temperatures compatible with enzyme catalysis (*e.g.* vegetable oil and methanol mixtures are monophasic in [C_{18}mim][NTf_2] at 50°C[7]). By cooling reaction mixtures based on these SLILs under room temperature, they are solid phases that can be fractionated by centrifugation, allowing the release (wringing out) of reaction products from the SLIL net, and resulting in two phases: a solid SLIL phase and a liquid phase containing synthetic products (flavour esters, biodiesel, etc.). This approach for product separation can be improved by using centrifugal filters, demonstrating the excellent suitability of this straightforward and green approach for carrying out synthesis and separation of pure products with full recovery and reuse of the reaction system (SLIL and biocatalyst).[6]

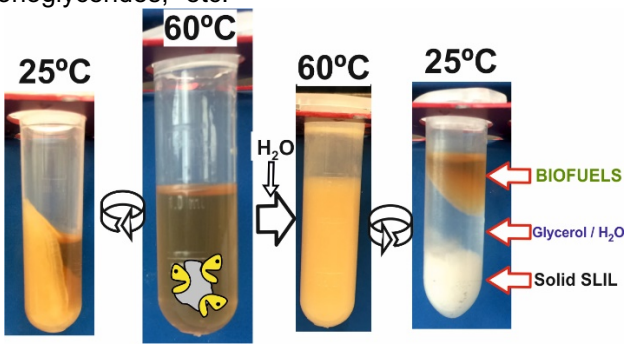


Figure 1. Schema of the protocol for the biocatalytic synthesis of biofuels in SLIL, and product separation with full recovery and reuse of the SLILs

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Sustainable integrated Algae Biorefinery for the production of bioactive compounds for Agriculture and Aquaculture (SABANA)

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Thematic Areas: 6. Biotecnología azul

Abstract: Microalgae production capacity worldwide is limited by (i) the low scale of current production facilities, in addition to (ii) excessive production costs of the technologies already available and (iii) the large amount of resources (mainly fertilizers) needed, competing with traditional agriculture. To solve these problems larger reactors involving improved technologies are required, in addition to combine the production of microalgae biomass with the treatment of effluents, both liquids and gases, being mandatory. On this way the microalgae production cost would be reduced and it would be improved the overall sustainability of the process. However, when using effluents, the actual regulation avoids the utilization of the resulting biomass for human related applications, it being possible to use it in agriculture and aquaculture, in addition to biofuels and other non-human related applications.
A market analysis shows that agriculture and aquaculture are highly interesting markets for microalgae biomass. Thus, SABANA project aims at developing a large-scale integrated microalgae-based biorefinery for the production of biofertilizers, biostimulants, biopesticides, in addition to aquafeed and feed additives for aquaculture. To this end, the utilization of non-usable water such as seawater or wastewater, in addition to nutrients from wastewaters (sewage, centrate and pig manure) is proposed. Moreover, technologies related with the production, harvesting and processing of the biomass are also proposed. The objective is to achieve a sustainable zero-waste process at a demonstration scale up to 5 ha.
Techno-economic analysis already completed demonstrated that to achieve production costs about 1 €/kg to use optimally designed and operated open reactors and to recover nutrients from wastewaters (urban, digestate or manure) is required. Additionally, the downstream processing of the biomass must be simplified and focused to transform the entire biomass into products without releasing wastes. Sustainability analysis confirm that proposed technologies are sustainable, minimizing the impact versus conventional wastewater treatment processes, thus microalgae related wastewater treatment processes allows to reduce the energy consumption up to 0.2 kWh/m^3 versus energy consumption of 0.5 kWh/m^3 of conventional systems. Optimized open raceway reactors allow minimizing the energy consumption below 5 W/m^2. Concerning harvesting, it was demonstrated that flocculation-sedimentation + centrifugation minimize the energy consumption and cost of microalgae biomass collection to lower than 0.1 kWh/$kg_{biomass}$. Concerning processing it has been demonstrated that to perform an initial cell disruption step is mandatory whatever the final application of the biomass.The utilization of high pressure homogenization is recommended. Anyway the energy consumption of the process must be keep below 2 MJ/$kg_{biomass}$ to be sustainable, for that the optimization of operation conditions being necessary. Final processing of the biomass by enzymatic hydrolysis or extraction using adequate solvents allows to obtain the final products suitable to be used. The quality and type of final products is largely a function of quality of produced biomass. Thus, on SABANA project up to 100 strains has been evaluated for their use in agriculture or aquaculture. In this sense, in addition to the biochemical composition (proteins, lipids, carbohydrates), the amino acids and fatty acids profile, in addition to the phytohormones content has been determined, the performance of these strains in different bioassays being also evaluated. Data shows as the concentration of phytohormones is much higher in some green algae than in cyanobacteria, it being also higher in freshwater strains that in seawater strains. In contrast, seawater strains are richer in polyunsaturated fatty acids highly demanded in aquaculture. Bioassays performed confirm the reliability of produced biomass to be used on both applications, agriculture and aquaculture.
Summarizing, SABANA project is providing real figures from a microalgae related biorefinery at demonstration scale, the reliability of the proposed technologies being confirmed at real outdoor conditions, accomplishing industrial standards, and closely with the market. Business plan demonstrate that the proposed technologies are profitable, it sustainability being mainly a function of the type of resources involved on the production step. SABANA is expected to conclude with real commercial processes demonstrated at commercial level.

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Exploiting bacterial metabolism for removal of aromatic pollutants and biowaste revalorization.

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Abstract: Aromatic compounds constitute the second most abundant carbon source in nature, and many of them represent major environmental pollutants and are present at high amounts in the biowaste generated by anthropogenic activities. Bacteria have evolved an extremely high versatility towards the degradation of a wide variety of aromatic compounds through complex metabolic and regulatory networks that function under aerobic and/or anaerobic conditions. Many different aromatic compounds are channeled through different peripheral pathways into a few central intermediates that enter into the central metabolism via some few aromatic central pathways. This "aromatic funnel" can be exploited for the bioconversion and revalorization of complex mixtures of aromatic compounds such as those derived from polluted environments or lignocellulose biowaste. Nevertheless, due to their complex metabolism and toxicity, aromatics are usually non-preferred carbon sources and bacteria have evolved regulatory mechanisms to shut-down the expression of the aromatic degradation pathways when more preferred carbon sources are available (carbon catabolite repression). Here some examples of systems metabolic engineering of the aerobic bacterium *Pseudomonas putida* to develop optimized biocatalysts for revalorization of lignin-derived compounds will be presented. The characterization of a novel redox-sensing two-component regulatory system that controls carbon catabolite repression of the anaerobic catabolism of aromatic compounds in the denitrifying bacterium *Azoarcus* sp. CIB will be also presented, and its use to design whole-cell redox biosensors will be discussed.

The impact of nanobiotechnology in the design of new medicines

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Thematic Areas: Nuevos retos en biotecnología

Abstract: Biological molecules including antigen and therapeutic proteins and monoclonal antibodies, are taking an increasing space in the industry pipelines. Despite their potency, the difficulties of these macromolecules for overcoming biological barriers and reach the intracellular targets have limited their full exploitation.

Fortunately, the continuously improved understanding of the biological barriers and the molecular biology associated to pathological conditions is paving the way for a rational design of formulations of biotech compounds based on the use of nanotechnology. Our laboratory has significantly contributed to this field. As an example, in the 90's we were the first to report that nanoparticles made of either PLA-PEG or chitosan were efficient vehicles for the transmucosal delivery of proteins and antigens. The result of our subsequent efforts is an array of nanotechnologies that can be used to deliver proteins across mucosal surfaces, and, also, to facilitate their intracellular delivery following parenteral administration.

In my presentation, I will focus on the design of protein carriers that could be used in different therapeutic areas: (i) oral delivery of peptides intended to treat either local or systemic diseases, (ii) delivery of mAb targeted to intracellular onco-proteins, as new oncological treatments, (iii) nanovaccines designed to prevent diseases, i.e. HIV or E. Coli infections as well as to treat diseases, i.e. diabetes and multiple sclerosis.

Overall, our experience in this field has benefited from integrative approaches adopted by specifically designed consortia. Hopefully, the results of these cooperative efforts will help to accelerate the progress of a rational design of protein-based nanomedicines.
More information about these projects can be found at:

http://www.usc.es/grupos/mjalonsolab/

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The following researchers have contributed to the work related to the oncology field: Jose Crecente, Tamara Gómez, Ana Olivera and Dolores Torres from the USC and Africa González from the University of Vigo.
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Modification of regulatory systems in *Streptomyces* to increase antibiotics production.

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Thematic Areas: Microbial biotechnology (keynote). Systems biotechnology: metabolic modeling and biosynthetic pathways.

Abstract: The sequencing of multiple genomes of bacteria of the genus *Streptomyces* has allowed to corroborate that all of them have genetic information to produce more than 20 different compounds with possible biotechnological interest. However, these strains only produce a very limited number of these molecules in laboratory cultures. So, finding ways to activate these silenced routes constitutes one of the big challenges in the research in these mycelial bacteria. Multiple strategies are being addressed to obtain this activation and, among others, our group is carrying out the study and modification of different regulatory systems as a way to increase the production of metabolites of interest and, if possible, to activate any of the silenced routes.

Our group is studying the regulation exerted by several two-component systems (TCS) and a pair of proteins with XRE/DUF397 domains on the production of antibiotics using the model organism *Streptomyces coelicolor*.

Throughout this study we have identified a special TCS containing two histidine kinases (HK) and a single response regulator (RR) to which the two HKs of the system seemed to control differently. This system, that acts as a positive regulator in the production of antibiotics and differentiation, directly controls the production of actinorhodin, the blue antibiotic produced by *S. coelicolor* (Rico, et al., 2014a; Rodriguez et al., 2015). In addition, the two kinases of this system control, in some way not still identified, an orphan response regulator (denominated Aor1) that is a master regulator whose deletion causes drastic changes in the expression of more than 1000 genes (Antoraz et al., 2017). We are also studying two TCSs that negatively regulate both antibiotic production and morphological differentiation and have obtained deleted mutants in both negative systems. The generation of deleted strains in the negative TCSs and their use as host strains for heterologous routes has made possible to verify that this is a good strategy to increase the heterologous production of already known compounds. Likewise, we have shown the potential that overexpression of the AbrC3 RR of the positive TCS in other organisms supposes to stimulate the production of their endogenous secondary metabolites (Rico et al., 2014b; Becerril et al., 2018).
Our regulatory study also includes a group of two proteins with XRE/DUF397 domains that have been proposed as possible toxin-antitoxin systems. The functional study of one of these pairs showed that, under the tested conditions, it did not behave as such systems, while its overexpression drastically increases the production of antibiotics in *S. coelicolor* and in other *Streptomyces* species (Santamaría et al., 2018).

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BIO TEC 2019

CONGRESO NACIONAL DE BIOTECNOLOGÍA

10 - 13 JUNIO VIGO

PREMIO SEBIOT JÓVENES INVESTIGADORES

Universidade Vigo

SEBiot Sociedad Española de Biotecnología

In vitro metabolic engineering at the interface between solid materials and enzymes

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Thematic Areas: Biocatalysis.

One of the major challenges of sustainable chemistry is expanding the palette of bio-based chemicals that can replace, or at least ameliorate, the exploitation of fuel-based chemicals. Cell-free or *in vitro* metabolic engineering using soluble enzymes is an emerging and versatile approach that seeks to increase the selectivity and productivity of chemical biomanufacturing processes. However, soluble and isolated enzymes present major issues in terms of efficiency, stability and re-usability that hamper industrial applications.
This talk will be a journey through the fascinating world of enzyme engineering to solve intrinsic problems of naturally evolved enzymes to work as industrial biocatalysts. In this journey, I will invite the audience to visit the scientific contributions that my collaborators, supervisors, lab mates and I have delivered to this field. I will offer a holistic view about the engineering of cell-free biosynthetic pathways starting from the molecular design of individual enzymes at the interfaces with artificial chassis (solid materials) to the systemic interactions of multi-functional biocatalytic systems confined into that artificial microenvironments. I have found in nature the inspiration to create synergies between enzymes and solid materials to make better biocatalysts. Along the journey, I will stop at different universities and research centers around the world to illustrate that both internationally and multi-disciplinary are required to advance in the fundamental knowledge that boosts the technological development of any system; and particularly the biocatalytic systems. Finally, I will provide a handful of applications that have been developed in my lab based on this holistic view and through an integrative approach that aims fabricating more efficient multi-functional heterogeneous biocatalysts for manufacturing highly added chemicals from renewable materials.

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Establishment of plant cell suspension cultures for the production of bioactive compounds from *Kalanchoe*.

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Thematic Areas: Plant Biotechnology.

Abstract: Plant cell suspension cultures constitute an efficient and simple biological system for the large-scale production of phytochemicals with interesting bioactivities and the study of initial cell density in suspension culture is an equivocally underestimated factor [1]. In this study, we develop the initial steps for the initiation of these cell systems, using three species belonging to the subgenus *Bryophyllum* (genus *Kalanchoe*, Crassulaceae): *Bryophyllum daigremontianum* (BD), *Bryophyllum tubiflorum* (BT) and *Bryophyllum* x *houghtonii* (BH). This plant genus presents an increasing interest because of their associated biological properties, acting as a potential source of chemopreventive and antioxidant agents [2]. We firstly tested different plant growth regulators (PGRs) treatments (using different combinations of 2,4-dichlorophenoxyacetic acid, 2,4-D, and 6-benzylaminopurine, BAP) in order to obtain dedifferentiated callus with convenient friable aggregation from different *Bryophyllum* explants cultured *in vitro*. Explants were placed in solid MS medium [3], supplemented with PGRs and 3% sucrose and solidified with 0.8% agar at pH=5.8, and incubated at 25 ºC in the dark. After 5 weeks, calli were obtained and they were maintained for successive subcultures, every 3 weeks, until a large cell mass was generated. Later, friable callus masses were transferred into liquid culture media and incubated under the same conditions with continuous shaking at 120 rpm. Different initial cell densities, ranging from 50 to 100 g $\cdot L^{-1}$, were tested in order to monitor culture growth along time using several physiological responses, such as fresh weight and medium conductivity and pH. Measurements were performed every 5 days during a total period of 30 days. As cultures grew, we observed an increase in fresh weight in parallel to a decrease in culture media conductivity and stable pH values, thus indicating that growth was favourable under the used conditions. Altogether, our results satisfy the initial steps on the establishment of cell suspension cultures for the production of valuable phytochemicals and give information about the growth kinetics (lag phase, exponential phase, stationary phase and death phase) of cultures, in order to characterise their behaviour towards large-scale metabolite biosynthesis.

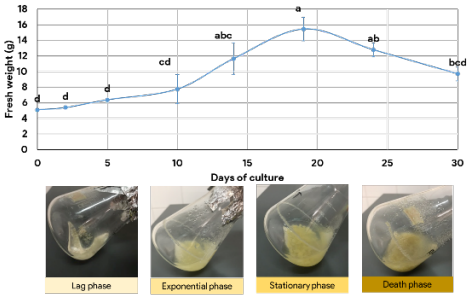


Fig. 1. Growth kinetic of *Bryophyllum* plant cell suspension. Initial cell density: 100 g per liter. Different letters indicate significant differences at $p<0.05$. Vertical bars indicate standard deviation.

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Microbial biopesticides to control fungal diseases in grapevine. Production, field monitoring and analysis of the plant response.

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Thematic Areas: BIOTECNOLOGIA VEGETAL

Abstract: Control of plant diseases caused by fungi is generally achieved through conventional chemical fungicides. However, the outcome of the public concern and the governmental restriction for their use has prompted a rising interest in the use of natural products. These products, such as microbial pesticides and natural extracts, are effective and many are considered of low risk for consumer health and the environment. Grapevine diseases such as grey mold, powdery mildew and downy mildew that are caused by *Botrytis cinerea*, *Erysiphe necator* and *Plasmopara viticola,* respectively, are responsible for important economic losses worldwide.

Strains of lactic acid bacteria and *Bacillus* species are potential candidates for their use as biocontrol agents of grapevine diseases due their potent antagonistic activity against plant pathogens. Strains of *Lactobacillus plantarum* UdG (1) and *Bacillus amyloliquefaciens* UdG (2) were selected from a wide collection of plant-associated bacteria to evaluate their performance to control fungal diseases in grapevine, and due to the production of several types of antimicrobial peptides.

We have optimized the production of these strains in bioreactor and different formulations have been prepared to obtain microbial biopesticides to be used in the field tests. After a preliminary *in vitro* and *ex vivo* screening, the best formulations were chosen for field experiments. In addition, we have developed molecular methods to monitor viable cell population based on the use of the nucleic acid-binding dye PEMAX, a strain-specific DNA marker, and quantitative PCR (3). These monitoring methods were previously validated in grapevine plant material and they are currently used in field experiments located in Penedès region (Catalonia, Spain) and Le Roussillon region (Occitanie, France). Furthermore, the objective of this work is to study at transcriptomic level the plant response upon the biopesticide application using the next-generation sequencing RNAseq. Consequently, it is expected that knowing the upregulated genes a quantitative PCR could be designed to follow the plant response over time.

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Phenolic compounds profiles of different parts of the plant *Cynara cardunculus* L. var. *altilis*

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Thematic Areas: Plant Biotechnology

Abstract: *Cynara cardunculus* L., commonly known as cardoon, is a perennial plant that belongs to the *Asteraceae* family. Native to the Mediterranean basin, it is also found in other regions of the world such as Argentina, Africa, Chile, China, United States, and Peru [1]. It has been used in folk medicine due to its diuretic and dyspeptic properties, such as liver tonic and in hepatic disease treatments [2]. Its flowers are the most commonly used part due to its milk coagulating properties (vegetable rennet in goat and sheep cheeses manufacture), but its immature heads are also widely consumed as food in several dishes. In recent years, the expansion of different industrial applications of crop residues (*e.g.* forage, paper pulp industry, or biodiesel and energy production) has been a key lever to the growth of Mediterranean cardoon cultivation [3].
Cardoon is considered a functional food as a result of its richness in compounds with high added-value and proved health benefits [1,4]. Several studies make reference to its high content in phenolic compounds, such as phenolic acids and flavonoids including anthocyanins. The polyphenolic content of different plant parts of this species is strongly influenced by environmental conditions and harvest stage. Therefore, studies regarding the influence of these factors are extremely important to better understand the benefits associated to this plant. In the present study, the phenolic compounds composition of different plant tissues of cardoon (petioles, blades, heads, bracts and seeds) was analysed. Samples of cultivated cardoon (var. *altilis)* were collected in Greece in June 2018. The phenolic composition was analysed in hydroethanolic extracts by HPLC-DAD-ESI/MS.
Cardoon petioles, blades, heads, bracts and seeds presented differences in the phenolic composition, as also in their contents of individual compounds. Blades showed the highest number of phenolic compounds, being identified thirteen molecules. In petioles and bracts, a total of eleven phenolic acids and flavonoids were found, while in heads and seeds a total of nine and seven molecules were profiled, respectively. Blades were the plant part with the highest concentration of phenolic compounds, followed by bracts, petioles, seeds and heads. Caffeoylquinic and dicaffeoylquinic acids were the main compounds present in all the studied tissues.
The results obtained demonstrated that phenolic compounds allocation varies among cardoon plant parts harvested at the same time period, with blades being the part that showed the highest abundance and variety of phenolic compounds.

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Preparation and validation of a DLC (Drug-in-Liposome-in-Cyclodextrin) system in cancer cell lines

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Thematic Areas: Biotecnología de la salud

Abstract: Camptothecin has been identified as a potential drug for treating cancer. It is a plant alkaloid obtained from *Camptotheca acuminata*, whose target is topoisomerase I, a nuclear enzyme that solves errors produced in DNA during the cell replication. This compound has a very low solubility (1), so some changes has been done in the original drug in order to solve that drawback. However, the problem is that these modifications reduce the potential cytotoxicity of the drug.

Therefore, this work presents a research whose aim is the synthesis of a carrier with a liposomal nature that captures camptothecin, by forming an inclusion complex (DLC system, Drug-in-Liposome-in-Cyclodextrin). This system increases the solubility and the stability of the drug.

For this purpose, the inclusion complex (carboximetil-β-cyclodextrin)-camptothecin (CMCD-CAMP) was synthesized at two different pHs (3,5 and 7,4) and the best results were obtained at pH 7,4.

Focusing on liposomal carriers, two different types were synthesized (by the addition or not of polyethylene glycol, PEG). Their stability and efficacy were compared. The results showed that both systems were equally stable during 21 days, and some differences were observed in size (140 nm in liposomes without PEG and 120 nm in liposomes with PEG).

After that, the inclusion complex was encapsulated into liposomes by different techniques (pH gradient and thin layer method). The most efficient one was the thin layer method (2) where the mixture of dried liposomes was put in contact with the solution previously formed (CMCD-CAMP inclusion complex) and the liposomes are able to self-assembly the inclusion complex.

Furthermore, the loading capacity in the liposomes was evaluated, and the results showed that liposomes without PEG have higher loading capacity compared with systems with PEG. Despite that fact, the system with PEG presents some important advantages in the organism (PEG is able to evade the immunology response and therefore, increase their circulation time). For that reason, both systems were tested in cellular cultures.

Due to the fact that camptothecin is a drug mainly used for the treatment of colon, breast and lung cancers, the *in vitro* assays were carried out in the three cellular lines: HTT-116 (colon), BT-474 (breast) and H-460 (lung). The new system was more effective in breast cell line, although it was highly effective in all cellular lines. Comparing the systems with and without PEG, the best results were achieved by DLC system with PEG.

Summing up, a new system has been developed, increasing the solubility of camptothecin, and therefore, less amount of drug should be added to reach the same result, reducing the side effects.

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Nanoencapsulation of controlled size superparamagnetic nanoparticles for biomedical applications

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Thematic Areas: Nuevos retos en biotecnología; Nanobiotecnología

Abstract: There has been an increased use of magnetic iron oxide nanopartícles (IONPs) with superparamagnetic properties for various biomedical applications being used as promising agents in detection and analysis, or in different therapies such as targeted drug delivery since they have a high magnetic moment only when an external magnetic field is applied [1–2]. The final properties of the IONPs are strongly influenced by the synthesis route and conditions due to its unique size dependent behaviour. A soft chemistry technique with a growing interest is the microemulsion reaction method due to the efficient control of size, shape, monodispersity and composition of NPs obtained. Water-in-oil (W/O) microemulsions consist on aqueous domains dispersed in a continuous oil phase, where the microdomains are stabilized by an interfacial film of surfactant molecules. Microemulsions work as nanoreactors where the synthesis of the desired NPs take place, involving a chemical reaction of co-precipitation.

In previous studies regarding novel point-of-care (POC) devices for cancer diagnosis, superparamagnetic IONPs were functionalized to specifically bind to Prostat specific antigen (PSA) in order to perform magnetic lateral flow immunoassays (LFIA) that were evaluated with a novel Scanning Magnetic Impeditive (SMI) sensor, which is highly sensitive to the time-varying magnetic field produced by the IONPs [4]. A clear correlation between the sensing variable and the protein concentration of the samples probed the ability of the method to quantify PSA within the clinical range of interest using the superparamagnetic IONPs as labels arising a promising proof of concept for a fast, easy-to-use, new analytical method for broad, POC screenings. However, future improvements in NPs amplification at the lateral flow strips and in sensor instrumental design will further improve detection limits. An alternative could be IONPs encapsulation using different type of nanocolloidal systems, such as nanovesicles. Nanovesicles (e.g., liposomes, niosomes or transfersomes) are an important family of organic NPs, produced by bottom-up nanotechnology. They are considered soft NPs because interactions among their molecular components are similar to those arising from biological systems. Moreover, nanovesicles entrapping magnetic IONPs are also promising therapeutic systems which can be guided to act in a specific site of action by an external magnetic field and used as an alternative to conventional chemotherapy through heat-triggered drug delivery or hyperthermia [2].

The feasability of the synthesis of magnetic IONPs with average diameters between 4 and 7 nm through precipitation in W/O microemulsion has been demonstrated. The microemulsion stability region was determined in a ternary diagram as a function of the aqueous phase (aqueous solution containing $FeCl_2$ and $FeCl_3$) using the titration method using CTAB as a main surfactant, 1-butanol as a cosurfactant and with 1-hexanol as the continuous oily phase. These monodisperse IONPs were further encapsulated using size-tuned nanovesicles obtained by using a modified ethanol injection method (EIM) controlling the operating conditions based in a previous study in which factorial experimental design and analysis of variance (ANOVA) was applied [5]. Systems formulated were characterized in terms of size and shape by DLS (Nanozetasizer from Malvern) and TEM.

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Minicircle Gene Delivery to Transiently modulate Mesenchymal Stromal Cell function in the context of angiogenesis-based regenerative medicine

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Thematic Areas: Health Biotechnology

Abstract: The modulation of angiogenesis is a potential therapeutic approach for many diseases, either targeting the stimulation of angiogenesis in ischemic heart disease, peripheral arterial disease (PAD), or inhibiting this process, namely in certain cancers. One promising way to treat PAD is to explore the potential of mesenchymal stromal cells (MSCs), which are able to setup a regenerative environment through secretion of soluble factors. The therapeutic potential of *ex vivo* expanded MSCs can be potentiated by introducing pro-angiogenic genes in cells prior to administration. Conversely, blocking their expression at post-transcriptional level, using RNA interference (RNAi) technology, might be a promising strategy to slow down tumor growth. Therefore, the transient modulation of the expression of the pro-angiogenic VEGF (vascular endothelial growth factor) gene in human MSCs could result in an improvement of their therapeutic features, while maintaining their intrinsic low-immunogenicity.

Our approach to modulate VEGF expression by MSCs was based on their transient modification by transfection with minicircles (small non-viral DNA vectors) to 1) promote angiogenesis by transfection with minicircles encoding VEGF and 2) inhibiting angiogenesis with small-interfering RNAs (siRNAs) expressed as short-hairpin RNAs (shRNAs) from minicircles.

Minicircles were designed to contain the adequate sequences, produced in *Escherichia coli* and purified by established chromatography-based processes.

One of the most innovative aspects of this work is the use of minicircle vectors that were rationally engineered to increase the transfection of MSCs by:

1) Promoting VEGF expression: MSCs transfected by microporation with minicircles showed similar cell viability (>90%), but higher recovery (70% vs 50%) compared to conventional plasmid vectors. Supernatant and gene expression analysis revealed that two days after transfection, MSCs modified with minicircles secrete 60-fold more protein and express 130-fold more VEGF mRNA copies than non-transfected cells. The increased angiogenic potential of those cells was also demonstrated *in vitro* by two different functional assays: cell tube formation assay and HUVEC migration assay. MSCs maintained their immunophenotypic profile and differentiation capacity after being genetically modified, assessed by flow cytometry analysis and multilineage differentiation studies, respectively.

2) Blocking gene expression: MSCs were lipofected with minicircles encoding VEGF interference RNAs. Overall, RT-qPCR results revealed a VEGF-mRNA knockdown of approximately 50% after synthetic siRNA transfection of MSCs. Similarly, ELISA results indicated a 40% decrease in VEGF secretion by transfected cells. Also, the transient modified MSCs induced a decreased potential of tube formation by HUVECs, suggesting a reduced angiogenic potential. Unexpectedly, the MSCs transfected with the minicircles encoding interference VEGF-RNA showed an increased VEGF expression (~3-fold increase on VEGF mRNA copies and a ~4-fold increase in protein production). Although further studies are required, these discrepancies appear to be related to the incorrect processing of the transcribed shRNA into a functional RNAi.

In conclusion, minicircle-mediated VEGF gene delivery, combined with the unique properties of human MSCs, could represent a promising *ex vivo* gene therapy approach with improved angiogenic potential

for numerous conditions, including PAD. This study also provides insights regarding the implementation of a RNAi-based system in MSCs, consequently diminishing their pro-angiogenic potential, aiming at slowing down tumor angiogenesis.

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Synthesis of potential prebiotic sugars by enzymatic extracts from the nectaries yeast *Metschnikowia reukafii*.

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Thematic Areas: Food biotechnology.

Abstract: Prebiotics are "substrates utilized by host microorganisms conferring a health benefit" [1]. The most widely accepted prebiotics are the non-digestible short chain carbohydrates with a degree of polymerization between two and sixty [2]. Currently, fructo-oligosaccharides (FOS), galacto-oligosaccharides (GOS) and isomalto-oligosaccharides (IMOS) are the most recognised bioactive carbohydrates and their production is a growing market. However, gluco-oligosaccharides, carbohydrates that contain glucose units linked by α (1 ☾1), α (1 ☾2), α-(1 ☾3) and α-(1 ☾6), have also potential prebiotic properties and industries has an increasing interest in this sort of compounds.

Metschnikowia reukaufii (Saccharomicetales: Metschnikowiaceae) is a predominant nectar-inhabiting yeast responsible for depletion of nectar sugar content and other important ecological functions on the flower nectaries. *M. reukaufii* has developed the capacity to grow under extreme conditions, characterized by a high osmotic stress, low nitrogen content and the presence of secondary compounds [3].

In this study, a novel transglucosylation activity from *M. reukaufii* extract was analyzed. The potential enzyme associated with this activity hydrolyzed sucrose to fructose and glucose, but also synthetized different transglucosylation products when a high concentration of sucrose was used. The new transfer compounds were identified by high performance anion exchange chromatography coupled with pulsed amperometric detection and purified using semi-preparative liquid chromatography. In addition, the structure of these products were characterized by mass spectrometry and 2D nuclear magnetic resonance techniques.

Interestingly, the characterized sugars contained a wide variety of glycosidic bonds, among them oligosaccharides that are natural components of honey [4].

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Sequential three-step biocatalytic process for the transformation of milk whey into the novel sweetener D-tagatose

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Thematic Areas: Biocatalysis. Food Biotechnology

Abstract: D-Tagatose is a rare and safe ketohexose; structurally, it is an isomer of D-galactose and C-4 epimer of D-fructose. D-tagatose is a potential candidate for sugar substitute since it has 92% sweetness as compared to sucrose with less than half caloric value and does not show any laxative effect and aftertaste. Various physiological studies on glycemic index have shown that D-tagatose does not elicit any increase in blood glucose level, thus making it ideal for diabetic patients. It does not cause tooth decay and supports the growth of healthy intestinal microflora.[1]

The current production process of D-tagatose is based on chemical isomerization of D-galactose, which may readily be obtained by hydrolysis of lactose. Due to the complexity and the low yields of the chemical production method and the environmental implications, several research groups have tried to develop an enzyme-catalyzed process for the production of D-tagatose. In particular, it has been found out that some variants of L-arabinose isomerase may catalyze the isomerization of D-galactose into D- tagatose.[2]

Our objective was to develop an efficient methodology for the transformation of a cheap and available feedstock, milk whey, into D-tagatose. Milk whey is the liquid portion obtained during cheese-making or during coagulation of the milk casein process as a byproduct, whose annual production is higher than 180 million tonnes. However, whey creates significant environmental and health issues due to its large volume production and high organic content. Therefore, proper management (treatment or re-use) of cheese whey, which would be economical and ecofriendly, is required.[3, 4]

In our work, three sequential reaction steps were developed to convert milk whey into D-tagatose. The first involved a commercial β-galactosidase from *Bifidobacterium bifidum* to break lactose into D-glucose and D-galactose. In the second step a microorganism was employed for the elimination of D-glucose. Finally, the arabinose isomerase L-AI US100 from *Bacillus stearothermophilus* isomerized galactose into D-tagatose. All reactions were analyzed by high performance anion-exchange chromatography coupled with pulsed amperometric detection (HPAEC-PAD).

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Production and characterization of a functional beverage obtained by fermentation of kiwifruit juice with kefir grains

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Thematic Areas: Biotecnología Alimentaria

Abstract: In recent years there is an increasing consumer interest in consuming functional foods containing biologically active food components with a positive effect on well-being and life prolongation (Homayouni et al., 2012). Among these functional foods, those containing probiotics, prebiotics and symbiotics can produced a great number of positive effects on overall health (Fujiya and Kohgo, 2010). In this way, a healthy-improving food with a high nutritional value named kefir, has reported to possess numerous health benefits, including the stimulation of the immune system, regulation of blood pressure, inhibition of tumor growth, metabolism regulation through improved digestion, among others (Chen et al., 2008).
Kefir has been described as a refreshing, slightly carbonated beverage with a low alcohol and acetic acid content, which is produced by fermentation of cow, goat, ewe and buffalo milk with kefir grains. These grains are composed by a matrix of protein and polysaccharide, which contain a complex microbial consortium composed of lactic acid bacteria (LAB, 10^8 CFU/g), yeast (10^6–10^7 CFU/g) and acetic acid bacteria (AAB, 10^5 CFU/g) (Teijeiro et al., 2018). However, in recent years, new kefir formulations have been produced by using other food sources including food wastes like whey, milks from soy, peanut, cocoa pulp, walnut included fruit juice. The kiwifruit, is one of the newer high value fruit crops that has gained national and international acceptance, due to its content in vitamin C, potassium, folate, dietary fiber and polyphenols and its attractive flavor, nutritional quality and healthy function (Lyu et al., 2018). Therefore, in the present work, we studied the fermentation kinetics of kiwifruit juice (*Actinidia Lindl*. spp.) with kefir grains for production of a fermented beverage with a high content of viable probiotic cells.
The repeated batch fermentations were carried out in duplicate in 4 consecutive subcultures of 24 h in Erlenmeyer flasks containing 60 mL of juice which were inoculated with 1.8 g of kefir grain (wet weight), with agitation (150 rpm) and without agitation. Every 24 h, the grains were separated from the fermented juice, weighed and used to inoculated newly 60 mL of fresh juice. The fermented samples from every subculture were characterized by determining the evolution of the following variables: pH, total free biomass, wet weight of the kefir grain, counts of viable lactic and acetic bacteria and yeasts, antibacterial activity and the concentrations (by HPLC) of sugars, organic acids and alcohols.
The results obtained showed the possibility of producing fermented beverages by fermentation of kiwifruit with kefir grains without agitation, containing relatively levels of viable cells (between 10^7 and 10^8 CFU/mL of lactic and acetic acid bacteria and yeasts) higher than the recommended dose of viable probiotic (10^6 CFU of probiotic per mL or g) necessary to observe beneficial effects (Lee et al., 2000). This could facilitate the subsequent commercialization of the probiotic beverage could contribute to valorize the fruits with low commercial value and increase the income of the producers.

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Innovative functional yogurt incorporating microencapsulated calcium lactobionate

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Thematic Areas: food biotechnology

Abstract: functional foods incorporate bioactive molecules which are usually sensitive to physical, chemical and biological factors. One of the substances that can be added to food stuff are prebiotic compounds, such as calcium lactobionate (CaLb). Prebiotics have the risk to be consumed by the own microflora of food stuff preventing the arrive to the gastrointestinal tract where they promote probiotic growth. One of the tools usually employ to protect this kind of compounds are microencapsulation techniques **(1)**.
As far as it is known, there are few prebiotic microencapsulated products in market. In this concern, the propose of the present research was to develop and characterize CaLb microparticles employing as coat material sodium caseinate (NaCas) and transglutaminase enzyme (TGase), trying to improve microparticle properties. Microparticles have been characterized by analyzing their encapsulation efficiency, solubility and morphology. After been characterized, microparticles were tested in yogurt as an example of food matrix. The aim was to know the protective effect of microparticles by the consume of the own yogurt microflora (*Streptococcus thermophilus* and *Lactobacillus bulgaricus*) along 15 days. As a result, there were some differences between NaCas and NaCas-TGase microparticles. Encapsulation efficiency was higher in NaCas-TGase (85.3±1.1%) than in NaCas microparticles (79.4±1.1%). Solubility was similar in both cases (NaCas, 69.4±8.2%; NaCas-TGase 68.8±8.1%) as well as microparticle morphology. Microparticles showed a visual appearance of white powder with a size ≤355 µm. SEM results showed that microparticles were rough, plain and with no pores. Concerning protective effect, NaCas and NaCas-TGase microparticles showed it (*Figure 1*). In both cases, the CaLb concentration is more than the double present in the only product which is in the market today **(2)**, so this kind of food stuff can be consider as an innovative functional food inside within a market in wide extension.

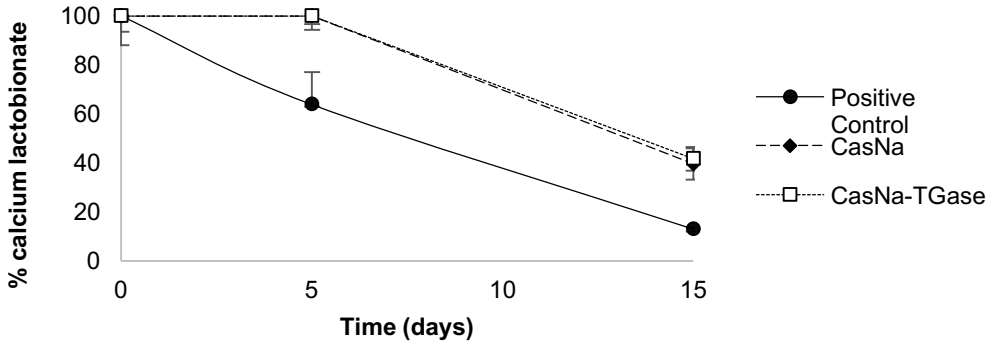


Figure 1. Evolution of CaLb percentage in yogurt with each type of microparticles along 15 days. Final point showed the protective efect against consumption.

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Nutritional value of different microalgae isolated in the south of Spain and Portugal on the basis of their amino acidic profile

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Thematic Areas: Biotecnología alimentaria.

Abstract: Aquaculture is, according to FAO, the fastest growing food-producing sector and now accounts for more than 50 % of the fish globally consumed. However, the sector is challenged by the lower availability and higher prices of fishmeal. These concerns have led to the search of novel nutritive feed alternatives of high quality which can provide essential vitamins, fatty acids and amino acids to aquaculture species (Gamboa-Delgado & Márquez-Reyes, 2018). Essential amino acids must be acquired through the diet and are vital for specific function such as protein synthesis, tissue repair and nutrient absorption.

Microalgae are photosynthetic organisms which interest for aquaculture has increased in the last years. Some microalgal species present high content of proteins, in addition to high content pigments and other high-value compounds, or good digestibility. Moreover, microalgae could also be an outstanding source of proteins, with levels similar to soybean or eggs (Troell et al., 2014).

In this work, the amino acid profile and protein content of more than 55 microalgae isolated from the coast of Cádiz, Huelva and Algarve was determined in order to find the species with the highest percentage of proteins and essential amino acids (mainly tryptophan, lysine and methionine).The microalgae were identified by morphological and molecular approaches and classified within clorophytes, haptophytes, bacillariophytes, cryptophytes, rhodophytes, ochrophytes, miozoa or cyanobacteria groups. The amino acids profile was determined by GC/MS chromatography, after an acid/basic hydrolysis and a derivatization with ethyl chloroformate so as to produce N-etoxicarbonil-ethyl-esters of amino acids.

The microalgae with the highest amounts of proteins and essential amino acids were *Tetraselmis sp.* and *Dunaliella salina* for chlorophytes; *Dicrateria sp.* and *Exantemachrysis gayraliae* for haptophytes; *Phaelodactylum tricornutum* for bacillariophytes; *Hemiselmis cf. cyclopaea* for cryptophytes; *Nannochloropsis gaditana* (CAL3) for ochrophytes; *Heterocapsa cf. Pigmaea* for miozoa and *Oscillatoria sp.* and *Synechococcus sp.* (green) for cyanobacterias. Only one rhodophyte (*Porphyridium cruentum*) was isolated and studied. The results also showed that glutamine/glutamate (Glx), asparragine/aspartate (Asx), alanine (Ala) and lysine (Lys) were the most abundant amino acids in most of the species studied.

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Antioxidant potential of *Kalanchoe* extracts for the preservation of water-in-oil emulsions

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Thematic Areas: Food Biotechnology.

Abstract: Lipid peroxidation encompass a loss of food quality and the formation of reactive by-products that have a negative impact in human health, as it is the case of aldehydes and conjugate dienes (CD) [1]. This autooxidative process has a higher impact in oil-in-water emulsions and, normally, synthetic antioxidants are used for preservation. Consumers' current trends are moving towards a natural-based diet, and it forces the application of new strategies to be performed by food industry. In this work, the addition of plant-derived extracts from the subgenus *Bryophyllum* (genus *Kalanchoe*, Crassulaceae) was tested in water-in-oil emulsions. The ratio water:oil was 9:1; aqueous phase consisted in 0.04 M citrate buffer (pH=3.0) and the oil phase consisted in fish oil. Tween-80 was used as surfactant and the hydromethanolic plant extracts were included in the aqueous phase. The antioxidant efficiency was determined by the delay on the accumulation of CD in the emulsion, that are measured fluorometrically. In order to compare the activity associated to different extracts, the time needed to reach a concentration of 0.5% of CD in the emulsion was recorded. The addition of 0.5 mM total phenolic compounds in the extracts caused a delay of 8 h, compared to control, in the case of *Bryophyllum daigremontianum* and *Bryophyllum tubiflorum* extracts; while 12 h were determined for *Bryophyllum* x *houghtonii* extracts (Fig.1). The differences between species could be attributed to their different phenolic composition. These findings open a novel, broad biotechnological strategy for the use of plant-derived products as food additives in order to replace synthetic antioxidant agents, to satisfy sustainable food industry demands.

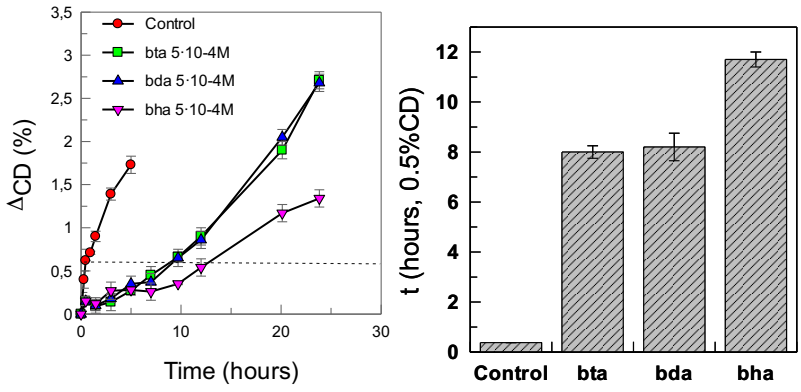


Fig.1. CD accumulation water-in-fish oil emulsions throughout time (left) and time needed to reach 0.5% CD concentration in the presence of *Bryophyllum* extracts (right). "bda" stands for *Bryophyllum daigremontianum* extract, "bta" stands for *Bryophyllum tubiflorum* extract and "bha" stands for *Bryophyllum* x *houghtonii* extract.

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Surfactants of microbial origin extracted from agro-industrial wastes: Agrifood applications

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Thematic Areas: Food Biotechnology

Abstract: During the last years, an increasing demand by consumers of the use of biodegradable and biocompatible ingredients and additives in food formulations has led the food industry to improve in the search of more natural compounds. Among them, surfactants of microbial origin, known as biosurfactants, have attracted a lot of interest as natural detergents. These biosurfactants are obtained by microbial production from natural sources and are composed of lipids, proteins and/or sugars. This composition provides them the advantage to be more compatible than surfactants produced by chemical synthesis. Moreover, they have good efficiency and low toxicity in addition to the fact that they are environmentally friendly. On the other hand, agro-industrial wastes, like corn steep liquor (CSL), can be considered as important sources for the extraction of biosurfactants. Therefore, in recent years, several publications have shown the capacity of a biosurfactant extract obtained from CSL to be applied in different areas of the cosmetic, pharmaceutical and food industry (1-4). López-Prieto et al., 2019 (4) showed that the biosurfactant extract from CSL could be incorporated to a drinkable yogurt promoting the growth of *Lactobacillus casei*. Moreover, previous studies have proven the antimicrobial ability of biosurfactants against pathogens, which is of important interest for the food industry in order to avoid possible risks and contaminations in food products (5). Furthermore, biosurfactants can be utilized as well to help in stabilization and solubilization of food formulations, enhancing their sensory and rheological characteristics (6).

In this study the biosurfactant extract obtained from CSL, using the protocol stablished by Rodriguez-López et al (2016), was subjected to several physico-chemical and microbiological analysis confirming its suitability for its application in the agrifood industry.

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Analysing the transferase activity of α-glucosidase GAM1 from *Schwanniomyces occidentalis*

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Thematic Areas: Food Biotechnology

Abstract: Isomaltooligosaccharides (IMOs) are mainly α(1-6)-linked glucose monomers with a degree of polymerization from 2 to approximately 10, such as isomaltose, panose or isomaltotriose, among others[1]. IMOs show low glycemic index and prebiotic potential. There is an increasing industrial interest for IMOs due to their versatile functionality as food ingredients, as well as cosmetic and pharmaceutical precursors[2].

The production of IMOs can be performed by two different kinds of enzymes, glycosyltransferases or glycosidases. The α-glucosidase GAM1 from *Schwanniomyces occidentalis* structurally belongs to the Glycoside Hydrolase family 31 (GH31) (www.cazy.org) with a characteristic $(\beta/\alpha)_8$ barrel structure. GAM1 is capable of hydrolysing α(1-4) glycosidic bonds liberating α-D-glucose residues and, in saturating conditions, possesses transglycosylation activity forming isomaltose, panose and maltotriose among others, using maltose as substrate[3,4].

Due to the biotechnological potential of new glycosylated compounds showing bioactive properties, the ability of GAM1 for the glycosylation of different glycosidic acceptors was evaluated. The enzyme was able to glycosylate several mono- and disaccharides, among them glucose, xylose and sucrose, providing new potential prebiotics. The transglycosylation reactions were performed in maltose saturating conditions and reaction products were analysed by high performance liquid chromatography with evaporative light scattering detector (ELSD).

GAM1 structural analysis and site directed mutagenesis were used in this work to improve the transferase activity of this enzyme and to evaluate the IMOs profile production. Obtained results sheds light on the enzyme specificity.

Acknowledgements: This work was supported by grants from the Spanish Ministry of Economy and Competitiveness (BIO2016-76601-C3-1-R/-2R/-3R). Moreover, it has received funding from European program Horizon 2020 (Blue Growth: Unlocking the potential of Seas and Oceans) under grant agreement Nº (634486; INMARE). We thank Asunción Martín-Redondo for technical support.

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Production of furandicarboxylic building block from renewable methoxymethylfurfural through a self-sustained enzymatic cascade

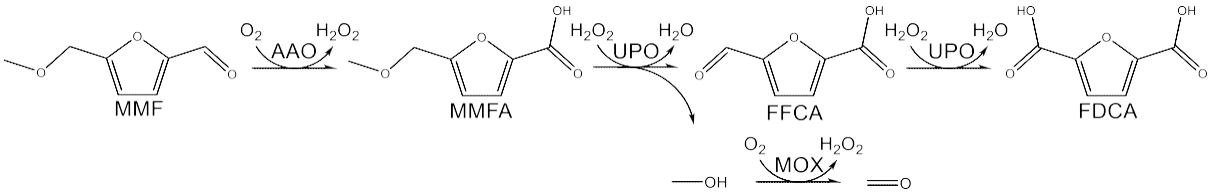
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Thematic Area: Biocatalysis

Abstract: 2,5-Furandicarboxylic acid (FDCA) is on the spotlight. It is a chemical building block of renewable origin that may give rise to a variety of chemicals of interest. Remarkably, when condensed with ethylene glycol, it produces poly(ethylenefurancarboxylate), biodegradable polyesters with a bright future in the bioplastic manufacturing industry, as they are regarded as substitutes for the ones derived from fossil fuels. FDCA results from the oxidation of furfurals such as 5-hydroxymethylfurfural (HMF) and 5-methoxymethylfurfural (MMF), which, in turn, are obtained by the acidic dehydration of hexoses present in plant biomass. MMF production and storage generates less by-products than HMF due to the presence of the methyl ether.

In this regard, we have developed a biocatalytic approach for the conversion of MMF into FDCA. It consists of an enzymatic cascade involving three fungal enzymes: two are oxidases —aryl-alcohol oxidase (AAO) and methanol oxidase (MOX)—, which reduce atmospheric oxygen to H_2O_2, while the last one is a H_2O_2-consuming peroxygenase (UPO). AAO performs the first oxidation on the MMF molecule supplying UPO with one equivalent of H_2O_2 to demethoxylate and hydroxylate the resulting molecule, thereby producing FDCA. MOX fuels the peroxygenase through the oxidation of methanol, the by-product of the demethoxylation catalyzed by UPO. In this way, the enzymatic cascade proceeds independently until attaining ~70% of conversion of the initial MMF into FDCA, as depicted in the Scheme below. Moreover, if less than one equivalent of methanol is added, the conversion is almost complete (98%).

All in all, the cascade developed proceeds autonomously with the only input of atmospheric O_2 and MMF to attain a high conversion into FDCA, which can be further improved by adding a modest amount of exogenous methanol.



Scheme. Representation of the self-sustained enzymatic cascade for the production of FDCA from renewable MMF.

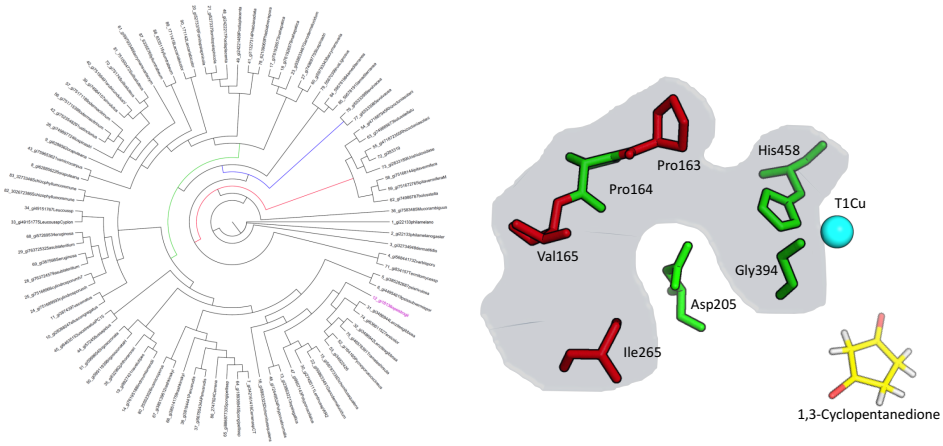
Travelling back and forth: Directed evolution of an ancestral high-redox potential laccase

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Thematic Areas: Biocatalysis

Abstract: Reconstruction of ancestral enzymes can be procured through the sequences of their extant counterparts. This methodology has recently arisen as a powerful tool to engineering enzymes, unlocking properties long time absent. The alliance with directed evolution results appropriate considering the possibility of boost these new features along with the availability of a finest model for allocate disruptive mutations [1].

This work addressed (i) the reconstruction via a Multiple Sequence Alignment (MSA) of 500 basidiomycetes laccases, (ii) the resurrection by cloning an expressing one of the ancestral nodes in *Saccharomyces cerevisiae,* (iii) the characterization of the purified enzyme in comparison to an extant *in vitro* evolved laccase [2] and (iv) the directed evolution of the ancestral laccase. The ancestral laccase paddle on the opposite direction of classical fungal laccases displaying improved heterologous functional expression, higher stability at acidic pHs and shifted activity towards the neutral/basic spectrum of pHs. Finally, the ancestral laccase was submitted to directed evolution for the efficient oxidation of initiators (β–diketones) involved in the polymerization of styrene and acrylamide [3].



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Towards the search of powerful enzymes by applying metagenomics and computational techniques

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Thematic Areas: Biocatálisis

Abstract: Nobody can deny the resource crisis we are immersed in. Our fossil fuel-based economy and the exponential growth of world population are taking us to a point of no return where our living way will be not supported by the planet. In order to get out of this crisis, new biotechnological strategies are emerging for reducing production costs and removing the toxic waste from our environment. Most of these strategies are based on using microbes or its enzymes.

Enzymes are versatile biocatalysts that can avoid using extreme conditions of pH or temperature typically used with inorganic catalyst because they have a specialized amino acidic environment, the active site, that energetically boost the reaction. But enzymes are not as implemented in industry as we may want due to its instability and the low number of them which are available. Another common disadvantage is that most naturally occurring enzymes, or at least those known, are converting a low range of substrates. This is the reason why promiscuous enzymes capable of accepting many substrates, and with potential to be usable in more than one industrial setting, are desired. If these enzymes can also show stringent stereo-specificity for certain industrial compounds and can also perform the reaction at very diverse harsh conditions, then, they will be ideal biocatalysts.

With this objective, our group (https://www.sysbio.csic.es/) has contributed creating one of the largest collections of enzymes, with primary used in biocatalysis, by applying metagenomics tools. Their extensive characterization with hundreds of chemical scaffolds using innovative screen methods was used to select Best Versatile Enzyme Candidates for application purposes, some of which behave better than commercial prototypes. The integration of experimental and structural data through new computational tools has laid the groundwork to understand enzyme properties, particularly, promiscuity, specificity, selectivity and stability and how to predict these properties from sequence data. Here, the outcomes of these efforts are summarized and discussed in the context of screening and designing our future biocatalysts.

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Production of neo-fructooligosaccharides by the b-fructofuranosidase from *Xanthophyllomyces dendrorhous* immobilized by different methodologies

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Thematic Areas: Food biotechnology. Biocatalysis

Abstract: The b-fructofuranosidase Xd-INV from the yeast *Xanthophyllomyces dendrorhous* is a highly glycosylated extracellular protein that catalyzes the release of fructose from substrates such as sucrose and synthesizes short-chain fructooligosaccharides (FOS). These FOS are fructose oligomers linked to the sucrose skeleton by different glycosidic bonds and exert several beneficial effects in human health, including the prevention of cardiovascular diseases, colon cancer or osteoporosis. In addition, they are non-cariogenic, non-caloric and have a sweet taste, which makes them an interesting ingredient for functional foods.

Inluin-type FOS are the most widely studied type of FOS and the only commercialized for human diet supplementation. However, some studies suggest that neo-FOS, in which fructose is β(2→6)-linked to the glucose moiety of sucrose, could display improved prebiotic and physicochemical properties, which makes them attractive for food and pharmaceutical industries [1]. In particular, this b- fructofuranosidase Xd-INV is unique in its ability to synthesize neo-FOS, mainly neokestose and neonystose.

To exploit the vast industrial potential of the b-fructofuranosidase Xd-INV, the immobilization of this enzyme is essential. For that reason, different immobilization strategies of Xd-INV were proposed in this work. In the first place, we immobilized the enzyme by entrapment in lenses of polyvinyl alcohol (PVA), obtaining lenses with 0.4 U/lens of activity and 85% of immobilization yield. We also studied the neo-FOS production with this biocatalyst starting from 600 g/L sucrose. We obtained 18.9 % (w/w) of FOS (59.1 g/L of neokestose, 30.2 g/L of 1-kestose, 11.6 g/L of neonystose and 12.6 g/L of blastose). The immobilized enzyme preserved most of its initial activity during at least 7 cycles of 26 hours at 30 ºC [2].

On the other hand, we carried out the immobilization of Xd-INV by ionic adsorption using two amino-activated resins with different spacer arms: Sepabeads EC-EA (ethylamino) and EC-HA (hexamethylamino). Both biocatalysts maintained their activity during 10 short reaction cycles of 20 minutes. We obtained the biocatalyst with the best activity using the Sepabeads EC-HA (56.9 U/g), whereas the activity of that in Sepabeads EC-EA was 29.7 U/g. The best biocatalyst (Sepabeads EC- HA) was packed into a column to design a fixed-bed bioreactor for the continuous production of neo- FOS. The reactor was tested during 12 days. In conclusion, this system could represent a starting point for the development of an industrial-scale biocatalyst for the production of neo-FOS.

Acknowledgements: This work was supported by grants from the Spanish Ministry of Economy, Industry and Competitiveness (Grants BIO2016-76601-C3-1-R and BIO2016-76601-C3-3-R). I thank the support of CSIC for the JAE-INTRO Scholarship.

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Characterization of a novel fungal laccase synthetized de novo and expressed in yeast by using directed evolution

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Thematic Areas: Biocatalysis

Abstract: Laccases secreted by Basidiomycete fungi are oxidases with a high biotechnological potential as environmentally-friendly biocatalysts with the ability to oxidize the lignin polymer and a wide range of aromatic compounds, using molecular oxygen as the only requirement. [1,2] Nevertheless, their difficult heterologous expression and acidic profile make their industrial applicability a challenge. On the other hand, we can increase the enzyme expression levels or modify its intrinsic features to adapt them to the operational conditions of the industrial processes by enzyme engineering [3–5].
In the current work we describe the characterization of a novel laccase from the saprophytic fungus *Agrocybe pediades* (ApL) synthesized *de novo.* The enzyme was selected among other laccases present in *A. pediades* genome because its production was induced under ligninolytic conditions, that is, when the fungus was grown on wheat straw as the only C source. Once the laccase CDS was cloned and expressed in *Saccharomyces cerevisiae,* we decided to rise the secretion levels by directed evolution. By using a mutated alpha pre-proleader previously evolved in our lab as signal peptide and after subsequent engineering of the enzyme, we obtained up to 1000 U/L of enzymatic activity (with ABTS) in flask cultures, thus enabling enzyme purification and characterization. On the other hand, the enzyme was hyperglycosylated and three N-glycosylation sites were predicted in the protein structure. With the aim to study the contribution of glycosylation to enzyme stability and the individual effect of each site, we constructed three single Gly-mutants, each without one of the putative N-glycosylation sites. After their production and purification, we concluded that N-glycosylation significantly affects ApL secretion but not enzyme stability. Kinetic constants with model substrates, T50 and optimum pH were determined for ApL. The enzyme showed superior catalytic efficiencies than other fungal laccases, good thermostability and quite good activity at neutral pH. These results, together with its good expression in yeast, make the enzyme a promising starting point to develop tailor-made biocatalysts.

Acknowledgements: This work has received funding from the Spanish National Project GENOBIOREF (BIO2017- 86559-R) and the Bio Based Industries Joint Undertaking (JU) under grant agreement No 792070. The JU receives support from the European Union's Horizon 2020 research and innovation programme and the Bio Based Industries Consortium.

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Immobilization of chitinase chit42 on chitosan beads and magnetic nanoparticles for chitooligosaccharides production

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Thematic Areas: Biocatalysis

Abstract: The immobilization of biocatalysts is a fundamental tool in biotechnology to facilitate the enzyme efficiency, the bioproducts synthesis and industrial applications of enzymes. Many different enzymatic immobilization methods have been described. Among others, the non-toxicity of chitosan materials makes this polysaccharide beads suitable for enzyme application in food and nutraceutical industry. In addition, the magnetic nanoparticles use a friendly material, show advantages as the easier and rapid separation of products and biocatalysts. In this study both, chitosan beads and magnetic nanoparticles were used for immobilization of chitinase chit42 (Fig.1), glutaraldehyde was used for covalent binding to supports and the systems effectiveness was evaluated by analysing the chitooligosaccharides production.
Chitinases are ubiquitous enzymes that have gained biotechnological attention due to their ability to transform biological waste from chitinolytic materials into valued chito-oligomers, which show wide agricultural, industrial and medical applications. The biological activities of chito-oligomers are related to their size and acetylation degree. *Trichoderma harzianum* is a soil fungus effective against many phytopathogenic fungi that produces several chitinases. Among them, chitinase chit42 (Glycoside Hydrolase family GH18) [1]. Different Chitin and chitosan were used as substrates for chitooligosaccharides production by the immobilized enzyme. The products were identified using HPAEC-PAD chromatography and mass spectrometry [2]. Structural analysis of chit42 is essential to understand the enzyme specificity and to address its biotechnological improvement.

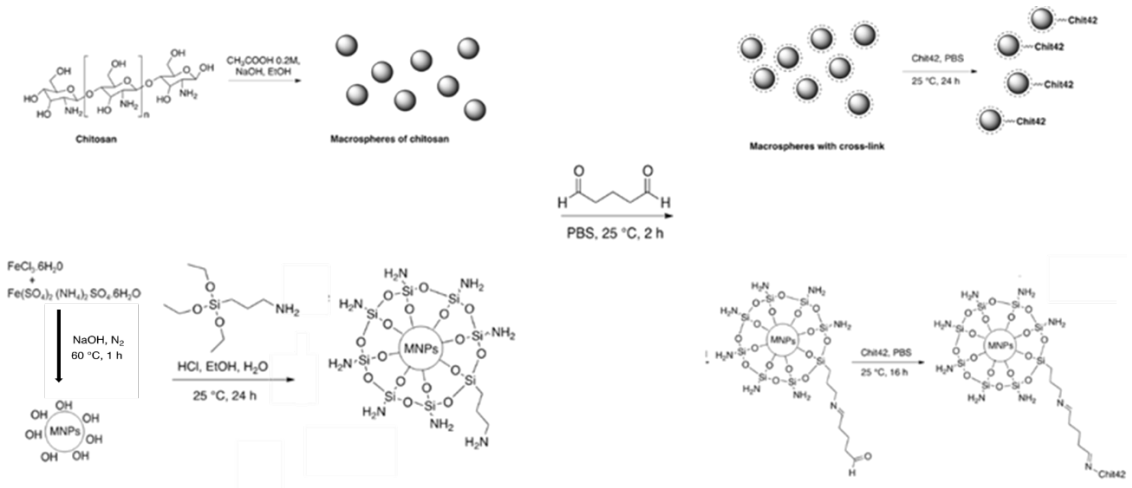


Fig1: Strategies used for Chit42 immobilization on chitosan beads and magnetic nanoparticles.

Acknowledgements: This work was supported by a grant from the Spanish Ministry of Economy and Competitiveness (BIO2016-76601-C3-1-R/-2-R/-3R).

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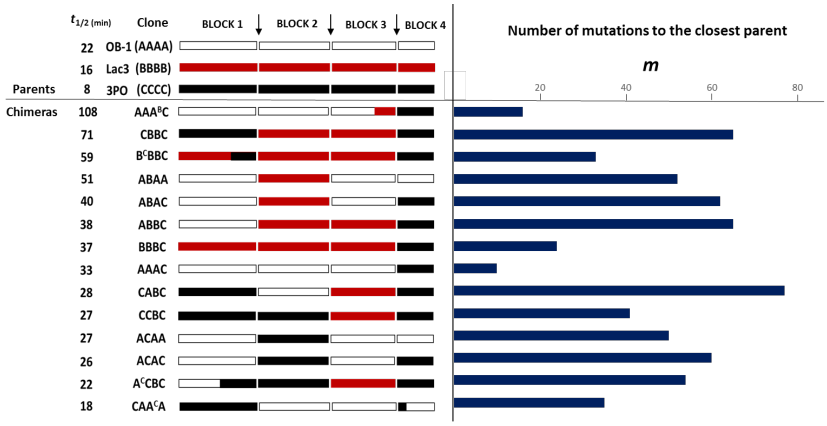
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The generation of thermostable fungal laccase chimeras by SCHEMA-RASPP structure-guided recombination *in vivo*

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Thematic Areas: Biocatálisis

Abstract: Fungal laccases are biotechnologically relevant enzymes that are capable of oxidizing a wide array of compounds, using oxygen from the air and releasing water as the only by product [1]. The laccase structure is comprised of three cupredoxin domains sheltering two copper centers -the T1Cu site and the T2/T3 trinuclear Cu cluster- connected to each other through a highly conserved internal electron transfer pathway. As such, the generation of laccase chimeras with high sequence diversity from different orthologs is difficult to achieve without compromising protein functionality. Here, we have obtained a diverse family of functional chimeras showing increased thermostability from three fungal laccase orthologs with 70 % protein sequence identity. Assisted by the high frequency of homologous DNA recombination in *Saccharomyces cerevisiae*, computationally selected SCHEMA-RASSP blocks were spliced and cloned in a one-pot transformation [2]. As a result of this *in vivo* assembly, an enriched library of laccase chimeras was rapidly generated, with multiple recombination events simultaneously occurring between and within the SCHEMA blocks. The resulting library was screened at high temperature identifying a collection of thermostable chimeras with considerable sequence diversity which varied from their closest parent homolog by 46 amino acids on average. The most thermostable variant increased its half-life of thermal inactivation at 70°C 5-fold (up to 108 min), whereas several chimeras also displayed improved stability at acidic pH. The two catalytic copper sites spanned different SCHEMA blocks, shedding light on the recognition of specific residues involved in substrate oxidation. In summary, this case-study through comparison with previous laccase engineering studies, highlights the benefits of bringing together computationally-guided recombination and *in vivo* shuffling as an invaluable strategy for laccase evolution, which can be translated to other enzyme systems.



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Design of sequential chemoenzymatic strategies towards optically active allylic alcohols and amines. Use of laccases in combination with alcohol dehydrogenases and amine transaminases

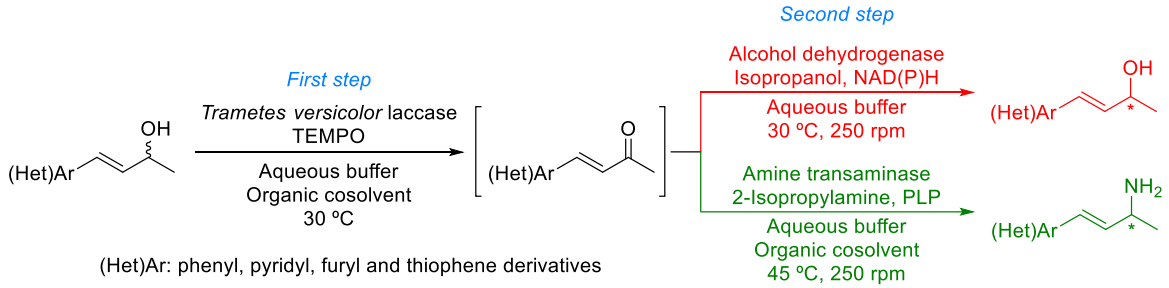
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Thematic Areas: Biocatalysis (Biocatálisis).

Abstract:

Multi-step one-pot enzymatic processes are receiving great attention in the last decade, since the avoidance of (unstable) intermediate isolation and the recovery of products with high yields and selectivities are possible due to compatibility of many classes of enzymes and the simplicity to modify the reaction conditions (substrate concentration, pH, temperature…).

In this communication, a series of sequential transformations will be developed based on the ability of the laccase–TEMPO system to oxidize racemic allylic alcohols in a non-selective fashion towards the formation of the corresponding α,β-unsaturated ketones (Scheme, first step), which will serve as potential candidates to react with alcohol dehydrogenases (top, second step) or amine transaminases (bottom, second step) for the production of optically active allylic alcohols or amines, respectively.



(Het)Ar: phenyl, pyridyl, furyl and thiophene derivatives

Laccases (EC 1.10.3.2) are multi-copper oxidative enzymes that consume molecular oxygen for the oxidation of activated substrates (e.g. phenolic derivatives) under mild conditions,[1] requiring chemical electron transfer mediators (i.e. TEMPO) for the oxidation of other types of alcohols such as allylic or benzylic ones.[2] Interestingly, laccase from *Trametes versicolor* has displayed excellent levels of activity for the oxidation of benzylic[3] and allylic[4] alcohols mediated by TEMPO, opening-up the possibility to develop more complex multienzymatic transformations.

Herein, the application of laccase from *Trametes versicolor* and TEMPO will be studied focusing on the oxidation of (3*E*)-4-(het)arylbut-3-en-2-ols in order to obtain the corresponding (3*E*)-4-(het)arylbut-3-en-2-ones, which will be substrates for bioreduction and biotransamination experiments using alcohol dehydrogenases and amine transaminases, respectively.

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Modelling the production of VFA from proteins by mixed culture fermentations

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Thematic Areas: Industrial Biotechnology

Abstract: Mixed-culture fermentations (MCF) are recognised as a valid process to yield added-value products from organic wastes in the form of volatile fatty acids (VFA). The use of mixed cultures is attractive due to their economical and operational advantages (e.g. no sterilisation is needed). However, they also pose a fundamental challenge: they are complex and their regulation mechanisms remain still unclear. One of the main limitations of the process is its low product selectivity: the productivity of the different VFA is highly dependant on operational conditions (e.g. pH, retention time or feeding characteristics). In consequence, developing bioprocesses based on this technology remains a challenging task. In this context, we aim at establishing a model-based methodology for developing novel bioprocess using MCF. The emphasis is placed on two aspects: to reach a high productivity and a high selectivity on the desired VFA(s). The improvement of selectivity is carried out by means of a metabolic energy-based model while the productivity is maximised using a general kinetic-based model.

Metabolic energy-based models assume that in energy-constrained environments, such as anaerobic fermentations, microorganisms produce those products associated with a higher ATP yield. This kind of models has been proven to predict the VFA conversion stoichiometry of carbohydrate MCF and mechanistically explain the effect of pH on the product spectrum (González-Cabaleiro et al., 2015). However, protein MCF has not been tackled yet from a modelling point of view, which impedes the design of processes using interesting protein-rich agro-industrial wastes (e.g. cheese way or canning industry wastewater). in this work we develop a metabolic model for the production of VFA from proteins in MCF following the methodology of González-Cabaleiro et al. (2015).

The conversion of 17 amino acids is simulated at different pH and retention time values. The model is capable of reproducing satisfactorily the product spectrum of gelatine MCF experimental data as well as the changes on the VFA yields with pH. The model offers for the first time mechanistic insight on the interactions among the different amino acids and on the pH effect on the product spectrum. This information can be exploited as we can now foresee how the pH value or the protein composition on amino acids affects the product spectrum and therefore direct the process towards one of more VFAs. This model will be the core of a tool for the early stage design of protein MCF ensuring a high selectivity on the desired VFA(s) (Fig. 1).

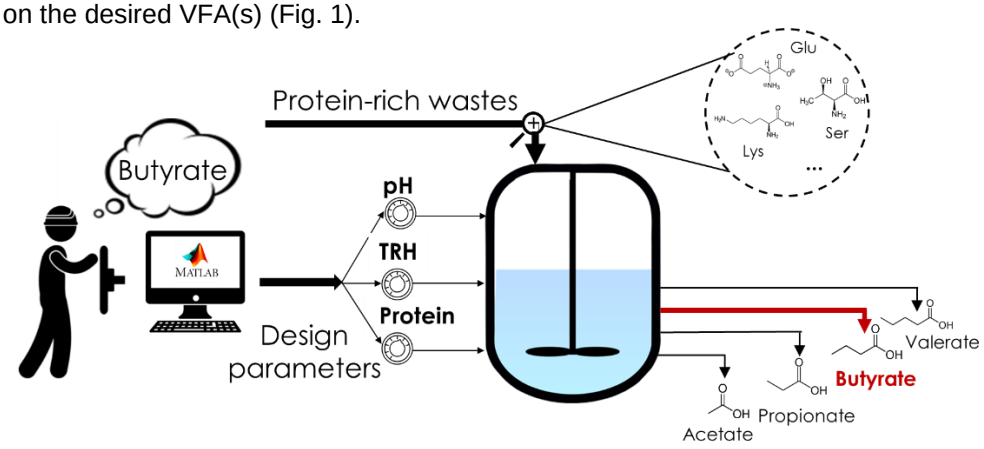


Fig. 1. Design parameters are selected with the metabolic model to maximise the targeted VFA production

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Scaling-up continuous lignocellulosic biobutanol production

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Thematic Areas: Industrial Biotechnology.

Abstract: Transport sector is still highly dependent on fossil energy carriers and responsible for a quarter of EU greenhouse emissions. In fact, the major energy consumer is road transport which represents 81.7%, followed by air (13.9%), rail (2%) and water (1.6%) [1]. Biofuels are considered to be the most promising substitutes for the decarbonisation of transport sector, especially heavy road and air transport. Even though its consumption has fallen down during the last years due to the lower oil prices and the existing sustainability constraints related to their environmental impact (i.e. iLUC). In fact, biofuel consumption in 2015 accounted for 14 Mtoe (19% bioethanol and 81% biodiesel) of which 91% was certified as sustainable [2]. For this reason new and more sustainable drop-in biofuel alternatives are being pursued with the aim of overcoming the current challenges and limitations exhibited by bioethanol and biodiesel through R&D projects as Next Generation Butanol (ButaNexT).
Biobutanol is an attractive advanced biofuel with superior fuel properties. It fits the existing fuel infrastructure; has a higher energy density (similar to petrol/gasoline) and has shown better performance properties than ethanol and biodiesel; however it has not yet been established in the market due to some technical and economic barriers.
The ButaNexT project aimed to demonstrate the techno-economic feasibility of the conversion of two sustainable lignocellulosic feedstocks (wheat straw and miscanthus) available at EU level, into biobutanol through the integration of different technology developments. The project ended in 2018 and the main achievements of the consortium have been the following: (1) design, construction and operation of a micronizing pilot prototype for controlled biomass particle size production up to 0.5 mm with a more reduced energy consumption and able to be scalable to industrial size; (2) development of a flexible and easy to adapt two-step fractionation process based on the combination of the micronizing prototype with a continuous thermochemical pretreatment; (3) development of tailored enzyme cocktails (MetZyme® SUNO™) that address harsh process-specific conditions and facilitate improved hydrolysis of pretreated biomass to monosaccharides; (4) development of a Clostridial strain able to ferment cellulosic sugars effectively (target butanol yield > 0.25 g/g pure sugar) and having 20% higher butanol tolerance than baseline strain; (5) development of a patent-pending “In situ product recovery process” based on improved pervaporation fluxes obtained during fermentation that allows continuous operation; (6) validation of the technical performance of the individual stages cited above in a pilot facility using wheat straw as feedstock from handling to product recovery in a 100 L bioreactor; (7) testing of properties and performance of butanol as a blend component in both diesel and gasoline fuels and (8) calculation of GHG emissions of biobutanol in a number of different scenarios based on the use of wheat straw and miscanthus as feedstocks and for the provision of heat, electricity and waste water treatment.
This work will show the final results from the project regarding the scale-up of ButaNexT process, from lab to pilot scale, to validate the continuous performance in a 100 L fermenter coupled to a pervaporation unit in order to increase lignocellulosic sugars (C6 and C5) conversion and obtain higher biobutanol productivities at CENER´s Biorefinery and Bioenergy Centre (BIO2C).

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ORAL COMMUNICATION

Dihydroxyacetone production from glycerol using *Gluconobacter oxydans*: a kinetic model

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Thematic Areas: Industrial Biotechnology

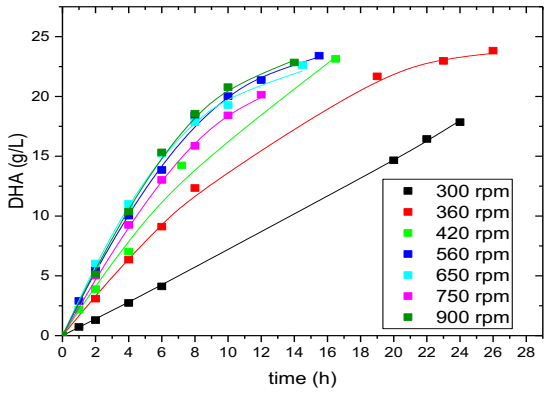
Abstract: Valorisation of raw glycerol (as a by-product from biodiesel production) is being highly studied within the integrated biorefinery concept development. Dihydroxyacetone (DHA) is a very interesting compound to be obtained due to its different applications: as skin self-tanning and, mainly, as a building block for polymer synthesis. Resting cells has shown to be the best cells state and operation way for carrying out this production process [1].

The aim of this work is to develop and to apply a kinetic model for the DHA production when using resting cells of *Gluconobacter oxydans* ATCC621 taking into account not only the biomass and substrate concentration but also the influence of the oxygen transfer rate, due to the highly oxygen dependence of this bioprocess.

The experimental work has been performed both at shaken flasks and bioreactor (STBR) scale in order to determine global rate limiting phenomenon: the oxygen transfer rate or the reaction kinetics itself. Different runs have been carried out changing the oxygen transfer rate as a function of the agitation speed; and also using different biomass concentrations as resting cells.

The proposed kinetic model has been fitted to experimental data using the software *Aspen Custom Modeller*. The kinetic model is able to describe runs conducted under several conditions, mainly with different fluid-dynamic conditions, and so with different relationship between OTR and OUR, which means that in some runs the controlling step is the mass transfer (OTR) while in other runs the biochemical reaction is the controlling step of the overall process rate. The parameter values calculated fulfill all the statistical tests and the values for different runs have a meaning relationship with the operational conditions.

Kinetic Model: OTR control: $\frac{dC_{DHA}}{dt} = \upsilon_{O_2} \cdot OTR$ Chemical reaction control: $\frac{dC_{DHA}}{dt} = \frac{k_p \cdot C_G}{K_G + C_G} \cdot C_X$



Acknowledgements: Ministerio de Ciencia, Innovación y Universidades. Project BIOREFINE. Ref: CTQ2017-84963-C2-1-R.

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A microfluidic platform for downstream process optimization

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Thematic Areas: 7. Biotecnología industrial. Bioprocesos: estrategias de producción y purificación de bioproductos, modelización, escalado

Abstract: The number of biotechnology-based pharmaceuticals in the late-stage pipeline has been increasing more than ever in particular mAbs represent a quarter of all biopharmaceuticals in clinical trials. As a result, there is an enhanced demand for more efficient and cost-effective processes. Here, the potential of miniaturization as a high-throughput screening tool to speed up process optimization is explored. The ATPS-microfluidic developed setup allowed the rapid screening of a wide range of concentrations inside the microchannel by varying the flow rates of the solutions while using sub-mL volumes for each ATPS-forming system. A Chip chromatography microfluidic developed platform allowed the effective screen of multiple adsorption and elution conditions within a few minutes for early stage multimodal chromatography optimization. This technique can be used with any target molecule or resin assuming a previous labeling procedure with a photostable and pH insensitive fluorophore.

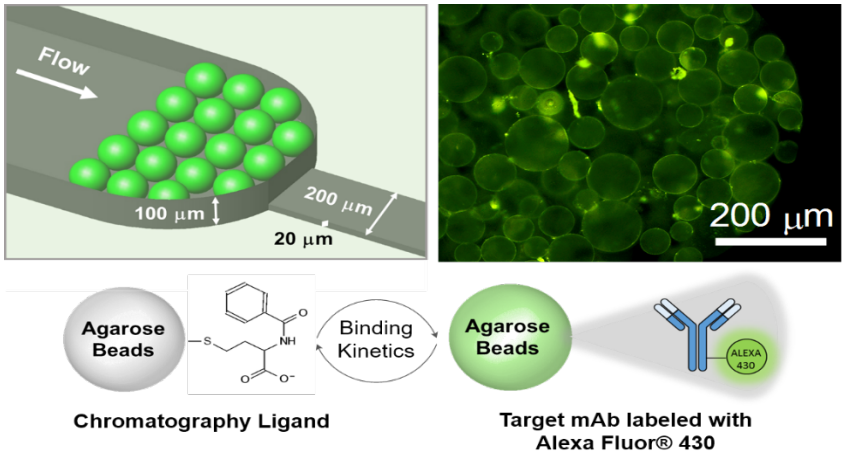


Figure: Chip Chromatography

Acknowledgements: iBB - Institute for Bioengineering and Biosciences acknowledges funding from FCT-Portuguese Foundation for Science and Technology (UID/BIO/04565/2013) and from Programa Operacional Regional de Lisboa 2020 (Project N. 007317).

Evaluation of batch ABE extractive fermentation with solvents of different nature

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Thematic Areas: Area 7. Industrial Biotechnology.

Abstract: The interest for the research and development of biobutanol production has increased over the last decades. The most applied system for producing biobutanol is the acetone-butanol-ethanol (ABE) fermentation by anaerobic Clostridia. However, to make the large-scale production of butanol economically viable it is still necessary to overcome some major drawbacks to improve the competitiveness of the ABE fermentation. It is known that Clostridia present a limited tolerance to ABE fermentation products, which can dissolve in the cell membrane, disturbing its integrity and affecting its specific permeabilization [1]. Thus, the main products of ABE fermentation are known to be toxic to Clostridia. A solution to this problem could be the implementation of *in situ* product recovery (ISPR) systems (e.g. liquid-liquid extractive fermentation) to transfer the toxic product from the culture broth to a non-miscible solvent as fast as it is produced.

Two solvents, a vegetable oil (pomace oil) and a beta-branched C12 Guerbet fatty alcohol (2-butyl-1-octanol) presenting opposite features in terms of biocompatibility towards *Clostridium acetobutylicum* and butanol recovery capacity were evaluated in batch extractive fermentations. These solvents were selected in previous small-scale ABE extractive fermentations in which not only extraction capacity (partition coefficient and selectivity), but also biological parameters (biocompatibility and bioavailability) were considered [2]. In this study, the concentration profiles of the substrate, intermediates and products were examined under the same experimental conditions, and subsequently compared with a solvent-free control. All the fermentations were carried out at anaerobic conditions in 250 mL sealed-bottle flasks filled with 100 mL of a synthetic culture medium. By concomitantly removing the inhibitory products from the broth, the total initial substrate was completely consumed with both solvents, while around 70% of the substrate was consumed in the solvent-free batch fermentation. Overproduction of butanol attained 60% and 100% with pomace oil (PO) and 2-butyl-1-octanol (2B1O) respectively. In the case of 2B1O extractive fermentation, 46% increase of butanol yield (mass of butanol per mass of glucose consumed) was also appreciated. However, with this solvent, a longer lag phase compared to solvent free or vegetable oil extractive fermentation was observed. Based on these results, it can be stated that the differences observed between solvents go further than those expected from thermodynamics of liquid-liquid extraction, and that culture metabolism and/or membrane-related functions seem to be influenced by the presence of 2B1O. The possible mechanisms for these effects are discussed.

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Bioprocessing of recombinant proteins using alternative purification platforms

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Thematic area: 7. Industrial Biotechnology (Bioprocesses: strategies for production and purification of bioproducts, modeling, scaling)

Preference of presentation format: Oral

Abstract: The advent of biopharmaceuticals in modern medicine brought enormous benefits to diverse human diseases and improved the well-being of many people worldwide. Since the FDA approval of humulin (recombinant insulin) in 1982[1], remarkable advances in the treatment of chronic diseases have been achieved with biopharmaceutical-based therapies[2]. This sector represents 40% of ca. 6000 products currently in clinical development[2], and is dominated by therapeutic proteins with over 200 protein drugs in the market[1]. Along the years, many improvements in the biopharmaceuticals upstream stage resulted in high titers of the desired product, and shifted the bioprocess bottleneck to the downstream processing, which is currently dominated by chromatography, accounting with more than 70% of total downstream costs[3]. Aiming at finding new cost-effective, efficient and sustainable technologies for proteins purification, novel polymer-polymer aqueous biphasic systems (ABS) with ionic liquids (ILs) as adjuvants are investigated as alternative purification platforms for the downstream of interferon alfa 2b (IFNα2b) from *Escherichia coli* BL21 cultures. Initial experiments showed that the production of IFNα2b is higher using the SOB culture medium and the western-blot analysis revealed that it is present in the inclusion body fraction. This fraction was washed, solubilized using a specific buffer and, finally dialyzed. After analyzing the stability of the target protein in several phase-forming components, the ternary phase diagrams of ABS composed by polyethylene glycol (PEG), polypropylene glycol (PPG) with ILs as adjuvants were determined at 25 °C, as well as the corresponding tie-lines, tie-line lengths and critical points. Chloride-based ILs combined with cholinium, imidazolium, pyrrolidinium, piperidinium, tetralkylammonium and tetralkylphosphonium cations were investigated.
In summary, this study reports effective IFNα2b purification platforms from *E. coli* based on polymer-polymer-ABS, being highlighted the beneficial role of ILs in the downstream processing of proteins, either as adjuvants in ABS or by exploring new ILs features in protein stabilization.

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Microorganisms for use as a biocatalyst for green chemistry processes

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Thematic Areas: 7. "Biotecnología Industrial".

Abstract: The development of green technologies in food production, biofuels and chemical intermediates for making systems available for effective industrial production, efficient and environmentally sustainable is a worldwide priority from the academic, industrial and government point of view. In this way, one of the most interestingly research lines is the use of efficient microorganisms as catalysts in different bioprocesses to transform different substrates, including by-products of other industrial activities.

Some microorganisms (*e.g. Saccharomyces cerevisiae*, *Escherichia coli*, lactic acid bacteria and *Clostridia* sp.) have been commonly used as fermentative entities for the production of high added-value products for the food, feed and pharmaceutical industry. However, the use of these microorganisms for an efficient production of compounds for low value products like chemicals and fuels, has some drawbacks such as: growth at relatively low temperatures, their requirements for expensive complex media to grow, their low ability to grow aerobically, etc.

Then, it is convenient to use more appropriate microorganisms to make these new industrial processes cost-effective. For this purpose, the selected microorganisms should be able to:

- grow aerobically to obtain an efficient growth phase and facilitate handling (*e.g.* for genetic engineering).
- grow anaerobically for an efficient production phase.
- grow on cheaper culture media, for example in strict mineral media.
- grow at high temperatures (mainly between 45 and 55 ºC) to prevent infections and reduce cooling costs. This implies the search for thermophilic microorganisms.
- grow at high substrate concentrations to be cultured in batch processes and avoid substrate inhibition of growth.
- grow at high product concentrations to avoid product inhibition.
- grow in presence of high CO_2 concentrations since CO_2 is often a co-product or a co-substrate. For example, in fermenters of 20 meters high, pressure values up to 3 bar can be reached.

In general, these microorganisms should be robust. In other words, they should be able to adapt to changing conditions.

Adaptation of current industrial strains to these conditions would be difficult because the genes involved in these complex traits are unknown. Therefore, it could be more efficient to use microorganisms with natural ability to survive at target conditions.

Since genetic accessible strains are lacking at the moment to optimize current bulk chemical production, the aim of this work was to isolate new strains from nature to produce bulk chemicals under ideal industrial conditions.

For an efficient isolation, a different method from the traditional plate isolation procedure was carried out. This allowed to obtain strains that fulfil all the above-mentioned desired requirements (except for the ability to grow at high concentrations of CO_2 because it was not checked yet). It stands out that 97 % of the strains isolated and characterized up to now, according to this method, show facultative respiration.

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Gene dosage and specific growth rate impacts on recombinant protein production in *Komagataella phaffi* regulated by the *AOX1* promoter

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Thematic Areas: 7. Industrial Biotechnology

Abstract: *Komagataella phaffi* (*Pichia pastoris*) is currently used as an efficient expression system for heterologous protein expression. This cell factory presents several advantages as a host such as the capacity to grow at high cell densities in defined media, the ability to secrete the product to the extracellular media and to perform post-translational modifications. One of the main reasons to use this yeast is the existence of a strong and tightly regulated promoter of the alcohol oxidase 1 gene (P_{AOX1}). Constructing producer strains from *P. pastoris* is highly likely to result in heterologous recombination and/or multiple insertion of the gene expression cassette, which usually expands the spectrum of clonal variability. Some authors have found gene dosage to play a central role in target protein production, and therefore how specific product formation rate (q_P) correlates with specific growth rate (μ), the so called "production kinetics". Using large numbers of gene copies results in increased productivity in some cases but has the opposite effect in others. In fact, as claimed, integrating several expression cassettes in the genome may have adverse effects owing to the physiological limitations in the transcription capacity of gene *AOX1*, which is governed by its transcription factors.

In this study, the effect of both gene copy number and μ on recombinant protein production in fed-batch cultures has been studied. Two *P. pastoris* clones expressing one and three copies of the *Candida rugosa* lipase 1 (*CRL1*) gene under the regulation of P_{AOX1} have been selected and tested as single- and multi-copy clones. The cultivation strategy consisted on performing different "carbon-limited fed-batches", in order to control the μ by means of adding methanol as a sole carbon source following a pre-programmed exponential feeding rate.

As an outcome, 3-fold higher values were obtained for the q_P of the multi-copy strain in comparison with the single-copy strain. However, no significant differences were observed for specific substrate and oxygen consumption rates and specific carbon dioxide production rate (q_S, q_{O2} and q_{CO2}, respectively) between the two clones. On the other hand, in relation with the behaviour of the cells at different μ, a similar pattern was observed in both clones, with a maximum of q_P when an intermediate μ was tested. Nevertheless, a linear correlation of q_S, q_{O2} and q_{CO2} with μ was observed.

As conclusion, all the results obtained in the present work indicates that both strain and bioprocess engineering could play an important role in the bioprocess development from an industrial point of view, representing a significant improve in the recombinant protein production.

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Extraction and Quantification of Antibiotics using Aqueous Two-Phase Systems based on Citrate Salts and Acetonitrile.

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Thematic Areas: Biotecnología Ambiental

Abstract: Use of antibiotics as growth promoters in animal husbandry at sustained low doses is a common practice in some countries and is of concern due to environmental enrichment of resistance genes (1). Antibiotics have been found in superficial water (2) and wastewater treatment plant (WWTP) effluents (3). Moreover, conventional wastewater treatment plants could be hotspots for enrichment of antibiotic resistance genes. Recently, liquid-liquid extraction based on the formation of two aqueous phase systems (ATPS) has attracted interest due to potential analytes enrichment up to 1000 fold (4). In the present study an ATPS based on sodium citrate and acetonitrile was evaluated for the extraction efficiency (%E) of the antibiotic chlortetracycline (CTC) in swine wastewater. The partition coefficient of antibiotic (K) was quantified using capillary electrophoresis (5). Determination of the phase diagrams is also presented in Fig. 1. Tie-lines were constructed measuring the conductivity of top and bottom phase of a given system. Accordingly to our results, CTC migrates preferentially to the acetonitrile enriched phase, with K = 4.8 ± 0.22 and %E = 87.5 ± 0.5. The electropherograms of the quantification of CTC in top phase (A) and bottom phase (B) are presented in Fig. 2. The presented method could be a reliable alternative for the quantification of antibiotics in animal wastewater, as it is fast, cheap and robust.

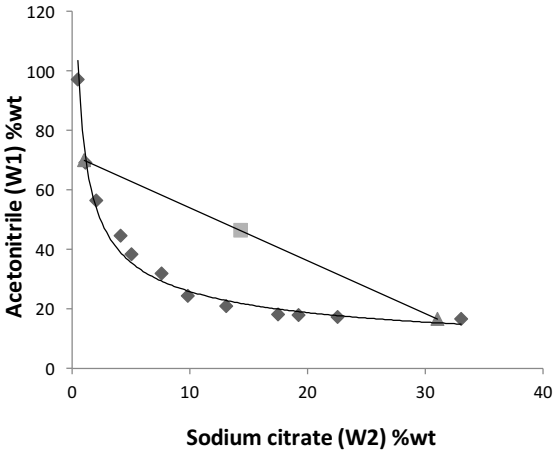


Fig. 1 Phase diagram of sodium citrate, acetonitrile and water at 298.15 K.

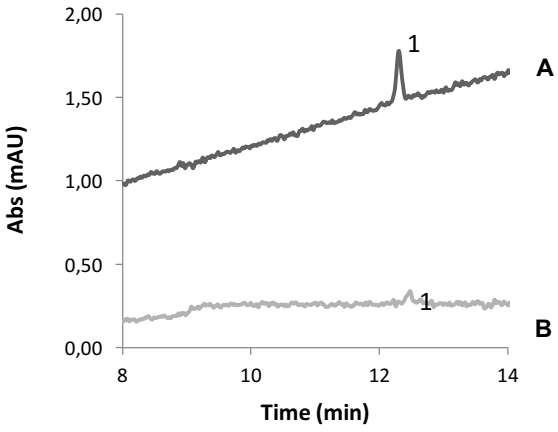


Fig. 2 Electropherograms of the quantification for CTC in wastewater; (1) in top phase (A) and bottom phase (B) at 270 nm. ATPS weight composition: 46, 39 and 14% for acetonitrile, water and sodium citrate respectively. C_{CTC} = 80 µg·mL^{-1}, sample dilution 1:10 in 50% v/v water methanol.

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Cheese whey, a Galician dairy industry by-product, as potential feedstock for VFA production using a mixed culture

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Thematic Areas: 5.Environmental Biotechnology

Abstract: Volatile fatty acids (VFA) are currently attracting much attention due to the great variety of by-products which can be obtained from those acids, such as biogas or biopolymers, but also pharmaceuticals or food industry products. Nowadays, industrial production of these VFA is mainly through chemical processes, although they are not fully sustainable. Recent research has mainly focused on the search of greener alternatives and biological processes. A major challenge of these technologies consists in making them as cheap as possible to compete with the chemical routes. Therefore, mixed microbial cultures instead of pure cultures as well as the use of agroindustrial wastes instead of synthetic substrates are proposed. Therefore, the present research reports the acidogenic fermentation of an industrial effluent typical of Galicia, i.e. the cheese whey (CW), for VFA production, following a similar approach as in some of our previous studies (Calero, 2018). Experiments were carried out in a sequencing batch reactor (SBR) using a mixed microbial culture as inoculum. The SBR configuration allows biomass retention without the need of any immobilization mechanism, which makes the process cheaper. The experimental design included shifts in solid and hydraulic retention times (SRT and HRT) in order to determine the best conditions favouring the process yield, besides coupling these shifts with high throughout amplicon sequencing analysis of the microbial community present in the reactor.
During the performance of the SBR reactor it was detected that increasing the SRT, from 5 to 10 and then from 10 to 15 days, had a stronger influence on yield and degree of acidification than changing the HRT. However, HRT yielded better results when it was decreased from 3 to 1 days. Other major effects involving SRT were also observed in terms of biomass concentration and VFA profile in comparison to HRT, as softer fluctuations were observed. The VFA profile might be one of the crucial parameters to be taken into account depending on the final application. Observations showed that lactate highlighted at shorter SRT (5 days), while butyrate was the dominant product when the SRT was established at 10 days. Finally, acetate formation started to prevail when the SRT was increased to its highest value (15 days). As the VFA profile was changing alongside SRT, the microbial community also did. *Bifidobacterium* and *Lactobacillus* genera were associated to the conditions where lactic fermentation highlighted. However, as the VFA profile turned to butyric acid production, the microbial community did also shift to *Megasphaera* genus as dominant organisms, which clearly showed the connexion between this VFA and this genus. Finally, microbial diversity was increased in the last conditions (SRT of 15 days) with the appearance of *Caproiciproducens*, *Pantoea* and *Sutterella* genus.
It can be concluded from this work that CW is a good agroindustrial effluent for VFA production due to its high and easy fermentable organic load. All the lactose was bioconverted into organic acids and good yields were reached no matter the applied condition. The increase of SRT improved the performance of the process. A similar trend was observed when decreasing the HRT. Finally, the microbial analysis of the reactor helped to understand the population involved in the fermentation processes.

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Hydrolysis of butcher´s waste by Alcalase

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Thematic Areas: Environmental biotechnology.

Abstract: The degree of compliance with European Directive 1999/31/CE and the Spanish transposition (RD 1481/2001) in the MBTPs of Castilla y León region could be calculated based on the obtained refuse derived fuel (RDF) characterizations and mass balances provided by the mechanical-biological treatment plants (MBTP) managers. According to these results, objective of 2006 (25% of reduction on biodegradable waste landfilled in 1995) was fulfilled mainly because of the built of 10 MBTPs in those years. However, objectives of 2009 (50% of reduction) and 2016 (65% of reduction) were not reached, in spite of the important reduction achieved in the amount of RDF which is landfilled (1). One possible solution for this problem is the separation in origin of easily recoverable biodegradable materials such as butcher's waste (rich in protein and lipids) for its transformation into products with higher added value. This was the aim of this work: butcher's waste collection and transformation for production of protein hydrolysates and fatty acids; waste reduction was also analysed.

Enzymatic hydrolysis was used to modify the butcher's waste. Alcalase 2.4 L from Novozymes A/S (Bagsvaerd, Denmark) was employed in the study. Hydrolysis experiments were carried out in a 0.5 L jacketed reactor in controlled hydrolysis conditions: pH = 8, temperature = 50ºC, time = 240 min and stirring speed = 300 rpm. Substrate (So) and enzyme (Eo) concentrations varied in the different tests covering the Eo/So range from 0.1 - 0.9 AU/g. Reaction was followed by monitoring the degree of hydrolysis (DH) according to the pH-stat method (2). After the enzymatic hydrolysis, samples were placed into boiled water at 95-97ºC for 20 minutes to deactivate the enzyme and centrifuged at 9000 rpm for 15 minutes to separate three phases: phase containing the un-hydrolysed waste, phase containing the protein hydrolysate and lipidic phase. Fatty acid distribution was analysed in the lipidic phase.

Hydrolysis curves showed a high reaction rate in the earlier time followed by a rate decrease that could be justified by the reduction in the concentration of peptide bonds available for hydrolysis along the process. A direct linear relationship between the Eo/So ratio and the degree of hydrolysis was observed, meaning that high Eo/So ratios favour the production of protein hydrolysates from meat waste. On the other hand, the Eo/So increase results in a higher amount of separated lipids and, consequently, in a higher amount of recovered fatty acids. Finally, the lower are the values of Eo/So, the greater is the amount of un-hydrolysed waste, therefore, it is convenient to use high Eo/So ratios.

Eo/So (AU/g)	DH (%)	RECOVERED LIPIDS (%)	NON-HYDROLYZED RESIDUE (%)
0.18	20,02	43.60	43.61
0.36	24,36	65.07	33.03
0.54	27,15	80.97	22.27
0.71	29,99	94.27	15.36
0.89	32,93	100.00	3.92

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Anaerobic populations and enzymes involved in the removal of organic micropollutants

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Thematic Areas: Environmental Biotechnology.

Abstract: Pharmaceuticals, personal care products, pesticides and other chemicals of emerging concern are known as organic micropollutants (OMPs) because they are present in the environment at trace concentrations, but they might exert a potential harm due to their bioaccumulation and toxicity [1]. Most OMPs are partially removed in the anaerobic bioreactors used in sewage treatment plants [2]. However, since the biotransformation mechanisms remain unknown, no efficient mitigation strategies can be designed to improve the removal of these pollutants. Therefore, the objective of this study is to get some insights about the main populations and enzymes contributing to the anaerobic biotransformation of OMPs.
Bioreactors with hydrolytic, acidogenic and methanogenic activities where operated in order to elucidate which are the main microbial populations biotransforming the OMPs in anaerobic processes. To find the responsible enzymes, in vitro assays were performed with purified enzymes and with a mixture of enzymes directly extracted from anaerobic biomass [2,3]. As summarized in Table 1 for some of the studied OMPs, several links between the experimental biotransformations and the enzymatic and microbial activities were found by combining the knowledge about OMP reactivities, biological processes and the identification of some transformation products. For instance, methanogenic microorganisms and, in particular, the methanogenic enzyme acetate kinase might be involved in the biotransformation of several compounds with carboxyl (e.g., naproxen) or hydroxyl (e.g., bisphenol A) groups. On the contrary, hydrolases synthetized by hydrolytic or fermenting bacteria might biotransform compounds such as erythromycin. In the case of sulfamethoxazole, several anaerobic populations might be able to biotransform it, since it presented a high removal efficiency in all anaerobic reactors. Since each OMP could follow several biotransformation pathways, apart from the enzymatic reactions shown in Table 1, other cometabolic routes are possible. This study aims to be a first approach to understand the biotransformation mechanisms of OMPs in anaerobic systems.

Table 1. Enzymatic reactions and main anaerobic populations involved in the anaerobic biotransformation of several OMPs.

OMP	Enzymatic Reaction	Candidate enzymes	Anaerobic population
Bisphenol A	Phosphorylation	Hexokinase (E.C. 2.7.1.1) Acetate kinase (E.C. 2.7.2.1)	Fermenters Methanogens
Erythromycin	Cleavage of cladinose Ester hydrolysis of macrolactone ring	Glycosylases (EC 3.2.-) Esterases (EC 3.1.-) Serine proteases (EC 3.4.21)	Hydrolytic bacteria Fermenters
Naproxen	Phosphorylation O-demethylation	Acetate kinase (E.C. 2.7.2.1) O-methyltransferases (EC 2.1.1.-)	Methanogens Acetogens
Sulfamethoxazole	Reduction of N-O bond in isoxazole ring	Membrane proteins: Cytochrome c, Ferredoxin hydrogenase (EC 1.12.7.2)	Several
Trimethoprim	O-demethylation	O-methyltransferases (EC 2.1.1.-)	Acetogens

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Pilot-scale composting of organic fraction of municipal solid waste for agricultural use

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Thematic Areas: 5. Environmental Biotechnology: Biotreatment and bioremediation processes

Abstract: Composting allows transforming the organic waste in nutrients that plants can use as a source for their own development and growth. It is a process carried out by decomposer microorganisms that, properly supervised, can result in a useful and beneficial product (compost) for agricultural lands and soil quality. According to the current waste generation volumes and rates, large scale composting of the organic fraction of municipal solid waste (OFMSW) might reduce the waste management problems and their hazard. Besides, it might revalue the MSW and reintroduce them in the economic cycle and the environment in a useful and safety way. However, large volumes and varying composition make it difficult to compost MSW due to temperature needs to be uniform along the whole process in order to both achieve the complete organic matter degradation and the final compost sanitation. In order to overcome this challenge in the near future and at the framework of the Life-NADAPTA Project (LIFE16 IPC/ES/000001) actions related to the development of innovative techniques to adapt agriculture to the climate change, OFMSW collected in Navarra was composted at pilot-scale. Two different technologies (static and dynamic open systems) and two different OFMSW: bulking agent proportions (1:1 and 1:2) were compared and the processes evolution was controlled both physicochemically and microbiologically.
After 100 days, 4000 kg of compost were produced and 1000 kg of impurity content were separated from the initial 5000 kg of OFMSW. Thanks to the thermophilic initial phase that lasted two weeks and to the mesophilic phase that followed during three to five more weeks, total coliforms and *Escherichia coli* concentrations were at least two orders of magnitude lower than initial values in all of the composting systems, complying with regulations (Royal Decree 506/2013). As pathogen occurrence indicators, these results proved a minimum environmental and health potential risk associated with the further agricultural use of the compost produced. Comparing technologies, microbiological pollution in dynamic systems (< 10^2 CFU/g *E. coli*) were lesser than in static ones (10^3 CFU/g *E. coli*), what was related to the more uniform temperature and water distribution throughout the dynamic system. Spot and mixed samples analysis also proved the difficulty of achieving a temperature uniformity while working with such large volume and varying waste. Regarding the OFMSW:bulking agent proportion, process evolution was similar though the different stages of 1:1 systems lasted a bit longer (Figure 1). Preliminary results showed compost had a proper quality, but upcoming germination tests and application on experimental farmland would help to verify it. Anyway, it could be claimed that more and better environmental awareness and education would be required in order to optimise the organic matter fraction separation and hence, the quality and efficiency of the composting process.

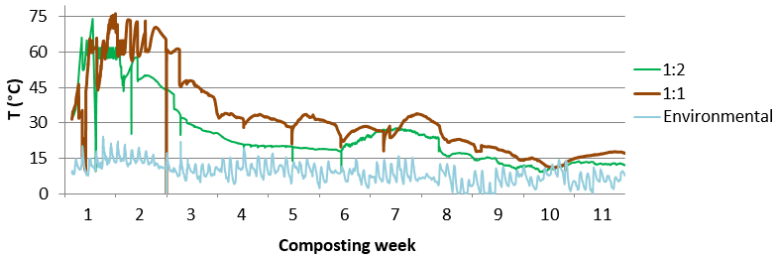


Figure 1: Temperature evolution of the composting processes developed in the static systems.

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Valorization of methane emissions into polyhydroxyalkanoates by methanotrophs enriched from *Sphagnum* mosses: Assessing the effect of temperature and phosphorus limitation

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Thematic Areas: biotecnología ambiental

Abstract: The increase in the concentration of greenhouse gases (GHGs) in the atmosphere is directly linked to this increase in the average temperature of the planet. Among them, methane (CH_4) concentration in the atmosphere (the second most important GHG (Desai, M. P., & Harvey, 2017)) increased at a yearly rate of 0.2-1%, mainly due to anthropogenic activities (landfilling, agriculture, livestock farming, waste management and energy production) ((EPA), 2017). In this context, the isolation of highly efficient methanotrophic communities is crucial for the optimization of methane bioconversion into products with a high market value such as polyhydroxyalkanoates (PHA) (Pieja et al., 2017).

The present study aimed at enriching methanotrophic consortia from two different inocula (*Sphagnum* peat moss and *Sphagnum* and activated sludge) able to accumulate polyhydroxyalkanoates (PHAs) while efficiently oxidizing CH_4. Moreover, the effect of temperature during enrichment and phosphorous limitation on the specific biodegradation rates of CH_4 and PHA accumulation potential was investigated.

Higher CH_4 degradation rates were obtained under P excess at increasing temperature (25, 30 and 37ºC). The biomass enriched from the mixed inoculum exhibited higher specific CH_4 biodegradation rates regardless of the temperature (a maximum value of 84.3 ±8.4 mg CH_4 h^{-1} g $biomass^{-1}$ was recorded at 37ºC). The results showed that phosphorus limitation was required to promote the accumulation of these biopolymers, the highest PHA content being observed with the *Sphagnum* inoculum at 25ºC (13.6 ± 5.6%). In this sense, the differential specialization of the microbial communities depending on the enrichment temperature could explain this finding. The genus *Methylocystis* increased its abundance from 0.24% up to 45% in the biomass enriched from *Sphagnum* at 25ºC, with a lower contribution observed at 30 and 37ºC. This likely mediated the higher PHA accumulations recorded at 25ºC. On the contrary, *Methylocystis* was less abundant in the community enriched from the mixed inoculum, which exhibited the highest abundance of *Methylocystis* at 25 and 30ºC, also resulting in the highest PHA accumulation at these temperatures.

This research demonstrated that *Sphagnum* is an appropriate inoculum for the enrichment of the genus *Methylocystis*, a type II methanotroph able to accumulate PHA (and more specifically PHB) using CH_4 as the only carbon source under phosphorus limitation. In addition, temperature during culture enrichment was identified as a key operating parameter influencing both the specific CH_4 biodegradation rate (with highest values recorded at 30ºC) and the PHA accumulation capacity.

Acknowledgements: This research was supported by MINECO (CTM2015-73228-JIN project) and the EU through the FEDER Funding. The ISP is partially financed by the regional government of Castilla y León and the EU-FEDER (CLU 2017-09).

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Power to gas technology by biological methanation.

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Thematic Areas: Industrial biotechnology.

Abstract: Renewable energy sources can cause overproduction of electricity in a very short time. One way of storing large amounts of energy and balancing the fluctuation is the use of power to gas (PtG) technology. PtG plays an important role in creating a reliable and economic power supply by means of the conversion of intermittent electrical energy into hydrogen or methane fuels.

The hydrogen can be combined with carbon dioxide, producing methane in either a catalytic process or biological methanation. It can be performed by hydrogenotrophic methanogenic archaea through direct transformation of H_2 and CO_2 or by means of an indirect transformation that involves homoacetogenic bacteria that transform CO_2 to acetate via the Wood-Ljungdahl pathway and acetoclastic methanogenic archaea that convert the acetic acid into CH_4. The biological method using hydrogenotrophic methanogens has the advantage of flexibility and lower investments.

Acetoclastic and hydrogenotrophic methanogens are prevalent in anaerobic biogas plants, in an approximate ratio of 20-80%, therefore, the use of a digested sludge as inoculum for the start-up of biological methanation reactor has advantages compared to the use of pure cultures that are not widely available. The reduction of costs and the simplicity for the start-up, because sterile conditions are not required, are the main benefits. However, the major disadvantage is the competition between hydrogenotrophic methanogens and homoacetogens for H_2 oxidation that can lead to the predominance of non-desirable routes.

The degradation of volatile fatty acids (VFA), mainly acetic acid, occur at low H_2 partial pressure in the liquid media, as the H_2 concentration increases, the VFA-degradation may be affected, affected perturbing the upgrading process.

Experiments were carried out to investigate the influence of hydrogen loading rate on biological methanation in a trickle bed reactor. H_2 (≥99%) and CO_2 (≥99.5%), with a stoichiometric ratio of 4:1, were introduced in different flows and final concentration was measured.

Two different periods were analysed. Period 1 (0-30 days) with an inlet gas flow rate <100 $mL \cdot min^{-1}$ and period 2 (30-70 days) with a gas flow rate >100 $mL \cdot min^{-1}$. Experimental data exhibited a steady increase of VFA concentration after the modification of the loading rate that could be explained by the development of homoacetogenic bacteria as dominant specie without acetoclastic archaea. When the VFA exceeded 1200 $mg \cdot L^{-1}$ for a prolonged period, the CH_4 composition in the gas drastically decreased down to 10%.

High output quality (~95%) of the methane could be reached when the loading rate is adjusted to the biomass present in the reactor. Identifying the parameters that favour VFA accumulation as the flow rate increases and analysing the pathways by which the H_2 is consumed by homoacetogenesis is crucial to ensure a high upgrading performance when mixed cultures are utilized.

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Gene expression study of *E. coli* growing in glucose and acetate as carbon sources

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Thematic Areas: Nuevos retos en biotecnología: Técnicas de análisis masivo (genómica, proteómica, metabolómica).

Abstract: *Escherichia coli* (*E. coli*) metabolism carbon fluxes are an important factor to take into account, since this bacteria is widely employed as a model organism in industrial and biotechnological processes. In this sense, *E. coli* can metabolize diverse carbon sources such as acetate, which is a poor carbon source, or glucose. Under glucose limitation conditions, *E. coli* develops carbon stress responses [1], so this bacteria exhibits an expansion in gene expression [2], the emergence of new pathways and alternative enzymatic activities. Thereby, diverse activities and new pathways could be part of underground reactions which may be connected to the existing network, increasing the potential of employing diverse and new carbon sources [3].

In this work, a gene expression study through a microarray assay has been carried out to know the differences between the genetic global expression in *E. coli* K12 MG1655 growing in minimal medium M9 supplemented with glucose or acetate. The transcription profiles of *E. coli* K12 MG1655 growing in acetate supplemented medium exhibited great differences in gene expression respect to the strain growing in glucose supplemented medium. This behavior may be attributable to the increase in the number of genes expressed which allow a large scale carbon-scavenging to metabolize "low quality" carbon source [2]. The adaptation process may involve a refined regulatory response to only activate genes of acetate metabolism (*acs*, *ackA*, *actP*), but we observed an increment in the expression of genes, which belong to other pathways, such as the glyoxylate shunt, methylglyoxylate shunt, TCA cycle, and gluconeogenic flux, suggesting that these pathways are at least partially controlled at the transcriptional level. Furthermore, *eutD* and *prcP* genes were overexpressed in acetate growing. These two genes belong to the ethanolamine and propionate metabolism, respectively, so in acetate growing EutD and PrcP must present other alternative enzymatic activities and could be part of the underground metabolism reactions.

The gene expression change in acetate compared to glucose was used to assess the metabolic flux distribution in key pathways. Furthermore, the results suggest difficulties to identify adaptive mechanisms only through examination of expression changes, since there are other mechanisms that regulate metabolism, such as post-translational regulation, enzyme kinetics and allosteric control [4].

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Synthesis, use and modelling of a gel containing Levan-capped silver nanoparticles for bactericidal purposes

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Thematic Areas: New challenges in biotechnology

Abstract: Silver has been proposed several times as a bacteriostatic product, and nowadays, their inclusion in nanoparticles is a field that is reaching in importance because it solves some of the traditional drawbacks (doses, tissue toxicity…). In this work, silver-coated nanoparticles with bactericidal effect were synthetized by using a green technique with levan (a fructose polymer) as reducing and capping agent (1). The nanoparticles obtained has a size of about 35 nm and a loading capacity of 29% (mass silver/mass polymer). The nanoparticles show a bacteriostatic effect with Gram positive and Gram negative bacteria where the lethal dose were calculated (12.6 ppm for *B. subtilis* and of 6.8 ppm for *E. coli*, as models for each one)

Polymer-silver nanoparticles were also introduced in an alginate gel to produce a further silver delivery system that can be useful for different applications. The gel was able to reduce the bacterial survival up to 20% in 5-10 h depending on the bacteria genera. From the data of survival and data for axial dispersion and nanoparticles diffusion, a mathematical model was proposed to explain the phenomena, taking into account all the factors involved. The model was validated with the experimental data for Gram positive and Gram negative bacteria, and it has been shown effective to predict the release and the effects on bacteria survival. A parameter estimation was carried out in order to determine the most interesting parameters of the model. Therefore, this model provides valuable knowledge for a potential use of a silver nanoparticle - gel for wound dressing applications.

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Controlled size nanocolloids production for bioapplications

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Thematic Areas: Topic 9: New Challances for biotechnology

Abstract: Nanovesicles (organic colloids) are particles formed by self-assembled amphiphilic molecules into closed bilayered structures with an inner aqueous core. Depending on the chemical nature of bilayer constituents, these particles are categorised into liposomes (lipids), niosomes (non-ionic surfactants) or polymersomes (block copolymers).
Niosomes exhibit unique advantages over the other types of vesicular systems due to their inherent characteristics of non-ionic surfactants. These advantages include; better chemical and physical stability of suspensions due to the absence of oxidation-related degradation, easy derivatization to introduce different functional groups for stability enhancement or bioconjugation, wide range of surfactant types available, high immunological tolerance, and cost effectiveness. Over the last 15 years niosomes applications have expanded to many fields: Food fortification, diagnostic agent, analytical chemistry, nanomaterial synthesis, and drug delivery are just some of examples. For all of these applications, a product with specific characteristics, homogeneity and reproducibility is desired, and in particular, controlled size and monodispersity are essential
Several techniques have been used in order to have nanovesicles of the eligible particle size and reasonable monodispersity. Being ethanol injection method the technique that offers monolayer smaller vesicles.
Microfluidics technology is very promising for precise control over input variables when mixing chemical species. Other advantages, include low consumption of chemicals (relevant in formulation optimization), scale-up possibilities for industrial production, on-line coupling to other processes (such as purification steps), and efficient control over temperature if required. Previous research works have used this method to examine various colloidal formulations. However, the production of niosomes through microfluidic routes remains less explored. In microfluidics a stream of lipids in organic phase is focussed between two aqueous streams in microchannels under laminar flow conditions, allowing the mixing of chemical species by molecular diffusion.
In this work, key operation and formulation parameters, involved in a microfluidic reactor with hydrodynamic flow focusing, were investigated in order to quantify their effects on niosomes morphology. Particular attention was given to temperature, which is both a requirement to handle non-ionic surfactants with phase transition temperature above room temeprature, and a tailoring variable for size and monodispersity control. High resolution and conventional 3D-printing technologies were employed for the fabrication of microfluidic reactor and thermostatic systems, since this additive technology has been essential for microfluidics development in terms of cost-effective and rapid prototyping. A customised device to control temperature and facilitate visualization of the process was developed, which can be easily coupled with commercial inverted microscopes.
Results demonstrated that niosomes with controlled particle size and required monodispersity can be easily produced by microfluidics technique. Results obtained were compared with those obtained by ethanol injection method.

ProBone - New tools for prospecting the marine bone-degrading microbiome for novel enzymes

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Thematic Areas: Nuevos retos en biotecnología.

Abstract: Due to the expected population growth in the coming decades, there is a growing demand of protein. Based on estimations of The Food and Agriculture Organization (FAO) of the United Nations[1] the consumption of beef, chicken and pork meat is projected to increase by 65%, 120% and 43%, respectively, from now to 2050. This will lead to a massive increase in mechanically deboned materials. Today, some of these by-products are used as feed ingredients or as fertilizers. These may however be upgraded by high-value products in nutrition, health, cosmetics or as specialty feed or scaffolds for chemical industry by establishing new value chains. Therefore, with a growing demand for protein, optimal utilization of these resources is increasingly important to solve global challenges. Enzymatic hydrolysis is an attractive approach to achieve hydrolysates and sediment fractions with desirable amino acid compositions for use in protein commodities and as platform chemicals. The enzymes that are commercially available are however limited and not tailored to hydrolyse the naturally recalcitrant collagen.

The ProBone Project (http://www.marinebiotech.eu/ProBone) and its partners want to explore naturally occurring enzymes that have evolved to degrade complex collagen. This will be done by firstly examining the diversity of free-living bacteria and symbiotic microorganisms that naturally colonize the bone interior and surface of whale carcasses but also cow, swine and turkey bones deployed on the sea floor in Byfjorden outside Bergen, Norway. Deep sequencing, which produces a wealth of metagenomic data and ideally reflecting the total microbial genetic content, will guide us in identifying enzymes potentially degrading each of the components of the recalcitrant bone materials. Then, by providing new screen methods and optimized expression tools, the enzymes with the desired specifications for hydrolysis of bone residues will be supplied.

The analysis of the first set of microbial samples extracted from bones deployed over several months has provided a first insight into a marine bone-degrading microbiome and the identification of more than 15000 sequences potentially encoding enzymes relevant to bone degradation. The sequences will be prioritized by a number of parameters and promising candidate genes will be chosen for expression trials to test their capacities for degrading recalcitrant bone material via innovative screening methods.

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Isolation and characterization of xylanolytic bacteria from Cachiyacu of Lupuna salterns in Peru

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Thematic Areas: Biotecnología microbiana.

Preferencia de formato de presentación: Comunicación oral y/o póster

Abstract: The xylanases are enzymes that participate in the hydrolysis of the β-1,4 glycosidic bonds present in hemicellulose. The interest in xylanases started around the 1950s due to its enormous potential to convert lignocellulose into glucose and soluble sugars. The importance of applications of xylanases in the food industry, bioethanol production, paper industry and others has been the cause of the search for native xylanases-producing bacterias with special characteristics **(1, 2)**. Firstable, samples from soil were collected from Cachiyacu of Lupuna salterns in Peru, followed by isolation of the bacteria using salt water agar from 7.5% supplemented with 0.5% yeast extract. The evaluation of xylanases production was made with culture media supplemented with 1% xylan **(3, 4)**. Physiological, biochemical and nutritional tests were made on the isolates that hydrolyzed xylan. In addition, the production of other hydrolytic enzymes such as lipases, amylases and proteases was evaluated. 67 bacteria were obtained of which 10 isolates hydrolyzed 1% xylan. These 10 isolates were selected to continue the study. The 80% (8/10) of the isolates are Gram-positive. The physiological, biochemical and nutritional characteristics were also evaluated, observing optimal growth of 30% (3/10) of the isolates in a range of NaCl between 0.5% and 20%, and 20% (2/10) in a range of pH between 6 and 9. Regarding the production of other hydrolytic enzymes 60% (6/10) of the isolates hydrolyzed amylases and 40% (4/10) lipases. There is a predominance of aerobic bacteria, halotolerant, Gram-positive rods, with growth in a wide range of pH and with hydrolytic activity against various substrates.
Keywords: Xylanases, hydrolases, halotolerant bacteria.

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Identification of target genes to reduce acetate yield by *Saccharomyces cerevisiae* under aerobic conditions

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Thematic Areas: Microbial Biotechnology

Abstract: Excessive acetic acid production is a key problem in winemaking processes aiming to reduce ethanol content of wine (1). It is related to a boost in acetate yields observed for *Saccharomyces cerevisiae* and some other yeasts under aerobic fermentation conditions. However, the biochemical mechanisms underlying this problem are unclear. We therefore used a genomic-scale modelling approach, combined with experimentally determined uptake/secretion rates, to gain insights into the metabolic rewiring of *S. cerevisiae* in response to oxygen under conditions relevant for winemaking. The model was also used to identify gene deletion targets affecting the production of acetic acid under aerobic conditions. Several gene knock-out with expected reduction in acetic acid production were thus identified as targets using MoMa and MiMBI algorithms.

The predictions were tested using strains from the prototrophic yeast knockout collection for the fermentation of natural grape must under aerobic conditions. Although many of the prediction did not show any reduction in acetic acid yield, and increased yield was observed in some cases, significant acetate reduction was obtained for three gene deletion strains, *ald6Δ*, *mdh3Δ* and *rip1Δ*. The involvement of *ALD6* on aerobic acetic acid production could be expected from results by other authors. However, the results for *MDH3* and *RIP1* could not be easily anticipated and thus provide new insights into yeast metabolism concerning acetic acid production, a major concern for the wine industry.

Acknowledgements:
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Random mutagenesis of *Rhodospirillum rubrum* to improve growth in syngas.

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Thematic Areas: Microbial Biotechnology.

Abstract: *Rhodospirillum rubrum* is a phototrophic anaerobic bacterium capable of growing under a broad variety of conditions and appears particularly well suited for growing on synthesis gas (a mixture of CO2, CO and H2) [1]. It is also a particularly attractive organism for the bioconversion of syngas into polyhydroxyalkanoates (PHAs), a biodegradable alternative to oil-derived plastics [2] . One of our major challenges is to enhance the growth rate of *R. rubrum* in moderate high pressures of CO, since this gas is toxic for *R. rubrum* at moderate doses, and has low solubility in water. For this purpose, **a strategy of random mutations of *R. rubrum* genome was carried out to increase genetic variability and eventually obtain an improved strain with tailored features for syngas uptake and tolerance.**

The procedure for mutating *R. rubrum* consisted in exposing the cells to UV radiation for a period of 15-20 minutes after which a protocol of five phases was followed for the adaptation, selection and isolation of interesting mutant clones. First, cells were recovered in rich media, after which they were transferred to anaerobic sealed bottles with SYN-acetate 10 mM under light [1]. An aliquot of the culture was transferred to a new bottle containing the same medium and 0,8 bar CO in the headspace. In the next step, the CO concentration was increased up to an inhibitory concentration (2,0 bar) in which we had never seen growth of the wild type strain in darkness. After one month of incubation, only five bottles turned turbid. 4-5 passages were made in SYN-acetate 10mM, CO 2,0 bar to enrich the culture with the fittest clones. The selective pressure was maintained by plating dilutions of the cultures into solid media contained in sealed bottles, with CO 0,8 bar in the headspace. Five clones of each bottle were isolated in rich medium and atmospheric gas and re-isolated twice in the same conditions.

A number of 18 surviving clones were evaluated based on their i) final biomass, ii) lag phase duration, and iii) gas production. Those clones with a lag phase shorter than 10 days (this is approximately half of the lag phase of the wild type) were further tested. No significant differences were observed in the final gas production/consumption of any clone. Two clones exhibited reproducible results in terms of a shorter lag phase, however, only one showed similar final biomass compared to the wild type. As the purpose of the random optimization strategy was to enhance growth sturdiness, this clone will be tested in future bioreactor assays with partial pressures of CO higher than those that can be tested in glass bottles.

Conclusions: The UV-mutagenesis strategy proved to be successful for the selection of new clones of *R. rubrum* with an enhanced CO-tolerance and adaptability. One of these clones clone will be further characterized in terms of its physiology, and their mutations will be unveiled.

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Induction of resistance in cell suspensions of pepper (*Capsicum annuum* L. var. *annuum*) by elicitation with extracts of *Moringa oleifera* leaves

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Thematic Areas: Plant Biotechnology

Abstract: Plant diseases caused by pathogenic organisms are responsible for great economic losses in the agricultural sector. The use of elicitors that induce the natural defenses of plants is an effective way to reduce the use of pesticides[1]. On the other hand, working with cell suspensions allows to reduce considerably the time and space necessary for the study of new eliciting compounds. In the present work, we studied the induction of resistance in cell suspensions of Padrón pepper (*Capsicum annuum* L. var. *annuum*) after elicitation with aqueous or ethanol extracts of *Moringa oleifera* Lam. In order to evaluate the induction of plant defenses, the phenolic content and antioxidant activity were determined, and the expression of two defense genes, *CaBPR1* and *CaSC1*, was analyzed by real-time PCR[2].
The phenolic content showed a significant increase in suspensions elicited with any of the two extracts of Moringa. On the contrary, induction of the antioxidant capacity was only observed in the suspensions elicited with the aqueous extract. The analysis of gene expression confirmed the defense-inducing effect of both Moringa extracts. A significant increase in the expression of *CaBPR1* and *CaSC1* was observed in cell suspensions within a few hours after elicitation with both extracts. This induction of the defense genes was also confirmed in whole pepper plants elicited with both Moringa extracts. Taken together, these results suggest that Moringa extracts have the potential to induce resistance in pepper.

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New molecular tools for the expression of foreign genes in microalgal nucleus

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Thematic Areas: Biotecnología Vegetal.

Abstract: The choice of an adequate promoter is essential to obtain good genetic transformation efficiencies. In microalgae, the best transformation efficiencies are generally obtained with endogenous promoters (León-Bañares et al., 2004). However, it is possible to express promoter-less genes in the nuclear genome of microalgae, as we have previously shown. Furthermore, we have demonstrated that the random insertion of a promoter-less marker gene and the subsequent isolation of the most robust transformants allow the identification of new strong promoters in microalgae (Vila et al. 2012). In the present work, using the freshwater microalgae *Chlamydomonas reinhardtii* as a host and the paromomycin-conferring resistance aminoglycoside 3`-phosphotransferase (*APHVIII*) gene from *Streptomyces rimosus* as marker gene, we have developed a new strategy to express transgenes in microalgae: Transformation with naked promoter-less genes. We chose the *BLE* gene, which encodes the Bleomycin resistance protein from *Staphylococcus aureus* as gene of interest. The *APHVIII* marker gene and the *BLE* gene have been randomly inserted into the nuclear genome, where their transcription relies on their adequate insertion into a region adjacent to an endogenous genomic promoter or in frame with a native gene. We have concluded that the *BLE* gene is, not only integrated into the genome, but also efficiently expressed in a high percentage of co-transformants. Moreover, by this method, transgenes are expressed under the control of endogenous promoters and integrated in the genomic environment of the promoter. This guarantees the presence of enhancers or other cis- or trans- regulatory sequences. We have demonstrated that the cotras formation with two promoter-less genes could be a valid approach for the expression of foreign genes, not only in microalgae, but also in other microorganisms, especially those with small compacted genomes with a high proportion of coding regions (Díaz-Santos et al., 2016).

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Antimicrobial and defence elicitor peptides in plant protection. From nature to the biotechnological product

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Thematic Areas: BIOTECNOLOGIA VEGETAL

Abstract: Losses due to pests account for near one third of the potential crop productivity, in spite of the actions taken for its control. In addition, plant food products safety is currently an important issue in food production. Pesticides have greatly contributed to improve crop productivity and food security, but with non-target effects due to adverse impact into environment and human health. The rising of new emerging plant pests, require novel pesticides for the new scenario of sustainable agriculture, and biopesticides from living organisms offer an alternative or complement to conventional pesticides for plant disease and pest control. Functional peptides have been proposed as novel biopesticides. Antimicrobial peptides (AMPs) are secreted by microorganisms to compete with other microorganisms, whereas those produced by animals and plants act as a first barrier of innate immune system to protect from microbial infection. Thus, AMPs are considered as promising compounds for plant disease control that meet current health and safety standards. However, natural sources of functional peptides have the disadvantage that the active compounds are at low concentrations, and generally require complex extraction procedures or may be toxic.

Syntethic analogs of natural peptides or the *novo* designed peptides with improved biological activity against many plant pathogens have been developed. Moreover, these peptides also possess advantages such as a minimized toxicity, better stability towards protease degradation and are biodegradable. Most of the AMPs have a lytic effect on pathogens, others can trigger plant defence mechanisms by inducing the pathways of resistance to pathogens or stress tolerance, and some peptides show combination between lytic and defence induction activity. Furthermore, there are certain peptides that show cell-penetrating properties without a lytic effect, and can be used as carriers to deliver conjugated moieties to the cell cytoplasm. Although synthetic peptides present several advantages, there is an important limitation for their use for agricultural purposes due to the production cost. To overtake this limitation, the biotechnological production using microbial or plant biofactories seems to be a good solution. Currently, several synthetic peptides have been expressed on plants, and a few have been produced using bacterial and plant biofactories.

In our research, we started with a synthetic library of AMPs (CECMEL 11). Further, we designed, synthetized and evaluated derivatives of the leader peptide BP100 for expression in plant systems (1). Afterwards, the BP178, a derivative of BP100 was expressed in the rice seed endosperm and we were able to produce and recover bioactive peptide using rice seeds as biofactories (2). Finally, were are testing AMPs for their antimicrobial activity and their capacity in eliciting plant defense responses, for controlling diseases caused by *Xylella fastidiosa*, a phytopathogenic bacteria which is devastating olive trees in Puglia region (Italy) and it is present in Balearic Islands and Alicante province.

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Influence of culture media composition and solvent aqueous ratio on phenolic content and antioxidant activity of *Kalanchoe* cultured *in vitro*.

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Thematic Areas: Plant Biotechnology.

Abstract: The subgenus *Bryophyllum* (genus *Kalanchoe*) includes a number of species used in the traditional medicine across Africa, Asia and South America, due to their phytochemical properties [1]. Phenolic compounds have been highlighted as the major compounds related to the antioxidant activity in *Bryophyllum*. This study aims to analyze the proper solvent for the extraction of phenolic compounds from *in vitro*-cultured plants belonging to this genus: *B. daigremontianum* (BD), *B. tubiflorum* (BT) and *B.* x *houghtonii* (BH). As phenolic compounds are usually extracted by hydromethanolic solvents [2], here we perform a phenolic extraction of both aerial parts and roots from adult *Bryophyllum* plants grown *in vitro*, using four solvents with the next methanol:water ratios: 40:60, 60:40, 80:20 and 100:0; named as M40, M60, M80 and M100. The extraction efficiency was determined by the total phenolic and flavonol contents and the antioxidant activity, in terms of their free-radical scavenging activity. Moreover, we tested the influence of two culture media concentrations: MS medium and half strength macronutrient MS medium, since nutritional deficiencies lead to an intensification of secondary metabolism [2,3]. In this communication, we will show that such reduction enhances the biosynthesis of phenolic compounds and enables a higher antiradical activity. We found that aerial parts showed higher phenolic rates and antioxidant activity than roots. Concerning different species, aerial parts from BD and BH recorded their maximum antiradical activity rate when using M80, while BT showed a stronger affinity towards M60. On the other hand, root-derived extracts were more efficient when treating with more polar solvents, such as M40. Antioxidant activity was highly correlated to total phenolic content and no correlation was found with flavonol content (Fig.1). These results will be discussed in the corresponding communication.

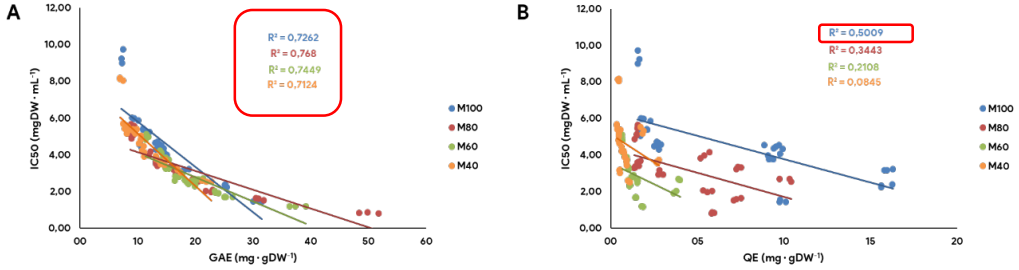


Fig.1. A) Correlation plot between antioxidant activity, IC50 (mgDW · mL^{-1}), and total phenolic content, expressed in equivalents of gallic acid, GAE (mg · gDW^{-1}). B) Correlation plot between antioxidant activity, IC50 (mgDW · mL^{-1}), and flavonol content, expressed in equivalents of quercetin, QE (mg · gDW^{-1}). Red squares indicate significant determination coefficients ($p<0.05$).

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Study of ectomycorrhizae communities in young chestnut trees using ITS sequences

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Thematic Areas: Plant biotechnology.

Abstract: Hifas Foresta (Forestry Department of Hifas da Terra) commercialized almost 40,000 chestnuts in the last campaign of 2017-2018. Each chestnut tree was inoculated, at least once, with fungal material from ectomycorrhiza fungi (ECM), but the inoculation was not always successful or the mycorrhizae were not always established properly on the client's plot and when it does, it does not always bear fruit. That is why we have studied the ECM mushroom community of different chestnuts of the company, to better understand which species of fungi we are facing. Specifically, we studied chestnut hybrids 111 and 125, grafted and ungrafted.

Root samples were collected during January-February 2018 from 1 and 2-year-old plots of chestnut tree. They were analysed following the procedure of Pestaña-Nieto & Santolamazza-Carbone (2009). We collected fine roots (<2 mm diameter) from 50 randomly selected chestnut samplings (approximately 120 cm of roots per tree). The morphological characteristics described by Agerer (1987-2002) were used to classify mycorrhized tips (Fig. 1). For every sampled tree, a subsample of each morphotype was analysed molecularly individually (Fig. 2). Once extracted and purified the DNA of each subsample, the basidiomycete specific primer pair ITS1F/ITS4B was used to PCR reaction. PCR products were purified and sequenced by Macrogen Laboratories. Sequences were edited and manually corrected before alignment to obtain a consensus sequence using the software BioEdit (version 7.0.8). All sequences were identified to genus or species level by querying the GenBank database, using the nucleotide–nucleotide (blastn) BLAST search option. The relative frequency and relative abundance of each taxon were calculated to find the Importance Value (ImpV) of each taxon. We calculated the Mycorrhizal Rate (MycoR) of each tree too.

The 125-ungrafted hybrid has a higher MycoR than the 111-ungrafted hybrid. Plant genotype did not influence mycorrhizal rate when saplings are grafted. Hybrid 111 tends to have a higher MycoR when grafted. In addition, the species with greater ImpV tend to be different between the hybrid 111 grafted and non-grafted. However, the graft does not influence the hybrid 125. On the other hand, the species with greater ImpV tend to change with the age of the chestnut tree inoculated.

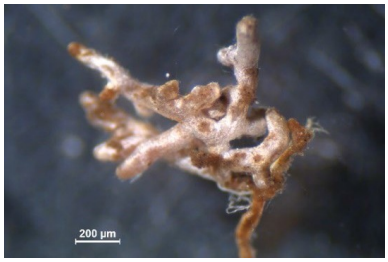


Fig. 1. Ectomycorrhiza identified as *Scleroderma citrinum*

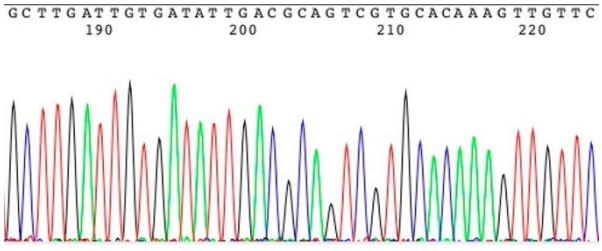


Fig. 2. Detail of a chromatogram of an ectomycorrhiza DNA sequence

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Identification of key factors to optimize plant tissue culture using neurofuzzy logic

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Thematic Areas: Plant Biotechnology

Abstract: Neurofuzzy logic is a powerful tool for identifying cause-effect relationships. Here it was used to discover the key factors that explain the observed variability during *in vitro* plant micropropagation, which involves the effect of several factors such as ion medium composition, plant growth regulators (PGRs), vitamins, etc. on the response parameters.

In this study, explants (axillary buds) from *Actinidia arguta* cv. Issai (hardy or kiwi berry) were cultured for 50 days in 4 different media: MS[1], St[2], B5[3] and R (an experimental medium designed by our group). Each media was supplemented with 20 different combinations of the PGRs: BAP (6-benzylaminopurine) and GA_3 (gibberellic acid). Several artificial neural networks models were built, in order to determine which are the key factors in the establishment of an *in vitro* proliferation culture medium for the cultivar used in this study. The influence of these factors on 2 response parameters (shoot number and leaf number) was studied.

Neurofuzzy logic was capable of accurately model both parameters. The generated model for the shoot number indicated that the variability of this parameter is explained by the effect of two factors, the plant growth regulator BAP and the ammonium ion (NH_4^+). The generated model for the leaf number, stablishes the interaction between phosphate ion (PO_4^{3-}) and GA_3 and between sodium ion (Na^+) and BAP, as the key factors for this parameter (Fig. 1)

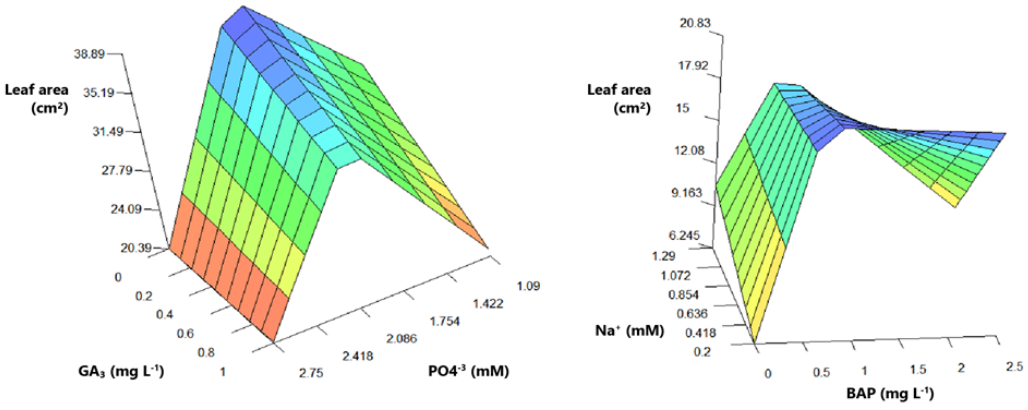


Figure 1. Neurofuzzy logic's 3D graphs with the interactions that produce a significative effect on the leaf area parameter.

The obtained results allowed us to determine which are the key factors for an adequate *Actinidia arguta* micropropagation, demonstrating the high prediction capacity and accuracy of neurofuzzy logic.

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Polydopamine nanoparticles: a study of the conditions that determine their size and loading-capacity of different metals

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Thematic Area: "Biotecnología de la Salud"/ "Nuevos retos en Biotecnología"

Abstract: Polydopamine, which is a synthetic melanin analogue, has acquired an important role in Nanomaterial Sciences in recent years, mainly thanks to its biocompatibility, coating ability and chemical versatility [1]. Thus, for example, this last property is related with the well-known polydopamine capacity for the chelation of diverse metallic cations [2], which has favored the use of this polymer for devising new diagnosis and therapeutic systems [3,4], among others numerous applications.

In this manner, polydopamine nanoparticles (200 nm) were synthesized in previous studies and their interaction with Fe^{3+}, Ca^{2+}, Na^{+}, Zn^{2+} and Cu^{2+} was analyzed [3]. As metal loaded-particles could be really useful for various research fields, the main goal of the work that is here summarized has been studying the polymerization-reaction conditions that determine polydopamine nanoparticles size and, therefore, their metal-loading capacity.

Thereby, different parameters have been studied in order to be able to control polydopamine nanoparticles' size in a reproducible manner. The ammonium hydroxide concentration that is necessary for the polymerization reaction has been varied, as well as the basic aqueous medium in which it takes place and the presence of oxygen. Such changes in the synthesis process have allowed obtaining nanoparticles with different diameters and, three of them (120, 180 and 530 nm), have been finally selected to determine nanoparticles' loading capacity of Fe and Cu depending on their size. For such purpose, absorption isotherms of both cations in the three distinct nanoparticles have been obtained and EDX TEM images have been taken to corroborate structural changes into loaded-nanoparticles.

As result, it has been proven that higher ammonium hydroxide concentrations enable to decrease nanoparticles size and that the smaller the size of the polydopamine nanoparticles, the greater loading-ability of both mentioned cations they present.

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Evaluation of salicylic acid permeation through silicone membrane in presence of biosurfactant produced from corn steep water

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Thematic Areas: Health biotechnology
Preferred poster presentation

Abstract: Salicylic acid is an active principle widely used in cosmetic and pharmaceutical formulations. It is a lipid-soluble phytohormone, with several properties in topical uses. For instance, its comedolytic and dermatolytic properties convert salicylic in an interesting active principle against acne vulgaris or xerotic disorders, among others[1,2]. On the other hand, in cosmetic and pharmaceutical formulations, the permeation of active compounds is a big challenge. Consequently, formulations contain surfactants, improving the solubilization of these principles actives[3]. However, chemicals surfactants can produce side effects like irritation or allergies. In this sense, the use of biosurfactants represents an interesting alternative to chemical surfactants because they are considered less toxic, more biocompatible, and with the same properties as their synthetic counterparts[4]. Therefore, the aim of this work was to study the effect of a biosurfactant extract obtained from a corn wet-milling industry stream in the permeation of salicylic acid through a silicone membrane called polydimethoxysiloxane (PDMS).

The biosurfactant evaluated in this work is produced in corn steep liquor by spontaneous fermentation and it was extracted following the procedure stablished by Vecino et al.[5]. For the permeation study, it was employed flow-through diffusion cells, with PDMS membranes, following the methodology employed by Bhuiyan et al [6]. In this case, the donnor solutions were saturated solutions of salicylic acid in PBS in presence or absence of biosurfactant extract. In order to determine the influence of micelles in permeation, two different concentrations of biosurfactant extract were tested: 5mg/L and 500 mg/L, below and above its critical micellar concentration (CMC), respectively.

The experiment carried out showed that salicylic acid improves its permeation in presence of biosurfactant from CSL, in comparison to the control without biosurfactant. Therefore in presence of 5 mg/L of the permeation of salicylic acid increased about 43% in comparison with the control.

It can be concluded that the biosurfactant, under evaluation, could be an alternative to chemical surfactants not only to solubilize hydrophobic compounds, in aqueous solutions, but also to increase the permeation of active principles through biological membranes.

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A biosurfactant extract obtained from corn as L-ascorbic acid stabilizer

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Thematic Areas: Health Biotechnology
Preferred presentation: Poster

Abstract: Vitamin C or L-ascorbic acid is a water-soluble carbohydrate essential for the human health. One of the most known properties of Vitamin C is its antioxidant capacity. In this sense, Vitamin C is able to regenerate other antioxidant species as the fat-soluble Vitamin E[1]. However, humans cannot produce this vitamin, what makes necessary to include it in health care products, in order to show an efficient effect over the skin. Moreover, Vitamin C is easily degradable making quite difficult to keep it as L-ascorbic chemical form during long time. Thus, cosmetic and pharmaceutical industries include dehydrated Vitamin C in its formulations, which must be rehydrated before use, leading to a tedious process.

Therefore, the aim of this work consists of avoiding the degradation of Vitamin C through the use of a biosurfactant extract obtained from corn steep liquor (CSL) that posseses surfactant and antioxidant properties[3]. The biosurfactant extract (BS) under evaluation was obtained from CSL, a residue of the corn wet-milling industry, where BS is produced in presence of lactic acid bacteria among other microorganisms, that growths spontaneously in CSL, following the protocol established by Vecino et al.[2].
For the evaluation of BS as stabilizer agent, this was added to an aqueous solution of L-ascorbic acid (AA) measuring its degradation within time by HPLC. In this regard, a 15-experiment Box-Behnken factorial design was established in order to predict the degradation of Vitamin C with the time, in presence and absence of biosurfactant, under different concentrations of both Vitamin C and biosurfactant extract.

In terms of L-ascorbic acid stability, different percentages of biodegradation were obtained depending on the concentration of both, L-ascorbic acid and biosurfactant extract, used in the experiments. In general, it was stated an improvement of L-ascorbic acid stability when the biosurfactant extract was present, increasing between 13% and 58% its stability. Moreover, there were observed low degradation values (24%) after 21 days of experiment, when the biosurfactant was present, making it suitable as stabilizer agent in formulations where Vitamin C is involved. The effect of the biosurfactant extract, under evaluation, on L-ascorbic acid stabilization can be also related, not only with the presence of antioxidants but also with the presence of palmitic acid in this extract, as it has been demonstrated to exert some stability over L-ascorbic acid in cream formulations[3-4].

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Engineering high copy number plasmids for pharmaceutical-grade pDNA production in Lactococcus lactis

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1. Introduction

The cost-effective production of high quantities of pharmaceutical-grade plasmid DNA (pDNA) is a key point for several biotechnological and pharmaceutical applications, such as the *ex vivo* production of DNA vaccines and recombinant proteins and also for the use of food-grade bacteria in live mucosal vaccination.
Lactic acid bacteria (LAB), particularly *Lactococcus lactis* are a safer alternative to the lipopolysaccharide (LPS)-producing *Escherichia coli* for pDNA manufacturing, since they are naturally LPS-free and have a food-grade and Generally Recognized As Safe status. In order to establish a cost effective *L. lactis*-based pDNA manufacturing platform it is imperative to engineer new high-copy number plasmids, since the highest copy number reported for the pAMβ1 replicon is around 100 copies.

2. Methods

A site-directed mutagenesis approach was used to modify the Ribosome Binding Site (RBS) of the *repDE* promoter of the pAMβ1-based pTRKH3 plasmid, in order to achieve different plasmid copy number (PCNs) in *L. lactis* LMG19460 cells. The PCN per chromosome was determined by real-time quantitative PCR. The mRNA Minimum Free Energy secondary structures and the correspondent Gibbs free energy values were predicted. The mRNA of the *repD* gene, along with the mRNA of its transcriptional repressors, were also quantified.

3. Discussion

With the exception of pTRKH3-a and pTRKH3-e, the average PCN of the modified plasmids at 10.5h of growth (i.e. late exponential/early stationary phase) were statistically higher ($p<0.05$ for pTRKH3-c and $p<0.01$ for pTRKH3-b, pTRKH3-d and pTRKH3-f) than the PCN obtained for the wild-type pTRKH3. The highest average PCN value was obtained for pTRKH3-b (215±38), representing a 3.5 fold increase over the wild-type plasmid (62±5) and much higher than the maximum of ~100 copies obtained for the pAMβ1 replicon in previous studies.
The pTRKH3-b plasmid has an unexpected 77 bp insertion, which led to the creation of an additional RBS sequence and two additional relevant start codons. Without that insertion, the mutant lost the ability to replicate with such a high PCN.
The *in silico* mRNA secondary structures analysis predicted the pTRKH3-b mutant as the one having the most negative ΔG value, which could promote the RepDE expression, leading ultimately to an increase in the PCN.
A principal component analysis performed with the mRNA quantification results showed that pTRKH3-b had an intermediate amount of CopF and CT-RNA repressors.

4. Conclusions

pTRKH3-b is a very promising high copy number shuttle plasmid that will contribute to bring LAB to the same level as *E. coli* as pDNA producers, with the additional advantage of being endotoxin free, which turns the process more industrially profitable.
Recently, an effort is being made in our research group in order to engineer knockout *L. lactis* strains that could improve even more the quality and quantity of the pDNA produced.

Peptide biomarkers for biogenic amine-producing bacterial characterization by LC-ESI-MS/MS.

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Thematic Areas: Food biotechnology

Abstract: Some bacterial strains have the ability to decarboxylate specific amino acids producing biogenic amines (such as histamine from histidine). The detection of these strains having amino acid decarboxylase activity is important to lower the risk of biogenic amine in food products, especially histamine. Histamine is regulated in fish (lower than 100 mg/Kg^{-1}, but it is generally recognized that the presence of other biogenic amines aggravate symptoms from histamine. Molecular methods for the detection of biogenic amine-producing bacteria in foods are based on the specific detection of decarboxylase genes by PCR allowing the detection before the biogenic amine is produced [1]. However, the difficulty is the availability of universal primers. Mass spectrometry [2] can provide a useful tool for the detection of strain-specific diagnostic decarboxylase peptides.
In this study, proteins from biogenic amine producing strains were isolated, after tryptic digestion, the resulting peptides were analysed by LC-ESI-MS/MS (Liquid chromatography-electrospray ionization-tandem mass spectrometry). A total of 100µg protein extraction was digested with trypsin, cleaned on a C18 microSpinTM, following by LC-MS/MS analyse. Proteomic data was processed by SEQUEST (Proteome Discoverer 1.4 package, Thermo Scientific) against Bacteria in the UniProt/ TrEMBL database. MS data was analysed to identify the peptides from the amino acid decarboxylases and then compared to the GenBank database using the Blast tool (NCBI) to achieve peptide/protein identification. Thus, decarboxylase peptides were detected in studied strains that could be used as biomarkers in further studies.

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Peptide biomarkers for mastitis producing *Streptococcus* spp. identification and characterization by LC-ESI-MS/MS.

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Thematic Areas: Food biotechnology

Abstract: Classical and culture-based methods for pathogen identification and characterization of their biochemical properties are slow and labour-intensive. Molecular microbial diagnostic methods based on genomics and proteomics allow a faster and more effectively species identification. Liquid chromatography-electrospray ionization-tandem mass spectrometry (LC-ESI-MS/MS) has been used for the analysis of bacterial pathogen strain-specific diagnostic peptides allowing the characterization of bacterial strains [1,2].

In this study, tryptic digestion peptides from mastitis producer *Streptococcus* spp. isolated from milk have been analysed by LC-ESI-MS/MS to search for specific biomarkers useful for a rapid identification of both, the bacterial species and the physiological and biochemical characteristics such as the expression of virulence factors including toxins, immune-modulatory factors, exoenzymes and so on. Thus, a total of 100 µg protein extraction was digested with trypsin, cleaned on a C18 microSpinTM, and then analysed by LC-MS/MS. Proteomic data was processed by SEQUEST (Proteome Discoverer 1.4 package, Thermo Scientific) against Bacteria in the UniProt/ TrEMBL database. Specific peptides from proteins such as bacteriophage proteins, toxins and M protein, were found and could be useful as specific biomarker peptides.

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Fish Parasite Biobank: supplier of sample and data for studying the genetic structure of zoonotic *Anisakis* spp. from European hake *Merluccius merluccius*

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Thematic Areas: Food Biotechnology

Abstract: Introduction: The Fish PARASITE Biobank (FPB), certified with ISO 9001, was implemented on 2013 as a traceable high-quality sampling platform for the largest surveillance plan ever conducted in EU fish production value chains. The Biobank collects and records fish zoonotic parasite samples and their associated information under strict conditions of traceability, which can be used for scientific, industrial and clinical studies. Among these zoonotic parasites, there are biobanked nearly one hundred thousand nematode larvae of the genus *Anisakis* coming from viscera and muscular tissues of different commercially important fish species from several geographic range. The focus of our study were the *Anisakis* specimens recovered from the European hakes *Merluccius merluccius* from the major fishing area FAO27, Northeast Atlantic Ocean, with the aim to elucidate eventual genetic differences among *A. simplex* parasites from nine sub-areas of FAO27 and several fish flesh sections. For this purpose, we identified the *Anisakis* spp. larvae by molecular methods and subsequently established the population genetic structure of the species *A. simplex* studying their cytochrome oxidase subunit 2 (COX2) gene.

Methods: A total of 675 *Anisakis* specimens biobanked from European hakes from nine sub-areas FAO27, IVa, VIIb, VIIc, VIIh, VIIj, VIIIa, VIIIc, VIId y IX, were included in this study. 75 *Anisakis* recovered of five hakes of each sub-area, including specimens from belly flaps, loins and tail, were selected to perform the molecular study. Genomic DNA of all larvae was obtained and each parasite was identified by PCR assay based on ITS markers and sequencing. The COX2 region was subsequently obtained for those specimens identified as *A. simplex* and the sequences achieved were analysed in the software DnaSP v. 6 to calculate the number of haplotypes, the haplotype diversity, nucleotide diversity, number of segregating sites and the average number of nucleotide differences. Joining haplotype networks using Nexus software (www.fluxus-engineering.com) were constructed.

Results and conclusions: The preliminary results of genetic identification of *Anisakis* parasites showed that the predominant species in hakes from European waters is *A. simplex* (95,3%) and the remaining parasites were *A. pegreffii* (0.95%) and F1 hybrids *A. simplex* x *A. pegreffii* (3,8 %). *A. simplex* (53 larvae), is the only species in sub-area VIIb (West of Ireland), whereas *A. simplex* and hybrids were identified in the other sub-areas: 68 *A. simplex*/3 hybrids from IVa (Northern North Sea); 60 *A. simplex*/2 hybrids from VIIc (Porcupine Bank) and 58 *A. simplex*/2 hybrids from VIIj (Southwest of Ireland). The sub-area XIIIa, corresponding to the North of Bay of Biscay, showed to be a sympatric area for *A. simplex* (63), *A. pegreffii* (3) and hybrids (5). The analysis of the *A. simplex* haplotypes will show the population genetic structure at the intraspecific level ant it could give information not only of epidemiological aspects of the parasite but also about the population genetic structure of its host.

Analysis of the composition of commercial fish feed by NGS: implications for parasite risk exposure assessment

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Thematic Areas: Food Biotechnology

Abstract: Introduction: Aquaculture feeds are formulated with a wide range of ingredients like fishmeal and fish oil from fisheries of small pelagic fish and by-products obtained from fish processing. The incorporation to the feeds of wild-fish and fishery by-products can introduce biohazards, which can be natural component in wild fish populations, such as parasites. Fish-borne larval nematodes belonging to the genus *Anisakis, Pseudoterranova, Contracaecum* and *Hysterothylacium* are cosmopolitan parasites that can accidentally infect human by consuming raw or undercooked seafood, causing gastrointestinal disorders as well as allergy. Clinical allergic response is only recognised to *Anisakis* larvae, but the evidences about the allergenic potential of the species of the genus *Hysterothylacium* are increasing. Currently, there are some evidences about the transmissibility of heat resistant allergens of *Anisakis* from feeds, which included fishmeal in its composition, to the animal farmed. Here, we have analysed the composition of ten dry commercial fish feeds by next generation sequencing (NGS), with the aim of evidencing for the first time the presence / absence of DNA from zoonotic and/or allergenic parasites in the composition of aquaculture feeds.

Methodology: Composition of ten fish feeds (FF1 to FF10), manufactured by 3 companies with international distribution, were analyzed by mtDNA-COI gene sequencing (150 bp) on an Illumina MiSeq platform. Sequences with percentage of similarity higher than 97% were simply clustered at unique sequence (operational taxonomic units -OTUs) using CD-HIT clustering program and OTUs were classify in taxonomic groups, phylum, family and genus using BLAST (database of NCBI).

Results: the sequences of ten fish feeds were assigned to 17 phyla, mainly Chordata and Streptophyta and in percentages lower than 1% to other phyla such as Nematoda. FF1 and FF4 was mainly composed by *Sprattus* (more than 61%); FF3 by *Ammodytes* (44%); FF6, FF7, FF8, FF9 and FF10 by *Clupea* spp. (between 87% and 60%); and FF2 and FF5 were mainly composed by vegetables of the genus *Triticum* (85% and 82%, respectively). Raphidascarids parasites were detected in the samples FF1, FF5, FF6, FF8 and FF9; and anisakids in the samples FF3 and FF10. Raphidascarid sequences showed a 100% of genetic identity with sequences of *Hysterothylacium aduncum* obtained from PARASITE Biobank (González *et al*. 2018); whereas Anisakidae sequences showed values of identity between 97% and 83% (query cover > 95%) with *Pseudoterranova, Anisakis* and *Contracaecum* species deposited in the GenBank.

Conclusion: Small pelagic species belonging to the genus *Sprattus*, *Clupea* and *Ammodytes* have been described as intermediate or paratenic host of *Anisakis* and *Hysterothylacium* species around the world. The detection of DNA from ascaridoid nematodes evidences for the first time its incorporation to the composition of the fish feeds and warns about the potential presence of heat resistant proteins involved in allergic reactions. Results suggest that this risk exposure should be taken into account when dietetic recommendations related to the consumption of farmed fish are formulated to sensitized patients.

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Putative probiotic lactic acid bacteria isolated from non-conventional sources

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Thematic Area: Food Biotechnology

Abstract: As defined by the Food and Agriculture Organization (FAO) and the World Health Organization (WHO), probiotics are known as live microorganisms that, when administered in adequate amounts, confer a health benefit on the host. They have different health-promoting functions preventing diarrhea, regulating the immune system, maintaining healthy gut microbiota, reducing hypertension, lowering cholesterol levels and improving lactose metabolism, among others. Probiotics typically have been added to fermented dairy products. However, milk allergies, high fat and cholesterol, lactose intolerance and the growing trend toward veganism have stimulated the research in the field of non-dairy probiotic products, particularly based on vegetable matrices. So far, only lactic acid bacteria isolated from human gastrointestinal tract are recommended by FAO and WHO to be used as probiotics. Nevertheless, more and more studies suggest that probiotic strains could be isolated from other sources. Since apart from probiotic properties, strains for this kind of products must be able to grow and/or survive in heterogeneous food matrices, and also satisfy different technological characteristics, these non-conventional strains could be of interest for the elaboration of more diverse non-dairy probiotic products (1, 2). The aim of this study was to evaluate the probiotic potential of two strains of lactic acid bacteria isolated from fruits.

Strain identification was done by sequencing the 16S rDNA gene. The probiotic properties studied (acid, phenol, and bile salt tolerance, resistance to different enzymes, hydrophobicity, auto-aggregation ability, bile salts hydrolysis capacity and antimicrobial and antioxidant activities) were tested by adapting protocols described previously (3).

The two isolates were identified by the 16S rDNA gene sequence as belonging to the genus *Lactobacillus*. Furthermore, their probiotic potential was evaluated. Both strains exhibited good growth capacity and high survival rate under simulated gastrointestinal conditions. They were also resistant to the treatment with lysozyme, pepsin, and pancreatin. In addition, the tested strains showed as well ability to autoaggregate, which may favor their adhesion to the host cells. The bile salt hydrolase activity displayed by the isolates could play an important role in the reduction of cholesterol. Antioxidant activity and antibacterial properties against different pathogens were also exhibited by the studied strains, thus enhancing their probiotic properties.

Taking into account the results obtained it can be concluded that the new LAB strains have a good potential to be used as probiotics, and due to their origin, can be proposed to be tested in the elaboration of non-dairy probiotic foods.

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Potential of surplus harvest vegetables as new sources of protein for human consumption

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Thematic Areas: Biotecnología alimentaria (Nuevos alimentos y tecnologías aplicadas a alimentación).

Abstract: The increase in protein demand, both for animal and human food, means that current sources of proteins are becoming unsustainable from an economic and environmental perspective. This leads to the search for new alternative proteins. This work suggests the use of surplus harvest vegetables as potential sources of low-cost dietary proteins for human consumption. RuBisCO, present in green leaves of fruits and vegetables, is the most abundant protein in earth[1] but remains largely underutilized in the food system. This non-allergenic protein contains not only a balanced composition of amino acids, whose nutritional value has been ranked as superior to those of some other food proteins[2], but also certain desirable functional properties that make it suitable in several formulated food systems.
Galician cabbage (*Brassica oleracea*) leaves were used as a model to optimise the extraction and purifying process. Leaves were processed with three different strategies: direct use of fresh leaves, leaves blanched for 3 min, and leaves treated with sodium metabisulfite. A green juice was obtained in each case pressing the leaves with a blender. Leaf proteins were extracted from the juice by heat precipitation (50 ºC, 30 min)[3]. The heated juice was centrifuged at 15,000 g for 30 min and carefully separated into supernatant and pellet.
An unique band was detected in the SDS-PAGE for supernatants from fresh leaves (SFL50) (Figure 1A). The protein determined by the BCA method in the SFL50 accounted for 34.89±1.99% of the protein determined by the Kjeldahl method in the leaves. The percentage of the protein in the supernatant of blanched leaves (SBL50) was lower (25.97±0,79). Probably an important protein fraction was denatured during the blanching process and eliminated with the pellets (PBL50), where an intense RuBisCO band was detected (Figure 1B). However, the SFL50 sample was quickly browning. Sodium metabisulfite was incorporated (SFLM50) to prevent sample browning, resulting in highly effective (Figure 1C).

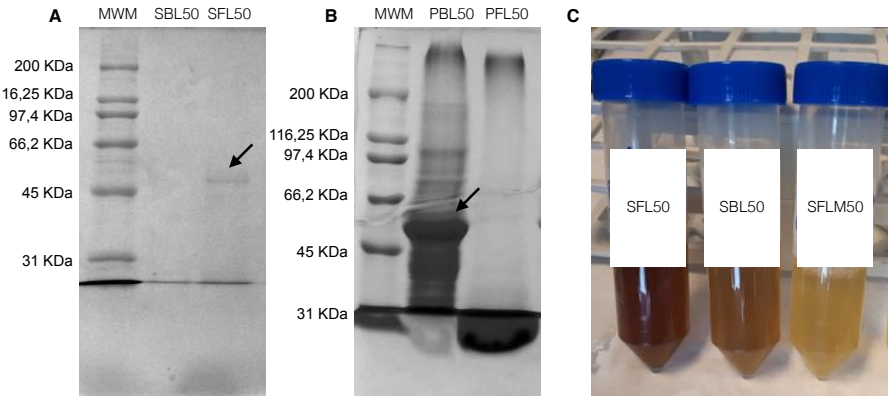


Figure 1. SDS.PAGE of: A) supernatant of blanched leaves (SBL50) and fresh leaves (SFL50); and B) pellets of blanched leaves (PBL50) and fresh leaves (PFL50). C) Supernatants obtained from fresh leaves (SFL50), blanched leaves (SBL50), and fresh leaves treated with sodium metabisulfite (SFLM50). The arrows indicate the band for RuBisCo subunit of 53 KDa.

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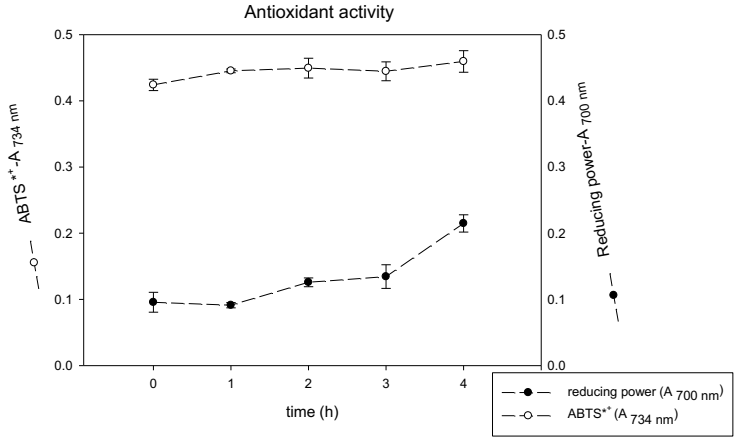
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Dairy hydrolysates obtained using an enzymatic extract from *Aspergillus niger*

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Thematic Areas: Biotecnología alimentaria (Nuevos alimentos y tecnologías aplicadas a alimentación).

Abstract: Enzymatic hydrolysis improve functional properties of dietary protein and may also lead to the development of biologically active peptides.[1,2] For example, antihypertensive peptides (ACE-inhibitory peptides) are generally achieved by the use of proteases from microbial, plant or animal origin. In this work, we evaluated a novel extracellular enzymatic extract produced by *Aspergillus niger* NRRL3 to yield hydrolysed milk.[3] To know the actual number of peptide bonds cleaved during the reaction, or degree of hydrolysis (DH) is a useful way of monitoring the extent of protein degradation.[4] We found that DH increases with time as well as with the percentages of extracellular enzymatic extract added, with a maximum of 85 % of GH at t=15 h with 10 % of extract. In addition, hydrolysates were evaluated as antioxidant agents and ACE inhibitors. The antioxidant activity of hydrolysates was measured with in vitro test $ABTS^{\bullet+}$ scavenging and reducing power by Fe^{3+}-Fe^{2+} transformation. The Figure shows the antioxidant activity at different hydrolysis reaction time. Finally, ACE-inhibitory activity of hydrolysates was determined, and these were able to inhibit ACE up to levels between 55 to 78 % depending on the hydrolysate. This work demonstrated the ability of the extract enzymatic produced by *Aspergillus niger* NRRL3 to obtain hydrolysates from milk and their application as a potential antioxidant and antihypertensive additives.



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Polyunsaturated fatty acid production by microalgae: optimisation and scale-up

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Thematic Areas: 7. Biotecnología alimentaria

Abstract: The consumption of polyunsaturated fatty acids (PUFA) and their importance has increased significantly in recent years due to the benefits associated with the reduction of cardiovascular risks and their role in the prevention and treatment of cardiovascular diseases.
Traditionally, the sources of these acids are related to fish oils or their by-products, but the interest and search for more sustainable sources has increased in recent years (1). The use of microalgae as a source of long-chain polyunsaturated fatty acids, especially docosahexaenoic acid (DHA), has attracted interest in recent years. These microalgae have proven to be sustainable alternatives to traditional sources of the aforementioned fatty acids, such as fish oils. For this reason, efforts are currently focused on the development of highly efficient microalgae production systems that improve the productivity of biomass as well as its quality (2).
The main objective of this work is the development of a system for the production of fatty acids from a known microalgae strain as *Crypthecodinium cohnii* under optimised conditions (salinity, carbon source and its concentration) and its scale-up to an airlift reactor.
Initially, the microalgae biomass and PUFA production were evaluated operating at several salinity levels (25, 50 and 75% of the sea salt concentration). As result of the experiments, a salinity of 75% presented the high microalgae biomass production rate. Moreover, the PUFA content after 10 days also showed the best results when the high salinity concentration was used. In the next step, the effect of carbon source (ethanol, glucose and sodium acetate) was assayed. Maximum biomass production was achieved when ethanol was used as unique carbon source with levels higher than 10 g/L. This value decreased on glucose cultures following to the attained when acetate was added. After that, the effect of concentration of ethanol and glucose was evaluated. The validation of the positive effect of salinity and ethanol was carried out by operating at 0.2 L-Erlenmeyer flasks (Figure 1) and bench scale bioreactor, operating in an airlift bioreactor. It was observed that the culture at optimal conditions in an airlift bioreactor allowed reaching similar levels to those recorded at small scale. Additionally, the kinetic characterization of the process by means of logistic model was performed, in order to elucidate the characteristics of the microalgae growth and synthesized PUFA.

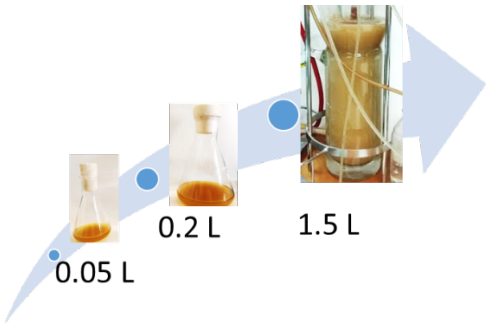


Figure 1. Scale-up from flask to airlift bioreactor

Acknowledgements: This research has been funded by Xunta de Galicia and ERDF Funds (Project ED431C 2017/47) and CDTI (Project IDI-20180390).

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Amelioration of Omega-3 docosahexaenoic acid production by microalgae

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Thematic Areas: Biotecnología alimentaria

Abstract: Omega-3 fatty acids docosahexaenoic acid (DHA) and eicosapentaenoic acid (EPA) have attracted worldwide attention for their benefits in the human health and the global demand is growing. However, their use for food supplements or additives is limited by the traditional commercial source of the product, fish oil. Mass-scale fisheries become an unsustainable option for achieving these products and a sustainable resource management is required. Among the different alternative sources (fungi, bacteria, algae,…), marine microalgae are plausible candidates for those fatty acid production. Several production system has been proposed, but a decrease in the production cost and increase in the DHA production are being demanded by the sector.
The aim of the study is the improvement of the Omega-3 fatty acids production process in a stirred tank reactor (STR) and the augmentation of the produced DHA. To that end, different aspects of the production related with the mechanical and process variables were tackled. The substrate inhibition in the production of DHA was avoided using a fed-batch system. At the beginning, the effect of the agitation blades (flat turbine type and radial propeller blades) were studied for this purpose. The obtained results showed that radial propeller blades leaded to a higher turbulence and biomass disruption in comparison with the flat blades, causing a decrease in the DHA production. Then, the stirring rate of the reactor was evaluated over the process time. At initial stage related with biomass growth, a lower agitation rate (0-100 rpm) resulted more favourable for the biomass production and a progressive increase in the rate was required in a second stage more related to the DHA production and consequent increase in the oxygen consumption by the microalgae. The source of carbon was also studied and ethanol and glucose were selected as plausible alternatives. Operating the bioreactor with glucose resulted in a higher biomass production (51.50 $g_{biomass}/L$) in comparison with the one using ethanol as carbon source. After that, the growth of the microalgae in the reactor was carried out without any pH control. The absence of pH regulation lead to the apparition of a red colour in the culture media, which could be related to the production of carotenes. The microalgae production also included other fatty unsaturated acids as omega-7 (vaccenic acid) and omega-9 acids (oleic and elaidic acid). Operating at the optimal conditions production levels of 65.78 mg DHA/$g_{fatty\ acids}$ were attained and 77.705 mg oleic and elaidic acids/$g_{fatty\ acids}$.

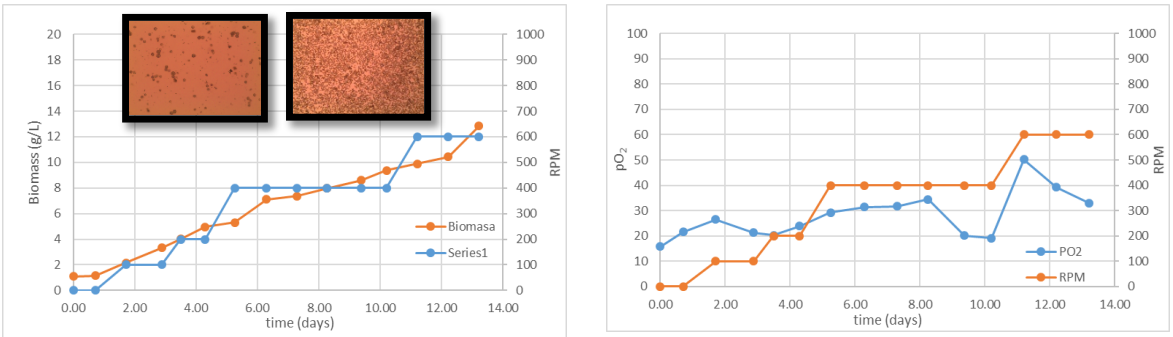


Figure 1. Biomass production, dissolved oxygen and stirring profile in a CSTR operating with ethanol as carbon source. Inset: microscopy picture of biomass (4x) after 1 and 10 days of growth.

Acknowledgements: This research has been funded Xunta de Galicia and ERDF Funds (Project ED431C 2017/47) and CDTI (Project IDI-20180390).

Effectiveness *of dietary supplementation of microalgae as aquafeed additives* for the juvenile white prawn, *Litopenaeus vannamei*

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Thematic Areas: Food Biotechnology.

Abstract: In this study, experimental diets were formulated with a mixture of three species of microalgae, *Tetraselmis suecica, Dunaliella salina and Chaetoceros muelleri* (1%, 2% and 3% of the dry weight of the food). Subsequently, juvenile shrimps *(Litopenaeus vannamei)* were fed with the diets to analyse improvements in survival, growth and immune response (1). Diets were isoproteic, isolipidic, and isocaloric (including the control) with an apparent difference in the pigment content between diets (Table 1). Results of quality and bromatological analysis show that diets meet the standards used in typical aquaculture food (2). After 23 days of feeding, a significant increase in weight (P>0.05) was observed in shrimps fed with diet 1% of microalgae. In contrast, diets enriched with 2 and 3 % of microalgae showed no difference with the control (Fig.1). On the other hand, infection bioassay with bacterium *Vibrio parahaemolyticus* showed that all diets enriched with microalgae increased survival rate 54% more than the control diet. This increased survival was related with microalgae consumption. These findings suggested that low inclusion of microalgaea in shrimp diets during a short period of time can result in healthier organism and therefore could contribute to the development of optimized diets for shrimp farming.

Table 1. Proximal composition of experimental diet.

Diet	% Protein	% Fat	% Carbohydrates	% Pigments
Control	47.067±0.20	10.23±0.09	15.87±0.62	ND
1%	46.098±0.80	10.07±0.20	14.89±0.80	0.264±0.05
2%	48.090±0.60	8.05±0.08	14.86±0.30	0.406±0.01
3%	48.092±0.21	9.94±0.25	15.02±0.15	0.523±0.01

ND.-No detected

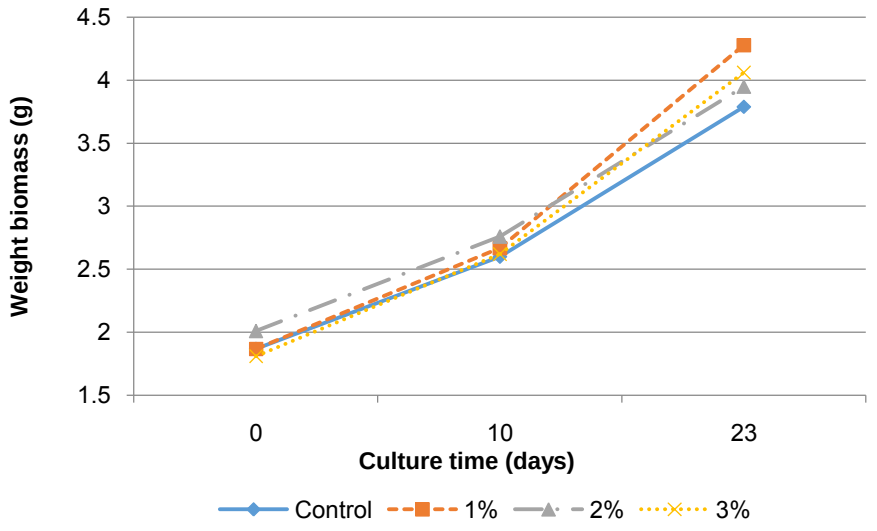


Fig.1. Mean of performance growth of *Litopenaeus vannamei* fed with different microalgae diets.

Acknowledgements: Authors express their gratitude to the Mexican National Council for Science and Technology (CONACYT).

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A NEW PROCEDURE TO PREPARE EDIBLE FILMS FROM PORCINE AND BOVINE BLOOD PROTEINS

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Thematic Areas: Biotecnología alimentaria.

Abstract: Over 300 million metric tons of plastics are produced in the world annually, the management of these plastic wastes has led to a serious ecological problem owing to their total non-biodegradability and the generation of toxic gases on combustion during incineration. Following the Bioeconomy Strategy guidelines for the EU, the research and production of new packaging materials using organic waste, residues and food discards is encouraged. The main objective of this work is to prepare and characterise new coatings and thin-layer packaging films using proteins from by-products of the meat industry (blood from slaughterhouses). For that purpose, bovine and porcine blood was gathered from slaughterhouses and centrifuged, then, the plasma fraction was undergoing to a new procedure to precipitate all the proteins contained in the raw plasma fraction. These proteins were finally solubilised in distilled water and the films were prepared via the dry-cast method using glycerol as plasticizer.

In order to characterize and understand the behaviour of these films, solubility, light transmission, mechanical properties, and other tests were performed. Films showed low solubility in water-based solutions with a pH close to 7 without necessity of using chemical crosslinkers, which usually show a high toxicity. The degree of solubility of films in aqueous solutions is a main parameter to characterize in these kind of packaging materials, since they have to stand in contact with food pieces that frequently have a high water content. Other films found in bibliography and also prepared using plasma proteins and without crosslinkers showed a very high solubility in water (close to 100%) (*1*). Furthermore, these new films resulted completely transparent (Figure 1), allowing the visual inspection of any product coated. In terms of mechanical properties, the results obtained suggest that these films are suitable to support the mechanical stress to which coatings foods are normally subjected.

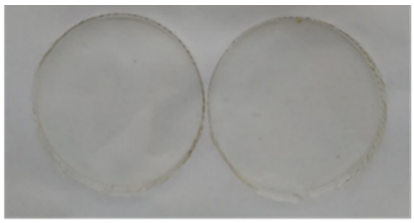


Figure 1. Edible films prepared using porcine (left) and bovine (right) plasma proteins.

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Influence of egg yolk proteins on the physical properties of gum arabic microparticles loaded with polyphenols

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Thematic Areas: Biotecnología alimentaria.

Abstract: Date palm pit extract represents an interesting source of polyphenols that provide antioxidant, anti-inflammatory, anti-bacterial and anti-viral functions. In addition, egg yolk is a valuable source of lipids, but its protein fraction (the granular fraction) is underused and shows different functional properties than the lipidic one. In this sense, the develop of new applications for these egg yolk proteins (EYP) could increase the commercial value of the egg yolk.

In this work, and with the aim of preserving the properties of the date pits extract, it was encapsulated using gum arabic (GA). Furthermore, the effect of the EYP on the properties of these microparticles was also tested. For that purpose, date pits polyphenols were encapsulated by freeze-drying using different ratios of GA and EYP as wall materials. The microparticles solubility, color, microstructure, Fourier transform infrared spectroscopy (FTIR), thermo-gravimetric analyses (TGA), encapsulation efficiency, antioxidant activity and their behavior into a food matrix (cheese, Fig. 1) were assessed.

The progressive incorporation of EYP to the wall material produced a decrease in the water solubility of these microparticles, which enables their addition into a food matrix with a high-water content. The EYP increased the antioxidant activity of the microparticles, in addition to the antioxidant activity already showed by the date pit polyphenols. TGA revealed that the microcapsules containing higher amount of EYP had a better thermal stability. FTIR confirmed the interactions between the date pits extract and the coating materials. The cheeses prepared maintained their textural properties when the microparticles were incorporated, and the microparticles with a high EYP content remained undissolved in the food piece.



Figure 1. A piece of cheese loaded with EYP-GA microparticles.

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Antioxidant capacity of macro and micro algae extracts of fresh and marine water with potential application in aquaculture.

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Thematic Areas: Food Biotechnology.

Food Biotechnology

Macro and microalgae have caught the attention of the aquaculture sector because they have a large number of compounds with biological activity. They produce phenols, polyphenols and carotenes known to exhibit antioxidant and antimicrobial activity. The antioxidant and antimicrobial capacity of 8 extracts from micro and macro algae was determined by the method DPPH (1, 1 Diphenyl 2-Picryl Hydrazyl) (1) and the method of a single disk (2), respectively. The microalgae *Nitzschia sp*., and *Spirulina sp*., presented the highest inhibition of the radical with 81.6 ± 3.1 and 76.4 ± 4.8% (Fig. 1A). A high antimicrobial capacity was observed by the microalgae *Tretaselmis suecica and C. muelleri*, which was significantly higher than the other samples with 2,382 ± 0.037 and 1.189 ± 0.629 cm, against *Vibrio parahaemolyticus* (Fig 1B). The antioxidant and antimicrobial capacity of microalgae demonstrates the potential for the use of these organisms in aquaculture, as a natural source of these types of compounds.

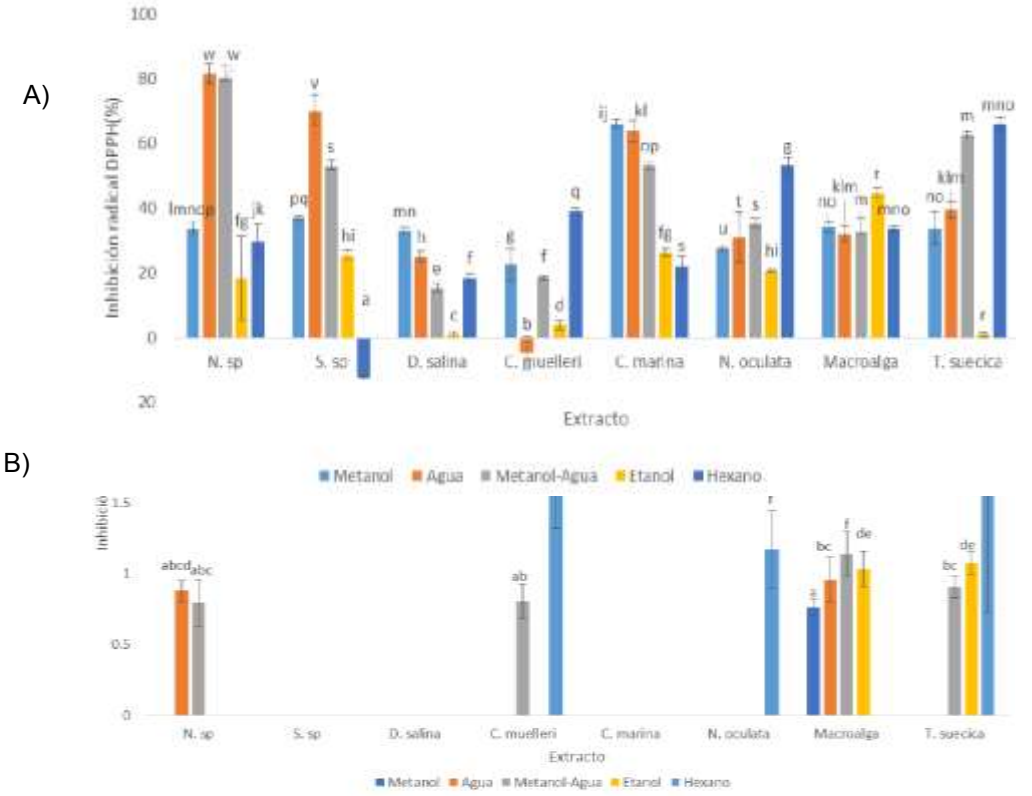


Figure 1. A) DPPH antioxidant activity and B) antimicrobial activity of macro and microalgaes.

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Improving the nutraceutical potential of *Eryngium viviparum* J. Gay through *in vitro* culture elicitation

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Thematic Areas: Food biotechnology.

Abstract: Food degradation by chemical oxidation represents one of the major problems for society. Antioxidants occur naturally in foodstuffs, but can also be added as preservatives to retard or prevent oxidation phenomena, since they are capable of breaking free radical-mediated chain reactions. Artificial antioxidants are the most commonly used compounds by the food industry, but the ingestion of many of these molecules have been linked to possible carcinogenic and toxic effects in humans (1). Therefore, it is necessary to search for new sources of natural antioxidant to help overcome these problems. Moreover, there are species that have interesting bioactive molecules thus they are in extinction and can disappear during time (2). Therefore, *in vitro* culture is a great biotechnological tool used both to conserve threatened plants as to improve the yield of secondary metabolites with antioxidant properties. Thus, this study was carried out to demonstrate the importance of the *in vitro* culture for conservation purposes and to elicit the production of phytochemicals with antioxidant properties in *Eryngium viviparum* J. Gay tissues. Explants of the threatened plant *E. viviparum* were cultured *in vitro* in solidified MS medium and a 3-level factorial design with 2 factors was implemented to studied the effect of cytokinins 6-benzylaminopurine (BAP) and kinetin (KIN) at 0, 1 and 2 mg L^{-1} on the elicitation of antioxidants. Hydroethanolic extracts (ethanol:water 80:20, *v/v*) were prepared from aerial parts and roots of *in vitro* culture by solid-liquid extraction (3). Two cell-based antioxidant activity assays were performed to assess the lipid peroxidation inhibition capacity, through the thiobarbituric acid reactive substances (TBARS) formation inhibition capacity using brain cell homogenates, and the anti-haemolytic capacity, through the oxidative haemolysis inhibition assay (OxHLIA) using sheep erythrocytes (3). The synthetic antioxidant trolox was used as positive control. The antioxidant activities were then correlated with the content of phenolic compounds, which were determined by HPLC-DAD-ESI/MS (4). The root extracts had higher antioxidant activity than aerial parts in both assays and both cytokinins were correlated with the increase of their antioxidant potential. The content of phenolic compounds was also higher in roots and strongly correlated with the recorded antioxidant activity. Regarding the aerial parts, only KIN increased the concentration of antioxidant compounds in the hydroethanolic extracts, but this activity was lower. Therefore, the best results were achieved in root extracts, which had similar antioxidant activity as trolox and the elicitation increased their phenolic compounds content by two times in comparison to the control sample without cytokinins. This study highlighted the interest of *in vitro* culture to obtain *E. viviparum* roots rich in phenolic compounds with high antioxidant activity. Hence, the extracts could be used in the food and nutraceutical industries for several applications as natural antioxidants.

Acknowledgements: To FCT (Portugal) and FEDER under programme PT2020 for financial support to CIMO (UID/AGR/00690/2019) and the research contracts of J. Pinela (Project AllNatt, POCI-01-0145-FEDER-030463), M.I. Dias and L. Barros. To FEDER-Interreg España-Portugal programme for financial support through the project 0377_Iberphenol_6_E. To IACOBUS research stay program for the financial support of M. Ayuso.

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Valorisation of a threatened plant species through *in vitro* culture elicitation of phenolic compounds

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Thematic Areas: Food biotechnology.

Abstract: *Ex situ* conservation strategies, such as *in vitro* culture, have been suggested for the recovery of threatened plants close to extinction. *In vitro* culture or micropropagation is a plant-multiplication technique that allows to obtain a large number of clones in a short period of time and limited space, which can be used in reintroduction programs and for research purposes (1). This biotechnological tool also allows increasing the production yield of secondary metabolites, such as phenolic compounds, thus emerging as an efficient system to obtain high added-value bioactive compounds (2). This approach allows valorising the culture of threatened plants for their commercial use in different industrial sectors interested in bioactive compounds, such as the food and pharmaceutical industries. Therefore, the aim of this research was to study the phenolic compounds elicitation by cytokinins in roots and aerial parts of the *in vitro* cultured endangered plant *Eryngium viviparum* J. Gay (Fam. Apiaceae).
E. viviparum explants were cultured *in vitro* in a solidified MS medium. A 3-level factorial design was implemented to study the effect of two cytokinins, 6-benzylaminopurin (BAP) and kinetin (KIN), at 0, 1 and 2 mg L^{-1}, on the elicitation of phenolic compounds. The obtained plant material was lyophilized, reduced to powder, and submitted to a solid-liquid extraction with ethanol:water (80:20, *v/v*) to obtain combined extracts (3). Then, phenolic compounds were identified and quantified by HPLC-DAD-ESI/MS (3) and the main phenolic compounds with known bioactivities were correlated with both cytokinins.
Seventeen phenolic compounds were identified in all *E. viviparum* hydroethanolic extracts, corresponding to 12 phenolic acids and 5 flavonoids. Rosmarinic and chlorogenic acids were the main bioactive phenolic acids. Regarding flavonoids, tectorigenin-*O*-glucuronide was identified and quantified only in the aerial parts. In the roots, the increase of phenolic acids was correlated with the presence of KIN in the culture media. Concerning the aerial parts, cytokinins did not correlate with phenolic acids, but KIN correlated with the increase of tectorigenin-*O*-glucuronide. In addition, the roots had a higher phenolic composition content than the aerial parts and the combination of both cytokinins promoted a doubling of the phenolic compounds concentration in some samples. Therefore, this study demonstrated the efficacy of *in vitro* culture in adding value to the threatened plant species *E. viviparum*, as the levels of valuable secondary metabolites were increased by elicitation, thus being a sustainable approach that can help save and valorise other endangered plants.

Acknowledgements: To FCT (Portugal) and FEDER under programme PT2020 for financial support to CIMO (UID/AGR/00690/2019) and the research contracts of J. Pinela (Project AllNatt, POCI-01-0145-FEDER-030463), M.I Dias and L. Barros. To FEDER-Interreg España-Portugal programme for financial support through the project 0377_Iberphenol_6_E. To IACOBUS research stay program for the financial support of M. Ayuso.

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Elaboration of films-based antibacterial plant extracts to preserve liquid food products.

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Thematic Areas: Biotecnología Alimentaria.

Abstract: The food industry tends to value bioactive substances of plant origin to satisfy consumer demand. Secondary metabolites have a large spectrum of antimicrobial activity and their effectiveness has been proven *in vitro* and in food products (1, 2).

The aim of this work was to develop films based on polylactic acid (PLA) containing phenolic extract from *Scirpus holoschoenus* and *Thymus fontanesii* essential oil to preserve fish soup and liquid white egg. To test the antimicrobial capacity of these films, the food liquids tested were previously contaminated with *Pseudomonas aeruginosa*. This pathogenic bacterial strain was selected due to its capacity to grow in a refrigerated medium and to contaminate a great variety of food pieces.

The monitoring of *P. aeruginosa* growth was done at 4 and 7 °C, and its physiological status was assessed by flow cytometry, using the double staining SYBR green and Propidium Iodide (PI). A significant decrease in the number of viable cells was noted when the food liquids tested were in contact with the PLA films. In addition, the double staining evidenced a rise in the cell membrane damage depending on the phenolic extract used.

Generally, the results indicate that these antimicrobial films are able to prolong the shelf life of liquid products conserved at low temperatures against psychrophilic bacteria.

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BIOFUNCTIONAL CHARACTERIZATION OF PROTEIN HYDROLYSATES OBTAINED FROM COD (*Gadus morhua*) BYPRODUCTS

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Thematic Areas: Food Biotechnology

Abstract: Cod by-products have been reported to contain an important percentage of protein, which can be applied in food industry to produce different valuable products; the production of fish protein hydrolysates can help to avoid fish stocks depletion and, at the same time, can enhance the functional and nutritional properties of end-products. The aim of this study was the production of cod hydrolysates to be incorporated into a restructured fish-based product for the improvement of its functional quality. In this work six fractions of fish hydrolysates were obtained from cod byproducts (skin and bones), using three different hydrolysis conditions: i) type and concentration of enzyme, ii) temperature and iii) hydrolysis time, followed by a fractionation protocol with three membranes (30 kDa, 10 kDa and 1 kDa). The biofunctionality of the selected peptide fractions was characterized according to their antioxidant, antihypertensive and anti-inflammatory activities and preventive effects against osteoporosis. Furthermore, chemical parameters, such as degree of hydrolysis, amino acid composition and molecular weight were studied. The best hydrolysates according to the chemical parameters turned out to be RB2-1 + 10, RB2-1-1, RB2-2-1 and RB2-3-1 fractions. However, the evaluation of the functional properties discarded the fraction characterized by containing larger peptides (RB2-1 + 10) and the fraction RB2-3-1, while confirming the RB2-1-1 and RB2-2-1 as the most functional ones.
With the objective of incorporating the hydrolysates as a functional ingredient for the development of new products, the organoleptic qualities of the peptide fractions have been taken into account. For instance, fraction RB2-1-1, although presented very interesting properties from a chemical and functional point of view, was not acceptable at organoleptic analysis, and was discarded for this purpose.
Finally, starting from six peptide fractions, only one RB2-2-1 (obtained in these conditions: alcalase enzyme, 0.5% v/w, 50ºC during 2 hours), was selected as the best one to be incorporated in a fish matrix for the development of a new functional product.

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Effect of the stages of the deodorization process of cochayuyo (Durvillaea antarctica) on the yield and minerals and dietary fiber content, of deodorized algae flour

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Thematic Areas: 2. Biotecnologia Alimentaria.

Abstract: *Durvillaea antarctica* is a seaweed endemic to Chile, rich in minerals and dietary fiber. Despite its high nutritional value and content of bioactive compounds, it is scarcely consumed by the population. A deodorization process, consisting of a consecutive series of washes, followed by drying, grinding and sieving, allows to obtain seaweed meal with a lower intensity of aroma and flavor, which can be included in bread making, up to 15%, without affecting the sensory characteristics of the final product. However, it could cause the reduction of some components of interest. The objective of this work is to evaluate the effect of the stages of the deodorization process, on the performance, and the content of minerals and dietary fiber. For each stage of the process, a sample of algae was extracted, dehydrated, ground and sieved, and reserved for analysis. Sodium, potassium and calcium were determined by Atomic absorption spectrophotometry, and dietary fiber was quantified by the AOAC 2011.25 method. The results show that the deodorization process significantly increases the yield of the flours, from 69% to 90%, considering as final product those particles with a size equal to or lower than 212 um. The washing steps cause significant reduction in the content of sodium, potassium and soluble fiber. The sodium: potassium ratio remains stable for all particle sizes, while the low molecular weight soluble fibers are retained in the 500 µm sieve. The deodorization process applied to the cochayuyo to obtain deodorized seaweed meal, implies an increase in the yield and the reduction of some soluble compounds such as sodium, potassium and soluble fiber, which should be considered when using it for food formulations enriched.

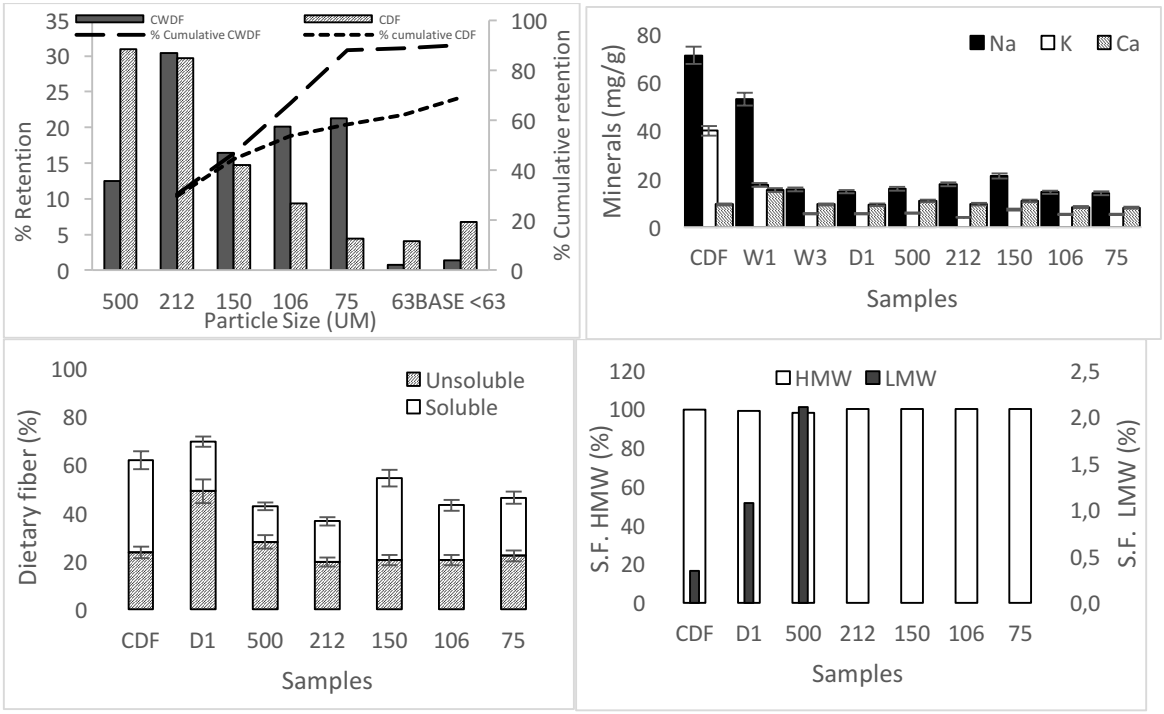


Figure 1. Effect of the stages of the deodorization process on: A) Performance; B) mineral content; C) dietary fiber; D) Soluble fiber, high and low molecular weight

Acknowledgements: to GRANOTEC CHILE S.A. by the analysis of Dietary Fiber and to the Universidad de Antofagasta, for support the Project of Undergraduate Thesis ATI17-1-08 and for Project [Semilleros 5310]

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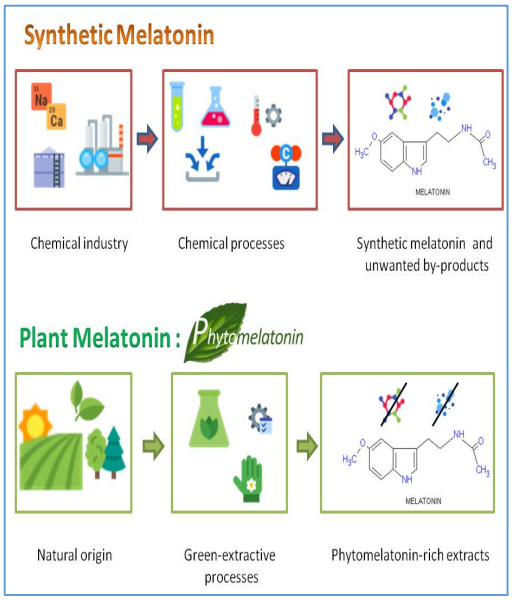
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Phytomelatonin as a natural alternative to synthetic melatonin in food supplements and others applications

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Thematic Areas: Biotecnología alimentaria

Abstract: Melatonin (*N*-acetyl-5-methoxytryptamine) is the main hormone synthesized in the pineal gland of animals. This indolic molecule has numerous cellular and physiological effects, acting as a biological modulator of circadian rhythms, mood, sleep, body temperature, locomotor activity, food intake pattern, retinal physiology, sexual behaviour, and the immune system. Also, melatonin has been widely used for therapeutic purposes and associated with the prevention of several diseases related to ageing and oxidative stress, including neurological, metabolic and cardiovascular disorders, and also as anti-tumoral agent. While melatonin is the term used to name to the compound of animal origin or obtained by chemical synthesis, the term phytomelatonin refers to melatonin to plant origin. Phytomelatonin was discovered in plants in 1993, and from this date, a large body of information on the levels of phytomelatonin in many plants, especially in edible and medicinal plants has been obtained. In general, phytomelatonin content in plants is highly variable, varying from a few picograms up to micrograms per gram of plant material analyzed. Aromatic and medicinal plants had higher levels of phytomelatonin than fleshy fruits, being very variable the content in leaves, stem and roots.
At present, commercial melatonin supplements are obtained from synthetic melatonin. The possibility of using phytomelatonin instead of synthetic melatonin in dietary supplements arises from the desire to avoid the consumption of a variety of unwanted by-products originated during the chemical process of melatonin synthesis, which can appear in synthetic melatonin preparations, generally tryptophan-related compounds. They include 1,1'-ethylidenebis-(tryptophan), so-called "*peak E*", which is sadly related to Eosinophilia-myalgia syndrome (EMS), an incurable disease that killed 37 people and 1,511 affected cases in the mid-1990s, due to defective criteria for the manufacture of tryptophan. Also, *peak E* and 3-(phenylamino)-alanine (another by-product) seem to be related in Spanish toxic rapeseed oil syndrome in 1981.
To completely avoid the consumption of these unwanted by-products, we set out to obtain phytomelatonin-rich extracts from plants. We have developed a proprietary protocol from a mixture of herbs, by organic culture, with high phytomelatonin content. From this herbal mix, and by green-extractive processes, we have been able to obtain a series of stabilized natural phytomelatonin-rich extracts with very interesting properties from the healthy point of view. In this work we have partially characterized these natural extracts and discussed their multiple applications such as the use as food supplements and in functional foods, formulation in cosmetics, and use in cancer experimentation.



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Rheological properties and development of Staphylococcus sp. in structured foods

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Thematic Areas: Food Biotechnology.

Abstract: Foodborne diseases, which includes a wide spectrum of illnesses resulting from the ingestion of food contaminated with microorganisms or chemicals, are a major public health concern worldwide. *Staphylococcus aureus* enterotoxins are a common source of foodborne diseases. *S. warneri* is an opportunistic pathogen, less harmful than *S. aureus*, which can be used as a surrogate with the advantage of safer management. Both bacteria can be found in various types of food like meat, egg products, milk and other dairy products.

The influence of food composition and storage conditions on bacteria development has been the focus of diverse research works. However, very few studies have analysed the effect of food structure, which has been also reported as a relevant factor regarding microbial growth. Food structure exerts constraints on oxygen availability, water and metabolites distribution and even may cause alterations on cell development, morphology and membrane permeability.

Hen egg yolk is widely employed as ingredient in food products due to its organoleptic characteristics and well-known functional properties. In addition, egg consumption has often been linked to food poisoning outbreaks. Polysaccharides like kappa-carrageenan are functional ingredients contributing to the structure, texture and stability of food systems. Hence, with the aim of analysing the growth of *S. warneri* in foods with the same nutritional composition but different structure, egg yolk/kappa-carrageenan dispersions have been prepared and inoculated with this bacterium. In particular, the media have been prepared with 10% (w/v) of egg yolk and concentrations of kappa-carrageenan between 0 and 1.75% (w/v). When the specific rates of cell growth were compared, very few differences were observed for kappa-carrageenan concentrations below 1.5%. Additionally, the textural and microstructural properties of these egg yolk/kappa-carrageenan dispersions are being studied by means of rheological assays and microscopic observations and results will be analysed in terms of bacterial growth.

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Is the correct identification of chimaera a chimera?

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Food biotechnology. New foods and technologies applied to food. Animal production and health; traceability.

Abstract: The lack of recognition of complex population structures in fisheries management can lead to the depletion of local subpopulations. The incorporation of genetic data into fisheries management can be useful to achieve the objective of sustainable management.
The molecular traceability of fishery products includes control tasks to improve the safety, quality and legality of products from aquaculture or fisheries. This requires the development of reliable and affordable analytical methods, useful for the control of food safety and commercial fraud (1, 2, 3).
The chimaeras of the Order Chimaeriformes are a very little known group of species with a high rate of erroneous morphological identification. They are fished as bycatch of other species of high commercial interest; therefore, an accurate morphological and molecular identification is necessary in this Order. The main objective of the work plan was to complete the identification of two species of chimeras, *Hydrolagus affinis* and *Harriota raleighana*, both morphologically and at the molecular level.
Samples were collected on board the B/O Vizconde de Eza, in three oceanographic surveys of fishing prospecting lead by the Instituto Español de Oceanografía. Type specimens of *Harriotta raleighana* and *Hydrolagus affinis* were conserved at - 20º C for morphological analysis, muscle preserved in absolute alcohol, and muscle, liver and gonad preserved in RNAlater at -80 C.
Genomic DNA was extracted from the entire individual using the FENOSALT method (4). Two mitochondrial markers will be analyzed. A fragment of Cytochrome Oxidase I (COI) amplified with the pair of universal primers FishF2 and FishR2 (5) and the 16S ribosomal gene amplified with the pair of primers 16S-RA and 16S-RB (6). The identification was made through the Basic Local Alignment Search Tool available in GenBank and the assignment of the sequences to the species was verified.
Molecular analyzes play a crucial role and are therefore fundamental for knowledge of the spatial structure of the different populations.

Acknowledgements: EU NAFO Groundfish surveys were supported by BIOPESLE project.

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Implementation of the traceability process in products of autochthonous Portuguese poultry breeds.

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Thematic Areas: Biotecnologia alimentaria - Producción y salud animal; trazabilidad

Abstract: At the beginning of the 21st century, the breeds of autochthonous chickens of Portugal, Pedrês Portuguesa, Preta Lusitânica, Amarela e Branca (present in this territory for so long that they can be considered as having originated here) were in a situation of complete abandonment, without any study or characterization work, preservation or promotion action that valued them. Autochthonous hens are produced in a smallholding context, mainly in Northwest Portugal. This territory presents a disturbing aging population, resulting from the great wave of emigration during the 60s and the continuous attraction for the coast, which has economically, socially and culturally shaped the landscape of the rural world of our times.
As the Portuguese chickens are considered as Grade A of threat of extinction (very high risk) by Administrative Rule no. 55/2015, of February 27, promotion actions are an essential tool for the dissemination and encouragement for the creation of these breeds. The identification and characterization of the genetic heritage is still insufficient and characterization, valuation, marketing and promotion strategies are fundamental tools for the attractiveness of their production and products.
In fact, the sustainability of production is becoming an increasingly strong consumer argument for the choice of products and producers that are part of their diet. The breeding system for indigenous species is balanced by the use of natural resources, land and water, environmentally “friendlier”, particularly adjusted in less favoured regions where these resources are scarce. The high quality of the meat, its mode of production, nutritional and sensorial quality, indicate the native hens as a differentiated product of value, suitable for market niches, such as the biological one, which also reveal a concern for agro-environmental sustainability.
Food safety and the protection of consumers' interests are a growing concern of food policy. As demand for local products increased, particularly autochthonous products, it was essential to implement processes that would provide a solid basis for traceability and safety. It is necessary to ensure that consumers have confidence in the decision-making processes underpinning food law, in their scientific basis and in the structures and independence of institutions protecting health and other interests.
The establishment of a grouping of producers of the autochthonous poultry breeds and the organization of a commercialization circuit, structured into a brand identifying the products, is now under way. Members are the breeders, licensed by the sanitary authority (DGAV), with groups selected and listed in the Genealogical Register and that meet the requirements of productive, sanitary managements and records of exploitation. Identification of the animals is the basis of the entire traceability process and animals reared and for sale on the market shall be marked by an earring placed on the front fold of the right wing and registered in the RG of the breed.
The collection of the animals on the farm, the slaughter in a selected slaughterhouse and its consequent processing and commercialization are carried out under the responsibility of the producer association, allowing the traceability of these products during all stages of the food chain, promoting consumer confidence. The promotion of the products identifying and valuating the nutritional and sensorial characteristics of the autochthonous poultry meat, already studied, are part of the strategy of valorization and sustainability of the Portuguese breeds.
Thus, recognizing the diversity of these breeds, admitting their unique qualities, and considering that the patrimony that they represent belongs to the whole society and, as such, must be supported and defended, it is therefore imperative that strategies be implemented to value them, from their territory of creation to consumers and society in general.

Implementation of a traceability system in the rooster production controlled by the “Confraria Gastronómica O Galo de Barcelos”

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Thematic Areas: Biotecnología alimentaria, Producción y salud animal; trazabilidad

Abstract: The “Galo de Barcelos” (Barcelos Rooster) have become the icon of the city due to its connection to the legend of the Way to Santiago, the reference image of the Barcelos and Portuguese pottery and the importance of the roasted rooster as the most important reference of the Barcelos cuisine.
The “Confraria Gastronómica o Galo de Barcelos” was created in 2016 with the purpose to value and promote the “Galo de Barcelos” in its cultural and gastronomic aspects. The Confraria has created a set of production rules that include feeding, density, housing and sanitary aspects. The goal is to have a rooster with a minimum of 120 days, with more than 4 kg, created according to those rules. To guarantee the traceability of the entire process, every animal is identified with a wing tag that as a serial number. This number allows to control the entire production process, since the chick is delivery to the farmer, until the rooster is presented in the restaurant.
The Confraria visits often the farms, checks the rearing conditions and weights the roosters. A random a sample is measured (five biometric measures, according to the FAO guidelines). The initial studies indicate an average daily gain of 29,7 g/day, the live weigth at the slaughter is 4196,5±281,76g, with a carcass weight of 3395,7±257,67g, representing a carcass yield of 81%. More studies are required including more farms as well as the analysis of the carcass.
The process will complete with the collection of the animals in the farms, the slaughter in a selected slaughterhouse, the processing and the commercialization being carried out by the Confraria, or under its control. Soon, the consumers will be able to consult all the information about the animal in the website of the Confraria.



Figure 1 – Rooster controlled by the Confraria



Figure 2 – Wing Tag with the serial number

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Study of the adsorption procedure of phenolics from *Kalanchoe* aqueous extracts onto activated carbon

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Thematic Areas: Food Biotechnology.

Abstract: Traditional extraction procedures involve time-consuming protocols and the use of organic solvents with a negative influence on the environment and human health [1]. Here, we report a *greener* alternative to be applied for the extraction and isolation of polyphenolics from aqueous extracts of a medicinal plant *Bryophyllum* x *houghtonii* (genus *Kalanchoe*, Crassulaceae) cultured *in vitro*, using active carbon (AC) as a selective adsorption matrix. In order to test AC selectivity towards polyphenols, gallic acid (GA) and its derivative propyl gallate (PG) were used. The adsorption of both compounds was determined by a first order constant and loading equilibrium onto AC was reached at 4 hours of contact (Fig. 1A and 1B). An increase in temperature, from 20 to 40 ºC, did not alter significantly the adsorption process. These findings suggest that phenolic adsorption occur selectively, being phenolic ring the binding site. Furthermore, the adsorption of phenolics from aqueous *Bryophyllum* extract at 20 ºC was monitored. The higher complexity of the extracts implies a delay on the time needed to reach the equilibrium, 7 h (Fig. 1C). All results can be explained thermodynamically by means of Freundlich isotherm, which can be efficiently applied to complex systems, as it is the case of plant extracts.
Altogether, in this work we combine two safe, environmental friendly methodologies for the production, isolation and purification of phenolic compounds with antioxidant activities from plant sources. In one hand, the establishment of plant *in vitro* culture brings a number of solutions for overcoming the common disadvantages of conventional breeding (geoclimatical dependence, low secondary metabolite biosynthesis rate) [2]. On the other hand, we present an efficient and fast methodology for the isolation and recovery of polyphenols from complex extracts with the purpose of proposing a low-cost and health-promoting approach in the study of plant bioactive compounds.

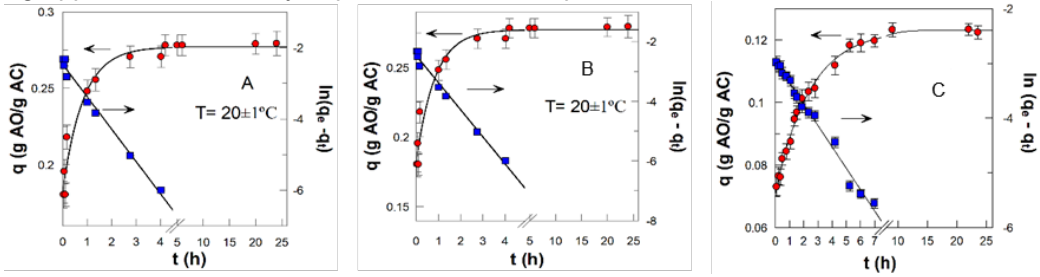


Fig.1. Variation of adsorption rate, q, by means of grams of antioxidant, g AO, per gram of AC at equilibrium (dots) and first-order constant fit (squares) at 20 ºC. A) GA adsorption, B) PG adsorption and C) aqueous *Bryophyllum* extract adsorption. q_e stands for AC loading rate at equilibrium and q_t stands for AC loading rate at measuring times.

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Re-using laccase for the production of oligorutin in a reaction media free of organic solvents

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Thematic Areas: Biocatalysis

Abstract:
Rutin (quercetin-3-O-glycoside) has been demonstrated to possess beneficial properties for preventing various diseases. However, this compound shows poor solubility in water, thus implying scarce bioavailability, and low thermal stability [1]. The enzymatic polymerization of rutin has been proved as an efficient method for obtaining products with enhanced properties, but toxic solvents that restrict the final product applications and negatively affect enzymatic activity are usually needed to increase the solubility of the substrate [2,3]. In our previous work, tests were performed using water as reaction media, starting the reaction at a concentration beyond rutin aqueous solubility. Rutin conversion and laccase stability, together with the antioxidant properties of the obtained products, led to selecting an enzymatic activity of 1000 U/L and 6 and 24 h as best reaction times at room temperature. Since a pH drop was observed during the reaction, a phosphate buffer pH 6.0 was selected as new reaction media. In this work, the possibility of re-using laccase from *T. versicolor* for the production of oligorutin without using organic co-solvents was studied in an enzymatic membrane reactor (EMR); after 6 and 24 h of reaction, the 90% of the volume of the reactors was retrieved after passing through an ultrafiltration membrane, and new substrate and reaction media was supplied to the reactor, in order to reach the initial values, without enzyme addition. The concentration of rutin and the enzymatic activity was monitored using the EMR configuration for three consecutive cycles of 6/24 h. Enzymatic activity did not drop during the oligomerization process but decreased during the ultrafiltration step. Nevertheless, enzymatic activity retained in the reactor was enough to achieve the partial conversion of the substrate added in each cycle for 6 h of reaction and total conversion in the case of 24 h. Products obtained after filtering were characterized in terms of antioxidant activity (FRAP test) and aqueous solubility. Future work will be performed regarding the increase of the initial substrate concentration in each cycle in order to optimize the production of oligorutin and reducing the loss of enzyme during filtering.

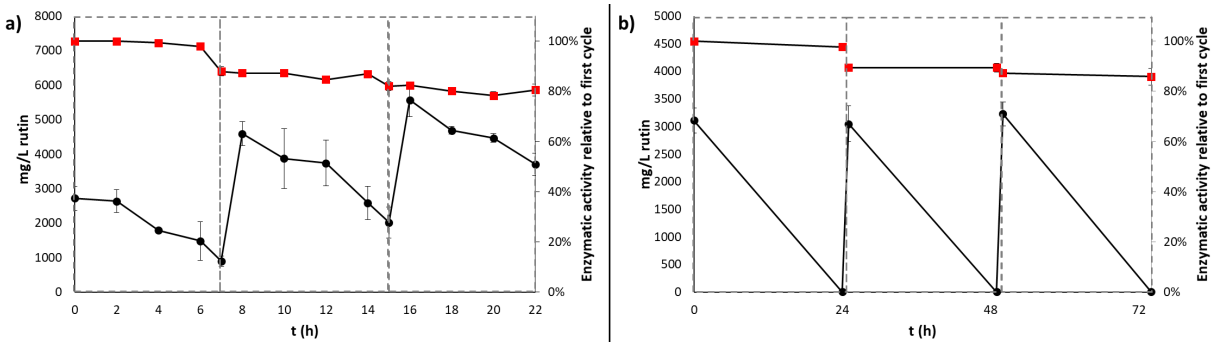


Figure 1.Enzymatic activity relative to the beginning of the first cycle (■) and rutin concentration (●) monitored during three consecutive cycles of reaction in an EMR for a) 6 and b) 24 h of reaction.

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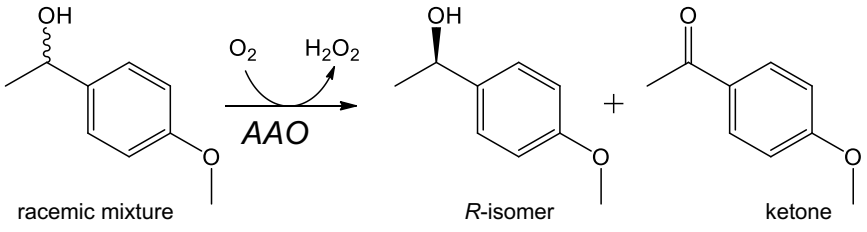
Changes in the substrate preference of aryl-alcohol oxidase allows a stereoselective oxidation of secondary alcohols

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Thematic Areas: Biocatálisis

Abstract: Production of pure enantiomers has become increasingly important in the pharmaceutical industry. For this purpose, microorganisms and enzymes have an enormous potential due to their high chemo-,regio- and enantioselectivities, being an interesting alternative to chemical synthesis. In this sense, aryl alcohol oxidase (AAO), a flavoenzyme that provides H_2O_2 to fungal peroxidases for lignin degradation in nature, is a promising candidate. This enzyme oxidises primary benzylic alcohols through a mechanism that consists in a concerted hydroxyl proton transfer and a stereoselective hydride abstraction from the pro-*R* benzylic position [1,2]. In the case of racemic secondary alcohols, the *R*-hydrogen abstraction would result in the selective oxidation of the *S*-enantiomer to the corresponding ketone. This stereoselectivity of AAO can be exploited for enzymatic deracemization of chiral mixtures and isolation of *R*-enantiomers of industrial interest if the enzyme activity on primary alcohols is switched to secondary alcohols.

The ability to oxidize secondary alcohols has been introduced in AAO by site-directed mutagenesis guided by computational simulations using as a starting point the F501A variant with a wider active site [2]. Adaptive-PELE simulations for the diffusion of (*S*)-1-(*p*-methoxyphenyl)-ethanol (used as a model of secondary aryl alcohols) in this variant allowed to identified key residues for the transition of this alcohol from the solvent to the active site. Thus, substitution of Ile500 produced more efficient variants for the oxidation of several secondary alcohols, and the I500M/F501W double variant was able to fully oxidize the *S*-enantiomer of (±)-1-(*p*-methoxyphenyl)-ethanol in only 75 min with high selectivity (*ee* >99%), while the *R*-enantiomer remained unreacted. Analysis of the resulting variants revealed that the mutations introduced facilitate the entrance and accommodation of bulkier secondary alcohols at the active site of AAO.



Scheme. Stereoselective oxidation of a racemic mixture of model secondary benzyl alcohol (1-[p-methoxyphenyl]-ethanol) by AAO leading to kinetic resolution.

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A designer natural deep eutectic solvent to recycle the nicotinamide cofactor in alcohol dehydrogenase-catalysed bioreductions

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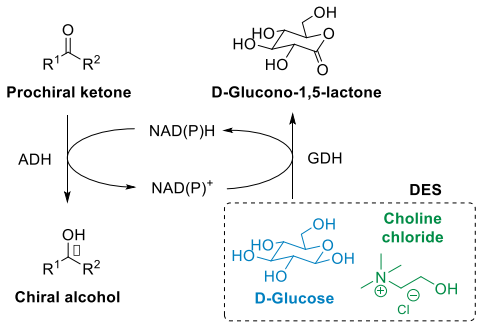
Thematic Areas: Biocatalysis (Biocatálisis).

Abstract: One of the difficulties found in the development of alcohol dehydrogenase-catalysed protocols at large scale is the need of coupling the cofactor recycling system. The use of glucose dehydrogenase (GDH) as a coupled-enzyme is one of the most popular methods, so that the GDH can assist the regeneration of the cofactor at expenses of glucose as cheap sacrificial co-substrate [1].

Recently, natural deep eutectic solvents (NADES) have appeared as a new generation of ionic liquids which can be employed as a sustainable alternative to traditional solvents in (bio)transformations due to their simple preparation, handling and availability form natural resources. Particularly, their use has quickly gained attention in enzymatic redox processes [2], choline chloride: glycerol (ChCl:Gly) mixtures being usually the choice for enzyme activity and selectivity studies.

Herein, we present a new methodology in which overexpressed ADHs are used in combination with a glucose-based NADES to reduce prochiral ketones. Therefore, the designer NADES can display a double role in the biotransformation. On the one hand, it acts as source of glucose for the cofactor regeneration system so there will be no need for adding external chemicals. On the other hand, the NADES can display remarkable properties as cosolvent to improve the solubility of lipophilic substrates (Scheme 1).

Scheme 1. Use of a designer deep eutectic solvent formed by D-glucose and choline chloride to recycle the nicotinamide cofactor [NAD(P)H] in alcohol dehydrogenase-catalysed bioreductions.



Five ADHs have been tested in NADES-aqueous buffer mixtures finding excellent activity levels with ADHs from *Lactobacillus brevis* (LbADH), from *Thermoanaerobacter* sp. (ADH-T) and from *Ralstonia* sp. (RasADH), which were superior to the results obtained in the absence of cosolvent or using only glucose. In addition, the use of a glucose-based NADES allowed to perform the bioreduction at higher substrate concentrations in comparison when the buffer system and the glucose/GDH was applied. Furthermore, after the optimisation of the reaction conditions, it was possible to scale-up the bioreductions and to perform recycling experiments with high success.

Acknowledgements: Financial support from the Spanish Ministry of Economy and Competitiveness (MEC, Project CTQ2016-75752-R) is gratefully acknowledged. A.M.-I. thanks MEC for a predoctoral fellowship inside the FPI program.

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Functional expression and characterization on a new chitinase from *Pseudoalteromonas tunicata* with nematicidal activity

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Thematic Area: Biocatálisis

Chitin is a linear β-1,4-linked homopolymer of N-acetyl-β-D-glucosaminide (GlcNAc), the second most abundant on earth after cellulose. Approximately 10^{11} tons are discarded every year. Chitin is present in crustacean shells, cell walls of fungi and in the exoskeletons of insects and nematodes. Chitinases are hydrolytic enzymes that cleave the the β-1,4 glycosidic bonds in chitin and are receiving increased attention due to their broad range of applications such as production of oligosaccharides, GlcNAc or as biopesticides. In fact, the nematicidal activity is by far the less explored utility up until now. Although several microbial chitinases have been studied and characterized, the diversity of applications and environments in which these enzymes must work, also demand a large number of different enzymes capable of acting in such conditions (1).
The aim of this work was to evaluate the functionality of a new gene (*Pt*chi18) from *Pseudoalteromonas* tunicate in *E. coli*, the characterization of the new chitinase and the study of its potential as bionematicide.
Cloning, expression, purification and enzyme characterization were done according to García-Fraga et al. (2). Nematicidal activity against *Caenorhabditis elegans* was carried out by adapting protocols described previously (3).
The *Pt*chi18 gene was cloned and expressed in *E. coli*. The mature recombinant chitinase *Pt*Chi18p with an approximate molecular mass of 90 kDa has a complex multidomain structure mainly characterized by the catalytic domain of glycosyl hydrolase family 18 (GH18) between residues 160 and 574 and a region between amino acids 784 and 870 where the carbohydrate binding module (CBM5/12) is located. The expression of the protein in *E. coli*, optimized using different host strains and according to the main parameters that affect gene expression, made it possible to demonstrate the functionality of the enzyme. The purified protein hydrolyzed colloidal chitin and p-NP-$(GlcNAc)_{2\text{-}3}$ showing the highest activity against p-NP-$(GlcNAc)_2$. *Pt*Chi18p exhibited a K_m of 0,235 mM and was active in a temperature range of 20-50ºC (with an optimum of 40ºC) and at pH values of 4,5-9,5 (with an optimum of 6,5). The enzyme maintained high stability particularly under suboptimal temperatures and in the presence of NaCl 2M, tolerating up to 4M. Finally, the possible nematicidal activity of the chitinase was also evaluated. Treatment of the nematode *C. elegans* with different concentrations of the studied chitinase showed that its effect was proportional to the amount of enzyme used causing a mortality above 76%, compared with the control, at the highest concentration assayed.
The results of the present study indicated that *Pt*Chi18p is a novel chitinase which has potential application as bionematicide, providing at the same time a theoretical basis for its application.

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Copper and polymerized lignin by laccase as wood treatment for marine uses

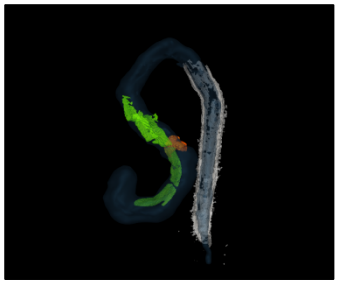
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Thematic Areas: 5.Biocatálisis.

Abstract: Wood in marine environment has been used throughout the history by different civilizations. Phoenicians knew well how harsh could be the sea to wood, so they developed several strategies to avoid biofouling and marine borers. Nowadays, the industry of preservatives for wood treatment is important in Europe, but many concerns about health and environment due to their leaching have forced the search of best preservative retentions and less harmful components. An example is CCA, which has been restricted just to a few uses and totally banned in marine environment mainly due to arsenic (1) in the European Union.
Lignin has been object of several patents (2) in order to apply it in presence of metal salts with the aim of improving metal fixation and reducing their leaching. Kraft lignin is suggested as an adequate material for this application because of its low cost and the possibility of reintroduce it in the wood industry as a revalorization approach. In our study, a one-step impregnation process was carried out in *P. sylvestris* pieces (80x80x10mm) with a solution of *Eucalyptus spp.* Kraft lignin and copper which was fixed with grafting and polymerization assisted by commercial laccase (Novozymes 51003). Subsequently, these treatments with its pertinent controls were tested in a marine trial for 1 year. Several determinations were performed: copper leaching before and after marine exposition by means of ICP-OES, copper distribution by ITRAX core scanner, biofouling coverage per each season and biomass retention by visual image analysis; and marine borers degradation by computed tomography scan analysis (CT scan).

Results show the neccesary improvement of the impregnation process and some other technical aspects like configuration and assembly of the sea trial structure. However, the analytical devices employed show an enhancement in the field of study, giving fast and accurate information.





Acknowledgements: the authors grateful acknowledge the ECIMAT for allow the marine trial in their facilities.

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Sustainable enzymatic synthesis of panthenyl monoesters in ionic liquids

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Thematic Areas: Biocatalysis

Abstract: Panthenol (2,4-dihidroxy-N-(3-hidroxypropyl)-3,3-dimethylbutamide, o provitamin B_5) and its derivatives (e.g. panthenyl triacetate) are compounds which present a great interest in the pharmaceutical and cosmetic industry due to their therapeutic properties and they are usually present in cosmetic products (e.g. hair conditioners, body cream, sun creams, etc.) and in pharmaceutical formulations because panthenol promotes wound healing. Panthenol esters are interesting compounds for the cosmetic industry because of its high ability to reside long time interacting with skin and hair.

Enzymatic catalysis is the most selective strategy for the synthesis of chemical compounds, being one of the pillars in the development of the Green Chemistry. In this context, it has been described a lot of applications in enzymatic catalysis for the synthesis of products with cosmetic and pharmaceutical interest.[1,2]

Nowadays, panthenyl esters are produced through chemist or enzymatic catalysis with carboxylic acids derivatives (e.g. acyl chlorides, etc.) in reaction media based on organic solvents. The enzymatic synthesis of esters by the esterification of a carboxylic acid with one alcohol (e.g. panthenol) is an easy, cheap and sustainable alternative against the classical organic synthetic protocol, where natural compounds (e.g. free fatty acids) can be used as substrates, and without the generation of undesirable products.

This work shows a sustainable methodology for the enzymatic synthesis of panthenyl monoacyl esters by the esterification of carboxylic acid (e.g. lauric acid, myristic acid, palmitic acid or oleic acid) with panthenol catalyzed by *Candida antarctica* B lipase in ionic liquids (ILs) as reaction media. The high yield and selectivity in the developed process, together the full recovery and reuse of the ILs allowed the production of panthenyl monoacyl esters with direct application for the cosmetic industry. Besides, the appropriate design of the reaction media (e.g. subtract concentration, reaction temperature) leads in the production of different panthenyl monoesters with yield up to 80% independently of the carboxylic acid employed.

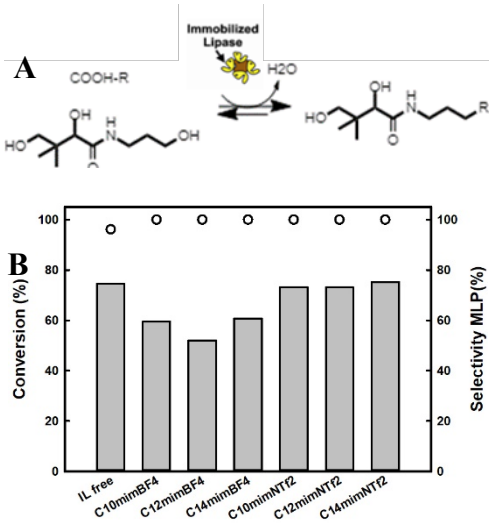


Figure 1. (A). Scheme of the enzymatic synthesis of panthenyl monoacyl esters (PMEs) by the esterification of carboxylic acids with panthenol. (B). Panthenyl monolaurate synthesis through esterification of lauric acid with panthenol in different reaction media.

Acknowledgments: Work partially supported by CTQ2015-67927-R (MINECO/FEDER) and Fundación Séneca (CARM) Ref. 20790/PI/18

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Sustainable approach for the synthesis of sorbitol monoesters in Ionic Liquids

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Thematic Areas: Biocatálisis en medios no convencionales.

Abstract: The aims of sustainability concern the improvement of resources exploitation, with cycles of reuse and wastes valorization, and the development of safer and non-contaminating processes.
Nature is the maximum exponent of processes that fulfill with the requirements of sustainability, atom and energetic economy, specificity and non-hazardous synthesis, thanks to its natural catalysts, the enzymes. Biocatalysts have become an essential tool to organic chemical synthesis because the increased efficiencies and selectivity achieved[1].
Sugar esters are non-ionic surfactants, which natural origin is highly appreciated for food, pharmaceutical and cosmetic applications. This work presents a smart protocol for the single esterification of sorbitol with long alkyl free fatty acids (FFAs) to produce monoesters. Sorbitol is a polyol with a marked hydrophilicity because its 6 hydroxyl free groups and a high melting temperature. These properties are responsible for its low miscibility with highly hydrophobic fatty acids. Till date, the processes developed for sorbitol esterification involve very high temperatures, intermediates of sorbitol dehydration, like sorbitan[2], and the use of organic solvents[3]. The use of ILs as solvents, provides the appropriate scenario for an efficient lipase performance under soft energy conditions.
Our data reveal the significance of the stoichiometry, the temperature, the methodology of incubation and the length of the FFA chain in the yield and selectivity of the reactions.
Finally, we have developed a smart and clean protocol for the purification of the products and the recovery of non-reacting substrates and solvent for setting a circular protocol of reuse.

ENTRY	Tª (ºC)	Sorb:FFA Ratio	FFA	Yield (%)	Specificity (%)
Incubation with agitation					
1	70	2:1	Lauric Acid	61,6	95,5
2	70	2:1	Miristic Acid	**52,3**	100
3	70	2:1	Palmitic Acid	37	100
4	70	2:1	Oleic Acid	41,9	100
Incubation with Ultrasounds					
5	45	2:1	Lauric Acid	40,2	98
6	45	2:1	Miristic Acid	**36**	100
7	45	2:1	Palmitic Acid	18,3	100
8	45	2:1	Oleic Acid	16,4	100
Ultrasounds pretreatment plus Incubation with agitation					
9	70	2:1	Miristic Acid	**75,5**	100

Table 1. **Data resulted from the esterification of sorbitol (1 mmol) with different chain length FFAs**. As observed, homogenization of the medium with ultrasounds before incubation considerably improves the yield.

Acknowledgements: This work has been partially supported by CTQ-2015-67927-R (MINECO/FEDER, Spain) and 20790/PI/18 (Fundación SENECA-CARM, Spain) grants.

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Cell-free immobilized multi-enzyme systems; from the design to the application

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Thematic Areas: Biocatalysis.

Abstract: Combination of several enzymes in one-pot opens a very attractive pathway to access more complex synthetic schemes to manufacture high added-value products in a more sustainable manner[1]. Nowadays, chemical biomanufacturing is dominated by microbial fermentations that use native pathways to mainly produce alcohols, acids or aminoacids. In the last decade, cell-free multi-enzyme systems have emerged as an alternative to whole cell biotransformations in chemical manufacturing since cell-free systems can work towards non natural substrates under non-physiological conditions often demanded by industrial processes[1]. However, enzyme isolation faces some challenges (productivity, robustness and costs) for implementing cell-free multi-enzyme systems into the industrial context. Scaling-up cell-free bioprocesses involving cofactor-dependent enzymes are even more challenging because they demand for exogenous cofactors that hamper the downstream processing and limit their economic feasibility of the process.

Inspired by the spatial organization and the molecular confinement of metabolic pathways within the living cells, our group has exploited immobilization techniques to spatially organized a variety of multi-enzyme systems (oxidoreductases, oxidases, transaminases) confined with their corresponding cofactors (NAD(P)H, PLP, FAD…) across the surface of synthetic porous carriers. Our work aims at creating *in vitro* and confined enzymatic pathways as self-sufficient and multi-functional heterogeneous biocatalysts, where cofactors and enzymes are successfully recycled and reutilized during several operational cycles to increase their total turnover numbers (TTN).
To illustrate this concept, four multi-functional heterogeneous biocatalysts have been tested for the synthesis of 1) enantiopure secondary alcohols[2,3] , 2) enantiopure non natural aminoacids[4], 3) amines[5] and 4) aminoalcohols. In this communication, we will disseminate the benefits of co-immobilizing enzymes and cofactors but also the limitations of this strategy. Finally, the performance of these immobilized multi-enzyme systems in flow-biocatalysis has been tested accompanied by some metrics that quantify the sustainability of the processes.

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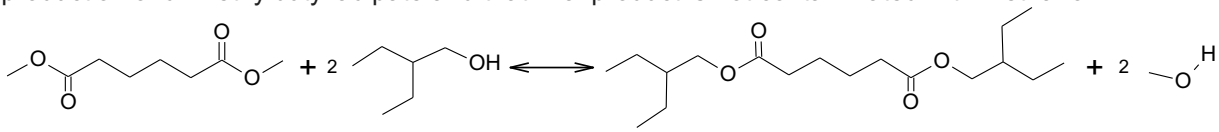
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Enzymatic alcoholysis of dimethyl adipate by 2-ethylbutanol. Clean synthesis of biolubricants in solvent-free medium.

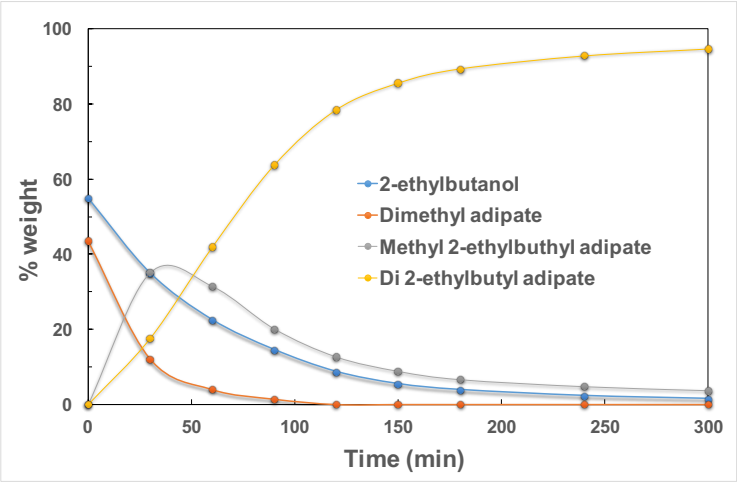
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Thematic Areas: Biocatalysis.

Abstract: Biolubricants have emerged as an alternative to traditional lubricants, mainly mineral oils, to reduce their footprint in our environment. Mineral oils are being replaced by new synthetic lubricants that, despite being more expensive, are biodegradable and less toxic, so that properly managed, they will contribute to reduce their environmental impact.
Long-chain compounds with branched or bulky moieties find uses in various commercial products, such us biodiesel, lubricants, plasticizers, cosmetics, and even edible fat replacers. Some branched esters are used in lubrication because of their excellent properties, i.e. low volatility, high flash point, good thermal stability, low toxicity and good biodegradability. One of the most important types of synthetic lubricants comprises esters of dicarboxylic acids, particularly adipic acid. The linear diacid moiety of the diester contributes to the good viscosity index, while the branched alcohol moiety gives a low pour point.
The processes of esterification in industry are still catalyzed by chemical catalysts, mainly sulfuric acid. However, difficulties in the recovery of catalyst, high energy consumption, corrosion of equipments, and the need for treatment of wastes are disadvantages in the chemically catalyzed synthesis. The use of enzymatic processes in solvent-free systems contributes definitively to the fact that the process (and not only the product) can be classified as sustainable. Among the enzymes, *Candida antarctica* lipase B (CalB) has shown a high catalytic activity for esterification of dicarboxylic acids.
Di-2-ethylbutyl adipate is an option to take into account in the search of new lubricants more respectful with the environment. The ester can be obtained by alcoholysis of dimethyl adipate with 2-ethylbutanol. At room temperature, it is a pale yellowish-green liquid, light, unctuous, and with a characteristic odor. It can be used as an additive for mineral lubricants, or as a lubricant itself.
This work shows the enzymatic production of di-2-ethylbutyl adipate, as well as the best reaction conditions. The objective thereof is to obtain only the pure product in the reactor, so that after the reaction cycle, and after decanting the catalyst, the product offers sufficient purity to be used directly without any other processing.
The low boiling point of methanol (65 °C) favors the balance of the alcoholysis reaction to shift to the production of di-2-ethylbutyl adipate and that final product is not contaminated with methanol.



Dimethyl adipate + 2-Ethyl butanol → Di-2-ethylbutyl adipate + Methanol



The evolution with time of the susbtrates, intermediate and product concentrations is shown in the figure. After 6 hours, at 50 °C, using a slight excess of 2-ethyl butanol and 2.5% w/w of Novozym® 435, a practically pure di 2-ethylbutyl adipate is obtained. Due to the temperature, methanol leaves the reaction medium. Process has been monitored by gas chromatography.

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Lipase comparison in solvent-free neopentyl glycol dilaurate synthesis.

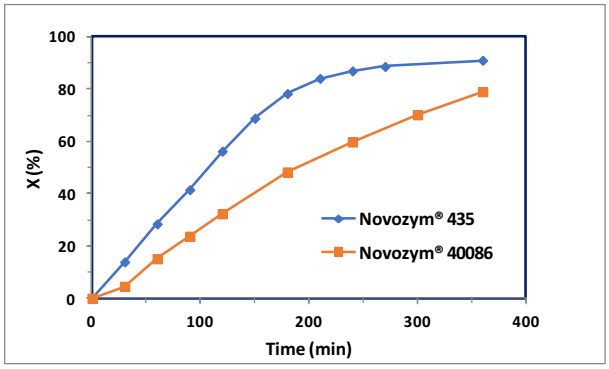
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Thematic Areas: Biocatalysis.

Abstract: Neopentyl glycol diesters are widely applied in different areas depending on the acid moiety. Frequently, fatty acid utilization is associated to cosmetic applications. Among these fatty acid/neopentyl glycol esters, neopentyl glycol dilaurate (NPGDL) highlights as emollient and viscosity increasing agent (1). During the last years, different green processes using enzymes have been proposed, being the lipase-catalyzed synthesis of esters one of the most frequently studied because of their ability to work under very soft and environmental friendly operation. Besides, the enzymatic synthesis presents the advantage that esters obtained can be labelled as "natural" according guidelines approved by the Scientific Committee on Consumer Safety (SCCS), which makes the biotechnological products more attractive to a market increasingly concerned with the personal and the environmental care.

In the present work, the optimization of the NPGDL synthesis using as catalyst two immobilized commercial lipases: Novozym® 40086 and Novozym® 435 and operating in a "solvent-free" system is studied. The atmospheric enzymatic synthesis was carried out in open-air jacketed reactors (50 mL), where 20 g of reactants were introduced in an equimolecular ratio and melted before adding the immobilized derivative. Biocatalyst amounts between 0.375 g and 1.5 g were assayed, while different temperatures were tested in a range of 50 to 80 °C (Novozym® 40086) and 60 to 90 °C (Novozym® 435). Results are summarized in the following table and figure.

	OPTIMAL CONDITIONS	
	Novozym® 435	Novozym® 40086
Amount of lipase (g)	0.75	1.5
T (ºC)	80	60



It can be observed that the two lipases differ in terms of optimal conditions and conversions reached. This different behaviour can be attributed to the lipase chemical structure which determines substrate specificity. In bibliography it has been described that *Rhizomucor miehei* lipase has wide alcohol binding cleft but a narrow acyl binding one, while *Candida antarctica* lipase B has opposite structure (2). However, *Candida antarctica* lipase B yields better conversions than *Rhizomucor miehei* lipase with branched polyols (3). In conclusion, the two lipases assayed are suitable to catalyze the esterification reaction studied, but Novozym® 435 yields higher conversions than Novozym® 40086.

Acknowledgements: Work funded with support from the Spanish MICINN (CTQ2015-66723-R) and the European Commission (FEDER/ERDF). We wish to acknowledge D. Ramiro Martínez Gutiérrez (Novozymes Spain S.A.) who kindly supplied lipases.

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Combined use of cyclodextrin glucanotransferase from *Geobacillus* sp. and amyloglucosidase from *Saccharomyces cerevisiae* to produce glucosylated derivatives of rutin

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Thematic Areas: Biocatalysis.

Abstract: Polyphenols are compounds of vegetable origin which have gained importance in the recent years because of their various biological properties. A number of polyphenols has been reported for their anticancer, anti-inflammatory, neuroprotection or antioxidant activities, among others [1]. However, these compounds tend to have a low stability and solubility, and this affects to their bioavailability. Rutin is a glycoside of quercetin which has demonstrated to be effective in ameliorating liver toxicity, neurobehavioral deficits, neuroinflammation and neuronal oxidative stress in rats [2,3]. Despite being glycosylated, rutin has a poor solubility in water. With the glucosylation of rutin, the solubility in aqueous mediums could be increased and it is important to expand its biopharmaceutical applications [4].
In this work, glucoside derivatives of rutin were synthetized using a cyclodextrin glucanotransferase (CGTase) enzyme. Soluble starch was used as glucosyl donor, and the reaction was carried out in carbonate buffer containing 20% (v/v) of acetonitrile to enhance the solubility of the rutin. As a series of polyglucosylated products was formed in the reaction mixture, the yield of monoglucosyde was improved by the treatment with an amyloglucosidase (STA1) from *Saccharomyces cerevisiae* (var. *diastaticus*) (**Figure 1**).
Reactions were monitoring by analytical HPLC and six different glucosides of rutin were isolated by semipreparative HPLC. The products were characterized by a combination of MALDI and 2D-NMR methods.

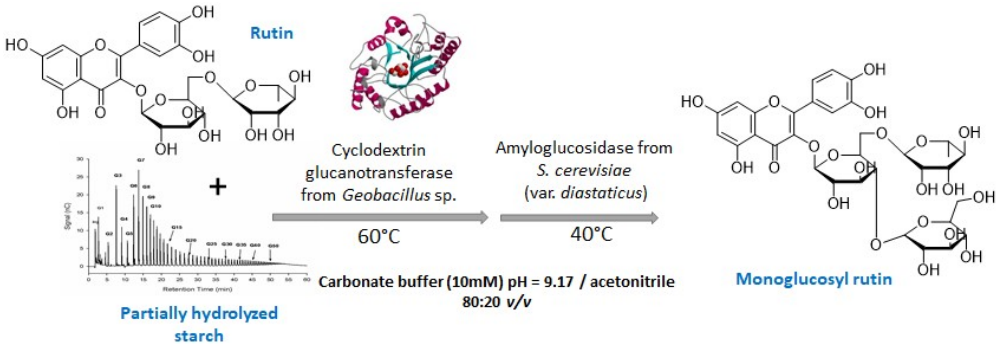


Figure 1. Reaction scheme for the monoglucosyde production.

Acknowledgements: This work was supported by a grant from the Spanish Ministry of Economy and Competitiveness (BIO2016-76601-C3-1-R). We thank the support of Spanish Ministry of Education, Culture and Sport through the National Program FPU (FPU17/00044).

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Nicotinate phosphoribosyltransferase *Escherichia coli* characterization

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Thematic Areas: Biocatálisis

Abstract: Nicotinate phosphoribosyltransferase (NaPRT) catalyzes the conversion of nicotinate to nicotinate mononucleotide. The product is then converted intracellularly into NAD^+ though NAD^+ salvage pathway (1). NAD^+ is an essential metabolite in all organisms that is involved in several metabolic routes. Thus, NAD^+ and derivates constitute promising drug targets for the development of new antibiotics and are relevant factors in biotechnology applications (2–5). NaPRT is a poor characterized enzyme, although it has been studied in a few organisms (6,7).
In this work, *Escherichia coli* (*E. coli*) NaPRT enzyme characterization is presented. NaPRT overexpression and purification was carried out and kinetic parameters for its three substrates ATP, nicotinate and 5-phospho-α-D-ribose-1-diphosphate have been calculated. Moreover, NaPRT thermodynamic behavior was also evaluated.
The results presented in this work constitute the first NaPRT *E. coli* characterization and open the way for future studies based on nicotinate phosphoribosyltransferase regulation as an essential enzyme in metabolism.

Acknowledgements: This work was supported by Ministry of Science and Innovation (BIO2014-54411-C2-1-R9 (which includes ERDF European co-funding) and the Seneca Foundation CARM (19236/PI/14).

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Characterization of a novel thermostable lypolitic hydrolase found by functional metagenomics from a hot spring

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Thematic Areas: Biocatalysis.

Abstract: A novel thermostable lypolitic enzyme was found by functional screening performed on a metagenomic library obtained from a hot spring in Ourense (Galicia, Spain). The library used a fosmid vector [1] to clone long metagenomic DNA fragments (up to 40kb) extracted from up to 25L filtered water samples, and *Escherichia coli* as the host organism. The substrate used for lypolitic activity detection was trybutirin [2]. A clone with lypolitic activity was isolated and subcloned in a smaller plasmid vector. A second round of screening was performed to select positive clones in the new vector system, and the positive clone was sequenced with a primer-walking strategy. The predicted ORF encoded a 198-aminoacid residues protein and blastp [3] predicted domains from the SGNH-hydrolase superfamily and GDSL-like lipases, but the percentage of identity to known sequences was relatively low (no higher than 65%) thus representing a novel member of these lypolitic enzyme families. In order to characterize the biochemical properties of the enzyme, the gene was cloned in an expression vector to increase the production yield and ease its purification.

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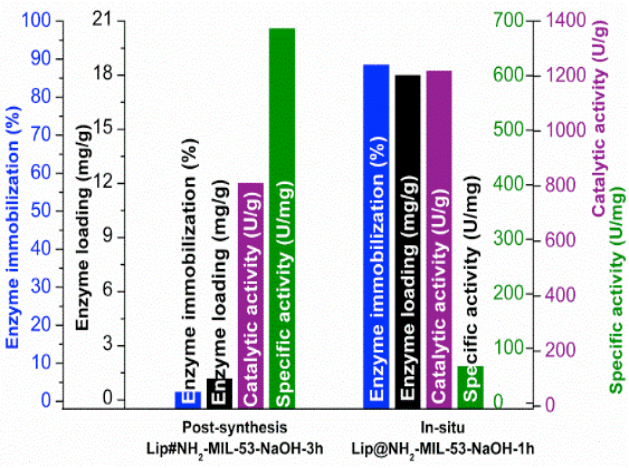
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In-situ and post-synthesis Immobilization of lipase CALB on MOF NH2-MIL-53(Al)

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Thematic Areas: 8. Biocatalysis.

Abstract: The potential application of MOF (Metal Organic Frameworks) as support materials for enzyme immobilization is based on their extraordinary compositional versatility and porous nature. However, their microporosity is not enough to encapsulate enzymes. Efforts to synthetize MOFs with larger pores have been done, with the result of expensive and unstable materials only capable to support extremely small proteins (trypsin, citochrom c [1]). Our groups developed a new methodology to prepare MOF materials under mild conditions (water as the unique solvent, room temperature, absence of corrosive reactants and no energy imput [2]. This method produces nanocrystalline MOF materials, whose crystals are aggregated/agglomerated describing more or less ordered intercrystalline mesoporosity. Besides, the mild experimental conditions are friendly with the enzymatic activity. All this allows carrying out a systematic comparison between the in-situ and post-synthesis immobilization of enzymes on these nanocrystalline MOFs materials.



In this work a comparative study between both methods is performed to immobilize lipase on NH2-MIL-53(Al), which had been previously tested as support for beta-glucosidase. The influence of the nature of deprotontant agent, time of synthesis or the presence of –NH2 groups are studied.
In situ: Deprotonant agents (NaOH, TEA y NH4OH) and time of immobilization of lipase on NH2-MIL-53(Al) were studied. Immobilization yields over 85% with enzyme loads around 20 mg/g were obtained. Immobilization yield increased with time as the specific activity decreased. The best activity values were obtained with NaOH, after 1 hour *Post synthesis*: The best values were obtained at an optimal time of 3 hours contact between MOF support and enzyme extract.

Enzyme loading is higher in "*in situ*" method whereas specific activity is higher in "*post-synthesis*" one. This is to be expected since conditions favoring MOF formation are priority in the in situ strategy.

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Esterification reactions in free solvent systems with cutinase covalently immobilized or only by adsorption.

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Thematic Areas: Biocatalysis

Abstract:

The objective of this work is to evaluate the catalytic capacity in solvent free reaction media of a recombinant cutinase when immobilized on different supports, as well as to evaluate the operational stability of these enzymes.
Many studies have indicated that an enzymatic catalysis in reactions as esterification or transesterification can be reached in the presence of organic solvents [1]. Because of the toxicity and flammability of organic solvents, enzymatic catalysis in a solvent-free medium is important in industrial applications. Several studies have reported that immobilized enzymes can effectively catalyze the hydrolyses and synthesis reactions [2].
Recombinant cutinase from *Escherichia coli* (from different batches M3, J1 and J2) was immobilized on hydrophobic supports (Amberlite XAD-2, XAD-4, XAD-7, XAD-1180, Chauny XAD-16 and DIAION HP20), inorganic support (alumina), ionic supports (Dowex 50W-X8, AG 50W-X8, AG 50W-X2, Amberlite IRC 50, IRA-400, IRA-458) and through covalent binding (on commercial supports epoxy/butyl methacrylate, epoxy methacrylate and amino methacrylate). The supernatant enzyme (enzyme that has not been immobilized) in contact with the supports was characterized by stereolytic activity (p-NPB) [3] and total protein (Bradford method) [4]. The esterification ability of immobilized cutinase on the different supports in the synthesis of propyl laureate and hexyl caproate was studied. The conversion rate of the acid was determined by titration with a NaOH solution.
The hydrophobic supports (Amberlite XAD-7 and Amberlite XAD-1180), inorganic support (alumina), ionic supports with pH adjustment (Dowex 50W-X8, AG 50W-X8 and AG 50W-X2) and covalent binding support (Epoxy/butyl methacrylate) showed a visible decrease in activity and protein values in supernatant, which represents a greater amount of enzyme immobilized in the respective supports. Immobilized cutinase (M3) on inorganic support alumina showed the best conversion (63%) of hexyl caproate in esterification reaction at 30ºC compared to hydrophobic and ionic supports, but less than Cal B (89%). In studies with immobilized enzyme (M3 concentrated) in alumina preequilibrated with water activity at 0.97 the synthesis reaction of hexyl caproate has a conversion of 83.3%. The activity of cutinase (M3) immobilized on alumina by adsorption showed 60% of the initial activity in the 2nd reaction and 0% in the 3rd, showing that enzyme desorption to the reaction medium must have occurred. Cutinase (J2) immobilized on the covalent epoxy/butyl methacrylate support showed a constant degree of conversion (around 90%) at the end of 29 reuses, which demonstrates a high operational stability of the cutinase when covalently immobilized.
We can conclude that the esterification reactions catalyzed by cutinase in solvent-free systems are promoted when the enzyme is covalently immobilized, whereas in the presence of organic solvents the cutinase shows greater activity and stability when immobilized by adsorption, as we have seen in previous studies [1].

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Chemoenzymatic synthesis of muramic and glucuronic acids derivatives and preparation of multivalent dendrimers as Dengue virus antagonists.

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Thematic Areas: 8. Biocatalysis.

Abstract: Carbohydrates are involved in many important pathological processes, such as cancer or bacterial and viral infections. Most of these roles depend on the carbohydrate binding activity of proteins. In this work, we present the preparation of novel derivatives of glucuronic acid and muramic acid as potential active compounds in the treatment of Dengue virus [1]. New functionalized analogs were prepared through optimized synthetic strategies, which allowed high yields under more sustainable conditions. In a second step, enzymatic approaches have been explored in order to achieve new disaccharides based on those structures. In this way, different glycosidases were screened, employing N-acetylglucosamine as acceptor and the muramic and glucuronic acids derivatives as donors in the presence of green solvents (Figure 1).
As it is well known, biomolecules exhibit multiple carbohydrates, which stablish multivalent interactions increasing the binding strengths [2]. With the aim of mimic the natural interactions and enhance the affinity towards the target protein, different glycodendrimers were prepared starting from those derivatives functionalized with an azide group (Figure 1). Surface Plasmon Resonance (SPR) studies have demonstrated that the materials obtained bind efficiently to the Dengue virus envelope protein. Finally, these interactions have been simulated and explained by molecular modelling studies.

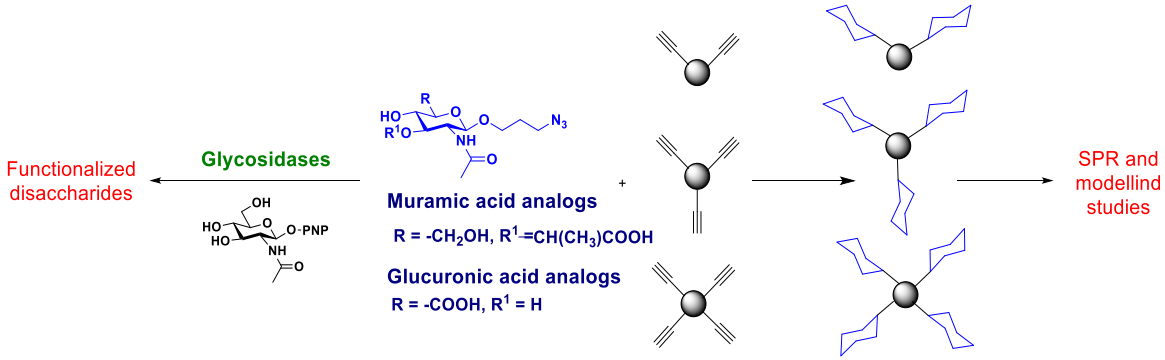


Figure 1. Preparation of muramic acid and glucuronic acid analogs

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Chemoenzymatic strategies for the preparation of glycoproteins of interest and molecular modelling studies of the enzyme-substrate recognition.

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Thematic Areas: 8. Biocatalysis

Abstract: As is well known, carbohydrates of the cell surface, conjugated in glycoproteins or glycolipids, play very important roles in different biological functions, such as cell communication. Thus, there is an increasing interest on the preparation of new multivalent glycoconjugates to facilitate the understanding of the functions of carbohydrates in nature as well as to promote development of new therapeutic glycans. However, the complexity of these structures hinder the development of efficient and general synthetic routes [1]. In this context, biocatalysis, due to the excellent regio-, chemo- and stereolectivity displayed by enzymes, offers alternative strategies which allow important improvements in this field. In this work we describe the chemoenzymatic preparation of new functionalized disaccharides, Gal-β-(1→3)-GlcNAcSCH$_2$CN and Gal-β-(1→6)-GlcNAcSCH$_2$CN, catalyzed by the β-galactosidase from *Bacillus circulans* ATCC 31382 and β-galactosidase from *Escherichia coli,* respectively (Figure 1). Reactions were conducted in the presence of biosolvents, which directly contribute to the sustainability of the process and allow the achievement of higher yields [2, 3]. Those cyanomethylthioglycosides can be further easily attached to a model protein after the activation of the group on the anomeric position with imino methoxyethyl (IME) reagent. Thus, the acceptor N-acetylglucosamine has been functionalized with this group that allow the conjugation with proteins. Molecular modelling studies performed can explain the results obtained and the catalytic behavior of the enzymes towards the functionalized N-acetylglucosamine.

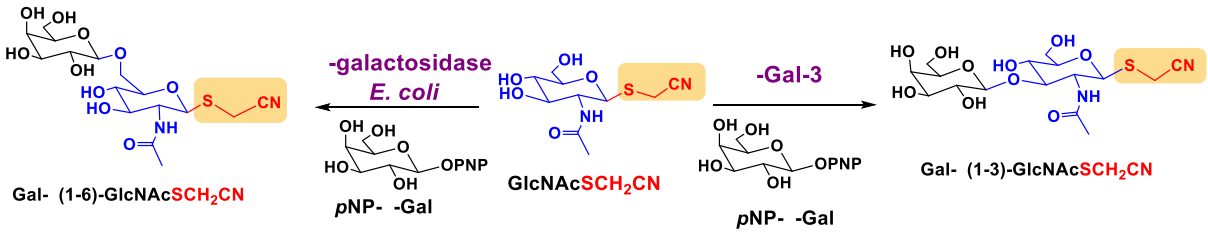


Figure 1. Enzymatic synthesis of functionalized disaccharides.

References

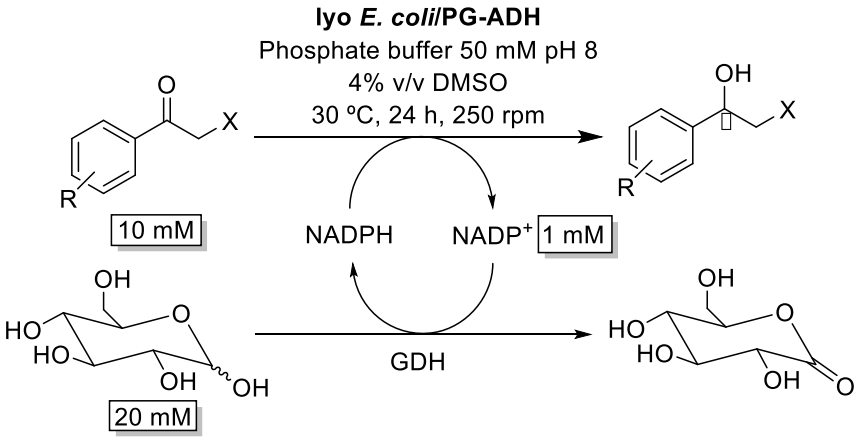
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Stereoselective reduction of halogenated ketones with an alcohol dehydrogenase from *Pichia glucozyma* overexpressed in *E. coli*

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Thematic Areas: Biocatalysis (Biocatálisis).

Abstract: Reduction processes catalyzed by enzymes are important synthetic tools for the synthesis of valuable compounds such as chiral alcohols, used as important building blocks [1]. In addition, fluorinated compounds like β-fluoro alcohols are precursors of natural products, *e.g.* steroids or carbohydrates [2]. In this work, we will show the stereoselective reduction of different fluorinated ketones with a lyophilized preparation of an ADH from *Pichia glucozyma* overexpressed in *E. coli* [3]. This enzyme showed pronounced Prelog stereorecognition in the reduction of acetophenone and propiophenone derivatives to yield the corresponding (*R*)-alcohols.



Scheme 1: Reduction of α-halogenated ketones catalysed by ADH from *Pichia glucozyma*.

Different aromatic alcohols fluorinated at α-position were obtained with a variety of substituents in the aromatic ring, showing quantitative conversions and *ee* values >99% in most cases. We have broadened the scope substituting the fluorine atom by a chlorine atom, and also using 2,2-difluorinated compounds, obtaining the corresponding alcohols with good conversions and *ee* values. Alcohols obtained in these reactions corresponded to (*R*)-enantiomeric series.
Finally, we also tested a different structure such as ethyl 4,4,4-trifluoroacetoacetate, achieving the alcohol with moderate conversion (67%) and high *ee* (90%).

Acknowledgements: Financial support from MEC (CTQ2016-75752-R) is gratefully acknowledged.

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Selective production and characterization of acidic xylooligosaccharides from beechwood xylan by an enzymatic cocktail

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Thematic Areas: Biocatalysis.

Abstract: Xylan is a hetero-polysaccharide formed by xylose subunits that can be highly branched with other monosaccharides -mainly arabinose-, acetyl side groups, methyl-glucuronic acid, glucuronic acid and/or ferulic acid. The degree, pattern and composition of these substitutions is strongly related to the xylan source. Xylan hydrolysis can be achieved by chemical or enzymatic methods but the latter ones are more efficient and eco-friendly. Nevertheless, due to xylan complexity, enzymatic procedures require the coordinated action of several hydrolases (mainly endoxylanases and β-xylosidadses) to accomplish the complete breakdown of xylan.

Xylooligosaccharides (XOS) are soluble oligosaccharides composed of 2-10 xylose units linked by β(1→4) bonds that display interesting properties such as antioxidant, antitumorigenic, antiallergic, anti-inflammatory, antimicrobial and prebiotic [1]. Acidic XOS or aldouronics are hetero-oligomers of xylose with uronic acid (glucuronic or methyl-glucuronic acids) residues randomly linked by α(1→2) bonds to the xylose moieties, which are produced by glucurono-xylan hydrolysis. Several studies have compared neutral and acidic XOS, concluding that the latter have stronger bioactivities and uronic acid ramifications are important determinants of these properties [2].

The aim of this work was to stablish a procedure for the production, separation and characterization of acidic XOS by hydrolyzing beechwood xylan with the enzyme cocktail Depol 670L. XOS were separated using anion-exchange chromatography cartridges giving two different pools: the neutral XOS and the acidic XOS. Both fractions were analyzed by HPAEC-PAD on a Dionex ICS3000 system [3] and MALDI-TOF mass spectrometry.

Following this methodology, we achieved the purification of an acidic fraction, which was significantly enriched in a compound with MW 495.2, corresponding to X2_MeGlcA (M+Na+). The structure was assessed employing a combination of 1D and 2D-NMR techniques. The compound was MeGlcAα-1,2-Xylβ-1,4-Xyl. In addition, the antioxidant activity of the purified product was analyzed measuring the $ABTS^{\cdot+}$ radical decoloration. Interestingly, neutral XOS showed no antioxidant activity whilst X2_MeGlcA exhibited higher activity than xylan.

Acknowledgements: This work was supported by a grant from the Spanish Ministry of Economy and Competitiveness (BIO2016-76601-C3-1-R/2-R/3-R). We thank the laboratory of Dr. Manuel Ferrer (ICP-CSIC) for technical assistance.

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Biocatalytic synthesis of oxygenated biofuels through the technologies of ionic liquids and supercritical carbon dioxide.

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Thematic Areas: Biocatalysis

The increase in the production of biofuels has generated in the market an excess of glycerol as a by-product. Some glycerol derivatives, such as solketal (1,2-isopropylidene glycerol), have been used successfully to synthesize new oxygenated fuels, such as fatty acid solketal esters (FASE), in order to increase the octane number of the gasolines. Biodiesel exhibits an excellent suitability as a liquid fuel (adiabatic flame temperature, viscosity, etc.), blending up to 20% volume fraction of ASE.[1]

Through the use of immobilized lipases, the biocatalytic synthesis of oxygenated biofuels can be carried out, such as fatty acid solketal esters (FASE), either by direct esterification of fatty acids (lauric, palmitic, oleic acid, etc.) with solketal, or by transesterification of different esters (eg vinyl laurate) with the same alcohol. However, the direct use of the solketal produces a strong effect of deactivation of the enzyme, being convenient to make an adequate design of the medium and the reaction systems to mitigate the possible disadvantages and improve the biocatalytic application.[2]

In this sense, it is worth mentioning the hydrophobic ionic liquids (IL), based on cations with long alkyl side chains (*e.g* [C_{18}tma][NTf_2]), have been shown as an excellent reaction medium to produce FASEs, with yields close to 100 %.[3] Alternatively, biphasic systems based on the combination of IL (or the use of supported Ionic Liquid Phases, SILLPs) and supercritical carbon dioxide ($scCO_2$) are also suitable reaction medium to develop new green biocatalytic processes.[4]

This paper presents the design of a new continuous flow biocatalytic reactor, based on the C. antarctica lipase immobilized on SILLP particles, for the production of solketyl esters in supercritical conditions (Fig.1), capable of producing a product pure with yields close to 100%, and without loss of activity during continuous operation.

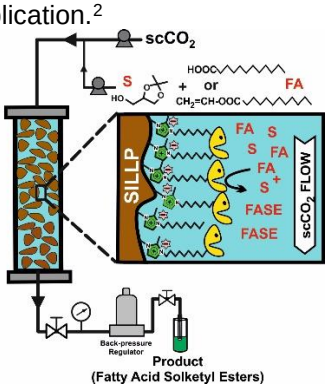


Fig.1. *Experimental set-up of the supercritical biocatalytic reactor.*

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Preparation of SBA-15 in different conditions to obtain optimal supports for laccase immobilization

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Thematic Areas: Biocatalysis.

Abstract: Ordered Mesoporous Materials offer wide application areas of chemistry and biotechnology. Particularly SBA-15 displays hexagonal arrangement of parallel unidirectional channels, advantageous as enzyme support where pore size and particle morphology are the controlling parameters for the protein molecule. The experimental conditions can be controlled to yield the desired structural and morphological features of the material, as well as the functionalization of its surface to improve chemical affinity with the enzyme. Functionalization with amine groups have been performed to immobilize laccase on NH_2-SBA-15.
Particles of SBA-15 with short channels and large pore diameter (1) were synthetized after a systematic study of the influence of every experimental parameter on the structural features of the materials: surfactant, micelle expanders and source of silica. Particles with hexagonal plate shape and channels between 200 and 500 nm length were obtained yielding SBA-15 pore diameters between 10 and 14 nm (2).Upon functionalization with amine groups, laccase (pI = 4.2) was immobilized on the -NH_2 gropups (pKa ~10) at a pH intermediate via electrostatic interactions. The enzyme was immobilized on all the supports tested (from 18 to 57 mg/g) with fair catalytic activity and with enzyme desorption below 9% after 48 hours in all cases.

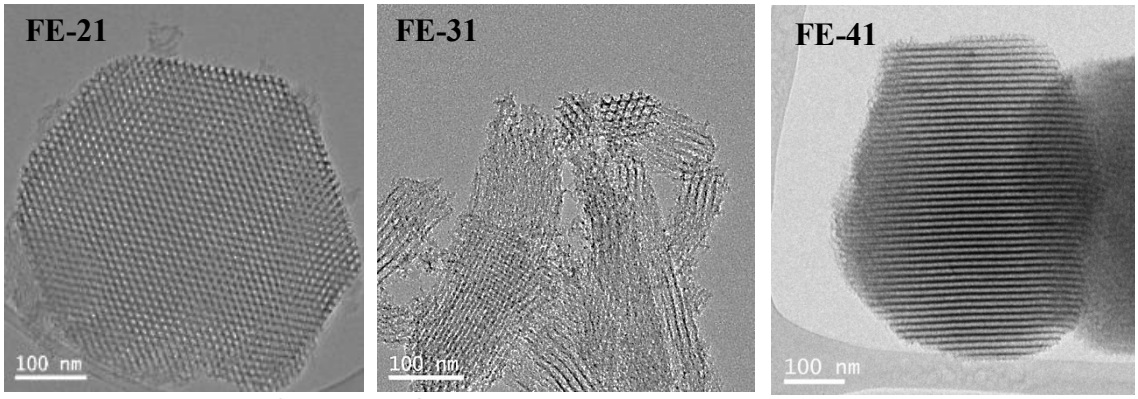


Figure 1. TEM image of optimized SBA-15 samples

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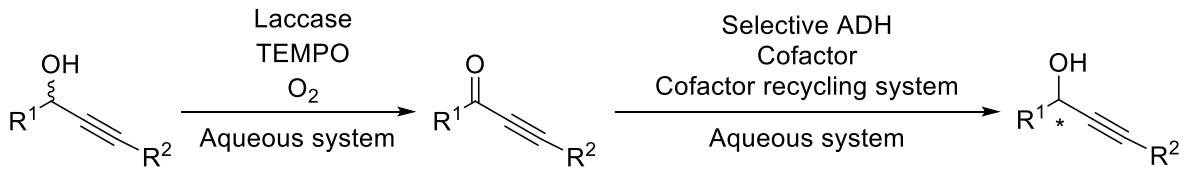
Laccase-catalyzed oxidations of propargylic alcohols. Application to deracemization experiments by combination with alcohol dehydrogenases

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Thematic Areas: Biocatalysis (Biocatálisis)

Abstract: (Chemo)enzymatic deracemization strategies are nowadays considered highly efficient and sustainable transformations for the conversion of a racemate into an enantiomerically pure product (alcohol, amine, etc).[1] Particularly attractive is the deracemization of secondary alcohols so many different approaches have been reported in recent years. These strategies generally involve a (selective) chemical or enzymatic oxidation of the corresponding racemic alcohols into the carbonyl intermediate, followed by an asymmetric bioreduction step, leading to the desired alcohols in high yields and optical purities.[2] In this context, our research group has recently taken advantage of the sequential use of chemical oxidants and alcohol dehydrogenases (ADHs) for the development of deracemization protocols.[3] Thus, the use of chemical agents (I_2/TEMPO)[3a] or a chemoenzymatic oxidizing system (*Trametes versicolor* laccase/TEMPO)[3b,c] provided straightforward and efficient approaches for the oxidation of benzylic alcohols. Subsequently, the ketone/aldehyde intermediates were stereoselectively and independently reduced to (*R*) or (*S*)-alcohols using ADHs.

Herein, the extension of this methodology applied to the chemoenzymatic oxidation and deracemization of propargylic alcohols has been investigated. Firstly, the oxidation of a large family of propargylic alcohols has been studied, comparing the efficiency of traditional chemical oxidations (Dess-Martin, MnO_2…) with the laccase-TEMPO system. Focusing on the development of a concurrent deracemization protocol, stereoselective bioreductions are disclosed using both (*R*) and (*S*)-ADHs. Finally, the combination of both redox processes in a sequential approach has been successfully addressed (Scheme 1), obtaining the enantiopure propargylic alcohols in excellent selectivities and good yields in semi-preparative transformations.



Scheme 1. Chemoenzymatic deracemizacion of propargylic alcohols.

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Autohydrolysis followed by ionic liquid treatment for *Eucalyptus globulus* wood fractionation

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Thematic Areas: Industrial Biotechnology.

Abstract: The 2030 Agenda for Sustainable Development recognises the need for a green and sustainable chemistry and engineering [1]. In this context, cleaner processes require the substitution of organic solvents by less harmful solvents. In addition, the solvent selection for a biomass processing is crucial for the environmental sustainability and feasibility of a biorefinery [2].
In this work, water and the ionic liquid 1-ethyl-3-methylimidazolium acetate ([C_2mim][OAc]) were chosen as green solvents for a selective fractionation of *Eucalyptus globulus* wood in order to valorise their main fractions (hemicellulose, cellulose and lignin). Two sequential stages were proposed for the biomass processing. First, an autohydrolysis step allowed the hemicellulose solubilisation and its recovery as oligosaccharides in the aqueous liquid phase, yielding a solid phase composed by 60 % glucan and 34 % lignin. Second, a treatment with [C_2mim][OAc] was evaluated for the delignification of the autohydrolysed biomass. Optimisation of operational conditions (temperature and time) of the ionic liquid treatment was performed to improve the enzymatic saccharification of cellulose remaining in the solid phase and to solubilise lignin. The proposed process configuration is expected to contribute towards the development of integrated biorefineries based on hardwood biomass.

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Preparative chromatography of recombinant nucleic acids using ionic liquids as multimodal ligands

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Thematic area: 7. Industrial Biotechnology (Bioprocesses: strategies for production and purification of bioproducts, modeling, scaling)

Preference of presentation format: Poster

Abstract: Nucleic acids are fundamental in many biological processes and the alterations on their expression pattern are associated to the progression of diseases, being therefore important targets in basic and applied research, but also for therapeutic purposes[1]. However, the current methodologies for the purification of nucleic acids, particularly RNA, are costly, laborious, time-consuming, require the use of organic solvents or imply the structural modification of RNA[2]. Indeed, the search on chromatographic ligands for RNA purification endowed of superior selectivity, robustness and reproducibility is still in high demand, and for which ionic liquids could be investigated as alternative/effective ligands. Ionic liquids are a group of organic salts, with great versatility to manipulate their cation/anion design, and presenting particular physicochemical characteristics (non-volatility, non-flammability, among others)[3]. These compounds have been applied in liquid chromatography in distinct ways[4], including as ligands in the stationary phases; moreover, it has been shown that they stabilize the conformational structures of nucleic acids, being therefore projected as proper solvents and preservation media. Therefore, the main aim of this work consisted on the development of new and effective macroporous-based matrices functionalized with ionic liquids for preparative liquid chromatography, envisaging the separation of nucleic acids. Initially, different ILs were covalently attached onto silica (SILs), which was used as the stationary phase for the screening and identification of the most suitable ILs for nucleic acids purification. In general, the obtained SILs allow the establishment of different interactions with nucleic acids, mainly hydrophobic and ionic interactions. Then, macroporous-based matrices were modified with the most promising ILs, in which the obtained results showed that these columns are able to selectively isolate ribosomal RNA from small RNAs and DNA in a single purification step, thus representing a step forward in nucleic acids isolation methodologies. These IL-based matrices present high robustness, high reproducibility and high dynamic binding capacity, even after regeneration steps. Overall, the successful separation of nucleic acids by IL-macroporous based chromatography may open news perspectives in nucleic acids purification methodologies, with a wide applicability for nucleic acids structural and functional studies and to prepare these samples for therapeutic-approaches.

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Medium chain length polyhydroxyalkanoates production from Camelina oil

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Thematic Areas: Industrial Biotechnology.

Abstract: Polyhydroxyalkanoates (PHAs) are biodegradable polyesters synthesized by some prokaryotic organisms from renewable sources whose application is still hindered by unpractical economics [1]. Middle chain length PHAs subclass (mcl-PHAs) with longer lateral side-chains show interesting properties as elastic and adhesive specialty polymers. mcl-PHAs producers such as *Pseudomonas* have demonstrated high yields on fats and oils [2]. Feasible processes for their obtention could be based on the use of lipid-rich agricultural byproducts and non-food vegetable oils due to their relative low cost and minimal processing required. *Camelina sativa* is a low-input, non-food chain competing crop, whose seed consists in about 43% oil in dry matter with about 90% of unsaturated fatty acids [3]. Camelina oil was for the first time tested for the production of mcl-PHA by different *Pseudomonas* strains. The use of *Pseudomonas resinovorans* enables direct fermentation of this substrate without prior hydrolysis. A first approach to process development in bioreactor has provided up to 40% polymer content, matching highest mcl-PHA titer reported from plant oils (13.2 g/L).

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MEP pathway optimization by directed enzyme evolution to produce highly valuable chemicals in microbes.

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Thematic Areas: Biotecnología Industrial

Abstract: Isoprenoids are a group of compounds that find applications in the synthesis of a wide variety of relevant compounds from commodities such as biofuels and rubber, to high-value compounds such as flavours, fragrances or pharmaceuticals (1). Currently, isoprenoids are manufactured by a petrochemical process. However, increasing concerns on environmental pollution have motivated a growing interest in developing sustainable processes to produce isoprenoids.

During last decades, microbes have emerged as suitable platforms for sustainable, environmentally friendly, and cost-effective processes to produce many relevant compounds. Along these lines, many endeavours have been made to engineer microorganism to function as microfactories by transferring a whole range of foreign enzymes (existing or even engineered with new functions) to tractable microorganisms to produce these relevant compounds (2).

Microorganism can biosynthesise the isoprenoids' precursors (isopentenyl pyrophosphate, IPP, and dimethylallyl pyrophosphate, DMAPP) through two different metabolic pathways: MEV (mevalonate pathway), and MEP (methylerythritol phosphate pathway). Despite the improved titers of MEV pathway, MEP pathway is attracting attention for isoprenoids biosynthesis for its higher efficiency in terms of theoretical carbon yield and its lower oxygen requirement compared to MEV pathway, which would decrease the need for aeration in an industrial scenario, and hence reducing the energy needed during industrial fermentation.

Unfortunately, strain construction and optimization to produce industrially relevant titers is usually hindered by several complications. For instances, enzymes from different species may not perform optimally in the desired host because of either the intrinsic activity/specificity, suboptimal environmental conditions or limited access to external cofactors. This is the case for the FeS enzymes (IspG and IspH) involved in MEP pathway. It has been demonstrated that FeS enzymes are the limiting steps in the MEP pathway. These FeS enzymes require a number of "supplying enzymes" for their activation named FeS cluster carrier proteins, FeS cluster biosynthetic pathways, and electron transporters (3). Thus, to achieve improved FeS enzyme activity and hence MEP pathway outcomes, it is critical to ameliorate the performance of these supplying enzymes.

In this work, directed evolution of supplying enzymes is used to overcome the limiting factors in the MEP pathway. In addition, to facilitate the detection of enhanced enzymatic variants a complementation assay has been used where the cell growth of an engineered *Escherichia coli* strain is linked to better performance of these enzymes. This platform can be further used to modulate other enzyme properties that would lead to increased compound production.

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Growth rate and final protein production can be dramatically increased by using a secretion signal that drives the proteins through co-translational translocation in *Pichia pastoris*.

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Thematic Areas: Industrial Biotechnology.

Abstract: During last decades, *Pichia pastoris* has been considered as one of the most widely used cell factories to efficiently produce heterologous proteins, many of them extracellular. This popularity arises from several advantages of *P. pastoris*, including the possibility to grow in both defined and complex media, the capacity to reach high cell densities of up to 130 g/L of dry cell weight, and the availability of the strong and tightly regulable AOX1 (alcohol oxidase 1) promoter (P_{AOX1}), which can be induced by methanol or repressed by glucose or glycerol.

Protein secretion is one of the most limiting process in heterologous protein production. The limitation could be related to the ER translocation process, and its effect could be minimized by using a new engineered secretion signal that enables the proteins to be translocated through the co-translational translocation. (**Fitzgerald et al.,2015; Barrero et al., 2018**).

The aim of this study was to compare the improved secretion signal and the commonly used alpha mating factor secretion signal from *Saccharomyces cerevisiae* in the recombinant protein production of the fluorescent reporter E2-Crimson, the *Bacillus thermocatenulatus* Lipase (BTL2) and *Rhizopus oryzae* Lipase (ROL) at a fermenter lab-scale under the control of AOX1 promoter. First simple batches were made to test all strains growth parameters on methanol as carbon source. Strains with the improved signal sequence had a better final titter production. In addition, these strains were able to grow at a significantly higher maximum specific growth rate than those with the conventional secretion signal.

These results were corroborated in a 5L fed-batch cultivation, using methanol as the inducer and sole carbon source. Methanol concentration was controlled at 3g/L by performing a predictive-adaptive PI control strategy. The final product concentration and volumetric productivity, appeared to be improved as well.

In conclusion, the new engineered secretion signal has demonstrated a real alternative to improve recombinant protein production in the cell factory *Pichia pastoris*.

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The new derepressible promoter P_2: a promising expression system for methanol-free recombinant protein production in *Pichia pastoris*

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Thematic Areas: Industrial Biotechnology

Abstract: The methylotrophic yeast *Pichia pastoris (spp. Komagataella phaffii)* is a widely used host for recombinant protein production (RPP) since it has several promising features that makes it a rising candidate for hosting the expression of heterologous proteins. Among these, *P. pastoris* is able to grow up to very high cell densities in defined media, to perform post-translational modificationsand to secrete the protein target to extracellular medium. Moreover, there are strong and tightly regulated promoters available, specially the methanol inducible *AOX1* promoter (P_{AOX1}) and the constitutive *GAP* promoter (P_{GAP}), which often assure high recombinant protein titers and yields.

Despite this, since the trends in bioprocess are turning to methanol-free strategies, the use of P_{AOX1} for RPP in *P. pastoris* seems to stepwise decrease.For that reason, huge research work is being performing to develop novel expression systems based on the modification of the classical promoters or on the identification of novel alternatives.In this context, the purpose of this work to characterizea brand-new expression system based on a new promoter, called P_2. It is derepressible upon glucose/glycerol depletion, and it could be further induced by methanol. Furthermore, the P2 expression system has been compared with the well-known P_{GAP} by expressing de *Candida antarctica* lipase B (CalB) under both expression systems.

To achieve a deep characterization of the new P_2 expression system and a proper comparison with the P_{GAP}, a set of chemostat cultivations of both strains were carried out using glycerol as sole carbon source. After that, some macrokinetic parameters were calculated, paying more attention on the specific CalB production rate (q_p), and its dependence on specfic growth rate. The profiles obtained determines the optimal operational conditions to maximize CalB production rate and a possible extrapolation to fed-batch cultures.

As major results, it has been demonstrated the P_2 expression system as a promising one for RPP with *P. pastoris*. Respect to the reference, P_{GAP}, a important increase of up to 4-fold was observed for q_p. Moreover, while the strain expressing CalB under the P_{GAP} showed an expectable linear relation between q_p and specific growth rate, in the case of the CalB expression under P_2, this relationship appears to be saturated beyond 0.1 h^{-1}, indicating potential bottlenecks on the folding-secretion pathways that needs to be further investigated.

Acknowledgements:
This work was funded by MINECO and FEDER under Project CTQ2016-74959-R. The authors' group is member 2017-SGR-1462 and the Reference Network in Biotechnology (XRB) of Generalitat de Catalunya J. Garrigós-Martínez a PIF scholarship from the Universitat Autònoma de Barcelona.

Oxygen-limiting conditions to improve recombinant protein production in *Pichia pastoris* regulated by the *AOX1* promoter

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Thematic Areas: 7. Industrial Biotechnology

Abstract: The yeast *Pichia pastoris (Komagataella phaffii)* is widely used as an effective cell factory for recombinant protein production (RPP). Within its advantages it can be included its versatility to grow up to very high cell densities on defined media; the high amount of information and techniques that are available to perform genetic modifications and the ability to perform post-translational modifications and to secrete the recombinant proteins.

The very promising features of this cell factory are leading to an increased interest, which is giving room to interesting studies covering the different aspects that contribute to improve the bioprocess efficiency, from strain engineering to bioprocess engineering and finally scale up. In this way, oxygen availability has been widely studied in *P. pastoris* recombinant production processes, because its limited transfer capacity can become a bioprocess-limiting factor. Strikingly, it has been described that oxygen-limited cultures can lead to an important increase on protein specific productivity (q_P) driven by different promoters.

In this work, it has been studied the main physiological and metabolic parameters of the cell factory within a wide range of oxygen availability in glucose-limited chemostat cultivations producing recombinantly the *Candida rugosa* lipase 1 (Crl1) under the regulation of constitutive GAP promoter. An important increase of the specific *Crl1* production rate (q_P), titer (P) and yield (Y_{PX}) was achieved at the optimal conditions. Several secreted by-products accompanied with a decrease on biomass yield were observed because of the metabolic shift from respiratory to respiro-fermentative pathways. Additionally, differences in the respiratory quotient (*RQ*) were also detected from normoxic to hypoxic conditions.

In conclusion, when applying oxygen-limited conditionsm, the use of reporting indicators such as RQ allows the control of the physiological and metabolic state of the cells. Therefore, the determination of the optimal conditions to enhance bioprocess efficiency can be fulfilled in a feasible and robust way, following an accurate system characterization. The present work also provide the platform to be used at different fermentations set-ups with different oxygen transfer capacities, alternative operating modes, and even for the production of other target proteins.

Acknowledgments: This work was funded by MINECO and FEDER under Project CTQ2016-74959-R.The authors' group is member 2017-SGR-1462 and the Reference Network in Biotechnology (XRB) of Generalitat de Catalunya. A. Gasset acknowledges a PIF scholarship from the Universitat Autònoma de Barcelona.

Evaluation of the bioreactor configuration in the production of microalgae *Crypthecodinium cohnii*

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Thematic Areas: 7. Biotecnología industrial. Bioprocesos: estrategias de producción y purificación de bioproductos, modelización, escalado

Abstract: As a result of climate change and the growing global demography, terrestrial food resources have been affected by huge consequences. Therefore, the search of sustainable alternatives that would allow to obtain the food products from new resources it is necessary. An illustrative example of this is the cultivation of algal biomass for generating value-added products for the food industry. Thus, in recent times microalgae have arisen a new source that allows to obtain valuable products such as proteins, lipids and polysaccharides (1). In this sense there is a special interest in the food industry on obtaining the omega-3 fatty acid docosahexaenoic acid, also known as DHA. Among the different microalgae that provide this product, for this study, it was selected *Crypthecodinium cohnii.*
This heterotrophic marine microalga is an excellent producer of DHA, and around 30 to 50% of lipids accumulate in its membrane are DHA (2). Therefore, its production could compete directly with the conventional source of production such as pelagic fish species.

The main objective of this research was the optimization of the biomass production in bioreactor of the microalgae *C. cohnii*. For this purpose, different reactor configurations at lab scale Stirred tank reactor (STR) and Air-Lift reactor (ALR) were evaluated (Figure 1). Furthermore, the culture media composition was analysed in order to evaluated its effect in the biomass and DHA production. Comparing both reactor the rate of biomass growing was slightly higher when ALR was used. In addition, it was observed that depending of the used reactor the growing of the microalgae showed different appearance (Figure 1 embedded). While in the ALR biomass aggregates were observed after the third day of culture in the STR no aggregates were appeared.
Finally based on the different obtained results, it was concluded which type of conditions are need in order to make this process more profitable at industrial scale.

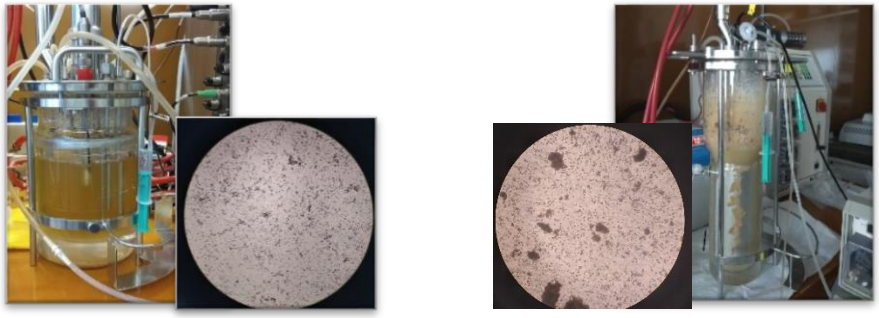


Figure 1. Stirred tank reactor (left) and Air lift reactor (right) used in the study. Embedded a detail of microalgae under the microscopy for each reactor.

Acknowledgements: This research has been financially supported by Xunta de Galicia (Project ED431C 2017/47) and CDTI IDI-20180390.

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Optimization of the culture medium for growth and DHA production of microalgae *Crypthecodinium cohnii*

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Thematic Areas: 7. Biotecnología industrial. Bioprocesos: estrategias de producción y purificación de bioproductos, modelización, escalado

Abstract: In the present study, it was accomplished the optimization of the production of fatty acids, concretely the omega-3 fatty acid docosahexaenoic acid (DHA), by the heterotrophic marine microalgae *Cryptecodinium cohnii*. This specific acid was studied because it is an essential substance for humans and we cannot produce it by ourselves. In addition, this microalga was selected because of its capability to produce high DHA concentrations, between 30 and 50% of the lipids that the microalga accumulates in its membrane (1).
The main objective of this study is to optimize the culture medium in order to improve microalgae biomass growth and DHA production at shake-flask scale. For this optimization, concentration of some medium components were evaluated. These components were: ethanol (carbon source), yeast extract (nitrogen source) and salt. The optimization was carried out by a response surface methodology using Central Composite Design (CCD). Two different responses, biomass growth and DHA production, were studied.
After doing the first experiments, it was observed that the highest biomass concentration was obtained by using the maximum ethanol and yeast extract concentrations (10 g/L in both cases) maintaining salt concentration at a constant value (25 g/L) (Figure 1). Moreover, it was observed that the used concentrations have effect on the microalgae morphology. Thus, operating at the optimal conditions, microalgae did not show any mobility and having a big size, while operating with less concentration of ethanol and yeast extract, microalga is able to increase its mobility and big aggregates appear. Similarly, the effect of the medium composition on DHA production was also determined. Therefore, based on the optimal conditions from maximizing both responses the numerical optimization applying Desirability function was determined.

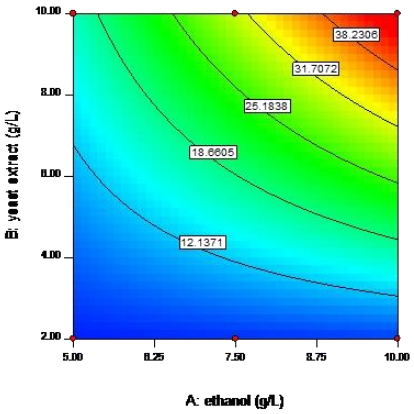


Figure 1. Biomass response surface obtained from using CCD.

Acknowledgements: This research has been financially supported by Xunta de Galicia (Project ED431C 2017/47) and CDTI (Project IDI-20180390).

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Simulation of biobutanol production at industrial scale

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Thematic Areas: Industrial biotechnology.

Abstract:

Butanol is a chemical compound of industrial relevance that is used in numerous processes of chemical transformations it can be used as fuel and as addictive to gasoline with great potential to develop on an industrial scale by Trindade et al [1].

Currently, butanol is mostly obtained through the petrochemical industry. However, the investigation of its biotechnological production is gaining further momentum due to environmental advantages.

The EU is developing policies to reduce climate change by supporting the development of research in the field of bioenergy against the use of fossil fuels, as shown in the Directive 2009/28/EC.

Biobutanol has advantages over other biofuels when used in conventional gasoline engines, as it does not involve additional modifications and its corrosive potential is lower than other biofuels like bioethanol.

Despite its interest, biobutanol production has not still been produced at industrial scale due to its higher production costs and low yields. Therefore, the implementation of efficient simulation tools can be crucial to learn about the global economics of the process and how much is still to be done to achieve a truly viable production process.
On the basis of the above, the design of the biobutanol production process was carried out based on the fermentation of ABE (acetone-butanol-ethanol) by *Clostridium acetobutylicum* microorganism using glycerol as raw material. The inherent production of by-products like acetone and ethanol and their role in the total benefits have been considered. The design has been carried out taking into account the recovery of process water and biomass so that the economic cost and environmental impact was minimized. The simulation of the process has been carried out in SuperPro Designer® v.8.5 (Intelligen, inc), a chemical engineering software specifically employed in bioprocesses.

After a careful revision of the existing literature data, the experimental conditions reported by Yadav et al [2] were selected as the basis for the scaling-up, leading to the design of a plant yielding 580 kg of butanol, 200 kg of ethanol and 17 kg of acetone per batch. This is translated into a plant able to produce 79-80 batch that would result in a total of 45-46 Tm of Biobutanol. The simulation of the process was successfully performed, thus easing the environmental and economic evaluation and setting the foundations of biobutanol production at industrial scale.

Acknowledgements: Thanks to Dr. Francisco Javier Deive Herva for contributing to this project and making it possible.

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Acetogenic bioconversion of greenhouse gases (CO_2, CO) and syngas to fuel-alcohols

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Thematic Areas: 1. Microbial Biotechnology, 7. Industrial Biotechnology.

Abstract: Ethanol and higher alcohols, such as butanol and hexanol, are compounds that can be used as fuels or platform chemicals. They can be produced through acetogenic fermentation of different gases and gas mixtures containing CO_2 and/or CO. Those gases are major compounds of syngas, which does also contain H_2, and they are also common in industrial emissions, among others in still industries (Van Groenestijn et al., 2013). H_2 is a suitable substrate that can also be metabolised by acetogenic bacteria together with CO_2 and CO. Ethanol and higher alcohols are possible end metabolites of the gas fermentation process in some acetogens. Syngas can be obtained from the gasification of renewable feedstocks or wastes, with the corresponding environmental benefit. The use of syngas or waste gases as carbon and energy sources in this process has several advantages, e.g. reduction of gas emissions, cost-effective technology, production of metabolites of interest.
The bioconversion usually proceeds in two stages; a first stage (acetogenesis) characterised by the accumulation of organic acids and a second phase (solventogenesis) during which the accumulated acids are converted into alcohols. A high, near neutral, pH is necessary for acetogenesis, while the second stage is stimulated by a lower pH. Different experiments were carried out using *C. carboxidivorans* as a biocatalyst for gas fermentation and production of higher alcohols. The main goal of this research was to check the effect of two different pH control strategies, applying either a constant pH of 5.75 or allowing natural acidification down to pH 4.75 during the fermentation process. The highest concentrations of acids and alcohols were obtained in the experiment at a constant pH of 5.75 (2.7 g/L ethanol, 1.9 g/L butanol and 0.85 g/L hexanol) whereas the highest production rates were observed at pH 4.75 (0.048 g ethanol/h*g biomass, 0.036 g butanol/h*g biomass, and 0.026 g hexanol/h*g biomass). However, natural acidification (pH 4.75) had a negative effect on biomass and the accumulation of acids, with lower growth rates at pH 4.75 (0.0057 h^{-1}) than 5.75 (0.072 h^{-1}).
This research shows the possibility to use syngas/waste gases to produce alcohols of commercial interest, under cost-effective and environmentally-friendly conditions.

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Citrus processing waste valorisation for the production of pigments by fungi fermentation

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Thematic Areas: Industrial biotechnology

Abstract:

The Region of Murcia is one of the most important fruit and vegetable producers in Spain and Europe. This industry generates a large amount of waste, which are mostly parts of processed raw materials that have no commercial interest. Most of this waste are no properly used or processed, only a small portion is commonly used for livestock feed[1]. However, due to the growing concern about the proper use of resources and the growing tendency to promote the circular economy model in industry, new strategies for waste valorisation in food sector are being addressed[2].

The main objective of the project AGROMAT, currently under development, is to investigate the potential of food waste extracts for their application in the development of raw materials for the furniture sector.

One of the strategies to address for reaching this aim is the application of biotechnological approaches to produce natural or bio-based materials from waste valorisation. Enzymes and microorganisms have the ability to transform, into new valuable products, those that have reached the end of their useful lifetime, via biocatalysis or fermentation processes[3,4,5]. According to this, in this project, the valorisation of industrial citrus processing waste will be developed as a source of nutrients in the fermentative production of pigments by different species of fungi.

To achieve this goal, the following methods have been planned. Some microorganisms that produce natural pigments, as secondary metabolites, will be studied and chosen by selective pressure methods, in order to reach good yields in the fermentation of citrus by-products. After obtaining stable culture lines of selected fungi, different fermentation methods will be used: solid-state, semi-solid or submerged fermentation. Each reaction will be controlled and analysed, studying different parameters and factors involved in the process: pre-treatment of citrus residues, culture media, temperature, time and incubation agitation, substrate moisture, amount of inoculum, reaction kinetics, generation of inhibitors and negative feedback, etc. High Performance Liquid Chromatography (HPLC) and Infrared Spectroscopy (FTIR) will be some of the analytical techniques used for this characterization.

The presence of different pigments will be estimated by spectrophotometry, measuring the absorbances in the wavelengths that coincide with the typical colours obtained from citrus. Different applications for these pigments will be studied. Its suitability will be evaluated to be used as dyes in paints, fabrics, and other materials applicable to the furniture industry, validating its potential to be exploited in a new sector.

As a result of the project AGROMAT, it is expected to obtain knowledge about the most efficient fungal strains in the fermentation of citrus residues, the optimum conditions to reach the maximum reaction yields, a suitable method to extract and purify the pigments and the way to apply them to products destined for use in the furniture industry, such as paints or fabrics.

Acknowledgements: We thank to Development Agency of the Region of Murcia by support offered in research funding.

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Steering volatile fatty acid production during protein fermentation by adjusting substrate composition and pH

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Keywords: protein fermentation; pH; VFA production; casein; gelatin

Thematic area. Industrial Biotechnologies

Introduction. Volatile fatty acid (VFA) production from waste and sidestreams is an appealing alternative to biogas generation because of the countless applications they have as precursors of other commercial compounds. Sugars conversion to VFA is relatively well studied, while not much is known about proteins fermentation and even available information is controversial (Ramsay and Pullammanappallil, 2001). For this reason, this study focuses on understanding the influence of protein composition (i.e. amino acid abundancies) and pH on fermentation outcome, in terms of protein conversion and VFA selectivity and productivity.

Materials and methods. Two continuous stirred tank reactors of 1 L working volume were operated at 25°C with a hydraulic retention time of 1.5 days. Two model proteins, casein and gelatin, were used as sole carbon source with an organic loading rate of 5.3 g COD/L·d. HCl and NaOH 3M were used to control the pH at three different levels: 5, 7 and 9. Nitrogen sparging (≈ 10 mL/min) was implemented to reduce hydrogen concentration in the liquid phase and maintain anaerobic conditions. Reactors inoculum came from a lab scale acidogenic fermenter (1.0 g VSS/L). Standard analytical methods were used to monitor reactors performance.

Results and conclusions. Results showed that different protein composition leads to different acidification degrees: casein always performed better than gelatin at all tested pH values, with the maximum conversion achieved at pH 7 (50% COD basis). VFA selectivity was affected by the protein composition as well, since casein fermentation at pH 7 is linked to higher n-butyric acid molar fractions while gelatin degradation is related to propionic and n-valeric acid production. Also, pH affects product spectrum (Figure 1). In general, low pH is linked to heavier molecular weight VFAs. These results will help to target specific VFAs production during protein-rich sidestreams fermentation processes.

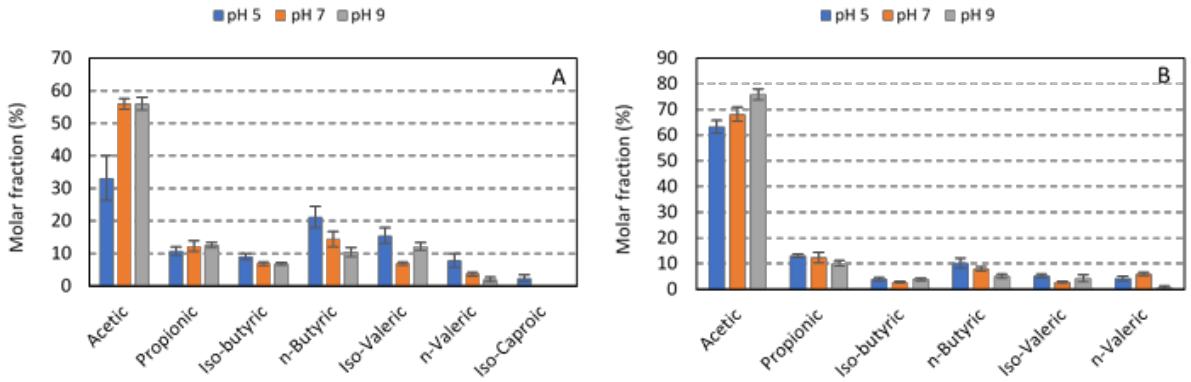


Figure 1. VFA spectra of casein (A) and gelatin (B) at different pH values.

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Proteins involved in the acquisition of the active structure of the secreted proteins in *Streptomyces lividans*

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Thematic Areas: Industrial Biotechnology

Abstract: *Streptomyces lividans* is a soil bacterium that efficiently secretes a vast array of hydrolytic enzymes and it has been used as a host for engineering extracellular overproduction of homologous and heterologous proteins of industrial application. The enzymes involved in secretory protein extracellular folding are the thiol-disulphide oxidoreductases and the peptidyl-prolyl cis/trans isomerases. The characterisation of these enzymes is of great importance when engineering *S. lividans* overproducer strains.
Bacterial peptidyl-prolyl *cis/trans* isomerases (PPIases) have been linked to protein folding of secretory proteins, due to their capacity to catalyse the *cis/trans* isomerization of peptide bonds preceding prolyl residues [1].

We have identified and characterized a Tat-dependent a *S. lividans* FKBP-like lipoprotein. Propagation in high copy number of the *sli-fkbp* gene encoding this enzyme results in a considerable increase in the extracellular activity of the two overproduced model proteins, the Sec-dependent alpha-amylase, and the Tat-dependent agarase [2].

The thiol-disulphide oxidoreductases catalyse the formation of disulphide bonds, which are required for secretory proteins to acquire their correctly folded fully active conformation [3]. We have identified four thiol-disulphide oxidoreductases encoding genes in the *S. lividans* genome. Two of these oxidoreductases, Sli-DsbA and Sli-DsbB, are required for the formation of the unique disulphide bond present in the Tat-dependent agarase, and the four of them, Sli-DsbA, Sli-DsbB, Sli-DsbC and Sli-DsbD, are required for the formation of the potential five disulphide bonds present in the Sec-dependent alpha-amylase. Therefore, the Sli-DsbA/ Sli-DsbB pair is involved in the formation of disulphide bonds right after the secretory protein translocation, while the second pair, Sli-DsbC/Sli-DsbD, appears to be active in repairing the wrongly formed disulphide bonds.

These findings could be of great relevance when engineering *Streptomyces* strains for the overproduction of stable, fully active secretory proteins.

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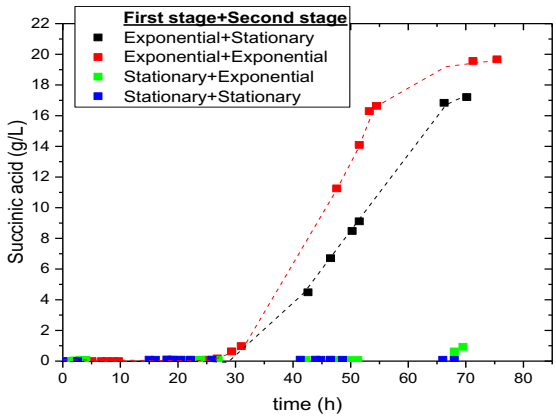
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Succinic acid production from xylose by *Actinobacillus succinogenes*: influence of inoculum built up

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Thematic Areas: Industrial Biotechnology

Abstract: Succinic acid is one of the main building blocks in which Industrial Biotechnology is focused on. This acid is a key compound for the synthesis of more than thirty important products such as surfactants, detergents, flavors, fragrances, biodegradable polymers, food additives, etc. [1]. Most of the studies regarding its bio-production are based on the use of glucose as carbon source. The concept of integrated bio-refinery carries on the use of all the fractions from the lignocellulosic materials, being the sugars obtained from the hemicellulose fraction (mainly xylose) those needing more attention. In this work, a study on the influence of the inoculum built up using xylose as carbon source for succinic acid production is performed. Inoculum build procedure is a matter of big importance in bioprocesses. Due to the succinic acid pathway it is necessary the use of carbon dioxide as co-substrate.
Actinobacillus succinogenes 130Z is the microorganism used in this study. TSB medium [2], supplement with 40 g/L of xylose has been used. The runs have been carried out at bioreactor scale. A 2L commercial bioreactor has been used at 37 ºC of temperature, a stirrer speed of 300 rpm and 0.1 vvm of CO_2 of gas-flow.
The inoculum has been built up using two stages considering the combination of exponential and stationary growth phases in the cell cultures. Four runs have been performed with cells with different growth time combining both stages (see combinations in the figure). In this way the best way for inoculum built up can be determined.
The results show that the best succinic acid production is achieved when first inoculum stage is carried out using cells collected from the exponential growth phase, scarcely influencing the cell age of the second inoculum stage. The figure shows the results for succinic acid production and it can be observed that there is no production when cells from a stationary growth phase are used in the first stage of inoculum built up.



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Scaling-up Ionic Liquid biodegradation: sequencing batch for fixed-bed reactor

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Thematic Areas: 5. Biotecnología ambiental: Biorremediación; procesos de biotratamiento y Biorrecuperación.

Abstract: Ionic liquids (ILs), defined as organic salts with a melting point below 100ºC, have attracted a great scientific interest in recent history. Among the reasons behind this appeal, the possibility of combining thousands of cations and anions makes it possible the design of task-specific ILs with unique properties. Although ILs are considered green solvents, studies have demonstrated that some cations are toxic and environmentally persistent, so their impact must be carefully evaluated [1]. A thorough search for more biocompatible and more biodegradable options has led to the proposal of choline-based ILs [2], since the biological degradation of this cation by a halophilic bacterium was already demonstrated in 2017 [3].

More specifically, in previous research works of our group, *Halomonas* sp. LM1C was successfully employed for cholinium biodegradation, and the promising potential of an alginate-polyvinyl-based immobilization strategy was demonstrated. Taking in account these results, the scale-up of this biodegradation process was approached. To do that, two different operation modes were employed: fed-batch and continuous culture. Additionally, several air flow rates were tested in order to avoid sudden changes in bioreactor dynamics. In all cases, choline biodegradation was monitored, and its relationship with decisive process variables like pH was inferred. In this particular case, the existence of pH drops below 6 was indicative of a loss in the biodegradation capacity of the selected halophilic microbial strain.

In general, the results obtained demonstrated the feasibility of a fixed-bed bioreactor to treat cholinium-based polluted effluents in fed-batch mode, and set the foundations for proposing a viable continuous biodegradation process at greater scale.

Acknowledgements: This work was supported by Xunta de Galicia (project ED431F 2016/007) and Spanish Ministry of Economy and Competitiveness (CTM2014-52471-R). F.J.D. acknowledges the Spanish Ministry of Economy and Competitiveness for funding through a Ramón y Cajal contract (RyC-2013-14225). A.C. is grateful to Xunta de Galicia for a predoctoral grant.

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Hugging a green approach to remove antibiotics from swine wastewater

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Thematic Areas: Industrial Biotechnology

Globally, antibiotics conquer an outstanding niche in the emerging contaminants family due to their wide use in the treatment of infectious diseases in both humans and animals [1]. Around 50–80% of these compounds are not metabolized and are thus found out in wastewater effluents, so is urged to hunt for competitive alternatives to remove them from the environment [2]. Aqueous Biphasic Systems (ABS) have long been considered as a competitive and versatile separation technique that can be applied to the extraction of a wide range of compounds like enzymes, metal ions, or antioxidants among others [3-5]. During the last years, the tunability of ionic liquids, salts that are liquid near room temperature, has contributed to boost their use for the implementation of new types of ABS. In this line, cholinium-based ionic liquids would be promising candidates for designing eco-friendly processes and removing pollutants as antibiotics due to their ability as salting out agents and biocompatible nature.

In this work, the capacity of choline dihydrogen phosphate (ChDHP) to salt out aqueous solution of non-ionic surfactant Tween 80 containing tetracycline and oxytetracycline as model of veterinary antibiotics in swine effluents has been proved [6]. The proposed strategy led to high levels of antibiotics removal in model aqueous solution (96%) and in a urine-type effluent and real swine (70%). Finally, the suitability of this process for implementation in a swine wastewater treatment was demonstrated after testing the antibiotics removal capacity in 5 cycles where both the ionic liquid and the non-ionic surfactant were reused.

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Wastes from hydrocolloid and fibre industry as *low-cost* adsorbents: adsorption and regeneration

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Thematic Areas: Biotecnología ambiental.

Abstract: Several contaminants of emerging concern including pharmaceuticals and personal care products (PPCPs) have been detected in surface waters. The impact of these substances in the environment is a matter of concern due to they may cause detrimental effects mainly in aquatic life and physiological effects in human life (1). One of the main characteristics of these compounds is their presence in small amounts, which makes it difficult their treatment by traditional wastewater treatment plants. Moreover, the removal of PPCPs by these compounds depends on the physicochemical properties of the pollutant such as the charge and polarity. Thus, adsorption becomes as a plausible alternative for the removal of the pollutants and new adsorbents are required for this purpose (2). Circular economy advocates the use of waste streams as a source of secondary resources and recovery of wastes for their reuse. Therefore, they become a potential source of raw material for adsorbents.
In this work, the application of biomass from hydrocolloid and fibre industry, their transformation in biochar as adsorbents for effective removal of emerging pollutants (PPCPs) and their regeneration were ascertained. Initially, the ability of several wastes as adsorbents was evaluated for the competitive removal of a mixture of two different PPCPs: sulfamethoxazole (SMX) and methylparaben (MePa). The wastes from fibre extraction process presented the best result attaining maximum uptake values around 3-5 mg/g for both PPCPs. Later, the characterisation of the selected waste was carried out and the adsorption process was studied in depth including the adsorption kinetics and isotherms. Langmuir model presented the best fitting to the data resulting in maximum uptake values 7.29 and 43.3 mg/g for MePa and SMX, respectively and the kinetics followed a pseudo-second order model and the simulation of an adsorption column was carried out. In spite of the good results obtained previously, the enhancement of the treatment was performed by the preparation of biochar derived from the fibre wastes. Several operational conditions for obtaining the biochar were evaluated from an economical and maximum removal point of view resulting the best conditions in temperature 320ºC and 30 min of treatment time under nitrogen atmosphere. The obtained results showed a sharp increase in the adsorption capacity reaching values between 10 and 20 times higher than the previously obtained for both pollutants with a maximum uptake around 60 and 140 mg/g for MePa and SMX according to the Langmuir model. Once the adsorption was finished, the regeneration of the spent adsorbent was evaluated by Fenton process. Operating with a 1:100 Fe:H_2O_2 ratio, five adsorption-regeneration cycles were carried out without any operational problem and reaching high removal levels of the pollutants.
Those results remarks the potential of the selected wastes as source of good adsorbents and feasible alternatives for the removal of PCPPs.

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Study of the sorption process and removal of diuron by *Trametes versicolor* immobilised in wood chips in a biodisc reactor operating in continuous mode

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Thematic Areas: Biotecnología ambiental.

Abstract: The occurrence of pesticides in water resources represents an issue of growing concern among the scientific community. Such substances are considered persistent, bioaccumulative and toxic, and have potential harmful effects on human beings and the environment, even at trace concentrations. The widespread application of pesticides on agricultural fields to control pests may lead to increase their presence in surface waters of agricultural basins, by runoff or leaching through the ground, as a side effect of their use (1).

Alternative bioremediation treatments have been proposed, being the bioaugmentation of fungi a feasible technology owing to they can degrade a wide range of pesticides. Among them, white-rot fungi (WRF) can biodegrade several types of pesticides as a result of the activity of their nonspecific enzymatic system (2). Long-term continuously operated fungi bioreactors of real wastewater under non-sterile conditions is a challenging issue since other autochthonous microorganisms, mainly bacteria, can compete to consume the existing substrate and decrease or inhibit fungi growth and its enzymatic activity over time. Given the low concentration of pesticides commonly detected in wastewater from agricultural areas, the addition of an external carbon source is normally required, and fungi inoculation over lignocellulosic materials could be an effective alternative, as it provides a complex and resistant carbon structure against bacterial growth, changes of the environmental conditions and mechanical stress, while reusing economic wastes (3).

The aim of the present work is to design and operate a rotary biodisc reactor of immobilized fungi on a lignin-cellulose support for treating polluted irrigation water. Experiments were performed by spiking real irrigation water with diuron and bentazone at HPLC detectable levels (10ppm). A systematic study of the adsorption process of diuron on wood was carried out in batch experiments and sequential batch experiments in order to determine the saturation point and the reversibility extent of the sorption process. The fungal degradation of the adsorbed diuron on wood has been assessed through biopile treatment. Preliminary results of the pollutants continuous removal by *Trametes versicolor* immobilised on pallet wood in a biodisc will be presented. The adsorption will be assessed through a control reactor with non-inoculated wood.

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Challenges in analysis and remediation of pharmaceutical pollution in water: A human concern.

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Thematic Areas: 5. Biotecnología ambiental. Nuevas herramientas de evaluación y análisis

Abstract: More than 3000 pharmaceutical products are globally authorized. From these, more than 600 have been shown to be present in the environment as resulting from the increasing use of human and veterinary pharmaceuticals that poses a threat to wildlife (aquatic and terrestrial) and also humans via drinking water supplies. Most of the worldwide Environmental Agencies highlight that international actions are needed in order to protect wildlife and humans from produced harm by pharmaceuticals (1). Several of these actions are required by the pharmaceutical industry with the design of less persistent pharmaceuticals in the environment ("greener pharmacy"), by the global education with adequate 'take-back' plans and the proper disposal of unwanted drugs (people are aware that flushing them down the toilet is not appropriate, Figure) and by the scientific community with the development of robust detection methods to identify those specific medicines below 0.05 μg/L and the implementation of advanced treatment techniques as effective remediation processes to reduce pharmaceutical residues from sewage/waste water.

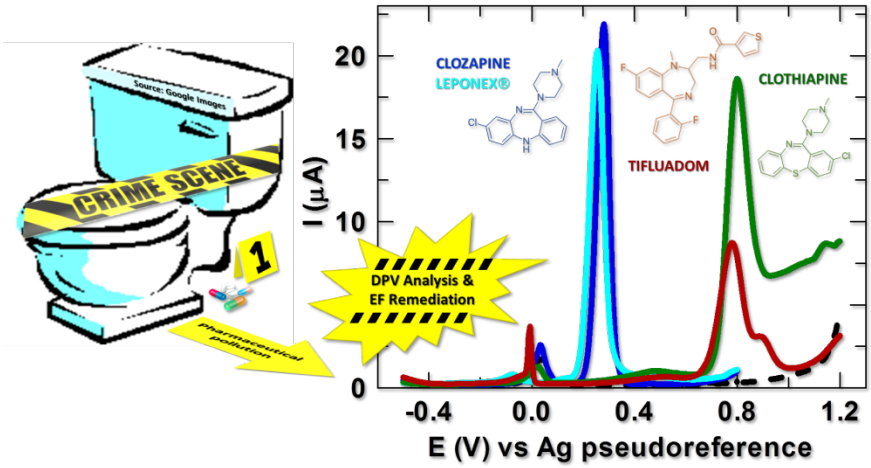


Figure. DP voltammograms of pharmaceuticals at initial time of the electro-Fenton treatment.

In this context, the goal of this work has been to investigate a combined electrochemical techniques (2), Differential Pulse Voltammetry (DPV) on screen-printed electrodes and electro-Fenton (EF) as Advanced Oxidation Process (AOP), for identifying, monitoring and removing two psychotropic and one analgesic drugs (Figure): Clozapine, sold under the brand name Leponex among others, and Clothiapine (an antipsychotic dibenzodiazepine derivative) and Tifluadom (a sedative benzodiazepine as Oxazepam). This combined methodology has been successfully applied to the elimination of these pharmaceuticals from tap water. Additionally, the OH* radical generated during the EF treatment has been monitored by fluorimetry using Terephthalic Acid as trapping reagent.

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Evaluation of the adsorption potential of biochar prepared from vine shoots for the removal of pirimicarb

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Thematic Areas: Biotecnología Ambiental

Abstract: In the last years, the presence of pesticides in aquatic environments has increased mainly as a result of the intensification of agricultural activities [1]. Pesticides represent an important class of pollutants due to their reported toxicity even at trace levels, therefore, their removal is an increasing concern. Among the different techniques that can be used to remove these contaminants, the adsorption is proposed as the best one due to its inexpensiveness, universal nature and ease of operation. In particular, biochar materials have been shown to be a promising solution, with high adsorption efficiency [2, 3].
According to the National Institute of Statistics (2009) [4], the annual amount of biomass generated in Portugal from vineyard pruning may attain 153020 t, and biochar production from these wastes is currently one of the more innovative and unexplored fields of research. In this work, it was evaluated the possibility of using vine shoots to produce biochar, which will be tested for the removal of the pirimicarb pesticide from aqueous solutions. For that, vine shoots were pyrolyzed (500ºC for 24 hours), milled and the moisture and ash content, as well as the volatile matter were determined. The results of the kinetic studies performed with the biosorbent showed that after the first fifteen minutes the equilibrium was achieved. During the kinetic assay, the pH variation was not significant, ranging from 9.30 to 8.27. Isotherm studies are being carried out to determine the effectiveness of the vine shoot biochar on pirimicarb adsorption. These first results suggest that the use of biochars prepared from vine shoots could represent a low-cost and eco-friendly alternative to other adsorbents for the pirimicarb removal from aqueous solution.

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Scaling-up Ionic Liquid biodegradation: immobilization approach for fixed-bed reactor

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Thematic Areas: 5. Biotecnología ambiental: Biorremediación; procesos de biotratamiento y Biorrecuperación.

Abstract: Ionic liquids (ILs), organic salts with a melting point below 100ºC, have been deeply investigated over the recent history due to their exceptional properties. Among them, tunability must be highlighted as it allows performing an *à la carte* solvent, by combining multiple anions and cations available. Despite ILs are generally considered as green solvents, their true environmental impact must not be underestimated (1). The search for more biocompatible and more biodegradable options has resulted in the bet in choline cation (N_{1112OH}) (1). Since this, choline-based ILs have been the subject of a growing interest (2) but there was not a first true biodegradation approach until 2017 (3), when the halophilic bacteria *Halomonas sp.* LM1C was successfully employed for such task.

In view of the promising results achieved with *Halomonas sp*., this study focuses its endeavour in the scale-up of the biodegradation process. For this purpose, an immobilization technique based on biocompatible hydrogels was selected and applied to the microorganism to be then used in a fixed-bed bioreactor. The experimental approach followed a four-step strategy: (I) study of Biomass $\%_{w/w}$ and Hydrogel $\%_{w/w}$ effects in N_{1112OH} diffusion on the selected hydrogels, (II) viability of *Halomonas sp.* entrapment into the hydrogels, (III) degradation studies at flask scale, and (IV) operation at bioreactor scale.

The results obtained at flask and bioreactor scale demonstrated the suitability of alginate-polyvinyl hydrogel in terms of cell leakage minimization and mechanical strength to be used as supporting matrix to perform *Halomonas sp*. entrapment.

Acknowledgements: This work was supported by Xunta de Galicia (project ED431F 2016/007) and Spanish Ministry of Economy and Competitiveness (CTM2014-52471-R). F.J.D. acknowledges the Spanish Ministry of Economy and Competitiveness for funding through a Ramón y Cajal contract (RyC-2013-14225). L.M. is grateful to the University of Vigo for a predoctoral grant.

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Bioelectro-Fenton process, a promising system for the removal of ionic liquids?

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Thematic Areas: 5. Environmental Biotechnology. Bioremediation, biotreatment and bioremediation processes.

Abstract: For the last years, ionic liquids have generated interest among the scientific and industrial communities. These compounds are characterised by a negligible vapour pressure, a high thermal and chemical stability and a low melting point, being a promising alternative to conventional solvents. However, it has been discovered that their potential toxicity, scarce biodegradability and great solubility makes them potential contaminants [1]. Thus, the direct application of a traditional biological treatment is not adequate, being necessary a previous step in order to increase the biodegradability of these compounds. In this context, advanced oxidation processes as electro-Fenton (EF) are gaining popularity for removing refractory and toxic pollutants [2]. Therefore, the goal of this work was the removal of the ionic liquid 1-buthyl-1-methylpyrrolidinium chloride ([bmpyr]Cl) by performing a combined system: an EF process followed by a biological treatment (Figure).

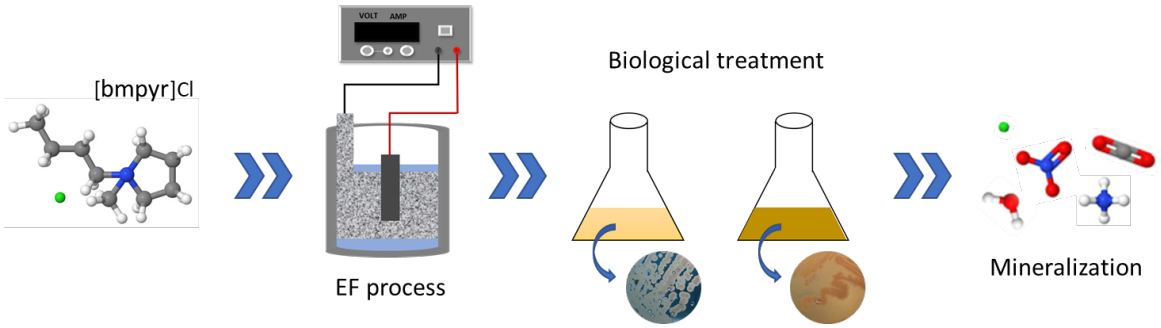


Figure. Scheme of the combined system for the removal of ionic liquid [bmpyr]Cl.

First, EF experiments were carried out in order to optimize the parameters involved in the process. These parameters were iron dosage (0.1-0.2 mM), current applied (50-100 mA) and initial ionic liquid concentration (0.075-2.5 mM). Based on the results, the optimal conditions selected were 0.1 mM of iron, 1000 mA and 1 mM of [bmpyr]Cl, achieving a total removal of the ionic liquid and a total organic carbon decay of 98% within 4 hours.
Then, the biological treatment was accomplished. To this end, a consortium of microorganisms has been employed and the experiments were carried out for 8 days. Also, a control of untreated [bmpyr]Cl with the microorganisms was performed. The evolution of carboxylic acids was followed in both cases, noting that no carboxylic acids were detected in the control, whereas an evolution of these compounds was seen in the solution with the ionic liquid previously treated by EF process. Hence, we can conclude that coupling a biological treatment after an EF process is a promising methodology for the removal of complex organic pollutants such as ionic liquids.

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Valorization of sewage sludge to produce volatile fatty acids though acidogenic fermentation: batch and continuous reactor experiments

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Thematic Areas: Environmental biotechnology

Abstract: Currently, the amount of sewage sludge (SS) generated in Wastewater Treatment Plants (WWTPs) is increasing worldwide. The management of SS represents more than 60% of total operating cost in WWTP (1) and its disposal has several environmental problems due to the high organic matter content and the presence of heavy metals, pathogens and xenobiotic compounds. An alternative to prevent its accumulation is the acidogenic fermentation of SS to produce volatile fatty acids (VFA). The VFA generated during anaerobic digestion have a wide range of applications in biotechnology, such as their use as carbon source for the biological removal of nutrients in WWTP or to produce Polyhydroxyalkanoates (PHA) using mixture cultures, among others (2).
The main objectives of this work were to study, at batch scale, the effects of: a thermal pre-treatment (120 ºC, 15 min) at different Substrate/Inoculum (S/I) ratios (1, 2, 4 and 6 g VS substrate/g VS inoculum) and the co-digestion with different winery wastes (grape pomace, and two distilled winery wastes) on the degree of acidification of SS and the VFA profile obtained. In addition, a continuous lab-scale reactor was operated to evaluate the effect of two ranges of organic loading rates (OLR),1300-1600 mg COD L^{-1} d^{-1} and 2400-3500 mg COD L^{-1} d^{-1}, on the SS acidogenic fermentation. Besides, a microbial community analysis was performed in the reactor.
From the batch studies it was observed that the thermal pre-treatment influenced positively the degree of acidification of the SS at low S/I ratios, reaching a maximum value of 45%. The main VFA products were acetic (40-50%) and propionic (15-25%) acids for all the S/I ratios tested. In the batch experiment of the co-digestion of sewage sludge with three different winery wastes, the maximum VFA production was obtained from the co-digestion with grape marc, reaching concentrations between 3000-3500 mg VFA_COD L^{-1}. Both distilled wastes reached concentrations in the range of 1500-2000 mg VFA_COD L^{-1}. The VFA profile was dominated by acetic (45-60%) and propionic (15-20%) acids in the three assays of co-digestion of SS with winery wastes. In the lab-scale reactor the highest degree of acidification (22%) was achieved at the lowest OLR (1300-1600 mg COD L^{-1} d^{-1}) applied. Analysis of the microbial community existing in the reactor revealed that OTUs most abundant present genes related with aminoacids and carbohydrates fermentation which are crucial for the biosynthesis of the volatile fatty acids.

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Vermicomposting increases bacterial diversity and changes composition and function yielding a high quality plant biostimulant

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Thematic Area: Biotecnología ambiental

Abstract: Wineries are one of the major agro-industrial sectors around the world. Grape marc, the major solid by-product derived from wine production, is nutrient rich but its application in its raw form into soil may cause phytotoxic and antimicrobial effects on plant growth due to the release of tannins and polyphenols (1). Vermicomposting, the mesophilic process by which organic waste is broken down through the synergistic actions of earthworms and microbial communities, has been shown to effectively convert grape marc to a value-added product (2, 3, 4). During vermicomposting, microbial communities experience drastic changes in composition associated to the decomposition of dead organic matter, which may affect the process in terms of reproducibility, performance and quality of the end product. An in-depth understanding and characterization of the temporal changes in bacterial communities throughout the process may help to predict the usefulness of grape marc vermicomposts in agriculture, soil restoration and bioremediation. The aim of the present study was therefore to evaluate the feasibility of grape marc derived from the red winemaking process of Mencía grapes in a large-scale vermicomposting system in order to yield a high-quality, polyphenol-free organic vermicompost that could be used as an environmentally friendly fertilizer. For this purpose, we monitored the changes in the content of macro- and micronutrients, as well as in microbial biomass carbon and microbial activity, assessed as basal respiration, in a pilot-scale vermireactor over a period of 91 days. The taxonomic and phylogenetic diversity and metabolic function of bacterial communities were also evaluated throughout the process by using 16S rRNA high-throughput sequencing. Moreover, the changes in the content of total polyphenols were determined as a proxy of phytotoxocity over the course of the process. The experimental set up and sample collection is explained in detail in literature (2).
Briefly, samples were collected at the beginning of the experiment and after 7, 14, 28, 42 and 91 days of vermicomposting, enabling us to capture multiple stages of the active and mature phases of the process. Overall, vermicomposting of grape marc followed the normal pattern of an accelerated decomposition process in which there was a significant reduction in microbial biomass and activity over time. The initial mass of the grape marc was considerably reduced, by approximately 78.5%, throughout the process. This mass loss was accompanied by a significant and constant increase in the initial nutrient content of the grape marc, except for P and K, during vermicomposting. The evolution of these parameters indicated a good performance of the process, adequate for studying the succession of bacterial communities during vermicomposting. Although α-diversity did not increase significantly between days 0 and 14, steady and significant increases were observed between days 14 and 91 at both taxonomic and phylogenetic levels. Of interest was the presence of nitrogen-fixing bacteria within the core microbiome of grape marc such as members from the family *Rhizobiaceae*. There was also a rapid reduction in the total polyphenol content of the initial grape marc in a period of 7 days, reaching their minimum level on day 70. In conclusion, vermicomposting of grape marc obtained from the red winemaking process of Mencía grapes resulted in stable but richer and more diverse bacterial community, with key functions to aid plant growth and development. All in all, these findings support the use of grape marc vermicompost for sustainable practices in the wine industry by disposing of this high-volume waste product and capturing its value to improve soil fertility.

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Biological treatment of anaerobic digestion liquor by white-rot fungi

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Thematic Areas: Environmental Biotechnology.

Abstract: Anaerobic digestion is increasingly being used to treat sewage sludge and other organic wastes because of its many advantages. The digestate obtained must be treated to remove water and concentrate nutrients, so usually a solid–liquid phase separation by centrifugation is carried out. The solid fraction can be applied to land, directly or after composting, and the liquid fraction needs additional treatment due to its high content in organic matter, ammoniacal nitrogen and deep colour. However, the treatment of this anaerobic digestion liquor is not easy by conventional biological treatments because of its low biodegradability and costly physic-chemical processes are necessary to obtain high efficacies. For these reasons, it is fundamental to look for alternative techniques that allow the treatment of these refractory effluents in an economical and environmental friendly-way.
The biological treatment by white-rot fungi has undergone a great interest in recent years. Specifically, several works have been published on the use of these fungi in the treatment of landfill leachates and effluents from the textile industry with good results. White-rot fungi are characterized by their high capacity to degrade complex contaminants such as phenol, pesticides, dyes or chlorinated insecticides due to its ability to produce enzymes such as lignin peroxidases (LiP), manganese peroxidases (MnP) and laccases.
In this work, a low-biodegradable liquor derived from the anaerobic co-digestion of sewage sludge and the organic fraction of municipal solid wastes was treated by *Phanerochaete chrysosporium* at 26° C during 10 days. The addition of different concentrations of glucose as an easily usable carbon source (3 g/L, 5 g/L and 8 g/L) and the control of pH were deeply studied. In all cases, the evolution of COD, biological oxygen demand (BOD_5), colour and pH were studied. The addition of glucose and adjusting the pH to 6.0 allowed degradations above 70% with respect to the initial COD and increasing the biodegradability of the effluent. These results show the possibilities of this fungus to be used in order to remove the refractory COD and/or increase the biodegradability in liquors derived from anaerobic digestions.

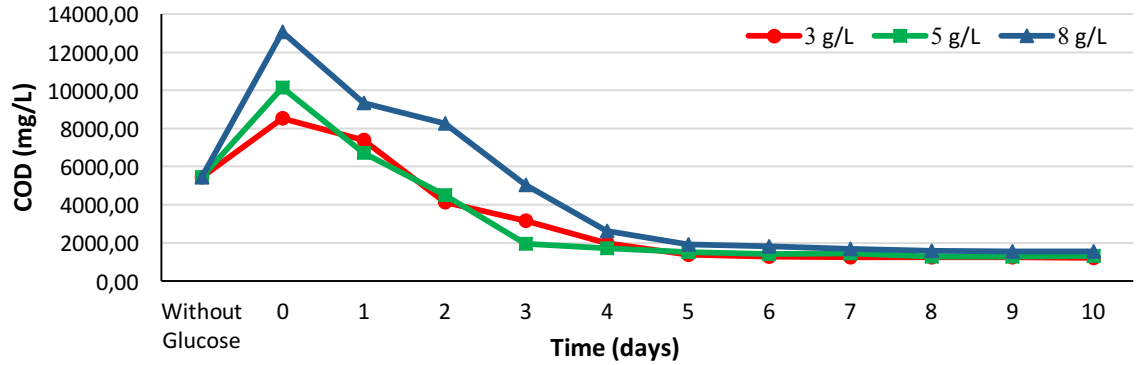


Figure 1. COD evolution during 10 days with the addition of 3 g/L (red), 5 g/L (green) and 8 g/L (blue) of glucose.

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Effects of different enzymes on the enzymatic hydrolysis of proteins from butcher's waste

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Thematic Areas: Environmental biotechnology.

Abstract: The region of Castilla and León (Spain) produces more than 1 million metric tonnes of butcher's waste by year (1). This waste (usually disposed in landfill) is a rich source of protein. In this work, we propose its transformation into products with higher added value, such as protein hydrolysates, by means of enzymatic hydrolysis.
Five different commercial proteases from Novozymes A/S (Bagsvaerd, Denmark) were evaluated for recovering protein: Alcalase, Neutrase, Protamex, Flaourzyme and PTN. Hydrolysis experiments were carried out in a 0.5 l jacketed reactor. The hydrolysis operating conditions were the same for all enzymes: pH = 8, temperature = 50ºC, time = 180 min, stirring speed = 300 rpm and enzyme/ substrate ratio = 0.54 AU/g. Reaction was followed by monitoring the degree of hydrolysis (DH) according to the pH-stat method (2).

On enzymatic hydrolysis of homogenized butchner´s waste, DH ranged from 7% to 30% (see table). Among different commercial proteases used, Alcalase showed the highest DH for protein hydrolysate. For the four endopeptidases assayed (Alcalase, Neutrase, PTN and Protamex), Neutrase shows a worse performance than the other endopeptidases that may be ascribed to a significant and critical role of the serine proteases opposite to the metalloproteases for the butcher's waste hydrolysis. This is demonstrated on table where Protamex (a preparation of serine protease and metallo-protease) is clearly inferior to Alcalase but superior to Neutrase if a high DH is desired. The DH obtained for Flavourzyme suggests the different behaviour of exopeptidases and their lower activity in the hydrolysis of the meat waste. DH obtained also show the influence of the enzyme source on the hydrolysis: serine endopeptidase from source animal (PTN) seems to be less suitable for hydrolysis of butcher's waste than endopeptidase from bacterial source.

From the above discussion it seems justified to assume that the serine endopeptidases from bacterial source are the most suitable proteases for the hydrolysis of the protein coming from butcher's waste

ENZYMES	SOURCE	CATALYTIC ACTION	CATALYTIC SIDE	DH (%)
Neutrase	*Bacillus amyloliquefaciens*	Endopeptidase	Metalloprotease	7.50
Flavourzyme	*Aspergillus oryzae*	Endo+exopeptidase	Amino peptidase	17.49
PNT	*Animal*	Endopeptidase	Serine protease	17.07
Protamex	*Bacillus protease*	Endopeptidase	Metalloprotease +Serine protease	26.95
Alcalase	*Bacillus licheniformis*	Endopeptidase	Serine protease	30.00

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Low cost biogas upgrading using ashes

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Thematic Areas: Environmental biotechnology.

Abstract:
Climate change is a very important problem nowadays, and because of this, different actions are being made in order to reduce its consequences. One of these actions is the promotion of circular economy by means of anaerobic digestion for organic waste valorisation, generating biogas that can be used as renewable energy. Typically, biogas is composed mainly by CH_4 and CO_2 but also by H_2S, NH_3 and other trace compounds, which vary in quantity depending on the substrate used for biogas production. After removal, most of the compounds different from CH_4, purified biomethane (around 95% CH_4) could be fed into the natural gas grid or used in mobility purposes. In general, the upgrading of biogas to biomethane can be accomplished by different physicochemical methods, with high performance but that are not profitable for small-scale biogas plants. This study investigates the application of different types of ashes, rich in calcium oxide (CaO), for biogas upgrading [1] by means of a process called accelerated carbonation, where CaO reacts with CO_2 to produce calcium carbonate ($CaCO_3$) as it is shown below:

$$\boldsymbol{CaO_{(s)} + CO_{2(g)} \xrightarrow{\Delta H_{278K} = -178\ kJ/mol} CaCO_{3(s)}}$$

A semi-continuous up-flow reactor with a volume of 30 L divided in two beds, supported by a metallic mesh, was used to evaluate the absorption capacity of different ashes and the effect of the mass distribution inside of the reactor. Experiments were performed at ambient temperature using an inflow gas rate of 350 Nml/min composed by a mixture 40 % CO_2 and 60 % N_2 (instead of CH_4 for safety reasons). Combustion ashes from mussel shell (named as 1), poultry manure (2), woody-biomass (3) and vine wood (4) were sieved to separate particles with a diameter higher than 8 mm. Absorption capacity was determined using 6 kg (wet basis; wb) of each type of ashes in a single bed whereas the effect of mass distribution inside of the reactor was determined in experiments using the same mass of ashes distributed in the two beds of the reactor. Temperature, pressure, outlet gas flow and composition were measured during each experiment. Results indicated that in these batch tests sorption capacity was affected by the different chemical composition of the ashes. When 6 kg of ashes were used in a single bed, obtained values and the time needed to reach 10% of CO_2 in the outlet gas were: 20.62 g CO_2/kg_{wb} and 230 min (4), 9.45 g CO_2/kg_{wb} and 155 min (3), 3.37 g CO_2/kg_{wb} and 36.5 min (1) and 3.34 g CO_2/kg_{wb} and 38.6 min (2). Due to the compaction and preferential routes, these values changed when the mass was distributed in the two beds. In this case, values for woody-biomass ashes (3) increased to 14.50 g CO_2/kg and for poultry manure ashes (2) decreased to 2.45 g CO_2/kg with time needed to reach 10% of 284 and 37.5 min, respectively. Therefore, these evaluated ashes were feasible for fixation of CO_2. However, the operation of the system needs to be optimized in order to achieve the highest performance. These values demonstrate that this novel and simple technology for biogas upgrading has a promising future and it is a good option to install at industrial scale.

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Anaerobic degradation of sulfamethoxazole using anthraquinone-2-sulfonate as redox mediator

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Thematic Areas: Biotecnología Ambiental

Abstract: The increasing use of pharmaceutical and personal care products (PPCPs) in medicine, on farms and in daily life means that significant amounts of them might enter the environment mainly through effluents. Among these substances, sulfamethoxazole (SMX), a sulfonamide bacteriostatic antibiotic, is widely used for treatment of several infections (e.g. of urinary tract). Thus, remarkable concentrations of SMX have been detected in different environmental matrices, which might represent a serious environmental issue since SMX presented a non-negligible ecotoxicity in tests carried out with *Vibrio fischeri* (Ortiz et al, 2016). Biological treatment of PPCPs, particularly the anaerobic one, is economically attractive for presenting lower costs than physico-chemical methods. However, anaerobic degradation of PPCPs is typically limited. Therefore, the use of quinone-based redox mediators might enhance this process (He et al, 2017). Hence, in this work, the impact of the redox mediator anthraquinone-2-sulfonate (AQS) on the anaerobic degradation of SMX was assessed in batch experiments.

The experiments were carried out, in duplicate, in 100-mL serum bottles (working volume of 50 mL) inoculated with anaerobic sludge from the municipal wastewater treatment plant of Valladolid (Spain) at a final concentration of 1 g TSS/L. All the bottles were filled with a synthetic medium containing SMX (100 µg/L), glucose as organic matter source (500 mg COD/L), macro and micronutrients, and sodium bicarbonate as buffer (1 g/L). Some bottles were supplemented with different concentrations of AQS (10, 50 and 100 µg/L), whereas others were not inoculated with the sludge in order to verify the chemical stability of SMX (chemical control). Subsequently, the bottles were sealed with butyl rubber stoppers and aluminium crimp seals, and their headspace was flushed with He in order to provide an anaerobic atmosphere. Finally, they were kept in an orbital shaker at 180 rpm in a temperature-controlled room at 35°C during the whole experiment (7 days).

Biogas production was determined by pressure change in the bottles. Liquid samples (1 mL) were withdrawn daily to determine sulfamethoxazole concentration in a ultra-high performance liquid chromatography (UHPLC) – tandem mass spectrometry (MS/MS) in selected reaction monitoring (SRM) mode. More specifically, chromatographic separation was carried out by an Sciex Exion LC and a Phenomenex reversed-phase column Kinetex EVO C18 (2.1 mm × 50 mm, particle size 1.7 µm), and mass detection was performed by a Sciex 6500+ QqQ.

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Ibuprofen, diclofenac and triclosan dissipation and associated microbial ecotoxicity in biopurification systems

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Thematic Area: Environmental biotechnology

Abstract: The presence of emerging contaminants in the environment is a growing worldwide problem. The inefficient disposal of pharmaceutical and personal care products (PPCPs) in wastewater treatment plants causes the entry of these pollutants into the environment (1). The effects of PPCPs are very diverse. Despite the concentrations of PPCPs detected in aquatic environment are very low, these pollutants can be bioaccumulative and cause ecotoxicological damage on microbial populations and risks for human health (2). For these reasons, it is necessary to develop new technologies for cleaning up contaminated water resources. Biopurification systems based on active biomixtures composed of soil and organic wastes are an efficient method to remove organic contaminants such as pesticides from wastewater (3).
The aims of this study were to evaluate the efficiency of the removal of ibuprofen, diclofenac and triclosan, three emerging PPCPs, in bioremediation systems composed of biomixtures of soil and vermicompost from olive mill cake generated by the olive-oil industry, to determine the ecotoxicologic effect of each contaminant, and to explore their impact on the composition of the bacterial community.
To achieve this goal, biomixtures were treated with each contaminant (separately and in mixture) and their fate monitored by HPLC. At different times, total genomic DNA was extracted and submitted for high throughput 16S rRNA gene amplicon sequencing. To determine the ecotoxicity of the PPCPs and the effect of each treatment on the bacterial population, the number of microorganisms in the samples treated with PPCPs and control samples without contaminants was estimated by quantitative PCR, colony forming units on solid LB medium and by using the LIVE/DEAD *BacLight* kit.
The results indicated that the biomixture harbours microbial populations with the ability to degrade PPCPs. Triclosan was the most persistent pollutant followed by diclofenac, while ibuprofen was removed completely. Triclosan also showed the strongest toxicological effect on the bacterial population. Observed changes within the microbial populations caused by these PPCPs are being presented.

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Removal of pharmaceutical compounds in wastewater using biochar from rice bran as adsorption matrix

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Thematic Areas: 5. Biotecnología ambiental. Biorremediación; procesos de biotratamiento y biorrecuperación

Abstract: The worldwide growing presence of micropollutants at low concentrations in effluents, as is the case of pharmaceutical compounds, has become an environmental problem since the wastewater treatment plants are not prepared for their elimination (1). With the aim of favouring decontamination and reducing the volume of water to be treated, a pre-treatment stage, adsorption, is necessary to concentrate these micropollutants and simplify their elimination. However, the use of efficient and cost effective adsorbents has become a milestone to reach for the scientific community
In recent years, biochar has been presented as a sustainable and cost-efficient adsorbent. Biochar is a solid material obtained from the dry thermochemical conversion of biomass in an oxygen-limited environment. It is typically produced from biomass feedstock such as agricultural residues, wood chips, manure or municipal solid wastes. As is described by *Rosales et al.* (2), numerous studies have demonstrated that biochar is a low-cost adsorbent for the removal of different kind of pollutants. The adsorption mechanisms for organic removal are influenced by the pollutant properties as well as biochar surface chemistry, which varies greatly with raw material and thermochemical conditions. Nowadays, the advances in their synthesis have encouraged remarkable efforts towards the development of biochar "on demand" that enables to obtain a great variability of biochar with different physicochemical properties such as surface area or microporosity, providing an avenue for biochar to maximize its efficacy to targeted applications.
In this work, the synthesis of biochar from rice bran is proposed. Initially, the temperature of pyrolysis was modified from 300 to 750 ºC and a deep characterization of the produced biochars was carried out due to the pollutants removal is governed by their interactions with biochar functional groups. The adsorption capacity of the synthetized biochars were evaluated using two model pharmaceutical pollutants, fluoxetine and prednisolone. The preliminary results indicate that the adsorption rates obtained range between 70-90% removal of both pollutants from wastewater showed better yields for fluoxetine. The best adsorption uptake was obtained when biochar was produced at higher temperature. This fact can be explained because high temperatures increase several properties such as surface area, aromaticity or hydrophobicity of biochar making it more accessible to hydrophobic organic pollutants.
Therefore, biochar can be produced from rice bran at different thermal conditions and its properties could be improved by controlling these variables. In addition, biochar is a good low-cost adsorbent and an ecological solution since it allows to close the cycle and promote a circular economy of resources.

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Water remediation by means of chestnut shell wastes

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Thematic Areas: 5. Biotecnología ambiental

Abstract: Adsorption has demonstrated to be an interesting treatment for water remediation due to its efficiency, versatility and easy handling[1]. However, one of the main drawbacks of this technique is the high cost of the typically used adsorbent material, *i.e.* active carbon. For this reason, in recent years increasing attention is being given to the application of wastes from forestry, agricultural or industrial activities. These low-cost adsorbents have the advantage of being eco-friendly, renewable, cheaper and easy to obtain compared with commercial alternatives[2]. Therefore, in this study, an agro-industrial waste as chestnut shells was selected as adsorbent. This is a lignocellulosic waste provided by the food industry Cuevas & Cía, S.A., located in Ourense (Spain), which presents no added value currently.
The adsorption capacity of the chestnut shells was tested by evaluating the removal of two ionic liquids from a water matrix: *1,3-Dicyclohexylbenzimidazolium chloride* (Imid IL) and *P-xylene-bis(N-pyridinium bromide)* (Pyrid IL). Previous to the adsorption treatments, the shells were dried in an oven at 60ºC for 48 h and then grinded. The adsorbent surface was characterized using Boehm titrations, obtaining that the acidic groups (mostly carboxilates) are the predominant in this material. Additionally, Fourier transform infrared spectroscopy analysis was performed in order to evaluate the modifications of the adsorbent surface functional groups when the ionic liquids are adsorbed. Moreover, the point of zero charge for the chestnut shells was determined to be 3.51. Taking into consideration that the main structure of both ionic liquids is positively charged, it is convenient to work at higher pH values so as to increase the adsorption affinity. In this regard, it was found that the maximum removal obtained after 2h was of 66% at pH 7.92 for the Imid IL and 75% at pH 8.23 for the Pyrid IL. Furthermore, kinetic studies were carried out concluding that for both compounds under study the pseudo-second order model fitted the experimental data, which means that the adsorption process is mainly controlled by chemisorption. Regarding the isotherm analysis, two parameter isotherm models were evaluated: Langmuir (Table 1) and Freundlich (Table 2). It was observed that Freundlich model showed the best fit for both ionic liquids, which implies that the adsorption takes place on heterogeneous surfaces or surfaces supporting sites with varying affinities. Finally, the adsorption behavior of a column at an industrial scale was simulated using the software FAST 2.1 Beta.
From this study, it can be concluded that chestnut shells is a low-cost material with good adsorptive properties for the treatment of complex organic pollutants, such as ionic liquids, and that shows promising results even for industrial applications.

Table 1. Parameters of the Langmuir model

	Imid IL	***Pyrid IL***
q_{max} *(mg/g)*	173.16	37.46
K_L *(L/mg)*	$6.0 \cdot 10^{-4}$	0.013
R^2	0.997	0.997
SEE	2.03	0.88

Table 2. Parameters of the Freundlich model

	Imid IL	***Pyrid IL***
K_F *(mg/g·(m³/mg)ⁿ)*	0.502	16.728
n	1.419	10.010
R^2	0.999	0.999
SEE	0.89	0.29

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Application of the invasive macroalgae *Undaria pinnatifida* in the synthesis of gold and silver nanoparticles

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Thematic Areas: 9. Nuevos retos en biotecnología. Nanobiotecnología.

Abstract: *Undaria pinnatifida* (Harvey) (UP) is a kelp-like brown macroalgae. In its native northeast Asia, *Undaria* is a winter annual species that inhabits rocky substrates from the low intertidal to 18 m depth. It has a global non-native range and is considered one of the world's "worst" invasive species(1). UP is considered the most widely eaten brown seaweed since it possesses large quantities of proteins, polysaccharides and fucoxanthin. However, as an invasive species, it is associated with changes in biodiversity in the coastal habitats. Since its eradication has been proved to be inefficient, there has been a trend towards its revalorization. UP contains valuable pharmacological compounds with a number of biological activities, such as prevention of hyperglycemia, suppression of chemically induced mammary tumors, inhibition of antihypertension and anti-obesity (2).
In this study, we proposed the application of UP in the synthesis of gold and silver nanoparticles. Firstly, an aqueous extract of UP was prepared and employed to lead the synthesis of nanoparticles by the reduction of $HAuCl_4$ and $AgNO_3$. Obtained nanoparticles were fully characterized by UV-Vis spectroscopy, Transmission Electron Microscopy (TEM), High Resolution Transmission Electron Microscopy (HRTEM) and Scanning Transmission Electron Microscopy (STEM); These techniques allow us to confirm the formation of spherical nanoparticles with mean diameters of 6.8 ± 1.0 nm for Au@UP and 14.1 ± 2.8 nm in the case of Ag@UP (Figure 1). Moreover, Fourier transform infrared spectra (FTIR) of UP extract, Au@UP and Ag@UP were measured in order to identify the functional groups of biomolecules that could be involved in the formation and stabilization of the nanoparticles. Finally, the reducing activity, total phenolic compounds and DPPH scavenging activity of the extract before and after the synthesis, were determined.

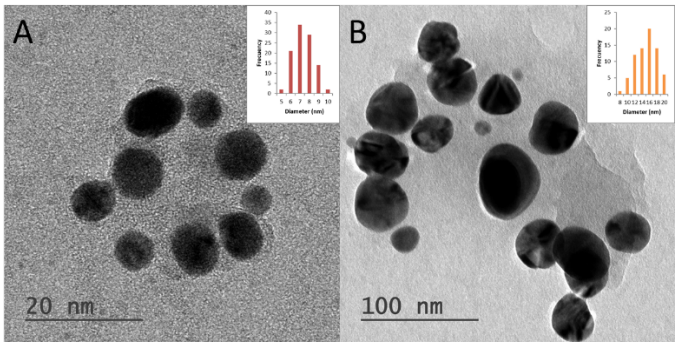


Figure 1 TEM images of A) Au@UP and B) Ag@UP and their corresponding size distribution histogram.

Acknowledgements: This work was supported by the Xunta de Galicia ED431C 2018/54 -GRC. NGB acknowledges a fellowship from Universidade de Vigo.

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Food waste application in the synthesis of gold nanoparticles. The case of Galician *Brassica oleracea* var. *acephala*

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Thematic Areas: 9. Nuevos retos en biotecnología. Nanobiotecnología

Abstract: In recent years, public attention has been increasingly focused on the limited capacity of local eco-environments to absorb the impact of food wastage. Therefore, there has been a trend towards the development of applications to reduce or reuse this waste. In this study we have focused our attention on the stem of *Brassica oleracea* var. *acephala* cv. Galega. This plant, dispersed throughout Galicia and the north of Portugal, produces leaves throughout the year. It forms part of the diet of people living in the region and is also grown as a forage crop. Previous studies have shown that *Brassica oleracea* contains a high content of antioxidant compounds, such as vitamins, phenols and carotenoids (1). This means that the plant may represent an eco-sustainable and low-cost source of natural antioxidants. Due to its high antioxidant capability it can also be used in the nanotechnology field where there is a growing interest in the synthesis of novel nanomaterials based on the concept of green nanotechnology. This integrates the principles of green chemistry and green engineering to produce eco-friendly, safe nanoparticles that do not use toxic chemicals in their synthesis protocol. In addition, the synthesis of metallic nanoparticles, such as gold, is of great interest due to the nanoparticles' unique physico-chemical properties and their applications in diverse fields, such as the food industry and medicine (2). Green nanotechnology offers an integrated approach to recycling, reuse or recovery of waste from agri-food based industries.
In this study, an aqueous extract of the plant stem was obtained and characterized by the determination of the reducing power, total phenolic content and DPPH scavenging activity before and after the synthesis of the gold nanoparticles. The gold nanoparticles were characterized by UV–visible spectrophotometry, Fourier transform infrared spectroscopy (FTIR) and transmission electron microscopy (TEM) confirming the formation of spherical nanoparticles with a mean diameter of 25.08 ± 3.73 nm (Figure 1).

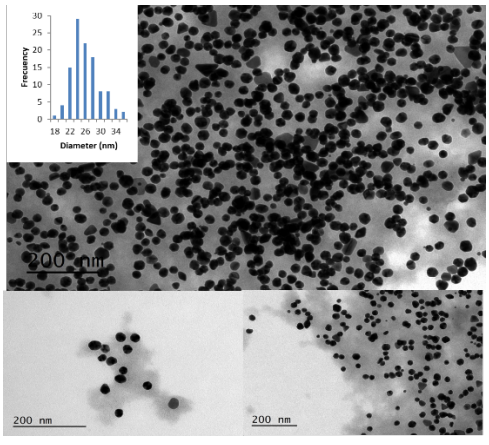


Figure 1- TEM images of the gold nanoparticles obtained.

Acknowledgements: This work was supported by the Xunta de Galicia ED431C 2018/54 -GRC. NGB acknowledge a fellowship from Universidade de Vigo

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A *Bacillus* sp. strain for biosurfactants production as biofungicides of pathogenic cocoa fungi *Moniliophtora perniciosa* and *Moniliphtora roreri*

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Thematic Areas: 1. Microbial Biotechnology: Multi-organization consortiums; communication systems and biotechnological relevance

Abstract: Cocoa is globally consumed and implies the livelihoods of millions of families. It only grows in areas near to the equator and crops are frequently affected by devastating diseases, as those caused by *Moniliophtora perniciosa* and *Moniliophtora roreri* fungi. Therefore, it is necessary to develop new control strategies. A sustainable and eco-friendly alternative was evaluated in this work using *Bacilus subtilis* (DSO3), a cocoa endophyte bacteria, able to produce biosurfactants with potential capacity of fungal growth inhibition.
B. subtilis (DSO3) was cultured in mineral medium and after 24 hours, cells were removed by centrifugation, obtaining a raw extract. It was acidified and the pellet was dewatered, freezed and lyophilized. Additionally, a molecular exclusion chromatography was performed in order to purify the active compound(s) according to polarity. Surfactant and antimicrobial activities were tested for the raw extract, the lyophilized product and the fractions eluted from the column.
Results proved that *Bacillus* sp. DSO3 is able to produce extracellular, acid and thermotolerant compounds with surfactant and *M. perniciosa* and *M. roreri* inhibition growth activities. Both, the raw filtered extract and the lyophilized product performed a 52,2 ± 5,4 % water surface tension reduction (from 70,9 ± 4,6 mN/m to 33,7 ± 1,6 nM/m). The IC_{50} of the filtered raw extract was 32,1 % and 19,9 % (v/v) for *M. perniciosa* and *M. roreri*, respectively (Figure 1). The IC_{50} of the lyophilized product was 48,7 $mg \cdot L^{-1}$ for *M. perniciosa* and 144,3 $mg \cdot L^{-1}$ for *M. roreri*.
Preliminary analysis suggested that both activities, surfactant and antimicrobial, were related. Hence, an eco-friendly biofungicide could be developed in order to tackle two of the most severe cocoa diseases. Moreover, biosurfactants might be a sustainable solution for other processes from the sanitary, agrofood and industrial sectors.

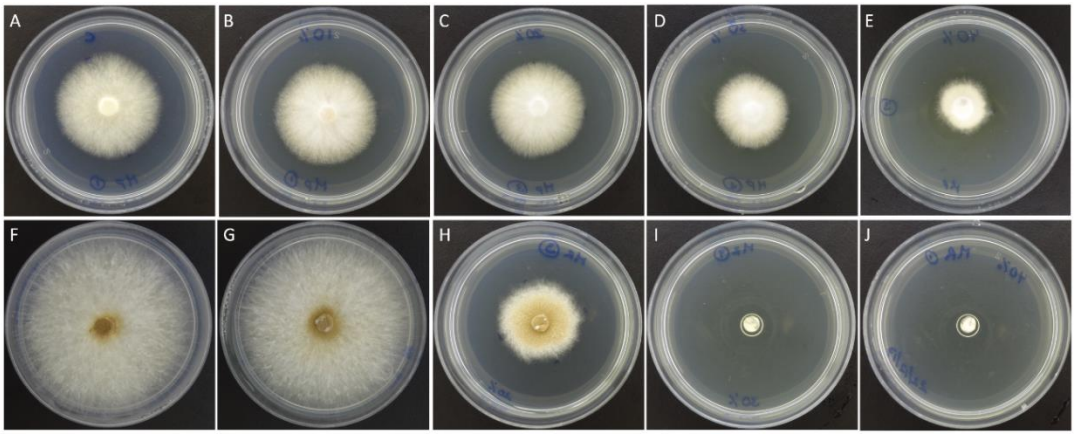


Figure 1: *M. perniciosa* (upper row) and *M. roreri* (lower row) growth in PDA after seven days of incubation in presence of different concentrations of the filtered raw extract; from left to right: 0, 10, 20, 30 and 40 % (v/v).

Acknowledgements: Authors wish to acknowledge EuroInkaNet Program.

References

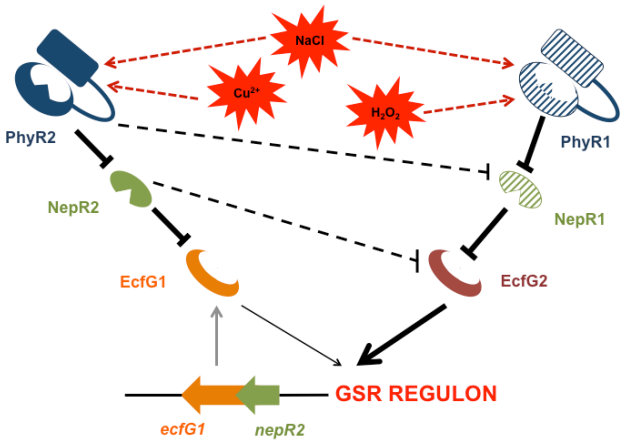
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Cross-talks between two paralogous cascades contribute to the regulation of the General Stress Response in *Sphingopyxis granuli* TFA.

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Thematic Areas: Microbial Biotechnology.

Abstract: In natural habitats, bacteria are forced to overcome many hostile conditions. In order to survive, they constantly adapt their expression profiles accordingly to the variations in the environment. Among other responses, bacteria can trigger the so-called General Stress Response (GSR), which responds non specifically to a wide variety of stresses, making them ready to thrive in conditions that otherwise would prevent their growth. In particular, α-proteobacteria have a unique signalling cascade to activate the GSR, involving three regulatory elements: an EcfG alternative σ factor, its cognate NepR anti-σ factor and a PhyR response regulator (1). Under permissive conditions, EcfG is bound to NepR, preventing the expression of the GSR regulon. Once some stress appears in the environment, PhyR receives a phosphoryl group and changes its conformation, showing a σ-like domain. This domain is able to sequester NepR, thus releasing EcfG to active the GSR.
Sphingopyxis granuli TFA is an α-proteobacterium able to grow with the organic solvent tetralin as carbon and energy source, and the first representative of its genus described as facultative anaerobic (2). After the analysis of its genome, two paralogous genes were identified for each GSR regulatory element, having EcfG1, EcfG2, NepR1, NepR2, PhyR1 and PhyR2. Once single and double mutants (when possible) were constructed at the three levels, their capability to activate the GSR and resist to different stresses were tested, allowing us to distribute the six elements in two cascades: the main pathway PhyR1-NepR1-EcfG2 and the accessory pathway PhyR2-NepR2-EcfG1. After this, combinatory double mutants in both cascades were constructed and tested again regarding their capability to activate the GSR to resist different stresses. Compared to the literature and to the experiments with the single mutants, we expected that mutations in the main cascade prevented the activation of the GSR. Surprisingly, these mutants did not behave as if both cascades were independent, thus indicating that there might be cross-interactions from PhyR to NepR and from NepR to EcfG between the two pathways and they might contribute to the tight regulation of the GSR in TFA.



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Characterization of an ibuprofen-eater bacteria

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Thematic Areas: Biotecnología microbiana.

Abstract: The presence of pharmaceutical compounds (PhCs) in drinking water, treated wastewater, groundwater, surface water, and soil around the world is an emerging challenge. It is clear that conventional wastewater treatment technologies are not meeting the challenge of the mounting presence of PhCs in wastewaters. The bioremediation of organic pollutants by microbial degradation is a proven method for counteracting the contamination caused by these recalcitrant substances.
In our laboratory, we have isolated a bacterium belonging to the genus *Sphingomonas*, able to grow with ibuprofen as sole carbon and energy source. This strain was isolated from a Wastewater Treatment Plant of a pharmaceutical company (Almirall). The analysis of its genome sequence showed the presence of genes previously described as necessary for the biotransformation of ibuprofen [1, 2]. The *ipf* gene cluster was 100% identical to the *ipf* cluster of the Ibuprofen-degrading strain, *Sphingomonas sp*. Ibu-2. The *ipf* cluster is flanked by tranposon sequences and its GC content is lower than the average of the genome, suggesting that this cluster could have been horizontally acquired.
To identify the genes necessary to grow in ibuprofen as carbon source, we performed a transposon mutagenesis with a miniTn5 derivatives and selected mutants unable to use ibuprofen as a nutrient. Three different phenotypes were detected among mutants with impaired grow in ibuprofen: (i) mutants unable to grow that do not modify the growth medium, (ii) mutants unable to grow that give a brown colour to the medium and (iii) leaky mutants able to poorly grow on ibuprofen. Sequencing of the DNA region flanking the insertions mapped the transposons in three main regions: the non-coloured mutants (i) were mainly located into the *ipf* cluster, the brown mutants (ii) mapped in a different genomic region that contains genes belonging to aromatics degradation pathway and the leaky mutants (iii) mapped in a Propionyl-CoA carboxylase carboxyl transferase subunit. We have constructed a genomic library to complement those mutant and the results suggest that only these regions are involved in ibuprofen degradation.
Based in our results we propose a complete ibuprofen degradation pathway that seems to have been formed by combination of different biodegradation pathways, some of which acquired by horizontal gene transfer, a process that is considered responsible for the evolution of the genome and the high catabolic versatility of this taxon.

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SfbR2, a pseudo-gamma-butyrolactone receptor from *Streptomyces filipinensis* represses filipin production

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Thematic Areas: Biotecnología microbiana.

Abstract: Gamma-butyrolactones (GBLs) are quorum sensing communication signals produced by Streptomyces species that coordinate de onset of secondary metabolism by binding to specific cytoplasmic receptors (GBRs), releasing them from DNA and allowing gene expression. This circuit is complicated by other regulators such as pseudo-receptors, GBR homologues whose function is not clearly understood.
Here, we have studied the GBL system of *S. filipinensis* DSM 40112, the industrial producer of filipin, a non-glycosylated polyene antifungal widely used for the detection and quantitation of cholesterol in cell membranes and for the diagnosis of type C Niemann-Pick disease.
The GBL system of *S. filipinensis* was identified by hybridization. Among the 9 genes present in the cloned cluster, two GBR encoding genes *sfbR* and *sfbR2*, and a gene homologous to GBL synthase encoding genes (*sfbA*) were identified.
Characterization of the pseudo-receptor SfbR2 was performed using gene replacement and complementation techniques. Gene deletion resulted in a significant increase in filipin production without affecting *fil* genes transcription while complementation restored parental production. Moreover, transcriptional analysis of the *sfbR2* deleted strain and binding assays using GST-SfbR2 purified protein showed that SfbR2 represses the transcription of *sfbR*, *sfbA* and its own expression by binding to conserved DNA sequences present in the promoters of these genes.
Our results indicate that the SfbR2 protein regulates both filipin production and the GBL system in *S. filipinensis*.

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Streptomyces tsukubaensis production of the immunosuppressant tacrolimus: negative effect of *adpA* regulator as revealed by the use of a microcultivation system

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Thematic Areas: Biotecnología microbiana.

Abstract: Tacrolimus (FK506) is an immunosuppressant drug used for the treatment of transplant patients. It is produced by *Streptomyces tsukubaensis*, a strain with low production levels for industrial requirements and high culture variability in flask cultures, which hinders the analysis of the effects of any genetic modification.

In order to increase tacrolimus production in *S. tsukubaensis*, we decided to overexpress *adpA*, a positive regulator of secondary metabolism and/or development in several *Streptomyces* strains (1,2). Previously, we have reported that the *adpA* gene shows a transcriptional profile correlated with that of the *fkbN* gene (3), the transcriptional activator of tacrolimus biosynthetic genes.

The *adpA* overexpression was carried out in pRAS, an integrative plasmid (phiC31 system), and under the control of the strong promoter SF14. After verifying the correct plasmid integration, tacrolimus production was analyzed in four exconjugants, cultured in 500 ml flasks. Unexpectedly, we observed a decrease in FK506 production, but with high variability between biological replicates in both control (wild-type strains with the empty plasmid) and the *adpA* overexpressed mutants. Plasmids based on the phiC31 system might integrate in more than one site, what might explain part of the variability. The rescue and analysis of plasmids from the control strains revealed integration in the expected chromosomal site, but plasmid rearrangements. However, the use of 3 ml cultures in 24-microwell plates enabled us to reduce the culture variability and confirm the negative effect of *adpA* overexpression on tacrolimus production.

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Deciphering the Regulation of safracin cluster in *Pseudomonas poae* strain PMA22

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Thematic Area: Biotecnología microbiana

Abstract: Non-ribosomal peptide synthase (NRPS) gene clusters usually encode the synthetic pathways of secondary metabolites of high industrial value. One of these high value molecules is safracin, an antibiotic with anti-tumor activity produced by *Pseudomonas poae* strain PMA22. The entire safracin synthetic gene cluster spanning 17.5 kb has been identified [1], and comprises 10 open reading frames (ORFs) encoding proteins for 3 NRPS, 3 safracin precursor biosynthetic enzymes, 2 safracin tailoring enzymes, 1 safracin resistance protein and 1 small hypothetical protein of unknown function. These genes are organized in 2 divergent operons and their expression is driven by 2 promoters: *Pa*, located upstream of the biosynthetic genes and *Pi,* located upstream of the tailoring genes.
This work examines the genes involved in the regulation of the biosynthesis of safracin in the wild type strain as well as in a safracin superproducer mutant (PMC-3) obtained by chemical mutagenesis. We have identified adjacent to the cluster a possible regulator gene annotated as *mexT*, which has been described as a pleiotropic regulator in other *Pseudomonas* strains [2]. The *Pi* promoter contains a putative operator region for MexT named “nod-box”. To study the possible regulatory role of MexT we have carried out transcriptional fusions of *Pa* and *Pi* promoters with a GFP reporter gene that were expressed *in trans* together with the *mexT* regulator in a different plasmid, in the strain PMA22
The resulting strains carrying the GFP fusion and the overexpressed *mexT* were analyzed by Flow Cytometry. We observed an increase of the expression controlled by the *Pi* promoter in the presence of the overexpresed MexT. The PMC-3 strain was transformed only with the GFP transcriptional fusions to compare the expression levels with the wild type strain, and the strain carrying the *Pi*-GFP fusion showed a higher expression of sixteen fold compared with the wild type strain, suggesting a similar transcriptional regulation by MexT.
The PMC-3 mutant strain was sequenced by PacBio to look for significant mutations that could be activating the safracin production. Three genes encoding cyclic di-GMP specific phosphodiesterases are mutated, one of them having an internal stop codon. This suggests that cyclic di-GMP intracellular levels could be involved in the regulation of safracin biosynthesis. In order to prove this, we monitorized the cyclic di-GMP levels by a swimming motility assay in PMA22 and PMC-3 strains. We observed in these two strains a positive correlation between the production of safracin and the cyclic di-GMP intracellular levels.

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Active and thermostable *E. coli* large-oligomer protein

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Thematic Areas: Biotecnología Microbiana

Abstract: *Escherichia coli* (*E. coli*) is a Gram-negative bacterium widely used in biology and biotechnology to produce high-value compounds such as aromatics compounds, terpenoids, sugars or heterologous proteins (1,2). Recombinant protein overexpression in *E. coli* still is the most employed strategy for large-scale protein production. However, many difficulties can occur during protein expression and purification, such as low expression, protein toxicity, difficult purification steps or inclusion bodies formation (3). To solve these drawbacks, many efforts have been done to find new expression and purification strategies (4). Protein inclusion bodies are insoluble protein aggregates which are formed when the aggregates are large enough to exceed their solubility. However, proteins can form soluble large-aggregates in response to diverse conditions, such as heat shock, other environmental stress conditions or a fast and high expression of recombinant proteins (5). Moreover, protein aggregation has been recently described as a native and regulated process in bacteria, yeast and mammalian cells (6).
In this work, the overexpression and purification of a soluble large-oligomer *E. coli* protein is presented. Native electrophoresis, HPLC size-exclusion chromatography, enzymatic activity measurement, Differential Scanning Calorimetry (DSC) and Isothermal Titration Calorimetry (ITC) were used to evaluate and compare two protein conformations.
Results showed that the *E. coli* protein CobB was purified in two different conformations: a dimer and a large-oligomer. The two protein conformations were stable and an equilibrium between them was not observed. Moreover, the large-oligomer conformation showed an enzymatic activity and a constant for substrate binding similar to dimer. Finally, the oligomer showed a higher thermostability than dimer, with a melting temperature almost 15 degrees higher. This result emphasizes the importance of these large-oligomer conformations for their use in different biotechnological processes with more extreme conditions (7).

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Brand-new ability of acclimated *Pseudomonas* strain in the presence of neoteric pollutants

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Thematic Areas: Microbial Biotechnology

Abstract: In the last years, environmental concerns have highlighted the extensive and increasing importance of implementing industrial processes using eco-friendly solvents. According to green chemistry tendencies, ionic liquids have been used as an alternative to traditional organic solvents. The outbreak of these neoteric solvents, with properties such as their negligible volatility, tunability, thermal stability and excellent solubilisation capacity [1,2] make these molten salts an attractive option in an array of sectors ranging from biotechnology, analytical chemistry or environmental engineering [3,4]. Due to the ongoing growth and high resistance to being degraded, like imidazolium-based ionic liquids, these chemicals are considered contaminants on the horizon. Thus, looking for microbial candidates with ability for remediating this kind of pollutants in the environmental could be presented as an efficient alternative. In this line, *Pseudomonas* genus has been highlighted in different research works as viable bioremediation agents[5].

Considering all the mentioned above, in this work, the role of an acclimated *Pseudomonas* strain in the presence of imidazolium-based ionic liquid [6] was tested to treat an effluent polluted with 3 model PAHs and a model diazo dye simultaneously. The optimum medium and operating conditions were selected by means of statistical design prior to demonstrating the viability of the strategy at bioreactor scale. All the experimental data were suitably modelled with logistic equations, allowing characterizing the kinetics of the biological reaction in order to ease process implementation at higher scale. The simulation of this one-step process applied at larger scale for the remediation of an effluent from a leather factory was compared with a conventional two-steps option, recording enhancements of treatment throughput near to 6 times while reducing the total cost in one order of magnitude.

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Evaluation of the extraction process of biotechnologically produced biosurfactant from *Lactobacillus pentosus*

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Thematic Areas: Microbial biotechnology

Abstract: In the last decades, the use of surfactants has been widely increased. Surfactants are surface-active compounds with several properties in environmental applications, food, cosmetic and/or pharmaceutical formulations. However, their use produces side effects such as irritation and allergies, among others[1]. For this reason, other alternatives have risen up. Biosurfactants are substances produced by microorganisms, able to reduce the surface tension of water, being more biodegradable and biocompatible than their synthetic counterparts[2]. Nevertheless, they have one important drawback, as their production is not profitable in comparison with chemical surfactants.
In this regard, the aim of this work was to evaluate different extraction conditions of a biosurfactant produced by *Lactobacillus pentosus*, a lactic bacterium Generally Recognised As Safe (GRAS). In this sense, some batch fermentations of *L. pentosus* were carried out, under the same controlled growth conditions. The culture media was based on glucose (11 g/L) and xylose (18 g/L) as carbon sources, as well as corn steep liquor (10 g/L) and yeast extract (10 g/L), as nitrogen sources. The fermentations were kept for 48 h, at 31 ºC and pH 5.85, stirring at 200 rpm. Afterwards, different extraction procedures were developed, using in all the cases a saline buffer phosphate (PBS), following the protocol previously established by Moldes et al[3]. The extractions were carried out with 50, 100 or 250 mL of PBS.
As a result, it was observed that the volume of PBS employed played an important role in the quantity of biosurfactant extracted. In this sense, the amount of biosurfactant picked up was higher as long as the volume of solvent increased as it has been reflected in **Table 1**.
This research demonstrates that the separation and purification of cell bound biosurfactants play an important role in their cost effective production, although these data should be corroborated at industrial scale.

Table 1. Amount of culture media and PBS employed, as well as the concentration and surface activity of the biosurfactant extract produced by *L. pentosus*

Fermentation	Volume Culture media (mL)	Volume PBS (mL)	Minimal Surface Tension (mN/m)	Concentration of biosurfactant extract (mg/L)
1	1500	200	55.7	604
2	1500	100	53.2	260
3	1500	50	53.0	215

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Study of the changes induced in the organoleptic properties of beer after genetic modification of yeast

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Thematic Areas: Microbial Biotechnology.

Abstract: The organoleptic properties of a product of the complexity of beer are due to the presence of a large number of molecules, whose concentration in the mixture is determined both by the raw materials used, and by the conditions of processing and fermentation. In this fermentation, to achieve an adequate product, the yeast used and the physical-chemical parameters of the production medium are critical. Thus, in the final mixture, we will have the presence of different families of polyphenols, organic acids, aldehydes and small peptides, all of them in a living process of chemical transformation where the pH and the redox balance will also influence.
In the present work, the profile of the molecules influencing the organoleptic properties of beer has been altered by overexpressing, through molecular biology techniques, the *ATF1* gene in a laboratory strain of the yeast *Saccharomyces cerevisiae*. The *ATF1* gene codes for the alcohol acetyltransferase I (EC 2.3.1.84) and its expression greatly affects the production of some esters such as ethyl acetate (apple flavor) and isoamyl acetate (banana flavor)[1]. The obtained strain was able to produce almost 1000 times more isoamyl acetate and more than 200 times ethyl acetate compared to a control strain at 30 hours of culture. These values represent an improvement of 5.5 times in the production of isoamyl acetate and 7 times the production of ethyl acetate with respect to the data obtained by other authors[2,3]. In addition, it has been verified by means of a trained tasting panel that the obtained concentrations of isoamyl acetate and ethyl acetate have been sufficient to modify the sensory profile of the culture medium.

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Characterization of extracellular vesicles produced by wine yeast strains under winemaking conditions

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Thematic Areas: Microbial Biotechnology

Abstract: This work describes the morphological, proteomic and physiological characterization of extracellular vesicles (EVs) produced by wine yeast. These are membranous structures ranging in size from 20 to 500 nm, that have been found in all three domains of the tree of life, including species with or without a cell wall. Fungal EVs are able to cross the cell wall and release different molecules such as proteins, lipids, nucleic acids, polysaccharides and pigments (1) into the extracellular medium. They have been attributed diverse functions such as cell-to-cell communication, response to nutrient availability, cell defence, RNA export, morphological transition and prion transmission (2). However, EVs have not been previously studied from wine yeast species.
Based on own and published results our hypothesis is that wine yeasts produce EVs as part of an interspecific interaction/communication mechanism. For the first time, we have isolated wine yeast EVs from the yeast culture supernatant in synthetic grape must. EVs produced by seven strains from six yeast species were characterized by transmission electron microscopy, including two different strains of *Saccharomyces cerevisiae* and five non-*Saccharomyces* isolates. A comparative proteomic analysis was run for three fractions of *S. cerevisiae* EC1118, cell content, EVs and vesicle-free secreted fraction. In addition, we have compared the proteome of the EVs and vesicle-free secretome fractions of *S. cerevisiae* and *Torulaspora delbruekii*. Finally, we have shown induction of cell mortality by EVs from some strains on different yeast isolates, and been able to discard some of the major protein cargos as responsible for this effect.

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Study of the antimicrobial activity of different species of microalgae extracts against pathogenic bacteria in aquaculture

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Thematic Areas: Blue Biotechnology

Abstract: Antimicrobial activity of eight microalgae species (*Tetraselmis chuii, C. calcitrans, Nannochloropsis, C. Salsugineus, Pavlova gyrans, Conticriba weissfloggi, Rhodomonas lens* and *Isochrysis galbana*) has been determined against two fish pathogens *Vibrio anguillarum* CECT 522 and *Aeromonas salmonicida* subsp. salmonicide CECT 894.
Microalgae ethanolic and aqueous extracts were obtained from the lyophilized microalgae previously growth in ANFACO-CECOPESCA. The antimicrobial activity of these extracts was tested using the agar diffusion technique and also by spectrometry in liquid medium in 96-well plate.
In the first case, microbial growth plates were incubated at 26°C and 30°C for 24 h, for *Aeromonas* and *Vibrio* respectively, before observing the presence of inhibition halos.
In the second case the growth of *Aeromonas* and *Vibrio* in the presence of the different extracts was determined over 24 hours by quantifying the optical density of the culture medium (absorbance at 600 nm) in a spectrophotometer.
Promising results have been obtained for *Aeromonas.* Ethanolic extracts produced a total inhibition of *Aeromonas* culture. Also the presence of the aqueous extracts of some microalgae reduced the speed maximum growth and increased latency phase significantly. For *Vibrio* only concentrated ethanolic extracts gave positive results.

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Simultaneous extraction and fractionation of lipids from the marine microalga *Nannochloropsis sp*. for the production of EPA rich polar lipid concentrates

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Thematic Areas: Blue technology. New products of commercial interest.

Abstract: This work deals with the development of an easy method for the simultaneous extraction and fractionation of saponifiable lipids (SLs), from the microalga *Nannochloropsis sp*., using low toxicity solvents, to produce eicosapentaenoic acid (EPA) concentrates in the form of polar lipids, either as glycolipids (GLs) or as phospholipids (PLs). These concentrates may have interesting applications in the field of food ingredients and pharmaceutical or cosmetics industry (1, 2), especially considering that with respect to traditional ones, based on glycerides or ethyl esters, are greater bioavilability, less prone to oxidation and have better integration in water-bases matrices. The raw material is microalgae biomass because it is a renewable feedstock, environmentally compatible and offers more security than other food sources such as fish or krill oil. The microalgal biomass used in this work contains 15.0% of SLs respect to biomass dry weight and 22.1% of EPA respect to total fatty acids. These SLs are constituted by 53.0% neutral saponifiable lipids (NSLs), 32.8% GLs and 14.2% PLs with 6.3, 49.4 and 18.3% of EPA respectively; so that 84.9% of total EPA is contained in the polar lipids. For the production of EPA rich polar lipid concentrates, a step-by-step extraction was developed. Firstly, and due to the selectivity of hexane toward NSLs observed in previous works (3), this solvent was used for remove in particular this lipid class from the biomass used. At the optimal temperature (40 ºC), the hexanic extract contained above 89.0% of NSLs of the biomass. Next, an EPA and polar lipids enriched fraction was achieved from the residual biomass using ethanol (96%) as extraction solvent. A SL extract with 87.3% of polar lipids (56.4% GLs, 30.9% PLs) and up to 35.2% of EPA was obtained. In this extract were recovered the 87.1% of polar lipids and 68% of EPA of the original biomass. Therefore, the proposed method allows a simple and simultaneous extraction and fractionation of the NSLs and polar lipids of *Nannochloropsis sp* and produces enriched EPA fractions.

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NAFEI – Un proyecto de movilización de estudiantes graduados para la promoción y valorización de la industria agroalimentaria y agroambiental

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Thematic Areas: Biotechnology and society (Bioeconomy)

Abstract: El Sector Agroalimentario es una área relevante de la economía europea y nacional, el cual ha experimentado una evolución considerable en los últimos años, adaptando los productos al gusto de los consumidores, tratando de procesarlos de forma más sana y presentando características innovadoras, a fin de hacerlos más competitivos[1]. Además, la valorización del conocimiento producido en centros de investigación ha sido un gran desafío para las universidades en las sociedades contemporáneas. Esta situación originó que las instituciones desarrollasen un nuevo objetivo en el ámbito de sus actividades, la facilitación de la transferencia de tecnología y la promoción del espíritu empresarial[2]. El objetivo de este trabajo es describir el "Northern AgroFood-Environment Innovation" (NAFEI) como un proyecto de promoción y valorización del sector agroalimentario y agroambiental.
El NAFEI se enmarca en el dominio de la Transferencia de Conocimiento Científico y Tecnológico y está especialmente orientado a la inversión en el Desarrollo de Nuevos Productos y Servicios, en el ámbito de los sistemas Agroalimentario y Agroambiental. Este proyecto propuso tres acciones que se articulan en: (i) Dinamización de la Innovación en los Sistemas Agroalimentaria y AgroAmbiental; (ii) Desarrollo de Nuevos Productos y Servicios y (iii) Promoción de la Transferencia de Conocimiento e Innovación.
De este modo, se promovieron acciones de sensibilización y motivación, involucrando medios de comunicación y un roadshow para movilizar competencias individuales, en que se crearon equipos capaces de desarrollar nuevos productos y servicios que atrajeron el interés en las cadenas de valor relacionadas. Estos nuevos productos y servicios se han desarrollado con enfoque en la producción primaria, en los productores de bienes y servicios intermedios (ingredientes, embalaje, equipos, etc.), productos y servicios medioambientales y, particularmente, productos y servicios alimenticios, incluida la valorización de sus subproductos. Este programa de dinamización fue acompañado por un Sounding Board compuesto por representantes de empresas, asociaciones, clusters institucionales, empresarios, inversores, etc. que se involucraron con la Escuela de Biotecnología - Universidad Católica Portuguesa, y en general con la Universidad Católica Portuguesa - Oporto y ofrecieron tanto a los candidatos como al Programa una orientación de prioridades y de valor para los mercados. Los Proyectos que participaron en el Programa fueron acompañados por Mentores Científicos y Empresariales.
Así, el NAFEI creó las bases para una cultura y una dinámica de innovación sostenible en la que los grupos de trabajo con competencias, centros de investigación aplicada especializados, actores clave del mercado y del tejido económico, contribuyeran para lo desarrollo de proyectos estructurados de nuevos productos y servicios de forma que a la creación de cadenas de valor asociadas a los sistemas agroalimentarios y agroambientales de la Región Norte.

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Newfood Project - Promoting the transfer of knowledge and innovation to the scientific community and society about traditional food products

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Thematic Areas: Biotechnology and society (Scientific dissemination and open science in Biotechnology)

Abstract: Scientific dissemination includes the use of resources, techniques, processes and products (vehicles or channels) for the dissemination of scientific, technological or innovation information to the general population[1]. Public and private higher education institutions offer mechanisms that allow the relations between universities and industry in the search for scientific and technological development. Therefore, it is necessary that this knowledge about science and technology will increase the population in general so that it can benefit from it.
In this sense, the NEWFOOD - Food Technologies Valorization[2] project arises. This, in addition to accelerate the processes of expansion and consolidation of the "Traditional Food Products" by promoting innovation as well as increasing the transfer of knowledge and technology around this products, aims to disseminate the results making them accessible to stakeholders. This project proposes three Actions: (a) develop a knowledge and skills map of the Region and an inventory of the needs of the sector to ensure the innovation process focused on the central axes of expansion and consolidation of the sector; (b) promote innovation projects aimed at generating new products / solutions of economic relevance in the "Traditional Food Products" sector and (c) actively disseminate knowledge, skills and resources to the entities involved, including stakeholders.
This work will focus on Action C which has the following tasks: Promotion of the transfer of knowledge to the economic tissue, extended to the whole value chain, through practical and demonstrative actions as well as through concrete projects with companies, of innovation for the availability of the capacities and resources existing in the Research Centers; Promotion of applied knowledge and innovation potential in the area to the national business community, the national and international consumer, and the technical and scientific community through a set of actions that include videos, campaigns on social networks, communications and publications of different nature at the level National and international.
In addition to the promotion of science and technology, scientific literacy will be promoted in a way that brings together stakeholders, including the economic tissue and the university. Scientific and technological benefits are often not available to the general population and are considered a privilege of higher classes. Therefore, it is hoped that with this project, it will be able to progress in this area by bringing science and technology closer to the general public, and also to promote traditional food products and innovation in them.

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