Study on Production of Levan by Bacillus subtilis DYU1 in a Fermentor

李奕宏、吳建一 ; 顏裕鴻

E-mail: 9806223@mail.dyu.edu.tw

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 DYU1 for the efficient production of the levan product, we studied and describe in this study the factors affecting the production of levan by this bacterium and the purification and characterization of the products. After cultivation for 24 h, 40-50 g/L of levan was produced in medium containing 20% (w/v) sucrose by B. subtilis DYU1. The product consisted of two fractions with different molecular masses (2 × 107 and 1 × 104 Da), and the high molecular mass product (2 × 107 Da) predominated during fermentation process (~ 24 h), which could be 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 DYU1, 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 DYU1 strain was found to be able to assimilate the two inorganic nitrogen substrates examined (NH4Cl and NaNO3), whereas it grew less efficiently in organic nitrogen substrates (especially yeast extract and sweet corn extract). The levan concentration and production rate (VL) were also influenced by the sucrose concentration. The sucrose was available utilized preferentially for levan synthesis. Maximum levan concentration (85 g/L) and VL (2.350 g/L/h) were attained at 25% sucrose concentration. Levan production from B. subtilis DYU1 was also affected by various environmental factors, i.e. pH and temperature, as pH 7 and 37°C were favorable for levan production.

Keywords : Bacillus subtilis DYU1、fermentation、fructan、levan

Table of Contents

圖目錄

表目錄

REFERENCES

1. Abdel-Fattah, A. F., Mahmoud, D. A. R. and Esawy, M. A. T. 2005. Production of levansucrase from Bacillus subtilis NRC 33a and enzymic synthesis of levan and fructo-oligosaccharides. Current Microbiology 51: 402-407.2.Allen, D. G. and Robinson, C. W. 1989. Hydrodynamic and mass transferr in Aspergillus niger fermentations in bubble column and loop bioreactors. Biotechnology and Bioengineering 34: 731-740.3.Allen, D. G. and Robinson, C. W. 1990. Measurement of rheological propertyes of filamentous fermentation broths. Chemical Engineering Science 45: 37-48.4.Ammar, Y. B., Matsubara, T., Ito, K., Iizuka, M., Limpaseni, T., Pongsawasdi, P. and Minamiura, N. (2002) Characterization of a thermostable levansucrase from Bacillus sp. Th4-2 capable of producing high molecular weight levan at high temperature. Journal of Biotechnology 99: 111-119.5.Archbold, H. K. 1938. Physiological studies in plant nutrition. VII. The role of fructoseans in the carbohydrate metabolism of the barley plant. Annals of Botany 2: 403-1938.6.Arrhenius, S. 1889. On the reaction velocity of the inversion of cane sugar by acids. Zeitschrift fur Physikalische Chemie. 4: 226-232.7.Arvidson, S. A., Todd Rinehart, B. and Gadala-Maria, F. 2006. Concentration regimes of solutions of levan polysaccharide from Bacillus sp. Carbohydrate Polymers 65: 144-149.8.Atkins, P. 2001. The elements of physical chemistry. (3rd ed.). Oxford University Press Inc., New York.9.Avigad, G. 1968. Levans. In Bikales, N. M. (Ed.), Encyclopedia of polymer science and technology. p. 711-718. Johb Wiley & Sons, Inc., New York.10.Bae, I. Y., Oh, IK. Lee, S. Yoo , SH. Lee, H. G. 2008. Rheological characterization of levan polysaccharides from Microbacterium laevaniformans. International Journal of Biological Macromolecules 42: 10-13.11.Barrow, K. D., Collins, J. G., Leigh, D. A., Rogers, P. L. and Warr, R. G. 1984. Sorbitol production by Zymomonas mobilis. Applied Microbiology and Biotechnology 20: 225-232.12.Beker, M. J., Shvinka, J. E., Pankova, L. M., Laivenienks, M. G. and Mezhbarde, I. N. 1990. A simultaneous sucrose bioconversion into ethanol and levan by Zymomonas mobilis. Applied Biochemistry and Biotechnology 24/25: 265-274.13.Bekers, M., Linde, R., Danilevich, A., Kaminska, E., Upite, D., Vigants, A. And Scherbaka, R. 1999. Sugar beet diffusion juice and syrup as media for ethanol and levan production by Zymomonas mobilis. Food Biotechnology 13: 107-119.14.Bekers, M., Upite, D., Kaminska, E., Grube, M., Laukevics, J., Vina, I., Vigants, A. and Zikmanis, P. 2003. Fructan biosynthesis by intra- and extracellular Zymomonas mobilis levansucrase after simultaneous production of ethanol and levan. Acta Biotechnologica 1: 85-93.15.Bekers, M., Upite, D., Kaminska, E., Laukevices, J., Grube, M., Vigants, A. And Linde, R. 2005. Stability of levan produced by Zymomonas mobilis. Procsee Biochemistry 40: 1535-1539.16.Bekers, M., Vigants, A., Laukevics, J., Toma, M., Rapoports, A., Zikmanis, P. 2000. The effect of osmo-induced stress on product formation by Zymomonas mobilis on sucrose. International Journal of Food Microbiology 55: 147-150.17.Belghith, H., Song, K. B., Kim, C. H. and Rhee, S. K. 1996. Optimal conditions for levan formation by an

overexpressed recombinant levansucrase. Biotechnology Letters 18: 467-472.18.Ben Tovim, R. And Hestrin, S. 1957. Patterns of levandegradation by extracellular enzymes in bacteria. Bulletin of the Research Council of Israel. Section A, 6A: 31019.Bender, M. M. and Smith, D. 1973. Classification of starch and fructosan-accumulating grasses as C-3 or C-4 species by carbon isotope analysis. Journal of the British Grassland Society 28: 97-100.20.Beveridge, T. J. and Graham, L. L. 1991. Surface layers of bacteria. Microbiological Revuews 55: 684-705.21.Biedrzycka, E. and Bielecka, M. 2004. Prebiotic effectiveness of fructans of different degrees of polymerization. Trends in Food Science & Technology 15: 170-175.22.Blake, J. D., Clarke, M. L., Jansson, P. E. and Mcneil, K. E. 1982. Fructan from Erwinia herbicola. Journal of Bacteriology 151: 1595-1597.23.Bodie, E. A., Schwartz, R. D. and Catena, A. 1985. Production and Characterization of a polymer from Arthrobacter sp. Applied and Environmental Microbiology 50: 629-633.24.Bothner, H., Waaler, T. and Wik, O. 1988. Limiting viscosity number and weight average molecular weight of hyaluronate samples produced by heat degradation. International Journal of Biological Macromolecules 10: 287-291.25.Calazans, G. M. T., Lopes, C. E., Lima, R. M. O. C., Franca, F. P. 1997. Antitumoural activities of levans produced by Zymomonas mobilis strains, Biotechnology Letters 19: 19-21.26.Ceska, M. 1971. Biosynthesis of levan and a new method for the assay of levansucrase activity. Biochemical Journal 125: 209-211.27.Chambert, R. and Petit-Glatron, M. F. 1989. Study of the effect of organic solvents on the synthesis of levan and the hydroysis of sucrose by Bacillus subtilis levansucrase. Carbohydrate Research 191: 117-123.28.Chambert, R., Treboul, G. and Dedonder, R. 1974. Kinetic studies of levansucrase of Bacillus subtilis. European Journal of Biochemistry 41: 285-300.29.Chung B. H., Kim W. K., Song K. B., Kim C. H. and Rhee S. K. 1997. Novel polyethylene glycol/levan aqueous two-phase system for protein partitioning. Biotechnology techniques 11: 327-329.30.Clarke, M. A., Roberts, E. J. and Garegg, P. J. 1997. New compounds from microbiological products of sucrose, Carbihydrate Polymer 34: 425.