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as hydrocolloids, since they delay the retrogradation of starch. This process occurs during the storage of the baked product and causes crumb hardening.
Celiac disease is a chronic autoimmune enteropathy that prevents people from eating gluten. This protein is found in wheat, oat, barley, and rye flours. Therefore, these flours must be avoided in the preparation of bread suitable for people suffering from celiac disease. As an alternative, cassava, potato, rice, corn, quinoa, and amaranth flours, among others, can be used, but they lack the viscoelastic properties of gluten.
The aim of this work was to characterize a recombinant CGTase and to evaluate its potential use to produce MOS from alternative flours for gluten-free bakeries.
The CGTase from *Paenibacillus barengoltzii* was expressed in *Pichia pastoris*, a GRAS (Generally Recognized As Safe) yeast suitable for making products for human consumption.
The recombinant enzyme was purified by affinity chromatography to α-CD. It showed an increased molecular mass due to N-glycosylations introduced by *P.pastoris,* as demonstrated by enzymatic treatment with PNGase F, followed by SDS-PAGE.
The highest CD formation activity was obtained in 50 mM phosphate buffer, pH 6.0, with an optimum temperature between 50 and 60 ºC. The enzyme was stable for one hour at pH 6 to 11 and temperatures below 50 ºC. The Km and Vmax values did not show differences from wild-type values.
To analyze enzyme microheterogeneity, a second purification step was performed by FPLC with a MonoQ 5/50 GL ion exchange column. Two fractions (A and B) with high amylolytic activity were obtained. Each fraction was analyzed by SDS-PAGE, MassSpectrometry (MS), and EDMAN degradation before and after deglycosylation with endoglucanase Endo H. The same amino-terminal sequence was observed for each fraction, but they both differed in their molecular mass, 87286 Da (fraction A) and 84277 Da (fraction B), from the wild type enzyme (74470 Da). This increase suggests the presence of six to seven glycosylated sites with high mannose residues in each fraction.
Finally, the production of MOS was determined by employing the affinity-purified recombinant CGTase on different gluten-free substrates. The highest production of MOS was obtained with starch from cassava, rice, corn and potato.
In sum, the recombinant CGTase obtained from *P. pastoris* shows microheterogeneity due to N-glycosylations introduced by the host. However, the enzyme is active over a wide range of pH and temperatures. The highest production of MOS, employed in the bakery industry to delay the retrogradation of the starch, is obtained when cassava, potato, corn and rice flours are used as substrates of the recombinant enzyme. The potential application of this recombinant enzyme in the production of gluten-free bakeries shall be explored further.

EP-9

Characterization of SdGA, a cold-adapted and salt-tolerant glucoamylase from *Saccharophagus degradans*

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Glucoamylases (GAs) are hydrolytic enzymes also known as amyloglucosidases, glucan 1,4-alpha-glucosidases or exo-1,4-α-glucosidases, EC 3.2.1.3. GAs hydrolyze glycosidic α-1,4 bonds (but also α-1,6 bonds) from the non-reducing ends of starch molecules and maltooligosaccharides releasing β-D-glucose. These are typically microbial enzymes present in archaea, bacteria and fungi but absent in animals and plants, and they are classified into the GH15 family of glycoside hydrolases (www.cazy.org). The main application of GAs (sometimes together with α-amylases and pullulanases) occurs in the process of saccharification of partially processed starch or dextrins to obtain glucose. Currently, there is strong interest in finding GAs with a better performance at low temperatures because these enzymes would avoid the heating requirement in some industrial processes such as starch saccharification among others, and, in this way, production costs could be minimized. *Saccharophagus degradans* is a gram-negative marine bacterium. It is the most versatile bacterium in terms of the degradation of complex polymers (CP) found to date. It is capable to degrade at least 10 complex polymers such as starch, agar, laminarin, cellulose, pectin, alginate, chitin, fucoidan, pectin, pullulan, and xylan at high rate. The objective of this work is to carry out the structural characterization and functional properties of SdGA, a novel

glucoamylase (GA) from *S. degradans*. The enzyme is composed mainly of a N-terminal GH15_N domain linked to a C-terminal catalytic domain (CD) found in the GH15 family of glycosylhydrolases with an overall structure similar to other bacterial GAs. The protein was successfully expressed in *Escherichia coli* cells, purified and its biochemical properties were investigated. SdGA showed maximum activity at 39°C and pH 6.0. The enzyme has high activity in a wide range, from low to mild temperatures, like cold-adapted enzymes. It showed the same maximum activity in the range of 0 – 1.0 M NaCl like salt-tolerant amylases. By thermal inactivation assays, we determined that SdGA is thermolabile at temperatures above 42°C and we found that glycerol 10% (V/V), acarbose 0.1 mM and NaCl 1 M stabilized the enzyme. Furthermore, we analyze the CD of SdGA, other cold-adapted, psychrophilic and thermostable GAs and we found that SdGA has a larger CD due to various amino acid insertions and a higher content of flexible residues compared to other thermostable GAs. These characteristics of SdGA allow it to be classified as a cold-adapted enzyme but also, a salt-tolerant enzyme. We propose that this novel SdGA, might have potential applications for use in different industrial processes that require an efficient alpha glucosidase activity at low/mild temperatures, such as biofuel production.

EP-10

Characterization of SdAmy, a novel alpha amylase from *Saccharophagus degradans*

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Saccharophagus degradans is a gram-negative marine bacterium. It is the most versatile bacterium in terms of the degradation of complex polymers (CP) found to date. The high rate of degradation of different polysaccharides that this bacterium has makes it a candidate to obtain and investigate the properties of the enzymes that degrade these polymers. The objective of this work is to carry out the structural characterization of SdAmy, a novel alpha-amylase from *S. degradans,* and the study of the CBM20 domain of SdAmy. The enzyme is composed mainly by a N-terminal catalytic domain (CD), a central AamyC domain (AamyC) and a carbohydrate-binding module family 20 at the C-terminal (CBM20). SdAmy and the CBM20 domain was successfully expressed in *Escherichia coli* cells, purified and its properties were investigated. The enzyme showed maximum activity at 40°C and pH 5.0. SdAmy showed a V_{max} and K_m values of about 0,04 UA.μg^{-1}.min^{-1} and 0,34 g.l^{-1} , respectively, when used potato starch as substrate. The activity and stability of SdAmy was improved in the presence of calcium. Carbohydrate binding assays showed that the CBM20 domain bind to amylose with a K_{ad} of 3.13 ± 0.25 ml/g. Our results allow us to propose that this novel SdAmy as an alternative amylase that could be used in processes involving enzymes that act at moderate temperatures.

EP-11

Evaluation of bioinformatic methods for the prediction of stabilizing mutations and its use in the design of a thermostable xylanase

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The design of active enzymes with increased stability is of high interest both for basic and applied research. There are a number of methods developed to predict changes in stability upon mutation

elongating α-glucans. The kinetic characterization showed that GlgSase482 is two orders of magnitude more active (53.1 ± 0.6 U/mg) than GlgSase471 (0.35 ± 0.01 U/mg), and both are specific for ADP-Glc, with similar apparent affinities (K_M ~0.16 ± 0.01 mM). Regarding glycogen as a substrate, the K_M value for GlgSase482 is 0.06 ± 0.01 mg/ml while for GlgSase471 0.032 ± 0.009 mg/ml. The addition of 10 mM Mg^{2+} was essential to reach their maximal activity, although in the absence of Mg^{2+} the enzymes were already actives (~60%). The addition of 10 mM Mn^{2+} to the reaction mixture inhibited the activity by more than 50%.
The genomic context analysis shows that the gene encoding GlgSase482 locates adjacent to the one coding for ADP-Glc pyrophosphorylase, then probably being part of a *glg* operon for glycogen synthesis. On the other hand, the gene coding for GlgSase471 locates elsewhere, together with *a priori* unrelated genes. This agrees with the fact that GlgSase482 is the enzyme with the highest efficiency for glucan elongation. Still, further studies need to be conducted to in detail comprehend the duplication of GlgSases (but not other enzymes from glycogen biosynthetic pathway) in *B. japonicum*. Results will allow further evolutionary understanding of carbohydrate-active enzymes that proportionate to the glucan its ultimate structure.

EP-13

Molecular insight of cellulose degradation by the phototrophic green alga *Scenedesmus quadricauda.*

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Generation of renewable energy resources and waste management are the major concern in twenty first century. Lignocellulosic agricultural and forest wastes are the promising feedstock for production of biofuel and value-added products due its high availability and low cost. Nevertheless, no commercial process has still been reported for the enzymatic hydrolysis of cellulose. The main reason is the high cost of the required enzymes, low specific activity, susceptible to inactivation and difficult to recycle.
A group of naturally occurring cellulases has been reported from heterotrophic microorganisms including bacteria and fungi. They secrete cellulases to utilize cellulose as sole carbon source. Bioconversion process involves the hydrolysis of cellulose to produce reducing sugars, further fermentation of the sugars to ethanol and other bioproducts. Cellulases hydrolyze the β-1,4 glucosidic bonds of the glucose polymer by two different ways, endoglucanases cut random positions along the cellulose chain, and exoglucanases progressively act on the terminal ends of the polymer, releasing either glucose molecules, or cellobiose. Finally, the cellobiose molecules produced are converted to glucose by intra- and extracellular beta-glucosidases (EC 3.2.1.21), celludextrinasas (EC 3.2.1.4), and celudextrin phosphorylases (EC 2.4.1.49), depending upon the characteristic of each cellulolytic species. Other than heterotrophs, cellulases belonging to glucoside hydrolase family (GH9) are also described from higher plants. However, it has been reported that plant cellulases participates in biosynthesis of cellulose rather than degradation.
Algae are phototrophs, ubiquitous with versatile metabolic pathways. Which have been well exploited to obtain multiple products through algal refinery. However, presence of cellulases and cellulolytic ability is poorly reported form algae. In 1970 Burczyk et al. reported the presence of extracellular cellulases in *Scenedesmus obliquus* by comparing the composition of their cell wall with that of the stem cell spore walls accumulated in the culture medium. In 1965, Dvořáková-Hladká et al. reported the presence of beta-glucosidase activity in *Scenedesmus obliquus*, which allows it to grow using cellobiose as a substrate. In 2012, Blifernez-Klassen et al. observed that the photoheterotrophic microalgae *Chlamydomonas reinhardtii* is also capable of degrading and assimilating exogenous cellulose. This interesting finding led us to search for cellulases in *Scenedesmus quadricauda*. It is freshwater, not mobile green algae usually forming colonies of four cells. It belongs to the same class of green algae (Chlorophyceae) as the genus *Chlamydomonas*. *S. quadricauda* has gained great

importance due to high capacity for effluent treatment, CO_2 capture and biofuel production as evident by our previous study also.
In the present work, we identified cellulases (GH1, GH5 and GH9) gene in the genome sequence of *S. quadricauda* LWG002611. Furthermore, we have carried out a comparative bioinformatic analysis in several available *Scenedesmaceae* algae genome (*Scenedesmus obliquus* EN0004 v1.0, *Scenedesmus obliquus* UTEX B 3031, *Scenedesmus obliquus* var. DOE0013 v1, *Scenedesmus* sp. NREL 46B-D3 v1.0, *Tetradesmus deserticola* SNI-2 v1.0 with and *S. quadricauda* LWG002611 to identify multiple homologs of endoglucanase, β-glucosidase and exocellulase genes. We were able to identify 57 endoglucanase sequences, 28 cellobiase sequences, and 12 exocellulase sequences. For each of them we identified the catalytic and carbohydrate-binding domains, analyzed by phylogeny their evolutionary relationship, and obtained 3D models of the most important catalytic modules and domains. The sequences were taken from phytozome or NCBI. Conserved domains, Signal peptide, and GH-family assignment were identified with Prosite patterns SignalP and predAlgo. For the phylogenetic analysis the sequences were aligned with Clustal Omega and the alignment submitted to MEGA 6 software. The 3D homology models were generated with RaptorX server. The regions implicated in substrate binding and activity were manually annotated using the pattern sequences or 3D structure of cellulase templates available in Prosite and pdb database. Our new finding would open the opportunity for future applications of algal cellulases for biotechnological applications.

EP-14

CBMs in trans: generating alternatives to improve the catalytic efficiency of enzymes

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Protein engineering by the addition of substrate binding domains is becoming a widely used strategy to improve enzyme properties. These binding domains are often used to increase the catalytic efficiency, as affinity tags to facilitate protein purification and also for targeting a protein to specific cellular locations. CBMs (carbohydrate binding modules) are non-catalytic protein domains that are naturally present in some enzymes and are associated with the ability to bind polysaccharides. Among CBMs, there is a subgroup called SBDs that have an evolutionary advantage due to the presence of two starch binding sites. The mechanisms of action of CBMs differ and are characteristic of the enzyme in which they are present. They can act by bringing the catalytic domain closer to the substrates, as a scaffold for protein-protein interaction and, additionally, they can break the structure of substrates increasing the catalytic efficiency of the enzyme. The latter is particularly important when the substrates are structured, such as starch granules or plant cell walls.
Typically, the fusion of CBMs *in cis* to generate chimeric proteins is used to evaluate constructs as possible biotechnological tools. Our laboratory has demonstrated the interaction of SBDs in cis with the glycogen synthase (GS) from *Agrobacterium tumefaciens*. The addition of the D3 domain from *Arabidopsis thaliana* starch synthase III (SSIII) to the GS conferred a higher capacity for glycogen biosynthesis, suggesting that the careful design of fusion proteins can led to the production of a fully active and conformationally stable molecule composed of domains that belong to different kingdoms, in this case, plants and bacteria. The presence of a polysaccharide-binding site outside the active site of the enzyme would lead to improve the binding capacity through multiple contacts, increasing the local concentration of non-reducing ends in the active site and resulting in a greater processivity of the enzyme.
While there are many studies on the effect of CBMs *in cis*, few studies have been conducted to evaluate their effect *in trans*. We analysed here the ability of different CBMs, coming from different families (a CBM20 from a *Ostreococcus tauri* protein (CBM20CP) and three xylan binding domains (XYL1-3, classified in the CBM22 family) from a xylanase from *A. thaliana* (AtXyn1) to act *in trans* on two commercial enzymes, an amylase (AmyC) and a xylanase (XYNA) respectively. The CBM20 is located in the central position of a protein without associated catalytic activity. Therefore, we

evaluated the effect of the recombinant CBM20 and the full CBM20CP. We found that the addition of CBM20 had a little impact on the activity of AmyC, while the addition of the full protein significantly increased (about 90%) the catalytic efficiency of the enzyme. On the other hand, of the three CBMs from AtXyn1 used, only XYL1 was able to increase the V_{max} of XYNA by 70% without significant differences in the K_m, suggesting that the increase in the catalytic efficiency is exclusively due to the increase in V_{max} and not in its affinity for the substrate. Our results show that the addition in trans of CBM20CP and XYL1 would be a useful strategy to improve the activity of some enzymes that use different polysaccharides as substrates.

EP-15

Mining an intertidal sediment metagenome for fucanases for the production of oligosaccharides from brown algae fucoidans

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Fucoidans are a sulfated polysaccharides present in the cell wall matrix of brown algae. These polysaccharides are generally composed of a backbone of α-L-fucose residues, but they are very diverse in terms of monosaccharide composition, sulfate content and linkage patterns, i.e. α-(1,3) or α-(1,3)/α-(1,4). Fucoidans have a variety of biological activities beneficial for human health, such as immune-modulatory, antiviral, anticoagulant, antitumor, antithrombosis and antioxidant. However, the low absorption of these complex carbohydrates and their low bioavailability limit their health benefits besides their prebiotic effect. Endo-acting enzymes called fucanases depolymerize these polysaccharides, generating bioactive fuco-oligosaccharides with various biotechnological applications. So far, only two CAZy families have been described: GH107 (endo-α-1,4-L-fucanase) and GH168 (endo-α-1,3-L-fucanase). In this work, we mined putative fucanase sequences in a metagenomic dataset from intertidal sediments of Ushuaia Bay (Tierra del Fuego Island, Argentina) exposed to brown algae detritus, and selected sequences for heterologous expression and characterization. Using a series of HMMs specific for these families, we identified eight sequences homologous to the GH107 family and 32 sequences related to members of GH168 family. The relative abundance of these genes in the metagenome was one every 17,000 sequences, although probable only part of the diversity of these enzymes is currently known. Overall, 26 sequences shared low identity values (<40 % at protein level), while 5 sequences had high identity values (>80 %) with reference sequences of the database. One of the scaffold contained both GH107 and GH168 homologs, and often the genomic context of the identified sequences contained genes potentially related to fucoidan degradation, including α-fucosidases (exo-acting) and sulfatases. These results provide further evidence of the predicted function of the identified sequences. In both families, the most abundant taxonomic assignment of the scaffolds containing the identified sequences was the Planctomycetes phylum (35 % of the sequences), for which fucanase enzymes have not yet been characterized. The second most abundant taxonomic assignment was the Terrabacteria group, followed by Bacteroidetes and Proteobacteria phyla. Besides the catalytic module, domains identified in these sequences include a pectin lyase fold, carbohydrate-binding module, beta helix and domains belonging to other GH families (GH10, GH13, GH15, GH31 and GH29). Four sequences related to the GH107 family were selected for heterologous expression in *Escherichia coli*, three probably from members of the Planctomycetes phylum and one from the Bacteroidetes phylum. Low temperatures during expression were needed for reaching high levels of protein expression in the soluble fraction. The enzymes were purified and characterized, and fucanase activity was evaluated by carbohydrate-polyacrylamide gel electrophoresis (C-PAGE). The assessed substrates were fucoidans extracted from four brown algae species of the Patagonian coast: *Macrocystis pyrifera*, *Undaria pinnatifida*, *Scytosiphon lomentaria* and *Adenocystis utricularis*. Among the four expressed genes, #113643 (potentially from a Planctomycetes) presented a high activity towards *M. pyrifera* fucoidan, with degradation products observed from a 30 s incubation time at 25 °C, faster than previously characterized members of the GH107 family. The enzyme was active in a wide range of temperatures

how minimal differences in the amino acid sequence impact on the regulatory signal transmission without affecting the global protein structure.

EB-11

Study of the properties of the frataxin from *Nannochloropsis gaditana*

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Fe-S clusters are among the oldest and most versatile cofactors used by most living organisms. They are formed by atoms of iron and inorganic sulphide. Although they can be synthesized non-enzymatically *in vitro*, their biosynthesis is catalyzed by specific enzymes. Three complete systems responsible for the biosynthesis of groups [Fe-S] were discovered in bacteria: NIF (nitrogen fixation), SUF (sulphur mobilization) and ISC (iron and sulphur cluster) and in eukaryotes the presence of a ISC and SUF homologous system in mitochondria and chloroplasts, respectively. In this work we address the initial characterization of this pathway in algae. First, we performed the identification *in silico* of gene and protein sequences possibly related to the biogenesis of Fe-S clusters in green and brown algae (*Nannochloropsis gaditana, Chlorella vulgaris* and *Ectocarpus siliculosus*) using the Phytozome database (https://phytozome.jgi.doe.gov). After performing sequence alignments and considering the high conservation found between the amino acid sequences, the frataxin from *N. gaditana* (NangaFH) was selected to perform its functional characterization. The sequence of NangaFH contains 204 amino acids, a mitochondrial targeting transit peptide of 82 residues and a frataxin domain comprised between residues 94 to 199. Within this domain the iron-binding sites are conserved (E95, D103, E104, D107, A111, D114, A115, D122 and E124). The recombinant protein was expressed in *E. coli* cells and purified to homogeneity. We evaluated their ability of NangaFH to attenuate the Fenton reaction by measuring the inhibition of malondialdehyde production after the addition of thiobarbituric acid. As previously suggested, frataxin could function as iron chaperone and in this way, its presence could attenuate oxidative damage by metals. Results showed that NangaFH did not attenuated the Fenton reaction in contrast to the frataxins from *C. vulgaris* and *A.thaliana* (both proteins attenuated the Fenton reaction about 23%). To determine whether NangaFH overexpression decreases the sensitivity of *E. coli* cells to oxidative and metal stress, bacterial cells overexpressing this protein were incubated in the presence of hydrogen peroxide, chromium and zinc. Our results showed that the expression of NangaFH allowed a better growth of *E. coli* cells under oxidative conditions. These results suggest that algae frataxin would have a protective role against oxidative stress in algae.

EB-12

Kinetic and structural characterization of a glycosyl phosphorylase from *Euglena gracilis*

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Euglena gracilis is a fresh water protist with a large metabolic capacity because it is able to grow photosynthetically or heterotrophically. *E. gracilis* is a microorganism of interest in biotechnology and biomedicine due to its ability to generate bioproducts such as polysaccharides, polyunsaturated fatty acids, vitamins, wax esters and other metabolites. Paramylon is the main reserve polymer of *E. gracilis*. It is a water-insoluble β-1,3-glucan with a high degree of polymerization. There is little information about the enzymes involved in the metabolism of paramylon. Recently, the presence of a protein in *E. gracilis* belonging to the family 149 of glycosyl hydrolases (*Eg*GH149) was reported. GH149 is a new family of "carbohydrate active enzyme" (CAZyme) and is thought to group glycosyl phosphorylase. Glycosyl phosphorylases can catalyze the