生物界面活性劑之菌株篩選及生產特性之探討=screening,characteristics and production of biosurfactant-lsolates in taiwan

戴全志、徐泰浩 ; 陳文章

E-mail: 9019876@mail.dyu.edu.tw

摘 要

生物界面活性劑 （biosurfactant） 為微生物所生合成的兩性界面物質，大部分是由細菌或真菌所產生，具有降低表面或界面張力，表面素 (surfactin) 即為枯草桿菌所生產的一種脂胜?界面活性劑，具有良好的界面活性。細胞固定化 (cell immobilization) 能使菌體和胞外產物分離，簡化下游產程。本研究的目的是由野外及中油嘉義煉製研究所內取得樣本，以M. S. M. （mineral salt media）培養液加煤油篩選菌種，了解其基本特性，並以固定化菌種及?酵槽生產生物界面活性劑。自野外成功篩選到菌株，以M. S. M. 培養，其表面張力值均在 30 dyne/cm 以下。以格蘭氏染色法的結果呈現紅色。其中新分離出來的菌株編號 110、111 的生長情形和枯草桿菌類似。新分離出來的菌種在不同培養基質的培養中，無論是添加不同碳氫化合物或是不同量的石油，均可利用分解。使用?酵槽培養枯草桿菌，所得到的表面素粗萃取量為 0.1713 g/L ，而新分離出來的菌株 110 培養的結果為 0.567 g/L 。固定化菌種以 sodium alginate 為擔體，固定新分離出來的菌種所得的表面張力值為 51.0 dyne/cm 、 62.6 dyne/cm 、 61.7 dyne/cm 。

關鍵詞 : 生物界面活性劑 ; 表面素 ; 生物反應器 ; 篩選 ; 固定化

目錄

參考文獻

1.吳清平、蔡芷荷、張菊梅、周小燕和姚汝華。1998。 表面活性素的結構特性及其生物合成機理。微生物 學通報 25 (6) : 351-353。 2.陳聰郁。1995。純化分離-超過濾。豐記科訊。58 (12) : 9-11。 3.張榮倫。1996。二階式培養結合休止細胞固定化技術應用 Bacillus subtilis 生物界面活性劑之生成。大葉工學院食品工程研 究所碩士論文。 4.賴振邦。1998。利用細胞固定化生物反應器系統生產界面活性劑及其分 離純化。大葉大學食品工程研究所碩士論文。 5. 魏賢卿。1986。膜分離技術的基本原理及其在乳品加工上之應用。食 品工業。18 (4) : 43-49。 6. 蘇遠志和黃世佑。1994。微生物化學工程學。台北市華香園出版社 頁 : 24-26。 7.小林敏幸。1987。生物反應器之應用。食品工業。30 (6) : 74-77。 8. Arima, K., Kakinuma, A., and Tamura, G. 1968. Surfactin, a crystalline peptidelipid surfactant produced by Bacillus subtilis : isolation, characterization and its inhibition of fibrin clot formation. Biochem. Biophys. Res. Commun. 31 : 488-494. 9. Banat, I. M. 1995. Biosurfactants production and possible uses in microbial enhanced oil recovery and oil pollution remediation : a review. Biores. Technol. 51 : 1-12.

10. Behlulgil, K., Mehmetoglu, T. and Donmez, S. 1992. Application of microbial enhanced oil recovery technique to a turkish heavy oil. Appl. Microbiol. Biotechnol. 36 : 833- 835. 11.Bryant, R. S. 1994. Microbial enhanced hydrocarbon recovery and its potential for application to north sea reservoirs. Trans IchemE. 72 : 144-150. 12.Champagne, C. P., Gaudy, C., Poncelet, D. and Neufeld, R. J. 1992. Lactococcus lactis release rom calcium alginate beads. Appl. Environ. Microbiol. 58 (5) : 1429-1434. 13.Cooper, D. G., Macdonald, C. R., Duff, S. J. B. and Kosaric, N. 1981. Enhanced production of surfactin from Bacillus subtilis by continuous product removal and metal cation additions. Appl. Environ. Microbiol. 42 (3) : 408-412. 14.Desai, A., Patel, R. M. and Desai, J. D. 1994. Advances in the production of biosurfactant and their commercial applications. J. Sci. Ind. Res. 53 : 619-629. 15.Fox, S. L. and Bala, G. A. 2000. Production of surfactant from Bacillus subtilis ATCC 21332 using potato substrates. Bioresource Technol. 75 : 235-240. 16.Grau, A., Fernandez, J. C. G., Peypoux, F. and Ortiz, A. 1999. A study on the interactions of surfactin with phospholipid vesicles. Biochimica et Biophysica Acta, 1418, 307-319. 17.Hubbard, D. W., Harris, L. R. and Wierenga, M. K. 1988. Scale up for polysaccharide fermentation. Chem. Eng. Prog. 8 : 55-61. 18.Huszcza, M. E. 1997. Surface properties of lipopeptides produced by bacillus coagulans. DAI-C 58/03, p990, Fall. 19.Khalid, Z. M. and Malik, K. A. 1988. Isolation of furnace oil utilizing bacterial capable of producing biosurfactant. Pakkistan J. Sci. Ind. Res. 31 : 714-717. 20.Kim, H. S., Yoon, B. D., Lee, C. H., Oh, H. M., Katsuragi, T. and Tani, Y. 1997. Production and properties of a lipopeptide biosurfactant from Bacillus subtilis C9. J. Ferment. Bioeng. 84 : 41-46. 21.Laycock, M. V., Hildebrand, P. D., Thibault, P., Walter, J. A. and Wright, J. L. C. 1991. Viscosin, a potent peptidelipid biosurfactant and phytopathogenic mediator produced by a pectolytic strain of Pseudomonas fluorescens. J. Agric. Food Chem. 39 : 483-489. 22.Lin, S. C., Carswell, K. S., Sharma., M. M., Georgiou, G. 1994. Continuous production of the lipopeptide biosurfactant of bacillus licheniformis JF-2. Appl. Microbiol Biotechnol. 41 : 281-285. 23.Lin, S. -C. 1996. Biosurfactants: recent advances. J. Chem. Technol. Biotechnol. 66 : 109-120. 24.Lin, S. -C., Lin, K. -G., Lo, C. -C. and Lin, Y. -M. 1998. Enhanced biosurfactant production by a Bacillus licheniformis mutant. Enzyme Microbiol. Technol. 23 : 267- 273. 25.Margaritis, A., Kennedy, K. and Zajic, J. E. 1980. Application of an air-lift fermentor in the production of biosurfactant. Dev. Ind. Microbiol. Chapter 30 : 285-294. 26.Mitsuru Wada, Jyoji Kato and Ichiro Chibata. 1980. Continuous production of ethanol in high concentration using immobilized growing yeast cells. Appl. Microbiol. Biotech. 11 : 67-71. 27. Morikawa, M., Ito, M. and Imanaka, T. 1992. Isolation of a new surfactin producer Bacillus pumilus A-1 and cloning and nucleotide sequence of the regulator gene, psf-1. J. Ferment. Bioeng. 74 (5) : 255-261. 28.Morin, N., Cardou, M. B. and Champagne, C. P. 1992. Production of concentrated Lactococcus lactis subsp. cremoris suspensions in calcium alginate. Appl. Environ. Microbiol. 58 (2) : 545-550. 29.Mulligan, G. N., Cooper, D. G. and Beufeldm, R. J. 1984. Selection of microbes producing biosurfactants in media without hydrocarbons. J. Ferment. Technol. 2 : 311-314. 30.Mulligan, G. N., Chow, T. Y. K. and Gibbs, B. F. 1989. Enhanced biosurfactant production by a mutant Bacillus subtilis strain. APPL. Microbiol. Biotechnol. 31 : 486-489. 31.Mulligan, C. N. and Yong, R. N. 1999. Metal removal from contaminated soil and sediments by the biosurfactant surfactin. Environ. Sci. Technol, 33 : 3812-3820. 32.Ochsner, U. A., Koch, A. K., Fiechter, A. and Reiser, J. 1994. Isolation and characterization of a regulatory gene affecting rhamnolipid biosurfactant synthesis in Pseudomonas aeruginosa. J. Bacteriology. 2044-2054. 33.Ohno, A., Takashi, A. and Shoda, M. 1992. Production of a lipopeptide antibiotic surfactin with recombinant Bacillus subtilis. Biotechnol. Lett. 14 : 1165-1168. 34.Ohno, A., Ano, T. and Shoda, M. 1995. Production of a lipopeptide antibiotic, surfactin, by recombinant Bacillus subtilis in solid state fermentation. Biotechnol. Bioeng. 47 : 209-214. 35.Peypoux, F., Bonmatin, J. M. and Wallach, J. 1999. Recent trends in the biochemistry of surfactin. Appl. Microbiol. Biotechnol. 51 : 553-563. 36.Razafindralambo, H. L. H. 1997. Contribution to the study of the surface-active properties of Bacillus subtilis lipopeptides. DAI-C 58/03, p916, Fall. 37.Roach, R. R. and Hoseney, R. C. 1995. Effect of certain surfactants on the starch in bread. Cereal Chemistry. 72 (6) : 578-582. 38. Rodriguez, S. B. and Mahoney, N. E. 1994. Inhibition of aflatoxin production by surfactants. Appl. Environ. Microbiol. 60 (1) : 106-110. 39. Roubin, M. R. de, Mulligan, C. N. and Gibbs, B. F. 1989. Correlation of enhanced surfactin production with decreased isocitrate dehydrogenase. Can. J. Microbiol. 35 : 854-859. 40. Sandrin, C., Peypoux, F. and Michel, G. 1990. Coproduction of surfactin and iturin A, lipopeptides with surfactant and antifungal properties, by Bacillus subtilis. Biotechnol Appl. Biochem. 12 : 370-375. 41. Schulz, D., Passeri, A., Schmidt, M., Lang, D., Wagne, F., Wray, V., Poremba, K. and Gunkel, W. 1992. Screening for biosurfactants among crude oil degrading marin microorganisms. Kiel Meeresfkorsch Sonderh. 322-326. 42. Scott, C. D., Woodward, C. A. and Thompson, J. E. 1989. Solute diffusion in biocatalyst gel beads containing biocatalysis and other additives. Enzyme Microbiol. Technol. 11 : 258-263. 43. Sen, R. 1997. Response surface optimization of the critical media components for the production of surfactin. Chem. Technol. Biotechnol. 68 : 263-270. 44. Sheppard, J. D. and Cooper, D. G. 1990. Development of computerized feedback control for the continuous phasing of Bacillus subtilis. Biotechnol. Bioeng. 36 : 539-545. 45. Sheppard, J. D. and Coope, D. G. 1991. The response of Bacillus subtilis ATCC 21332 to manganese during continuous-phased growth. Appl. Microbiol. Biotechnol. 35 : 72-76. 46. Sheppard, J. D., Jumarie, C., Cooper, D. G. and Laprade, R. 1991. Ionic channels induced by surfactin in planar lipid bilayer membranes. Elscvier Science Publishers B. V. 1064 : 13-23. 47. Takashi Mano, Shinjiro Mitsuda, Eltaro Kumazawa and Yasuyoshi Takeshita. 1992. New immobilization method of mammalian celles using alginate and polyacrylate. J. Ferment. Bioeng. 73 (6) : 486-489. 48. Thompson, D. N., Fox, S. L. and Bala, G. A. 2000. Biosurfactants from potato process effluents. Appl. Biochem. Biotech. 84-86, 917- 930. 49. Tomas, A., Courthaudon, J. L., Paquet, D. and Lorient, D. 1994. Effect of surfactant on some physico-chemical properties of dairy oil-in-water emulsions. Food Hydrocolloids. 8 (6) : 543-553. 50. Updegraff, D. M. 1990. Early research on microbial enhanced oil recovery. Dev. Ind. Microbiol. 31 : 135-142. 51. Van Dyke, M., Lee, H. and Trevors, J. T. 1991. Applications of microbial surfactants. Biotechnol. Adv. 9 : 241-252. 52. Van Walsum, G. P. and Cooper, D. G. 1993. Self- cycling fermentation in a stirred tank reactor. Biotechnol. Bioeng. 42 : 1175-1180. 53. Vogel, E. M. L., Hagerdal, B. H. and Vogel, H. J. 1995. Phosphorus-31 and carbon-13 nuclear magnetic resonance study of glucose and xylose

metabolism in agarose-immobilized Candida tropicalis. Appl. Environ. Microbiol. 61 : 1420- 1425. 54. Vollenbroich, D., Ozel, M., Vater, J., Kamp, R. M. and Pauli, G. 1997. Mechanism of inactivation of enveloped viruses by the biosurfactant surfactin from Bacillus subtilis. Biologicals. 25 : 289-297. 55. Vollenbroich, D., Pauli, G., Ozel, M. and Vater, J. 1997. Antimycoplasma properties and application in cell culture of surfactin, a lipopeptide antibiotic from Bacillus subtilis. Appl. Environ. Microbiol. 63 (1) : 44-49. 56. Wasko, M. P. and Buatt, R. P. 1990. Properties of a biosurfactant produced by the fuel contaminant Ochrobacterium. Inter. Biodeterioration 27 : 265-273. 57. Wei, Y. -H and Chu, I. -M. 1998. Enhancement of surfactin production in iron-enriched media by Bacillus subtilis ATCC 21332. Enzyme Microbiol. Technol. 22: 724-728. 58. Yakimov, M. M., Timmis, K. N., Wray, V. and Fredrickson, H. L. 1995. Characterization of a new lipopeptide surfactant produced by thermotolerant and halotolerant subsurface Bacillus lichiformis BAS50. Appl. Environ. Microbiol. 61 (5) : 1706-1713. 59. Yang, S. -S., Hung, H. -C. and Lin, Y. -T. 1991. Microbial contamination of fuel oil in the Taiwan area. J. Chinese Agri. Chem. Soc. 29 (4) : 370- 381. 60. Yurkov, V. and Beatty, J. T. 1998. Isolation of aerobic anoxygenic photosynthetic bacteria from black smoker plume waters of the juan de fuca ridge in the pacific ocean. Appl. Environ. Microbiol. 64 (1) : 337-341. 61. Zhang, Y. -M and Miller, R. M. 1995. Effect of rhamnolipid (biosurfactant) structure on solubilization and biodegradation of n-alkanes. Appl. Environ. Microbiol. 61 (6) : 2247-2251.