Production of Biopolymers by Commercial Natto Spores

游芸悌、施英隆

E-mail: 9314401@mail.dyu.edu.tw

ABSTRACT

Bacillus subtilis natto Takhashi strain, a commercial natto starter commonly used to prepare fermented soybeans products Natto, was manipulated by cultivating in appropriate media for simultaneous or selective production of poly (γ -glutamic acid) and levan. The simultaneous production of poly (γ -glutamic acid) and levan was obtained when the bacteria was cultivated in a basal medium containing both sucrose and L-glutamate. The selective production of the poly (γ -glutamic acid) or levan was obtained when the bacteria was cultivated in the medium without the presence of sucrose or L-glutamate, respectively. The products were well characterized by GPC, NMR, amino acid analysis. Bacillus subtilis natto Takhashi strain selectively produced a large quantity of extracellular γ -Poly glutamic acid (γ -PGA) when it was grown on 100 ml Medium E culture (Glutamic acid：2% , C3H4(OH)(COOH)3：1.2%, Glycerol：8%, NH4Cl：0.7%, FeCl3・6H2O：0.004%, MnSO4・7H2O：0.0104% , CaCl2・2H2O：0.015%, MgSO4・7H2O：0.05%, K2HPO4：0.05%). The optimum pH, temperature and agitation speed were 7.4, 37℃, and 175 rpm respectively. Under such condition Bacillus subtilis natto Takhashi strain produced a maximum of 1.7 g of γ -Poly glutamic acid (γ -PGA) in 100 ml of Medium E culture. Bacillus subtilis natto Takhashi strain selectively produced a large quantity of extracellular levan, when it was grown on 100 ml of 20% sucrose solution (Sucrose：20%, MgSO4・7H2O ：0.05%, NaH2PO4・2H2O：0.3%, Na2HPO4・12H2O：0.3%). The optimum pH, temperature and agitation speed were 7, 37℃ and 175 rpm respectively. Under such condition Bacillus subtilis natto (Takhashi strain) produced a maximum of 5.06 g of levan in 100 ml medium. When Bacillus subtilis natto Takhashi strain was grown on 100 ml medium containg both sucrose and glutamic acid (Sucrose：5%, Glutamic acid：1.5%, MgSO4・7H2O：0.05%, NaH2PO4・2H2O：0.3%, Na2HPO4 ・12H2O：0.3%), it produced 1.2 g of a mixture containg levan and γ -PGA after cultivation at 37℃, 175 rpm, pH 7 for 21 hr. The levan product consisted of two fractions of different molecular weight (1,790,000 and 11,000 Da), which was easily separated by ethanol fractionation. The effects of sucrose concentrations, initial pH, fermentation temperature, agitation speed on the levan productivity were also described.

Keywords : levan ; γ -Poly glutamic acid (γ -PGA) ; Bacillus subtilis natto Takhashi strain ; fermentation ; biopolymers ; shaking flask

Table of Contents

REFERENCES

1. 范宜琮(2001)以苔蘚桿菌生產聚麩胺酸之研究。大葉大學環境工程系碩士論文。 2. 高馥君、呂美玲、陳文亮、蔡維鐘(1998)黃豆副產物之保存-豆渣之乳酸發酵。食品科學（中華民國八十七年十二月/第二十五卷第六期:第787至798頁） 3. 中村巧(1983)公開特許公報, 昭58-16641. 4. 加藤丈雄,志賀一三,寺尺和弘(1986)日本食品科學工學會誌 33, 837. 5. Avigad, G., Levans. In Encyclopedia of polymer Science and Technology, Microbial Polysaccharides Vol. 8, ed. N. M. Bikales and J. Conrad. John Wiley & Sons, New York, (1968), pp. 711-718. 6. Bovarnick, M., (1942). The formation of extracellular D(-)glutamic acid polypeptide by Bacillus subtilis. J. Biol. Chem. 145, 415-424. 7. Cheng, C., Asada, Y., Aaida T., (1989). Production of γ-polyglutamic acid by Bacillus subtilis A35 under denitrifying conditions. Agric. Biol. Chem. 53, 2369-2375. 8. Choi, H. J., Kunioka, M., (1995). Preparation conditions and swelling equilibria of hydrogel prepared by γ-irradiation from microbial poly (γ-glutamic acid). Radiat. Phys. Chem. 46, 175-179. 9. Choi, H. J., Yang, R., Kunioka, M., (1995). Synthesis and characterization of pH-sensitive and biodegradable hydarogels prepared by γ-irradiation using microbial poly (γ-glutamic acid) and polu (ε-lysine). J. Appl. Polym. Sci. 58, 807-814. 10. Clarke, M. A., Roberts, E. J., Tsang, W. S. C., Godshell, M. A., Han, Y. W., Kenne, L., and Lindburg. B. (1988). Proc. Sugar Process. Res. Conf., New Orleans. La. 11. Daninippon Parmaceutical Co, Ltd., (1972). Ice cream stabilizer, JP Patent 19735/72. 12. Dearie, L., Toncheva, V., Dubruel, P., Schacht, E. H., Barrett, L., Seymour, L.W., (2000). Poly-L-glutamic acid derivatives as vectors for gene therapy. J.Control. Release 65, 187-202. 13. Dedonder, R. (1966). Levansucrase from Bacillus subtilis. Methods Enzymol. 8, 500-505. 14. Elisashvili, V. I., (1984). Levan synthesis by Bacillus sp. Appl. Biochem. Microbiol. 20, 82-87. 15. Evans, T. H., and Hibbert, H. (1946). Bacterial polysaccharides. Adv. Carbohydr. Chem. 2, 253-277. 16. Fujii, H., (1963). On the formation of mucilage by Bacillus natto. PartIII. Chemical constitutions of mucilage in natto (1). Nippon Nogeikagaku Kaishi 37, 407-411. 17. Goto, A., and Kunioka, M., (1992). Biosynthesis and hydrolysis of Poly (γ-glutamic acid) from Bacillus subtilis IFO3335. Biosci. Biotechnol. Biochem. 56, 1031-1035. 18. Han, Y. W., Microbial levan. Adv. Appl. Microbiol. 35 (1990). 171-194. 19. Han, Y. W., and Clarke, M. A., (1990). J. Production and Characterization of Microbial Levan. J. Agric. Food Chem. 38 (2), 393-396. 20. Han, Y. W. Levan Production by Bacillus polymyxa. J. Ind. Microbiol, (1989), 4, 447-451, in press. 21. Hara, T., Ueda, S., (1982). Regulation of polyglutamate production in Bacillus subtilis (natto); transformation of high PGA productivity. Agric. Biol. Chem. 46, 2275-2281. 22. Hara, T., Fujio, Y., Ueda, S., (1982). Polyglutamate production by Bacillus subtilis (natto).J. Appl. Biochem 4, 112-120. 23. Hasebe, K., Inagaki, M., (1999). Preparation composition for external use containing gammapolyglutamic acid and vegetable extract in combination. JP Patent 11240827. 24. Hayashi, H.；Takiuchi, K.；Murao, S.；Arai, M. Structure and insecticidal activity of new indole alkaloids, okaramines A and B, from Penicillium simplicissimum AK-40. Agric. Biol. Chem. (1989), 53, 461-470. 25. Hehre, E. J., (1955). Polysaccharide Synthesis from Dusaccharides. Methods Enzymol. 1, 178-192. 26. Hestrin, S., Feingold, D. S., and Avigad, G. The mechanism of polysaccharide production from sucrose. 3. Donor-acceptor specificity of levansucrase from Aerobacter levanicum. BiochemicaljOURNAL, (1955). 64, 340-351. 27. Ito, Y., Tanada, T., Ohmachi, T.,Asada, Y., (1996). Glutamic acid independent production of Poly(γ-glutamic acid) by Bacillus subtilis TAM-4. Biosci. Biotechnol. Biochem. 60, 1239-1242. 28. Ito, H., and Ito, H., (1985) Manufacture of snack foods. US Patent 4,509,942. 29. Ivanovics, G., Bruckner, V., (1937). Chemische und immunologische Studien uber den Mechanimus der Milzbrandinfektion und der serologisch identischen spezifischen Substanz des Bacillus mesentericus. Z Immunitatsforsch 90, 304-318. 30. Ivanovics, G., Erdos, L., (1937). Ein Beitrag zum Wesen der Kapselsubstanz des Milzbrandbazillus. Z Immunitatsforsch 90, 5-19. 31. Kang. K. S., and Cottrell, I. W. (1979). In “ Microbial Technology ”（H. J. Peppler and D. Perlman, eds.), 2nd Ed., Vol. 1, pp, 417-481. Academic Press, New York. 32. Khare, S. K.；Jha, K.；Gandhi, A. P. Citric acid production from okara (soy-residue) by solid-state fermentation. Bioresour Technol. (1995a), 54, 323-325. 33. Khare, S. K.；Jha, K.；Sinha, L. K. Preparation and nutritional evaluation of okara fortified biscuits. J. Dairying, Foods Home Sci. (1995b), 14, 91-94；Biol. Abstr. 103, 20755. 34. Kinnersley, A., Atrom, D., Meach, A. R. V. and Koskan, C. P. (1994). Composition and method for enhanced fertilizer uptake by plants. Patent WO94/09628. 35. Kubota H, Nambu Y, Ento T (1993b) Convenient and quantitative esterification of poly (γ-glutamic acid)produced by microorganisms. J. Polym. Sci. Part A Polym. Chrm. 31:2877-2878. 36. Kunioka, M., (1993). Properties of hydrogels prepared by γ-irradiation in microbial poly (γ-glutamic acid)aqueous solutions. Kobunshi Ronbunshu. 50, 755-760. 37. Kunioka, M., Goto, A., (1994). Biosynthesis of Poly(γ-glutamic acid) from L-glutamic acid, citric acid, and ammonium sulfate in Bacillus subtilis IFO3335. Appl. Microbiol Biotechnol. 40, 867-872. 38. Kunioka, M., (1995). Biosynthesis of Poly (γ-glutamic acid)from L-glutamine, citric acid, and ammonium sulfate in Bacillus subtilis IFO3335. Appl. Microbiol Biotechnol. 44, 501-506. 39. Kunno, A., Taguchi, t., Yamaguchi, T., (1988). New use of polyglutamic acid for foods EP 0284386. 40. Kunno, A., Taguchi, t., Yamaguchi, T., (1988b). Bakery products and noodles containing polyglutamic acid. US Patent 4, 888, 193. 41. Leonard, C. G., Housewright, R. D., Thorne, C. B., (1958). Effect of metal ions on glutamyl polypeptide synthesis by Bacillus subtilis. J. Bacteriol. 76, 499-503. 42. Leonard, C. G., Housewright, R. D., Thorne, C. B., (1958). Effect of metal ions on the optical specificity of glutamine synthetase and glutamyl transferase of Bacillus licheniformis. Biochem. Biophys. Acta 62, 432-434. 43. Li, C., Yu, D. F., Newman, A., Cabral, F., Stephens, C., Hunter, N., Milas, L., Wallace, S., (1998). Complete regression of well-established tumors using novel water-soluble poly(L-glutamic acid)-paclitaxel conjugate. Cancer Res. Res 58, 2404-24009. 44. Li, C., Price, J. E., Milas, L., Hunter, N. R., Ke, S., Tansey, W., Charnsagavej, C., Wallace, S., (1999). Antitumor activity of poly (L-glutamic acid)-paclitaxel on syngeneic and Xenografed tumors. Clin Canser Res. 5, 891-897. 45. Lindberg, B., Lonngren, J., and Thompson, J. J., (1973). Methylation studiem on levans. Acta Chem. Scand. 27, 1819-1821. 46. Matsuo, M. In vivo antioxidant activity of okara koji, a fermented okara, by Aspergillus oryzae. Biosci., Biotechnol., Biochem. (1997a), 61, 1968-1972；Chem. Abstr. 128, 166597. 47. Matsuo, M. Preparation and components of okara-ontjom, a traditional Indonesian fermented food. Nippon Shokuhin Kagaku Kogaku Kaishi (1997b), 44, 632-639；Chem. Abstr. 127, 277461. 48. McLean, R. C., Wolf, D. C., Ferris, F. G., Beveridge, T. J., (1990). Metal-binding characteristics of the gamma-glutamyl capsular polmer of Bacillus licheniformis

ATCC9945. Apply. Environ. Microbiol. 56, 3671-3677. 49. McLean, R. C., Beauchemin, D., Beveridge, T. J., (1992). Influence of oxidation state on iron binding by Bacillus licheniformis capsule. Apply. Environ. Microbiol. 58, 405-408. 50. Mitsuiki, M., Mizuno, A., Tanimoto, H., Motoki, M., (1998). Relationship between the antifreeze activities and the chemical structures of oligo- and poly (glutamic acid)s. J. Agric. Food Chem. 46, 891-895. 51. Miyamura, H.；Takenaka, Y.；Takenaka, T. The utility of okara, a byproduct of the soybean processing industry. II. Fibrinolytic activity of okara fermented Bacillus subtilis. Nippon Shokuhin Kagaku Kogaku Kaishi (1998), 45, 100-107 {Japanese} ；Chem. Abstr. 128, 166412. 52. Noguchi, A. Method for the preparation of textured soybean draff. U.S. Patent US 4 642 241, (1987)；Food Sci. Technol. Abstr. 87-08-V0076. 53. Ohno, A.；Ano, T.；Shoda, M. Production of the antifungal peptide antibiotic, iturin by Bacillus subtilis NB22 in solidstate fermentation. J. Ferment. Bioeng. (1993), 75, 23-27. 54. Ohno, A.；Ano, T.；Shoda, M. Use of soybean curd residue, okara, for the solid-state substrate in the production of a lipopeptide antibiotic, iturin A, by Bacillus subtilis NB22 Process Biochem. (1996), 31, 801-806. 55. Oku, T., Tokunaga, T, Hosoya N. Nondigestibility of new sweetener ‘ Neosugar ’ in the rat. J Nutr (1984)；114(9)；1574-1581. 56. Otani, Y., Tabata, Y., Ikada, Y., (1996). Anew biological glue from gelatin and poly(L-glutamic acid). J. Biomed. Mater. Res. 31, 157-166. 57. Otani, Y., Tabata, Y., Ikada, Y., (1996). Rapidly curable biological glue composed of gelatin and poly(L-glutamic acid). Biomaterials 17, 1381-1391. 58. Otani, Y., Tabata, Y., Ikada, Y., (1998). Effect of additives on gelation and tissue adhesion of gelatin —poly(L-glutamic acid),. Biomaterials 19, 2167-2173. 59. Otani, Y., Tabata, Y., Ikada, Y., (1998). Hemostatic capability of rapidly curable from gelatin, poly(L-gllutamic acid), and carbodiimide. Biomaterials 19, 2091-2098. 60. Pontis, H. G., and Del Campillo, E. (1985). In “ Biochemistry of Storage Carbohydrates in Green Plants ” (P. M. Dey and R. A. Dixon, eds.), pp. 205-227. Academic Press, New York. 61. Potter, M., Oppermann-Sanio, F. and A. Steinbuchel (2001). Cultivation of bacteria producing polyamino acids with liquid manure as carbon and nitrogen sources. Appl. Environ. Microbiol. 67, 617-622. 62. Sakai, K., Sonoda, C., Murase, K., (2000). Bitterness reliving agent. JP Patent WO0021390. 63. Sawamura, S., (1913). On Bacillus natto. J. Coll. Agric. Tokyo 5, 189-191. 64. Shih, I. L., and Van, Y. T., (2001). The production of poly (γ -glutamic acid)from microorganisms and its various applications. Bioresource Technol. 79, 207-225. 65. Stivala, S. S., and Zweig, J. E., Physicochemical parameters of partially hydrolysed Streptococcus salivarius levan fractions. Biopolymers, (1981). 20, 606-620. 66. Tanaka, T. O., Susumu, and T. Yamamoto, (1979). Synthesis of levan by levansucrase. Some factor affecting the rate of synthesis and degree of polymerization of levan. J. Biochem. 85, 287-293. 67. Tanimoto, H., Sato, H., Kuraishi, C., Kido, K., Seguto, K., (1995) High absorption mineral-containing composition and foods. US patent5447, 732. 68. Tanimoto, H., Sato, H., Karasawa, M., Iwasaki, K., Oshima, A., Adachi, S., (2000). Feed composition containing poly (γ -glutamic acid). JP Patent WO96353399. 69. Thorne, C. B., Gomez, C. G., Noyes, H. E., Housewright, R. D., (1954). Production of glutamyl polypeptide by Bacillus subtilis. J.Bacteriol. 65, 307-315. 70. Troy, F. A., (1973). Chemistry and biosynthesis of the poly (γ -D-glutamyl) capsule in Bacillus licheniformis .1.Properties of the membrane-mediated biosynthetic reaction. J. Biol. Chem.248, 305-316. 71. Yamanaka, S., (1991). New gamma-polyglutamic acid, production therefore and drinking agent contamining the same. JP Patent 3047087. 72. Yokoi, H., Natsuda, O., Hirose, J., Hayashi, S., Takasaki, Y., (1995), Characteristics of a biopolymer flocculant produced by Bacillus sp. PY-90. J. Ferment. Bioeng. 79, 378-380. 73. Yokota, H., Arima, T., Hirose, J., Hayashi, S., Takasaki, Y., (1996), Flocculation properties of poly (gamma-glutamic acid)produced by Bacillus subtilis. J. Ferment. Bioeng. 82, 84-87.