Production of Levan by Bacillus subtilis Natto

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ABSTRACT

Levan is a polymer of fructose linked by β-(2→6) fructofuranosidic bonds present in many plants and microbial products. Levan offers a variety of industrial applications in the fields of cosmetics, foods and pharmaceuticals. Although many investigations on levan formation have been reported, they suffered the disadvantages of low yields and the contamination of impure products. Thus, levan has great potential if it can be produced less expensively. To further investigate the possibility of the use of Bacillus subtilis natto for the efficient production of the levan product, we studied and described in this study the factors affecting the production of levan by this Bacillus sp.; the purification and characterization of the products were also investingated. After cultivation for 24 h, 40-50 g/L of levan was produced in medium containing 20% (w/v) sucrose by B. subtilis natto. The product consisted of two fractions with different molecular masses (2 × 107 and 9 × 103 Da), and the high molecular mass product (2 × 107 Da) predominated during fermentation process (~ 24 h), which were easily separated by fractionation using an ethanol gradient. The products were well characterized by Gel permeation chromatography (GPC), Fourier transform infrared (FTIR), and Nuclear magnetic reasonance (NMR). B. subtilis natto, which was washed with sterile water, was used to produce levan from a variety of nitrogen substrates, including yeast extract, peptone, urea, NH4Cl, NaNO3 and sweet corn extract. The B. subtilis natto strain was able to assimilate the two inorganic nitrogen substrates examined (NH4Cl and NaNO3), whereas it grew less efficiently in organic nitrogen substrates (esp., yeast extract and sweet corn extract). The levan concentration and production rate (VL) was also influenced by the sucrose concentration. The sucrose was utilized preferentially for levan synthesis. Maximum levan concentration (71 g/L) and VL (2.383 g/L/h) were attained at 25% (w/v) sucrose concentration. Levan production from B. subtilis natto was affected by various environmental factors, such as pH and temperature; initial pH 7 and 37°C were favorable for levan production. Additionally, levan production by PVA immobilized-cell beads was tested. PVA with immobilized bacteria can be used as the general producer of levan.

Keywords : Bacillus subtilis natto ; fermentation ; fructan ; levan ; immobilization

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