

Optimization of Feeding Solution Concentration and Feeding Time for Poly(*glutamic acid*) Production by *Bacillus licheniformis*

陳宥瑾、張耀南；吳建一

E-mail: 9417917@mail.dyu.edu.tw

ABSTRACT

In this study, the optimization of feeding solution concentration and feeding time for poly-*glutamic acid* (PGA) by *Bacillus licheniformis* CCRC 12826 was investigated by using two-level factorial design. It was found that the optimal volume of the suitable feeding solution comprising 40.0g/L glutamic acid, 42.0g/L citric acid, 158.0g/L glycerol, 1.0g/L NH₄Cl, was 25mL and the optimal feeding time was at 20h of cultivation. When 25mL of the suitable feeding solution was added to the originally designed medium at 20h of cultivation, the yield of PGA production was 27.4g/L at 120h of cultivation. It was little less than the yield (28.3g/L) without any feeding solution. When the volume of the feeding solution was reduced from 25mL to 5mL, the component concentrations were calculated to be 200.0g/L glutamic acid, 210.0g/L citric acid, 790.0g/L glycerol, 5.0g/L NH₄Cl. When this concentrated feeding solution was fed at the optimal feeding time described above, the yield of PGA production was 31.2g/L at 120h of cultivation. The PGA production was increased by 10.21% from 28.3 to 31.2g/L, while the yield was 37.7g/L after 144h of cultivation and it was increased significantly by 33.25%. With these feeding processes, the culture time for the highest yield of PGA production was delayed. However, this research demonstrated that the feeding processes with the two-level factorial design were worth using to improve the yield of PGA production by *B. licheniformis* CCRC 12826.

Keywords : poly-*glutamic acid* ; fed-batch ; *Bacillus licheniformis*

Table of Contents

封面內頁 簽名頁 授權書 iii	中文摘要 iv	英文摘要 v	誌謝 vi	目錄 viii	圖目錄 x	表目錄 xi								
第一章 前言 1	第二章 文獻回顧 4	2.1 聚麩胺酸(Poly- <i>glutamic acid</i>)之生成 4	2.2 苔蘚桿菌(<i>Bacillus licheniformis</i>)生產聚麩胺酸 8	2.3 聚麩胺酸之應用 13	2.3.1 醫藥應用領域方面 13	2.3.2 食品應用領域方面 14	2.3.3 環境保護應用領域方面 14	2.3.4 化妝品應用領域方面 15	2.4 回應曲面法 15	2.4.1 原理 16	2.4.2 優點 20			
第三章 材料與方法 23	3.1 儀器設備 23	3.1.1 儀器 23	3.1.2 材料 23	3.2 培養方法 25	3.3 回應曲面法之設計 27	3.3.1 部分因子之實驗設計 28	3.4 結果與討論 32	3.4.1 部分因子實驗設計 32	3.4.2 菌體生長量之探討 35	3.4.3 pH值變化之探討 35	3.4.4 產量之探討 41			
第四章 結論 45	參考文獻 46	圖目錄 圖2.1 聚麩胺酸之化學結構 5	圖2.2 D-form 麩胺酸之化學結構 6	圖2.3 L-form 麩胺酸之化學結構 7	圖2.4 桿菌生成聚麩胺酸之假設途徑 12	圖2.5 二水準因子設計圖 17	圖2.6 回應曲面圖 22	圖3.1 A25與CA5饋料溶液在20h培養時間饋加對苔蘚桿菌菌體生長(OD660)變化之影響 37	圖3.2 B15與CB5饋料溶液在30h培養時間饋加對苔蘚桿菌菌體生長(OD660)變化之影響 38	圖3.3 A25與CA5饋料溶液在20h培養時間饋加對培養基pH值變化之影響 39	圖3.4 B15與CB5饋料溶液在30h培養時間饋加對培養基pH值變化之影響 40	圖3.5 B15饋料溶液在30h培養時間饋加後苔蘚桿菌菌體生長(OD660)與培養基pH值之變化 42	圖3.6 A25與CA5饋料溶液在20h培養時間饋加對聚麩胺酸產量之影響 43	圖3.7 B15與CB5饋料溶液在30h培養時間饋加對聚麩胺酸產量之影響 44
表目錄 表1.1 不同菌株之生產聚麩胺酸所需成份及產量之比較 2	表3.1 修改培養基E之修飾培養基 24	表3.2 因子設計之中心點饋料溶液組成基質及其濃度 26	表3.3a 26-2因子設計之變數及層階 29	表3.3b 26-2因子設計之變數及層階 30	表3.3c 26-2因子設計之變數及層階 31	表3.4a 26-2因子設計之實驗結果 33	表3.4b 26-2因子設計之實驗結果 34	表3.5 不同饋料溶液的組成分濃度與饋料體積 36						

REFERENCES

- 1.洪哲穎。1998。回應曲面品質工程技術。工業局八十八年度人才培訓計畫研習班。私立義守大學。高雄縣大樹鄉。
- 2.洪哲穎、陳國誠。1992。回應曲面實驗設計法在微生物酵素生產上之應用。化工。39(2):3-18。
- 3.范宜淙。2001。以苔蘚桿菌生產聚麩胺酸之研究。大葉大學環境工程學系碩士班論文。彰化縣大村鄉。
- 4.徐敬衡。1994。生物分解性微生物塑膠之開發。食品工業。26(7):30-35。
- 5.黎中正。1998。實驗設計與分析。高立圖書有限公司，台北市。
- 6.Birrer, G. A., Cromwick, A. M. and Gross, R. A. 1994. *γ*-Poly(*glutamic acid*) formation by *Bacillus licheniformis* 9945a : physiological and biochemical studies. *Int. J. Biol. Macromol.* 16(5):265-275.
- 7.Bovarnick, M. 1942. The formation of extracellular D(-)-*glutamic acid* polypeptide by *Bacillus subtilis*. *J. Biol. Chem.* 145:415-424.
- 8.Box, G. E. P. and Wilson, K. B. 1951. On the experimental attainment optimum conditions. *J. Roy. Statist. Soc. Ser. B Metho.* 13:1-45.
- 9.Cheng, C., Asada, Y. and Aida, T. 1989. Production of *γ*-poly*glutamic acid* by *Bacillus licheniformis* A35 under denitrifying conditions. *Agric. Biol. Chem.* 53(9):2369-2375.
- 10.Cromwick, A. M., and Gross, R. A. 1995a. Effect of manganese() on *Bacillus licheniformis* ATCC9945a physiology and *γ*-poly(*glutamic acid*) formation.

Int. J. Biol. Macromol. 17:259-267. 11.Cromwick, A. M., and Gross, R. A. 1995b. Investigation by NMR of metabolic routes to bacterial poly(glutamic acid) using ¹³C labeled citrate and glutamate as media carbon source. Can. J. Microbiol. 41:902-909. 12.Cromwick, M., Birrer, G. A. and Gross, R. A. 1996. Effects of pH and aeration on poly(glutamic acid) formation by *Bacillus licheniformis* in controlled batch fermentor cultures. Biotechnol. Bioeng. 50:222-227. 13.Fujii, H. 1963. On the formation of mucilage by *Bacillus natto*. Part Chemical constitutions of mucilage in natto. Nippon Nogeikagaku Kaishi (in Japanese). 37:474-477. 14.Goto, A., and Kunioka, M. 1994. Biosynthesis of poly(L-glutamic acid) from L-glutamic acid, citric acid, and ammonium sulfate in *Bacillus subtilis* IFO3335. Appl. Microbiol. Biotechnol. 40:867-872. 15.Ivanovics, G. and Erdos, L. 1937. Ein Beitrag zum Wesen der Kapsel-substanz des Mil. Z. Immunitätsforsch Exp. Ther. 90:5-19. 16.Ivanovics, G. and Bruckner, V. 1937. Chemische und immunologische Studien über den Mechanismus der Milzbrandinfektion und Immunität; die chemische Struktur der Kapselsubstanz des Milzbrandbazillus und der serologisch identischen spezifischen Substanz des *Bacillus mesentericus*. Z. Immunitätsforsch Exp. Ther. 90:304-318. 17.Ito, Y., Tanaka, T., Ohmachi, T. and Asada, Y. 1996. Glutamic acid independent production of poly(L-glutamic acid) by *Bacillus subtilis* TAM-4. Biosci. Biotech. Biochem. 60(8):1239-1242. 18.Kubota, H., Matsunobu, T., Uotani, K., Takebe, H., Satoh, A., Tanaka, T. and Taniguchi, M. 1993. Production of poly(glutamic acid) by *Bacillus subtilis* F-2-01. Biosci. Biotech. Biochem. 57: 1212 – 1213. 19.Kunioka, M. 1997. Biosynthesis and chemical reactions of poly(amino acids) from microorganisms. Appl. Microbiol. Biotechnol. 47:469-475. 20.Ko, Y. H. and Gross, R. A. 1998. Effects of glucose and glycerol on poly(glutamic acid) formation by *Bacillus licheniformis* ATCC9945a. Biotechnol. Bioeng. 57:430-437. 21.Leonard, C. G., Housewright, R. D. and Thorne, C. B. 1958a. Effects of some metallic ions on glutamyl polypeptide synthesis by *Bacillus subtilis*. J. Bacteriol. 76:499-503. 22.Leonard, C. G., Housewright, R. D. and Thorne, C. B. 1958b. Effect of metal ions on the optical specificity of glutamine synthetase and glutamyl transferase of *Bacillus licheniformis*. Biochem. Biophys. Acta. 62:432-434. 23.Murao, S. 1969. On the polyglutamic acid fermentation. Kobunshi. 16:1204-1212. 24.Ontni, Y., Tabata, Y. and Ikada, Y. 1996. Rapidly curable biological glue composed of gelatin and poly(L-glutamic acid). Biomater. 17:1381-1391. 25.Perez-Camero, G., Congregado, F., Bou, J. J. and Munoz-Guerra, S. 1999. Biosynthesis and ultrasonic degradation of bacterial poly(L-glutamic acid). Biotechnol. Bioeng. 63:110-115. 26.Sawamura, S. 1913. On *Bacillus natto*. J. Coll. Agric. 5:189-191. 27.Shih, I. L. and Van, Y. T. 2001. The production of poly(L-glutamic acid) from microorganisms and its various applications. Biores. Technol. 79:207-225. 28.Thorne, C. B., Gomez, C. G., Blind, G. R. and Housewright, R. D. 1953. Synthesis of glutamic acid and glutamyl polypeptide by *Bacillus anthracis*. Factors affecting peptide production in synthetic liquid media. J. Bacteriol. 65:472-478. 29.Thorne, C. B., Gomez, C. G., Noyes, H. E. and Housewright, R. D. 1954. Production of glutamyl polypeptide by *Bacillus subtilis*. J. Bacteriol. 68:307-315. 30.Troy, F. A. 1973. Chemistry and biosynthesis of the poly(L-D-glutamyl) capsule in *Bacillus licheniformis*. Properties of the membrane-mediated biosynthetic reaction. J. Biol. Chem. 248:305-315. 31.Ward, R. M., Anderson, R. F. and Dean, F. K. 1963. Poly(glutamic acid) production by *Bacillus subtilis* NRRL B-2612 grown on wheat gluten. Biotechnol. Bioeng. 5:41-48. 32.Yokoi, H., Natsuda, O., Hirose, J., Hayashi, S. and Takasaki, Y. 1995. Characteristics of a biopolymer flocculant produced by *Bacillus* sp. PY-90. J. Ferment. Bioeng. 79:378-380. 33.Yokoi, H., Arima, T., Hirose, J., Hayashi, S. and Takasaki, Y. 1996. Flocculation properties of poly(gamma-glutamic acid) produced by *Bacillus subtilis*. J. Ferment. Bioeng. 82:84-87.