

以納豆菌生產生物性高分子之研究

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摘要

本研究探討並發現市售納豆菌(*Bacillus subtilis natto Takhashi strain*)可於不同培養基中生產不同型態之生物性高分子。當市售納豆菌(*Bacillus subtilis natto Takhashi strain*)於100 ml Medium E培養基 (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%) 中培養，可生產大量胞外聚合之聚麴胺酸(-PGA)產物，其培養基最適之pH值為7.4，最適之溫度為37，最適轉速為175 rpm。於此條件下培養6天可生產出大量的產物(1.7 g/100 ml)之聚麴胺酸(-PGA)。該產物經由氨基酸分析及H1—NMR分析可確定產物全是聚麴胺酸而無菌果聚醣之產物。當市售納豆菌(*Bacillus subtilis natto Takhashi strain*)於100 ml添加蔗糖但未添加麴胺酸之培養基 (蔗糖 : 20% , MgSO₄ 7H₂O : 0.05% , NaH₂PO₄ 2H₂O : 0.3% , Na₂HPO₄ 12H₂O : 0.3%) 中，可生產大量胞外聚合之菌果聚醣產物，其培養基最適之pH值為6，培養基最適之溫度為37，最適轉速175 rpm。於此條件培養21 hr可生產出大量的產物(5.06 g/100 ml)之菌果聚醣。該產物經由C13—NMR、H1—NMR及霍氏轉換紅外光譜儀(FTIR)分析，得知其產物僅有菌果聚醣(Levan)而無聚麴胺酸之產生。當市售納豆菌(*Bacillus subtilis natto Takhashi strain*)於100 ml添加蔗糖及麴胺酸之培養基 (蔗糖 : 5% , Glutamic acid : 1.5% , MgSO₄ 7H₂O : 0.05% , NaH₂PO₄ 2H₂O : 0.3% , Na₂HPO₄ 12H₂O : 0.3%) 中，可生產大量胞外聚合之菌果聚醣及聚麴胺酸之混合物。於pH值7，溫度37，轉速175 rpm，條件下培養21 hr可生產出大量的產物(1.2 g/100 ml)之含有菌果聚醣之聚麴胺酸產物。該產物經由氨基酸分析及C13—NMR分析，可得知其產物為含有菌果聚醣及聚麴胺酸之產物。本實驗亦發現市售納豆菌(*Bacillus subtilis natto Takhashi strain*)所生產之菌果聚醣之分子量有兩群，一為分子量為1,790,000之產物，另一者分子量為11,000之產物。此二群產物可以逐步添加冷凍酒精之分段沉澱法加以分離。

關鍵詞：菌果聚醣；聚麴胺酸；納豆菌；發酵；生物性高分子；搖瓶培養

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參考文獻

- 范宜琮(2001)以苔蘚桿菌生產聚麴胺酸之研究。大葉大學環境工程系碩士論文。
- 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)
- 中村巧(1983)公開特許公報,昭58-16641.
- 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.
- 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.
- Bovarnick, M., (1942). The formation of extracellular D(-)glutamic acid polypeptide by *Bacillus subtilis*. *J. Biol. Chem.* 145, 415-424.
- Cheng, C.,

Asada, Y., Aaida T., (1989). Production of -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 -irradiation from microbial poly (-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 hydargels prepared by -irradiation using microbial poly (-glutamic acid) and poly (-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 Pharmaceutical Co, Ltd., (1972). Ice cream stabilizer, JP Patent 19735/72. 12. Dearie, L., Toncheva, V., Dubrule, 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*. Part . Chemical constitutions of mucilage in natto (1). *Nippon Nogekagaku Kaishi* 37, 407-411. 17. Goto, A., and Kunioka, M., (1992). Biosynthesis and hydrolysis of Poly (-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 gammopolyglutamic 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(-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 über den Mechanismus der Milzbrandinfektion und der serologisch identischen spezifischen Substanz des *Bacillus mesentericus*. *Z. Immunittatsforsch* 90, 304-318. 30. Ivanovics, G., Erdos, L., (1937). Ein Beitrag zum Wesen der Kapselsubstanz des Milzbrandbazillus. *Z. Immunittatsforsch* 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. *Bioreour 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 (-glutamic acid)produced by microorganisms. *J. Polym. Sci. Part A Polym. Chrm.* 31:2877-2878. 36. Kunioka, M., (1993). Properties of hydrogels prepared by -irradiation in microbial poly (-glutamic acid)aqueous solutions. *Kobunshi Ronbunshu.* 50, 755-760. 37. Kunioka, M., Goto, A., (1994). Biosynthesis of Poly(-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 (-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 Xenografted tumors. *Clin Cancer 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. . 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 (-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 (-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 (-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.