Tercer Encuentro & Primer Workshop de la Red Argentina de Tecnología Enzimática

Septiembre 8 - 9 - 10, 2021 ▸ VIRTUAL

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Thrusday September 9

17:00 - 19:00 **E-Posters sessions at *Gather Town***

17:00 - 17:30 **Session I** *(cont.)*

DE-5
Laccase immobilization methodology for enzymatic fuel cells design
Coria-Oriundo, L.L.; Wirth, S.; Battaglini, F.

EB-1
A genetic selection method for discovering new methionine sulfoxide reductases
Manta, B.; Sinah, A.; Ozisik, D.; Mariotti, M.; Gladyshev, V.N.; Berkmen, N.

EB-3
Multi-domain flavodiiron proteins from *Trichomonas vaginalis*
Birocco, F.; Guerrero, S.A.; Iglesias, A.A.; Arias, D.G.

EB-5
A different catalytic mechanism in bacterial Ferredoxin-NADP+ reductases due to a particular NADP+ binding mode
Monchietti, P.; Ceccarelli, EA.; Catalano Dupuy, DL.

EB-7
Optimization of the liquid fermentation conditions for the secretion of mycolytic enzymes in the Trichoderma koningiopsis POS7 strain
Amerio, N.S.; Barengo, M.P.; Bich, G.A.; Zapata, P.D.; Castrillo, M.L.; Villalba, L.L.

EB-9
Structural dynamics and the consolidation of protein function by quaternary addition of nanobodies
Pignataro, M.F.; Pavan, F; Sewell, K.E.; Arce, L; Militello, D; Gentili, H.G.; Ibañez L.I.; Santos, J.

EB-11
Study of the properties of the frataxin from *Nannochloropsis gaditana*
Marchetti-Acosta N.S.; Barchiesi J.; Gomez-Casati D.F.; Busi, M.V.

EB-13
Influence of carbon and nitrogen sources on GABA production by *Levilactobacillus brevis* CRL 2013 in chemically defined media
Cataldo, P.G.; Ríos Colombo, N.S.; Savoy de Giori, G.; Saavedra, L.; Hebert, E.M.

EB-15
Glucansucrase approaches in novel homopolysaccharide producer lactic acid bacteria isolated from Argentinean fruits
Lobo, R.E.; Cataldo, P.G.; Hébert, E.M.; Torino, M.I.

EB-17
C4 decarboxylases: different evolutionary innovations to feed rubisco
Tronconi, M.A.; Gerrard Wheeler, M.C.; Saigo, M.; Alvarez, C.E; Maurino, V.G.; Drincovich, M.F.

degradation / synthesis of β-1,3-glucan. To obtain information about the function of this enzyme, we produced it in recombinant form, soluble and active.
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 (laminaribiose or Lam2) with inorganic phosphate and we found that the enzyme had its maximum activity at pH 7.5 and at 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. *Eg*GH149 efficiency decreases with an increasing degree of polymerization (PD), lacking activity with laminarin (PD ~ 30) or paramylon (PD ~ 3000).
On the other hand, *Eg*GH149 catalyzed the condensation of glucose with glucose-1-Phosphate (Kcat 1.32 s^{-1} and Km 1.81 for the glucose). We also carry out promiscuity tests for sugar phosphate and free sugar, finding that it is specific for glucose-1-phosphate. We also show determined that *Eg*GH149 is capable of using Lam2 and 2-deoxy-glucose with lower affinity than glucose. We do not detect enzymatic activity when evaluating other acceptors.
In order to know the quaternary structure of the enzyme we perform a gel filtration chromatography, showing that the *Eg*GH149 forms homodimers in agreement with previous reports for the same enzyme from other sources. With this information and the crystallized structure of another protein belonging to GH149 we made a 3D model of the protein in which we detected a laminarihexose binding surface away from the active site. In this regard, we decided to study the ability of enzyme to bind paramylon and laminarin, but we did not observe binding of the *Eg*GH149 to the substrates tested.
We use the recombinant protein to obtain specific serum against *Eg*GH149. Through western blot assays we show the presence of the protein in cells grown under autotrophic and heterotrophic conditions. In order to obtain information about its intracellular location, we performed a confocal microscopy assay: we observed signal recognition in the cytosol, forming hotspots near the paramylon granules regardless of the culture condition analyzed. This work provides information about the kinetic and structural behavior of this enzyme, as well as its distribution pattern in *E. gracilis* cells.
Granted by ANPCyT (PICT´16 1110 and PICT´19 0349).

EB-13

Influence of carbon and nitrogen sources on GABA production by *Levilactobacillus brevis* CRL 2013 in chemically defined media

Cataldo, P.G.; Ríos Colombo, N.S.; Savoy de Giori, G.; Saavedra, L.; Hebert, E.M.
Centro de Referencia para Lactobacilos (CERELA- CONICET), Argentina;
e-mail: pcataldo@cerela.org.ar

Gamma-aminobutyric acid (GABA) is a non-protein amino acid widely distributed in nature, having diverse physiological functions and great potential health benefits. Due to its relevance, GABA is becoming recognized as an essential nutrient for a healthy and balanced diet. *Levilactobacillus brevis* species, constitute the most competitive and technologically relevant group of microorganisms used to synthesize GABA since they are able to produce high levels of this compound within a variety of food matrices. Glutamic acid decarboxylase (GAD) system is responsible for glutamate decarboxylation and GABA secretion and consists of two important elements: a glutamate/GABA antiporter GadC and a GAD enzyme, either GadA or GadB. In addition, most *L. brevis* strains encode a transcriptional regulator, *gadR*, immediately upstream of the *gadCA* operon which positively regulates its expression. Previously, we demonstrated that CRL 2013 is able to grow and produce high amounts of GABA in fructose-supplemented MRS rich medium with a conversion rate of glutamate to GABA ~99%. Furthermore, GABA synthesis was impaired when this strain was grown at the expense of pentoses as the only carbon source. This impairment on GABA production was partially overpassed by the addition of ethanol to the culture media. Interestingly, CRL 2013 was unable to produce GABA after incubation in a chemically defined medium (CDM). Thus, the aim of this study was to analyze the effect of the carbohydrate and nitrogen source on the growth and GABA production by *L. brevis* CRL 2013 using a CDM. The addition of different nitrogen sources such as casitone (C), vegetal peptone (VP) and yeast extract (YE) stimulated the growth of CRL 2013 in both hexose and pentose- based CDM. Nevertheless, GABA synthesis could only be triggered in the presence of hexoses and YE, C or PV being highest in YE. Conversely, with xylose as the only carbon source, the supplementation of the basal CDM with several nitrogen sources did not allow GABA production and only low GABA levels were detected after 72 h of incubation in the presence of YE. In an attempt to restore GABA production, ethanol (0,25 g/L) was added to the xylose-CDM. Interestingly, the addition of ethanol could restore partially GABA production in the presence of YE. In order to gain insight to the transcriptional changes that could be associated to GABA production,

the expression of genes encoding key enzymes and regulatory elements within the GAD system and of the *ccpA* gene, involved in catabolite repression, was assessed through RT-qPCR using *recA* as the housekeeping gene. In the hexose- CDM, the expression of *gadR* and *gadA* was significantly increased in the presence of C and YE in both exponential and stationary growth phases in comparison with the non-supplemented CDM. At 24 h, *gadR* was overexpressed 112 and 90 times in the presence of YE and C respectively, when compared to the control CDM. Moreover, the expression levels of *gadA* were 2800 and 1000 times higher in the presence of YE and C respectively, when compared to the same control. However, when transcription levels were compared against the YE-supplemented xylose CDM (YE-xCDM) at 24 h, *gadA* and *gadR* were upregulated 1000 and 200 times respectively, in the presence of hexoses. The addition of ethanol to the YE-xCDM was translated into significantly higher *gadA* and *gadR* transcript levels compared with the same medium without ethanol (190 and 275- fold change respectively). Furthermore, no significant differences were observed for *gadB* while *ccpA* was 50 times upregulated in the presence of glucose and fructose when compared to the YE-xCDM, which was compatible with an active catabolite repression. Additionally, the expression profiles revealed that *gadA* and *gadR* were upregulated and *gadB* downregulated in the stationary growth phase in the presence of hexoses, whereas the opposite behavior was observed in the presence of xylose. This pattern was reversed after the addition of ethanol to YE-xCDM. To our knowledge, this is the first report regarding the impairment of the GAD system in the presence of pentoses as sole carbon sources and the restoration of GABA production upon ethanol supplementation in a CDM context within lactic acid bacteria. Taken together, these results contribute to the understanding of the regulation of the GAD system in LAB and highlight the potential use of alternative carbon sources to produce high GABA levels.

EB-14

Glycolysis in *Agrobacterium tumefaciens* revisited: characterization of a pyrophosphate dependent 6-phosphofructokinase

Brezzo E, Iglesias AA, Figueroa CM, Asencion Diez MD
Lab. de Enzimología Molecular, Inst. de Agrobiotecnología del Litoral, Argentina;
e-mail: masencion@fbcb.unl.edu.ar

The interconversion of fructose-6P (Fru-6P) and fructose-1,6-bisP (Fru-1,6-bisP) is a critical node in all organisms, where three enzymes participate in the latter. Phosphofructokinase (EC 2.7.1.11, PFK) phosphorylates Fru-6P by means of ATP while fructose-1,6-biphosphatase (EC 3.1.3.11) releases Pi from Fru-1,6-bisP. These two enzymes catalyse irreversible reactions, with coordinated regulation at different levels, allowing glycolysis and gluconeogenesis to proceed. A third reversible activity is catalysed by a 6-phosphofructokinase (EC 2.7.1.90, PFP) using pyrophosphate as a Pi donor. First described in trypanosomatids, PFPs were extensively characterized in plants but little is known regarding the properties from prokaryotic sources.
Members of the *Agrobacterium* genus were reported to metabolize glucose (mainly) through the Entner-Doudoroff pathway. Accordingly, previous studies demonstrated the absence of the "canonical" PFK activity in *Agrobacterium tumefaciens* crude extracts. However, *A. tumefaciens* genomic analysis reveals the presence of genes for almost all enzymes from the Embden–Meyerhof–Parnas pathway. Notably, at the Fru-6P/Fru-1,6-bisP node only one sequence (*Atu2115*) was found putatively encoding a pyrophosphate-dependent phosphofructokinase. Then, the main goal of this work was the biochemical characterization of the *A. tumefaciens* putative PFP enzyme. The *Atu2115* gene from *A. tumefaciens* strain C58 was *de novo* synthesized, cloned into the pET28 expression vector, expressed in *Escherichia coli* BL21 (DE3) and after IMAC purification, the enzyme was obtained pure.
The enzyme was confirmed concerning its PFP activity in *in vitro* assays. Remarkably, no other phosphorylated sugars were utilized as a substrate and PPi was the specific Fru-6P phosphoryl donor. Kinetic constants were determined for the forward (glycolytic) and reverse (gluconeogenic) reactions. The *Atu*PFP activity was 2-fold higher in the gluconeogenic direction (280 U/mg) than in the glycolytic one (144 U/mg), and showed higher affinity towards Fru-1,6-bisP (K_m 0.03 mM) than for Fru-6P (K_m 1 mM). Therefore, *Atu*PFP depicts better catalytic efficiency (~65-fold) in the