Molecular and biochemical characterization of a fructosyltransferase from *Burkholderia phymatum*.

Vera F., López-Munguía A. and Olvera C. Instituto de Biotecnología, UNAM, Cuernavaca, Morelos AP. 62210; fcovera@ibt.unam.mx

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Introduction. Fructans are fructose polymers synthesized from sucrose by fructosyltransferases (FTFs). Inulin is composed of β2-1 linked fructose residues, while levan is a β2-6 linked fructose polymer. These polymers have been reported in species such as *Erwinia herbicola, E. amylovora, Gluconoacetobacter diazotrophicus*, among others (1). The gender *Burkhorderia* has been reported to produce levan to obtain an advantage in the colonization of new ecosystems as symbiotic or pathogen agent (2).
The main objective of our work was the identification and characterization of the FTF present in *Burkholderia. phymatum*, which the presence of this kind of enzyme has not been reported.

Methods. The gene coding for a FTF was identified in the *B. phymatum* genome database of the NCBI. This gene (*KesL*) was amplified, cloned and expressed in *E.coli*. The resulting protein was purified by affinity chromatography with Ni^{2+}, and biochemically characterized. Enzymatic reactions were performed incubating the enzyme with sucrose (usually 12% w/v), and measuring the release of reducing sugars (3) at different temperatures and pH. The kinetic constants were obtained under optimal reaction conditions. The product specificity was defined by analysis of the reactions products by high performance liquid chromatography (HPLC).

Results. The *Burkholderia phymatum* FTF gene was identified exploring the annotated genome in the GenBank database (Ref. No. YP_001862155.1) defined as *KesL*. The *KesL* gene encodes for a peptide sequence of 57,3 kDa and an isoelectric point of 5.99. After isolation, of the gene, it was cloned and expressed in *E.coli*. After the extract purification, it was possible to obtain an enzyme preparation that contained 247.4 U/mg of protein, with a yield purification of 37% and a purification factor of 22.4. It was found that KesL has an optimum pH of 6.0 and an optimal temperature of 50 °C. After experiments where the initial rate data were obtained at various sucrose concentrations, it was found that the enzyme follows Michaelis Menten kinetics with a Km for sucrose of 27.09 mM and a turnover number of 309 seg^{-1}. A thin layer chromatography of the reaction products after 69 % sucrose conversion demonstrated the presence of polymer and fructooligosaccharides (FOS) with different degree of polymerization. However, when the reaction products were analyzed and quantified by HPLC, it was found that, in average, 80% of the substrate was hydrolyzed, with only 20% directed to polymer synthesis. Nevertheless, the resulting polymer has an estimated molecular weight of 3500 kDa, while nuclear magnetic resonance studies confirmed that the synthesized polymer is levan. Low molecular weight products (FOS) also observed during the synthesis were analyzed in samples after 69 and 77% of sucrose conversion, and compared to standards of 1-nystose, 1-kestose and 1-furanosyl nystose (fig. 1). It may be observed in this figure that the major FOS product in the reaction is 1-kestose. We were able to calculate around 6,0 g/L of this compound after 77% of sucrose conversion of reaction under optimal conditions.

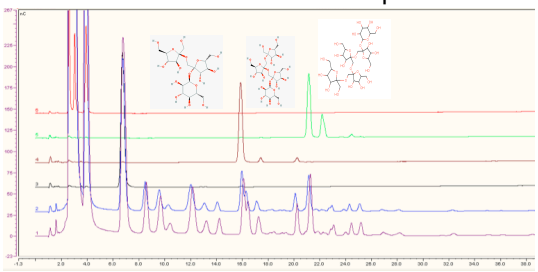

Fig.1 Chromatography FOS. Standars: Red sucrose, glucose and fructose, in green: 1-furanosyl nystose, brown: 1-nistose, black 1-kestose. Purple and blue colors represent reaction products at 69 and 77% of sucrose conversion respectively.

The presence of fructan gives the possibility of adhesion to Burkholderia, In order to avoid this virulence factor a molecular modeling assay showed that oligo-chitosan binds to the catalytic site (fig. 2) with a binding energy of -13.41 kcal/mol a which is a higher binding energy than the one calculated for sucrose (-10.3 kcal/mol).

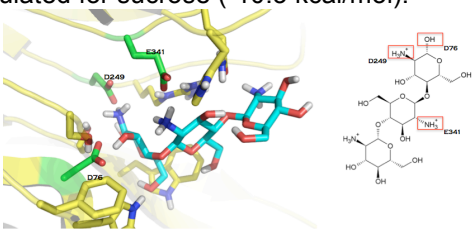

Fig.2 Molecular modeling of chitosan oligosaccharide in the active site of KesL.

Conclusions. It was found that the strain of *Burkholderia phymatum* contains a gene encoding an active fructosyltransferase able to produce fructan polymer and fructooligosaccharides

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