FRUCTOOLIGOSACCHARIDES PRODUCTION FROM SUCROSE BY IslA4, A TRUNCATED INULOSUCRASE FROM *Leuconostoc citreum*

Arlen Pena, María Elena Rodríguez, Clarita Olvera, Agustín López-Munguía; Departamento de Ingeniería Celular y Biocatálisis, Instituto de Biotecnología UNAM, Cuernavaca 62210; arlen@ibt.unam.mx

Key words: fructosyltransferase, fructooligosaccharides, inulosucrase

Introduction. Fructosyltransferases (FTFs) are enzymes that synthesize fructose polymers from sucrose by transfer of a fructosyl unit to a growing polymer chain. The fructosyl unit may also be transferred to water resulting in sucrose hydrolysis. Fructans function as soluble fiber, but fructooligosaccharides or FOS (fructans that containing between two and ten fructose moieties), are of particular interest due to their prebiotic properties (1). FTFs are classified as inulosucrases (EC 2.4.1.9) which synthesize β2-1 linked fructans (inulin), and levansucrases (EC 2.4.1.10) which produce fructans with β2-6 linkages (levan). IslA4 is a truncated form of inulosucrase (IslA) derived from *L. citreum* that contains only the catalytic domain. It has been shown that IslA4 produces high-molecular-weight inulin and a minimal quantity of FOS; however, it has a higher hydrolytic activity than the wild enzyme IslA (2). It has also been shown that product specificity is a strong function of reaction conditions (3). Therefore, in the present work we explore the effect of substrate and enzyme concentration in order to increase FOS productivity.

Methods. The reaction specificity was determined by measuring the amount of free glucose and fructose by HPLC. The amount of glucose reflects the total activity while fructose is the result of hydrolytic activity; therefore, the difference between glucose and fructose concentration allows the calculation of fructose present in transfructosylation products. Inulin was analyzed and quantified by gel permeation chromatography. Fructose incorporated only into FOS is determined as the difference between fructose involved in transfructosylation reaction and fructose present in the polymer. HPAEC-PAD was used to identify FOS profile.

Results. IslA4 reactions were carried out using three sucrose concentrations (292, 1170 and 2046 mM) and three enzyme concentrations (1, 5 and 10 U/mL) and allowed to proceed to approximately 90% substrate conversion. The reaction specificity found for each reaction condition is reported in Fig. 1, where it may be observed that at a given enzyme concentration, the transferase activity increases with substrate concentration, probably due to the lower water concentration. The effect of enzyme concentration is also clear in this figure, with a higher hydrolytic activity found at higher enzyme activity values. Therefore, the combination of higher sucrose concentrations (as 2046 mM) with lower enzyme activities (as 1 U/mL) result in the highest FOS synthetic activity (65% yield) with lower hydrolysis proportions (30% of the substrate).

The highest hydrolytic activity (89% of the substrate) was obtained in reactions with 292 mM of sucrose and 10 U/mL of enzyme activity.

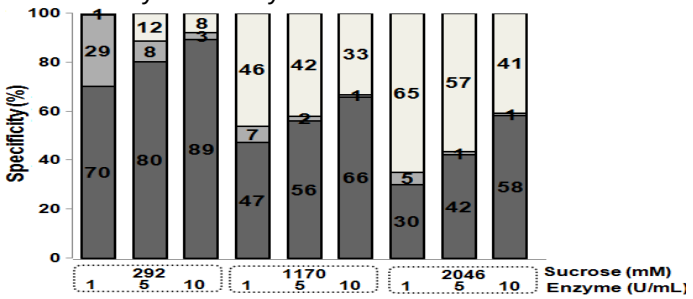

Fig.1 Reaction specificity as a function of the sucrose and IslA4 inulosucrase concentrations. □, FOS; ▣, polymer; ■, hydrolysis.

HPAEC-PAD allowed the identification of FOS produced in the reaction with the higher transference towards FOS (Fig. 2). We identified FOS possibly ranging from GF2 to GF9. The factors involved in the switch from inulin to FOS synthetic activities are still the subject of research.

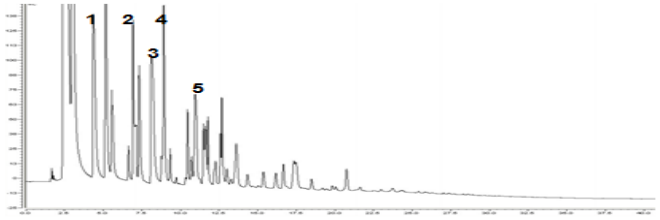

Fig.2 HPAEC analysis of FOS products synthesized by 2046 mM of sucrose and 1 U/mL of enzyme. FOS identified with standards numbered 1, 2, 3, 4 and 5 correspond to 1-kestose, 6-kestose, neokestose, nystose and fructofuranosyl-nystose.

Conclusions. In contrast with the high polymer (inulin) synthetic activity of the wild enzyme (IslA), the truncated IslA4 form becomes highly hydrolytic. However, appropiate sucrose and enzyme concentrations results in high transferase activity and FOS synthesis, while conditions may also be defined to hydrolyze the substrate. Therefore these two variables are key parameters controlling reaction specificity. Although the process may be optimized, the maximum production of FOS in these experiments was found 2046 mM of sucrose and 1 U/mL of IslA4.

Acknowledgements. Financial support by PAPIIT-UNAM No. IN212311.

References.

1. Kolida S, Tuohy K, Gibson GR. (2002). *Br J Nutr*. 87.(2.):S193-7.
2. Del Moral S, Olvera C, Rodríguez ME, López-Munguía A. (2008). *BMC Biochem.* 9.(6.).
3. Falconer D, Mukerjea R, Robyt J. (2011). *Carbohydr Res*. 346: 280-4.