Study on Production of Poly(glutamic acid) Using Bacillus subtilis DYU1 and Its Flocculation Property

陳右承、吳建一

E-mail: 9806225@mail.dyu.edu.tw

ABSTRACT

In this study, we use B. subtilis DYU1 as γ-Polyglutamic acid (γ-PGA) producer to investigate the effect of medium compositions and incubated conditions on γ-PGA production in flask. The maximum γ-PGA production (32.1 g/L) was obtained when it was grown in the medium containing 30 g/L L-glutamic acid. The applicable temperatures for producing γ-PGA were between 35 – 38 °C. The addition of amino acid solution to the medium could not enhance the γ-PGA production. On the other hand, we also investigate the effect of agitation speed and aeration rate on the γ-PGA production in a 5-L fermentor. Furthermore, in order to reduce the production costs, the molasses was used as basic production medium, at the same time the waste could be reused. The results suggest that the molasses has high potential for producing γ-PGA. The rheological properties of γ-PGA fermentation broth were studied using a rotational viscometer at several pHs and temperatures. The mathematical models were developed for determining the apparent viscosity of γ-PGA fermentation broth as affected by temperature and γ-PGA concentration. Potential applications of γ-PGA as humectant and flocculant was also study in this research and, the results show that γ-PGA has high moisture-absorption and moisture-retention capacities. Furthermore, the γ-PGA solution also has high moisture-retention capacity. Three kinds of γ-PGA (as flocculant) and the polyaluminum chloride (PAC) (as coagulant) were use to perform the flocculation test of E. coli. The γ-PGA produced from B. subtilis DYU1 was found to have excellent flocculating ability for E. coli suspension. Moreover, mechanisms describing the flocculation process with γ-PGA were proposed based on the experimental observations.

Keywords : Bacillus subtilis DYU1、 γ-PGA、fermentor、molasses、rheological properties、flocculation

Table of Contents

圖目錄

表目錄

REFERENCES

1.劉燊元，2005，篩選聚麩胺酸生產菌株及其絮凝性質之研究。大葉大學生物產業科技研究所碩士論文，彰化。2.呂媚君，2007，利用篩選之菌株Bacillus subtilis DYU1生產具麩胺酸之研究。大葉大學生物產業科技研究所碩士論文，彰化。3.楊思廉，1993，工業化學概論

，五洲出版社，台北，pp. 431-443。4.Afendra, A. S., Yiannaki, E. E., Palaiomylitou, M. A., Kyriakidis, D. A., Drainas, C. 2002. Co-production of ice nuclei and xanthan gum by transformed Xanthomonas campestris grown in sugar beet molasses. Biotechnology Letters 24: 579 – 583.5.Akagi, T., Higashi, M., Kaneko, T., Kida, T. and Akashi, M. 2005a. In vitro enzymatic degradation of nanoparticles prepared from hydrophobically-modified poly(gamma-glutamic acid). Macromolecular Bioscience 5: 598 – 602.6.Akagi, T., Kaneko, T., Kida, T. and Akashi, M. 2005b. Preparation and characterization of biodegradable nanoparticles based on poly(gamma-glutamic acid) with l-phenylalanine as a protein carrier. Journal of Control Release. 108: 226 – 236.7.Allen, D. G. and Robinson, C. W. 1989. Hydrodynamics and mass transfer in Aspergillus niger fermentations in bubble column and loop bioreactors. Biotechnology and Bioengineering. 34: 731- 740.8.Allen, D. G. and Robinson, C. W. 1990. Measurement of rheological properties of filamentous fermentation broths. Chemical Engineering Science. 45: 37- 48.9.Arrhenius, S. 1889. On the reaction velocity of the inversion of cane sugar by acids. Zeitschrift fur Physikalische Chemie. 4: 226- 232.10.Ashiuchi, M. and Misono, H. 2002a. Poly- γ -glutamic acid. In Biopolymers Vol. 7, S.R. Fahnestock, and A. Steinb?chel, eds.(Weinheim, Germany: Wiley-VCH) pp. 123 – 174.11.Ashiuchi, M., and Misono, H. 2002b. Biochemistry and molecular genetics of poly-gamma-glutamate synthesis. Applied Microbiology and Biotechnology 59:9 – 14.12.Ashiuchi, M., Kamei, T., and Misono, H. 2003a. Poly-gamma-glutamate synthetase of Bacillus subtilis. Journal of molecular catalysis. B, Enzymatic 23:101 – 106.13.Ashiuchi, M., Kamei, T., Baek, D. H., Shin, S. Y., Sung, M. H., Soda, K., Yagi, T., and Misono, H. 2001a. Isolation of Bacillus subtilis(chungkookjang), a poly-gamma-glutamate producer with high genetic competence. Applied Microbiology and Biotechnology 57:764 – 769.14.Ashiuchi, M., Nawa, C., Kamei, T., Song, J.J., Hong, S.P., Sung, M.H., Soda, K., and Misono, H. 2001b. Physiological and biochemical characteristics of poly- γ -glutamate synthetase complex of Bacillus subtilis. European Journal of Biochemistry 268:5321-5328.15.Ashiuchi, M., Shimanouchi, K., Nakamura, H., Kamei, T., Soda, K., Park, C., Sung, M. H., and Misono, H. 2004. Enzymatic synthesis of high-molecular-mass poly- γ -glutamate and regulation of its stereochemistry. Applied and Environmental Microbiology 70:4249 – 4255.16.Ashiuchi, M., Soda, K., and Misono, H. 1999a. Characterization of yrpC gene product of Bacillus subtilis IFO 3336 as glutamate racemase Isozyme. Bioscience, biotechnology, and biochemistry 63:792-798.17.Ashiuchi, M., Soda, K., and Misono, H. 1999b. A poly-gamma-glutamate synthetic system of Bacillus subtilis IFO 3336: Gene cloning and biochemical analysis of poly-gamma-glutamate produced by Escherichia coli clone cells. Biochemical and Biophysical Research Communications 263:6 – 12.18.Ashiuchi, M., Tani, K., Soda, K., and Misono, H. 1998. Properties of glutamate racemase from Bacillus subtilis IFO 3336 producing poly-??glutamate. The Journal of Biochemistry 123:1156-1163.19.Atkins, P. 2001. The elements of physical chemistry. (3rd ed.). Oxford University Press Inc., New York.20.Atkinson, B., Matvituna, F. 1983. Biochemical engineering and biotechnology handbook. ISBN: 0333332741; 978-033333274-021.Avichezer, D., Schechter, B., Arnon, R. 1998. Functional polymers in drug delivery: carrier-supported CDDP (cis-platin) complexes of polycarboxylates-effect on human ovarian carcinoma. Reactive and Functional Polymers 36: 59-69.22.Bajaj I. B., Lele, S. S., and Singhal, R. S. 2009. A statistical approach to optimization of fermentative productionof poly(γ -glutamic acid) from Bacillus licheniformis NCIM 2324. Bioresource Technology100: 826-83223.Bai, F., Zeng, C., Yang, S., Zhang, Y., He, Y. and Jin, J. 2008. The formation of a novel supramolecular structure by amyloid of poly-L-glutamic acid. Biochemical and Biophysical Research Communications 369: 830-834.24.Baranska, H., Labudzinska, A., and Terpinski, J., “ Laser Raman Spectroscopy-Analytical Applications, ” Ellis Horwood, Chichester, UK (1987).25.Beaulieu, M., Beaulieu, Y., Me?linard, J., Sithian, P., Goulet, J. 1995. Influence of Ammonium Salts and Cane Molasses on Growth of Alcaligenes eutrophus and Production of Polyhydroxybutyrate. Applied and Environmental Microbiology 61(1): 165-169.26.Bender, H., Rodriguez-Eatun, S., Ekanemesang, U. and Phillips, P. 1994. Characterization of metal-binding bioflocculans produced by the cyanobacterial component of mixed microbial mats. Applied and Enviromental Microbiology 60: 2311-2315.27.Bhattacharya, S., Bhat, K. K. and Raghuver, K. G. 1992. Rheology of bengal gram cicer arictinum flour suspensions. Journal of Food Engineering 17: 83-96.28.Birrer, G. A., Cromwick, A. M. and Gross, R. A. 1994. γ -Poly(glutamic acid) formation by Bacillus licheniformis 9945A. physiological and biochemical studies. International Journal of Biological Macromolecules 16: 265-275.29.Borb?ly, M., Nagasaki, Y., Borb?ly, J., Fan, K., Bhogle, A., and Sevoian, M. 1994. Biosynthesis and chemical modification of poly(γ -glutamic acid). Polymer Bulletin 32: 127-132.30.Borst A. H., Haverich, G., Walterbush, G., Maatz, W., Messmer, M. 1982. Fribin adhesive: an important hemostatic adjunct in cardiovascular operation. Journal of Thoracic and cardiovascular surgery 84: 548-553.31.Bovarnick, M. 1942. The formation of extracellular D(-)glutamic acid poypeptide by Bacillus subtilis. The Journal of Biological Chemistry 145:415-424.32.Bratby, J. 1980. Coagulation and flocculation, Ch. 8, Upland Press, Croydon, UK.33.Buescher, J. M. and Margaritis, A. 2007. Microbial biosynthesis of polyglutamic acid biopolymer and applications in the biopharmaceutical, biomedical and food industries Criterion Review Biotechnology 27: 1-19.34.Candela, T. and Fouet, A. 2006. Poly-gamma-glutamate in bacteria. Molecular Microbiology 60: 1091-1098.35.Cejka, A. 1985. Preparation of media. In: Rehm, H.-J., Reed, G., Brauer, H.(eds.). Biotechnology,Vol. 2. Weinheim(Germany): VCH, 629 – 698.36.Cell Therapeutics Inc. 2000. Company press release. www.cticseattle.com.37.Cheng, C., Asada, Y., and Aaida T. 1989. Production of γ -polyglutamic acid by Bacillus subtilis A35 under denitrifying conditions. Agricultural and Biological Chemistry 53:2369-2375.38.Chen, G. Q., and Page, W. J. 1997. Production of poly- β -hydroxybutyrate by Azotobacter vinelandii in a two-stage fermentation process. Biotechnology Techniques 11: 347 – 350.39.Choi, H. J., Kunioka, M. 1995a. Preparation conditions and swelling equilibria of hydrogel prepared by γ -irradiation from microbial poly(γ -glutamic acid). Radiation Physics and Chemistry 46: 175-179.40.Choi, H. J., Yang, R., Kunioka, M. 1995b. Synthesis and characterization of pH-sensitive and biodegradable hydarogels prepared by γ -irradiation using microbial poly(γ -glutamic acid) and poly (ε -lysine). Journal of Applied Polymer Science 58: 807-814.41.Choma C., Clavel T., Dominguez H., Razafindramboa N., Soumille H., Nyugen C., Schimtt P. 2000. Effect of temperature on growth characteristics of Bacillus cereus TZ415, Int. J. Food Microbiol 55: 73 – 77.42.Cromwick, A. M., Birrer, G. A., and Gross,

R. A. 1996. Effects of pH and aeration on γ -poly(glutamic acid) formation by Bacillus licheniformis in controlled batch fermentor cultures. Biotechnology and bioengineering 50: 222-227.43.Cumming, R. H., Robinson, P. M., and Martin, G. F. 1996. Flocculation of E. coli wuth cationic polymers. Bioseparation 6: 17-23.44.Daninippon Parmaceutical Co, Ltd. 1972. Ice cream stabilizer, JP Patent 19735/72.45.Dearfield, K. L., Abernathy, C. O., Ottley, M. S., Brantner J. H. and Hayes, P. F. 1988. Acrylamide: its metabolism, developmental and reproductive effects, genotoxicity and carcinogenicity. Mutation Research 195: 45-77.46.Deng, S. B., Bai, R. B., Hu, X. M., and Luo, Q. 2003. Characteristics of a bioflocculant produced by Bacillus mucilaginosus and its use in starch wastewater treatment. Agricultural and Biological Chemistry 60:588-593.47.Do, J. H., Chang, H. and Lee, S. 2001. Efficient recovery of poly(glutamic acid) from highly viscous culture broth. Biotechnology and Bioengineering. 76: 219-223.48.Du, G.., Yang, G.., Qu, Y., Chen, J. and Lun, S. 2005. Effects of glycerol on the production of poly(γ -glutamic caid) by Bacillus licheniformis. Process Biochemistry 40: 2143-2147.49.Dubois, M., Gilles, K. A., Hamilton, J. K., Rebers, P. A. and Smith, F. 1956. Colorimetric method for determination of sugars and related substances. Analytical Chemistry 28: 350-356.50.Editorial. 2006. China launches huge water quality study, Chemical & Engineering News 84: 8.51.Esser K., Kues U. 1983. Flocculation and its implication for biotechnology. Process Biochemistry 18:21 – 3.52.Fabi?n, S. G., Amelia M. A., and Juan M. D. 2004. Activated carbon fibers from Nomex by chemical activation with phosphoric acid. Carbon 42:1419 – 1426.53.Ferreira, I. M. P. L. V. O., Gomes, A. M. P. and Ferreira, M. A. 1998. Determination of sugars, and some other compounds in infant formulae, follow-up milks and human milk by HPLC-UV/RI. Carbohydrate Polymers 37: 225-229.54.Friedman, B. A. and Dugan, P. R. 1968. Concentration and accumulation of metallic ions by the bacterium Zoogloes. Developments in Industrial Microbiology 9: 381-388.55.Fujii, H. 1963. On the formation of mucilage by Bacillus natto. Part III. Chemical constitutions of mucilage in natto (1). Nippon Nogeikagaku Kaishi: 37, 407-411.56.Fujita, M., Ike, M., Tachibana, S., Kitada, G.., Kim, S. M., and Inoue, Z. 2000. Characteristics of a bioflocculant produced by Citrobacter sp. YKF04 from acetic and propionic acids. Journal of Bioscience and Bioengineering 89: 40 – 46.57.Gao, H., Jiang, T., Han, B., Wang, Y., Du, J., Liu, Z. and Zhang, J., 2004. Aqueous/ionic liquid interfacial polymerization for preparing polyaniline nanoparticles. Polymer 45:3017-3019.58.Gardner, J. M. and Troy, F.A. 1979. Chemistry and biosynthesis of the poly(γ -D-glutamyl)capsule in Bacillus licheniformis. Activation, racemization, and polymerization of glutamic acid by a membranous polyglutamyl synthetase complex. The Journal of Biological Chemistry 254:6262 – 6269.59.Goldman J. C., Carpenter E. J. 1974. A kinetic approach to effect of temperatureon algal growth. Limnology and Oceanograi 19: 756 – 766.60.Goto, A. and Kunioka, M. 1992. Biosynthesis and hydrolysis of poly(γ -glutamic acid) from Bacillus subtilis IFO 3335. Bioscience, Biotechnology, and Biochemistry 56: 1031-1035.61.Gouda, M. K., Swellam, A. E., Omar, S. H. 2001. Production of PHB by a Bacillus megaterium strain using sugarcane molasses and corn steep liquor as sole carbon and nitrogen sources. Microbiological Reviews 156: 201 – 20762.Gough, S., Flynn, O., Hack, C. J., Marchant, R. 1996. Fermentation of molasses using a thermotolerant yeast, Kluyveromyces marxianus IMB3: simplex optimization of media supplements. Applied Microbiology and Biotechnology 46:187 – 190.63.Gross, A. 1998. Bacterial γ -poly(glutamic acid). In Biopolymers from renewable resources, D.L. Kaplan, ed. (New York: Springer Berlin Heidelberg) 195 – 21964.Gross, R. A., McCarthy, S. P., Shah, D. T. 1995. Gamma-poly(glutamic acid) esters. US Patent 5: 378-807.65.Hantula J., and Bamford D. H. 1991b. Bacterial phage resistance and flocculation deficiency of Flavobacterium sp. Are phenotypically interrelated. Applied Microbiology and Biotechnology 36:105 – 8.66.Hara, T. 2002. Deser greening. Greening by utilization of microbial macromolecules. Kobunshi 49: 367-370.