IMMOBILIZATION OF INVERTASE FROM THE OSMOTOLERANT YEAST *Candida lactis-condensi* MpIIIa ON NYLON-6 FOR THE PRODUCTION OF INVERT SUGAR

Alejandro Santiago-Hernández, Miguel Angel Plascencia-Espinosa, María Eugenia Hidalgo-Lara.
Departamento de Biotecnología y Bioingeniería, Centro de Investigación y de Estudios Avanzados México D.F. CP 07360. E-mail josealex64@yahoo.com

Key words: invertase, immobilization, invert sugar

Introduction. Invertase (ɓ-D-fructofuranosidase E.C. 3.2.1.26) hydrolyzes sucrose into an equimolar mixture of glucose and fructose, known as invert sugar syrup (ISS). This syrup is widely use in food and brewing industries because minimize the crystallization and avoid the solubilization process of solid sugar. Most of industrial invertases displayed substrate inhibition at low sucrose concentration (10%). To overcome this problem, the osmotolerant yeast *Candida lactis-condensi* MpIIIa, able to growth at high concentrations of sucrose (60%), was used as a source of invertase. A system based on immobilized invertase could be used for the affordable production of ISS.

.

The aim of this work was the covalent immobilization of the invertase from *C. lactis-condensi* MpIIIa on Nylon-6 microbeads for the production of ISS.

Methods. The invertase from *C. lactis-condensi* ñINVMpIIIaò was purified by ion exchange chromatography, and the pure enzyme was covalently immobilized to Nylon-6 as previously described (1). Enzymatic activity was determined by the release of reducing sugar. Reducing sugar were quantified by the DNS method (2). One unit of activity was defined as the amount of enzyme that produces reducing sugar equivalent to 1 ɛmol of glucose per min. Protein concentration was estimated by the Lowry method (3).

Results. INVMpIIIa was covalently immobilized on Nylon-6 with an immobilization efficiency of 89 %. The immobilized enzyme showed an invertase activity of 5299 U/g of Nylon-6, one of the highest activities reported so far. The Nylon-immobilized invertase showed optimal activity at pH 5.5 and 60ºC in 50 mM acetate buffer. The km and Vmax were measurement as 67.15 mM of sucrose and 9550 ɛmol/min/g of Nylon-6, respectively. The enzyme activity was stimulated by Ca^{2+} (40 %) and Mn^{2+} (84 %) but completely inhibited by Hg^{2+} and Zn^{2+} **(Fig. 1**)

.

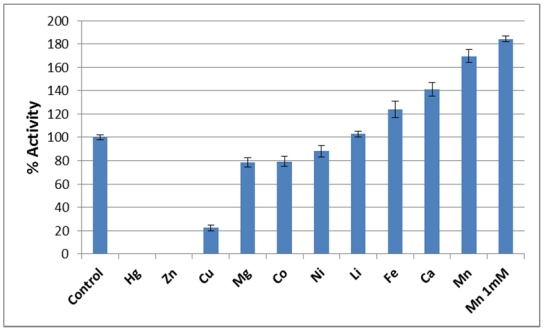

Fig.1 Effect of metals on the activity of immobilized invertase MpIIIa. The assay was carried out with 50 mM acetate buffer pH 5.5, 10 % of sucrose, added with 10 mM of metal ions.

Nylon-immobilized INVMpIIIa showed great stability retaining 50 % of its original activity even after 550 cycles of repeat use in presence of 10 % of sucrose at 25ºC. Immobilized enzyme showed great storage stability a 4ºC, retaining 75 % of its original activity after 10 months.

Conclusions. INVMpIIIa was successfully immobilized on Nylon-6. The biocatalyst obtained showed excellent catalytical properties, and good operational and storage stabilities. For these reasons, the INVMpIIIa from *C. lactis-condensi* immobilized on Nylon-6 represents a good candidate for the production of ISS.

Acknowledgments.
Research was funded by CINVESTAV, México. M. A. Plascencia received a scholarship from CONACYT, México.

References.

1. Amaya DL, Hidalgo LME, Montes HMC. (2006). *Food Chem.* 99:299-304
2. Miller GL. (1959). *Anal Chem.* 31:426-428.
3. Lowry OH. (1951). *J Biol Chem.* 193: 265-275.