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EN-P02
ANALYSIS OF SUBSTRATE SPECIFICITY OF GALACTINOL SYNTHASE FROM *BRACHYPODIUM DISTACHYON*

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Raffinose (Raf) is an α-1,6-galactosyl extension of sucrose that plays a key role in the stabilization of membranes during seed desiccation. Plants from the family Cucurbitaceae also use Raf for transporting carbon from photosynthetic to heterotrophic tissues. Additionally, Raf accumulates in tissues exposed to several abiotic stress conditions, such as heat, cold, salinity, and drought. Considering the importance of Raf for plant physiology and biochemistry, the number of studies dealing with enzymes involved in Raf biosynthesis are relatively scarce. To better understand the kinetic and regulatory properties of the enzymes involved in Raf metabolism, we cloned the genes encoding UDP-sugar pyrophosphorylase (USPPase, EC 2.7.7.64), galactinol synthase (EC 2.4.1.123), and Raf synthase (EC 2.4.1.82) from *Brachypodium distachyon*, a model grass evolutionary related to several economically important species, including rice and wheat. USPPase catalyzes the production of activated sugars, mainly UDP-galactose (UDP-Gal), the natural substrate of galactinol synthase, which in turn produces galactinol from UDP-Gal and *myo*-inositol. Afterward, Raf synthase transfers the Gal moiety from galactinol to a preformed sucrose molecule to produce Raf. The genes encoding USPPase, galactinol synthase, and Raf synthase from *B. distachyon* were synthesized *de novo*, and the recombinant proteins were expressed with an N-term His-tag in *Escherichia coli* cells and purified in a single step by IMAC. The activity of USPPase with different hexoses-1P was as follows: Gal-1P ≅ glucose-1P > glucuronic acid-1P > mannose-1P > glucosamine-1P. We found that galactinol synthase uses UDP-glucose as an alternative substrate, although with a significantly lower catalytic efficiency (196 M^{-1} s^{-1}) than with UDP-Gal (2.6 x 10^5 M^{-1} s^{-1}). Our results suggest that galactinol synthase might use other UDP-sugars *in vitro*, thus leading to novel *myo*-inositol derivatives different from galactinol. These molecules could then be used by Raf synthase to produce original oligosaccharides with multiple applications in biorefineries.

EN-P03
A FIRST EVIDENCE OF A GLUTAREDOXIN-LIKE PROTEIN IN *ENTAMOEBA HISTOLYTICA*

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Entamoeba histolytica, an intestinal parasitic protozoan, is the causative agent of amoebiasis. The parasite usually lives and multiplies within the human gut, an environment of reduced oxygen pressure. During tissue invasion, *E. histolytica* is exposed to elevated amounts of exogenous reactive oxygen species (ROS), which are highly toxic for the parasite. The metabolic pathway for ROS detoxification in this organism is a matter of controversy. Because neither glutathione nor its associated enzymes were found to occur, it has been proposed the cysteine as a main intracellular thiol and one of the compounds responsible for maintaining the intracellular redox balance. In this work, we present the functional characterization of a glutaredoxin-like protein from *E. histolytica* (*Eh*Grx1). Biochemical assays showed that *Eh*Grx1 was able to catalyze the *in vitro* reduction of GSH-derivate low molecular mass disulfides and cystine. The protein obtained by recombinant expression in *Escherichia coli* presented an apo-monomeric structure; however, a holo-protein form was obtained from supplemented culture media with ferric citrate and cysteine. The ability to ligate iron-sulfur centers (ISCs) was evaluated by UV-Vis spectroscopy and gel filtration chromatography, showing evidence that *Eh*Grx1 could bind ISCs. The Grx activity was not detected in holo-*Eh*Grx1, suggesting that its catalytic cysteine residue would be linked to ISC. We also evaluated by western blot the relative abundance of *Eh*Grx1 in *E. histolytica* cells exposed to exogenous oxidative species and metronidazole (the preferred drug for amoebiasis treatment). The results showed that the protein level increases respect to no-treated cells. Similar behavior was observed in the subcellular localization analysis, carried out for different oxidative conditions by confocal immunofluorescence microscopy. Altogether, the results suggest that *Eh*Grx1 could be involved in oxidative and nitrosative stress protection in the parasite. To the best of our knowledge, this is the first characterization of this type of protein in *E. histolytica*. Granted by ANPCyT (PICT2016-1778 and PICT2017-2268).

EN-P04
KINETIC AND STRUCTURAL CHARACTERIZATION OF A GLYCOSYL PHOSPHORYLASE FROM *EUGLENA GRACILIS*

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Euglena gracilis is a freshwater 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 how *E. gracilis* is able to metabolize this polymer. 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 degradation/synthesis of β-1,3-glucan. The kinetic parameters of the enzyme in both senses (and for several substrates) were determined. We have studied the partition of a disaccharide of glucose with β-1,3 bond (Lamianribiose or Lam2) with inorganic phosphate and we found that the enzyme had its maximum activity at pH 7.5 and 40°C (*Kcat* of 9.1 s^{-1} and a *Km* values of 1.57 mM for inorganic phosphate and 1.24 mM for the Lam2). We observed that the enzyme had no activity when testing other types of disaccharides. The influence of polymerization degree (PD) was evaluated, being the efficiency of the enzyme 10-fold lower for both laminaritetrose (PD = 4) and laminarihexose (PD = 6). No activity using laminarin (PD = 30) as a substrate was detected. On the other hand, *Eg*GH149 catalyzed the condensation of glucose with glucose-1-Phosphate (*Kcat* 1.32 s^{-1} and *Km*