

TITLE: PRODUCTION AND CHARACTERIZATION OF LEVANSUCRASE FROM *Bacillus subtilis* NATTO CCT 7712 FREE AND IMMOBILIZED

AUTHORS: BERSANETI, G.T.; SUWA, R.E.; SILVEIRA, V.A.I.; FONTOURA, I.C.C.; CELLIGOI, M.A.P.C.

INSTITUTION: UNIVERSIDADE ESTADUAL DE LONDRINA (RODOVIA CELSO GARCIA CID - PR 445 KM 380 - CAMPUS UNIVERSITÁRIO, CEP 86.057.970 – LONDRINA – PR, BRAZIL)

ABSTRACT:

Levansucrase (EC. 2.4.1.10) belongs to the family of glycoside hydrolases (GH68), enzyme produced by *Bacillus subtilis*, synthesizes fructooligosaccharides and levan using sucrose as substrate. The *B. subtilis* has potential for application due to its high capacity for multiplication and secretion of extracellular proteins. The immobilization is important to stabilize and reduce the inactivation of the enzyme by temperature and pH. It is also possible to reuse the biocatalysts immobilized, which becomes a great option for the industrial application. The objective of this study was to produce and immobilize levansucrase of *B. subtilis* natto and evaluate the pH and temperature effects on enzyme activity. Levansucrase was produced by *Bacillus subtilis* natto CCT 7712 in the medium containing (g L⁻¹): sucrose, 420.7; yeast extract, 2; KH₂PO₄, 1; (NH₄)₂SO₄, 3; MgSO₄(7H₂O), 0.6; MnSO₄, 0.2; ammonium citrate, 0.25, in Erlenmeyer flasks (2 L) containing 500 mL culture medium by 24 h. Cultures were harvest by centrifugation and supernatant was used as the source of enzyme free. Levansucrase transfructosylation activity was assayed by estimating the reducing sugar released after hydrolysis of the polymer. One unit activity (U) was defined as the amount of enzyme that released 1 µMol reducing sugars per 1 min under the experimental conditions. The supernatant was dialyzed, lyophilized and used in the immobilization process with a 3 % chitosan solution, with stirring at 5 °C for 3 h. After, free and immobilized enzymes were characterized in pHs (3.0 at 9.0) and temperatures (30 °C at 90 °C), the thermal stability for 8 h was studied in 30, 50, 70 and 90 °C. The activities were 21.5 U/mL and 19.1 U/mL for free and immobilized levansucrase. The enzyme exhibited 60.7 % of efficiency in the immobilization with chitosan support. The pH 6.0 was the best for free and immobilized levansucrase, reaching 18.3 U/mL and 19.0 U/mL of activity, respectively. At the extreme pH (3.0 and 9.0) there was a decrease in the enzymatic activity for both free and immobilized. The temperature 50 °C was better for immobilized enzyme (18.9 U/mL) and 60 °C for the free enzyme (19.9 U/mL). Levansucrase showed thermal stability at 70 °C for 8 h, with reduction of only 15.7 % for free and 9.6 % for immobilized. Levansucrase of *B. subtilis* demonstrated to be thermally stable and the immobilization process was important to produce fructooligosaccharide, molecules classified as prebiotics.

Keywords: enzyme, *Bacillus subtilis* natto, thermal stability, immobilization, sucrose.

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