限磷條件下培養菌株Burkholderia sp. Yu-4生合成PHBV

陳皓崴、吳淑姿、余世宗

E-mail: 321389@mail.dyu.edu.tw

摘 要

本研究於限磷條件下培養Burkholderia sp. Yu-4，並添加戊酸鈉為PHBV前驅物，探討對菌體生質量與PHBV生合成量的影響。於搖瓶培養中，隨著磷源濃度降低，其菌體生質量、最終pH值及營養源消耗量均下降。究其原因，磷酸氫二鈉與磷酸二氫鉀在培養基中扮演緩衝溶液的角色，因而有較穩定的pH環境供菌株生長。雖然提高培養基之起始pH值，可延長菌株生長時間，但起始pH值為9.0時，菌株的生長情況亦不佳，無法將培養基中磷源耗盡。以限磷培養基於批次發酵槽中培養Burkholderia sp. Yu-4，並調控pH值為7.0，結果發現，當培養至32小時，有最高菌體生質量與PHB生合成量，分別為10.1與3.89 g/L，PHB佔菌體量的38.5%；培養至48小時，培養基中磷源耗盡，達到限磷狀態，而培養至120小時，仍有氮源殘餘。以發酵槽批次培養，於第12小時添加戊酸鈉 (2.0 g/L)，菌株可穩定生長，且HV生合成量為最高；菌株於開始培養後6小時，大量生長；培養至8小時，菌株開始生合成PHB；培養至20小時，菌株逐漸生合成PHBV；培養至64小時，培養基之磷源耗盡，達到限磷狀態；培養至84小時，有最高的HB與HV產量，分別為2.29與0.42 g/L，HB佔菌體量的33.8%，HV佔菌體量的6.19%，HV含量佔總PHBV的15.5%。於培養第6小時添加戊酸鈉 (4.0 g/L)，菌株生長遲緩，可能是添加時間較早，菌體仍未適應培養環境，因此抑制菌株生長所致，故需延緩添加時間。若於培養第20小時才添加戊酸鈉 (4.0 g/L)，菌體則逐漸凋亡，可能是添加時間較晚，因菌體已老化，無法承受高濃度有機酸鹽所造成的危害，因此，添加時間需居中調整，也必需降低添加量。

關鍵詞 : 限磷條件、批次發酵槽

目錄

參考文獻

1. Akiyama, M., Crooke, E. and Kornberg, A. 1992. The polyphosphate kinase gene of Escherichia coli isolation and sequence of the ppk gene and membrane location of the protein. J. Biol. Chem. 267: 22556 – 22561. 2. Akiyama, M., Crooke, E. and Kornberg, A. 1993. An exopolyphosphatase of Escherichia coli the enzyme and its ppx gene in a polyphosphate operon. J. Biol. Chem. 268: 633 – 639. 3. Albertsson, A.C., Andersson, S.O. and Karlsson, S. 1987. The mechanism of biodegradation of polyethylene. Polym. Degrad. Stab. 18: 73 – 87. 4. Aldor, I.S. and Keasling, J.D. 2003. Process design for microbial plastic factories: metabolic engineering of polyhydroxyalkanoates. Curr. Opin. Biotechnol. 14: 475 – 483. 5. Amirul, A.A., Yahya, A.R.M., Sudesh, K., Azizan, M.N.M. and Majid, M.I.A. 2008. Biosynthesis of poly(3-hydroxybutyrate-co-4-hydroxybutyrate) copolymer by Cupriavidus sp. USMAA1020 isolated from lake Kulim Malaysia. Bioresour. Technol. 99: 4903 – 4909. 6. Anderson, A.J., Haywood, G.W. and Dawes, E.A. 1990. Biosynthesis and composition of bacterial poly(hydroxyalkanoates). Int. J. Biol. Macromol. 12: 102 – 105. 7. Augusta, J., M?荆ler, R.J. and Widdecke, H. 1992. Biologisch abbaubare Kunststoffe: Testverfahrenund Beurteilungskriterien. Chem. Ing. Tech. 64: 410 – 415. 8. Beccari, M., Majone, M., Massanisso, P. and Ramadori, R. 1998. A bulking sludge with high response selected under intermittent feeding. Water Res. 32: 3403 – 3413. 9. Bitar, A. and Underhill, S. 1990. Effect of ammonium supplement on production of poly-3-hydroxybutyric acid by Alcaligenes eutrophus in batch culture. Biotechnol. Lett. 12: 563 – 568. 10. Bohlmann, G.M. 2006. Polyhydroxyalkanoate production in crops. ACS. Symp. Ser. 921: 253 – 270. 11. Byron, D. 1992. Production of poly-b-hydroxyvalerate copolymers, FEMS. Microbiol. Rev. 103: 247 – 250. 12. Carucci, A., Dionisi, D., Majone, M., Rolle, E. and Smurra, P. 2001. Aerobic storage by activated sludge on real wastewater. Water Res. 35: 3833 – 3844. 13. Castilho, L.R., Mitchell, D.A. and Freire, D.M.G. 2009. Production of polyhydroxyalkanoates (PHAs) from waste materials and by-products by submerged and solid-state fermentation. Bioresour. Technol. 100: 5996 – 6009. 14. Chee, J.W., Amirul, A.A., Tengku Muhammad, T.S., Majid, M.I.A. and Mansor, S.M. 2008. The in?uence of copolymer ratio and drug loading level on the biocompatibility of P(3HB-co-4HB) synthesized by Cupriavidus sp. (USMAA2-4). Biochem. Eng. J. 38: 314 – 318. 15. Chen, C.W., Don, T.M. and Yen, H.F. 2006. Enzymatic extruded starch as a carbon source for the production of poly(3-hydroxybutyrate-co-3-hydroxylvalerate) by Haloferax mediterranei. Process. Biochem. 41: 2289 – 2296. 16. Chen,G.Q., Konig, K.H. and Lafferty, R.M. 1991. Production of poly-d(—)-3-hydroxybutyrate and poly-d-(—)-3-hydroxybalerate by strains of Alcaligenes latus. An tonie van Leewenhock 60: 61 – 66. 17. Chen, G.Q. and Wu, Q. 2005. The application of polyhydroxyalkanoates as tissue engineering materials. Biomat. 26: 6565 – 6578. 18. Chen, L.J. and Wang, M. 2002. Production and evaluation of biodegradable composites based on PHB-PHV copolymer. Biomat. 23: 2631 – 2639. 19. Choi, G.G., Kim, M.W., Kim, J.Y. and Rhee, Y.H. 2003. Production of poly(3-hydroxybutyrate-co-3-hydroxyvalerate) with high molar fractions of 3-hydroxyvalerate by a threonine-overproducing mutant of Alcaligenes sp. SH-69. Biotechnol. Lett. 9: 665 – 670. 20. Choi, J.I. and Lee, S.Y. 1999a. High-level production of poly(3-hydroxy- butyrate-co-3-hydroxyvalerate) by fed-batch culture of recombinant Escherichia coli. Appl. Environ. Microbiol. 65: 4363 – 4368. 21. Choi, J. and Lee, S.Y. 1999b. Efficient and economical recovery of poly(3- hydroxyl butyrate) from recombinant Escherichia coli by simple digestion with chemicals. Biotechnol. Bioeng. 62: 546 – 553. 22. Chung, C.W., Kim, Y.S., Kim, Y.B., Bae, K.S. and Rhee. Y.H. 1999. Isolation of a Pseudomonas sp. strain exhibiting unusual behavior of poly(3-hydro- xyalkanoates): biosynthesis and characterization of synthesized polyesters. J. Microbiol. Biotechnol. 9: 847 – 853. 23. Dirck, K., Henze, M., van Loosdrecht, M.C.M., Mosb_k, H. and Aspegren, H. 2001. Storage and degradation of poly-b-hydroxybutyrate in activated sludge under aerobic conditions. Water Res. 35: 2277

– 2285. 24. Du, G. and Yu, J. 2002. Green technology for conversion of food scraps to biodegradable thermoplastic polyhydroxyalkanoates. Sci. Technol. 36: 5511 – 5516. 25. Dyhrman, S.T., Chappell, P.D., Haley, S.T., Moffett, J.W., Orchard, E.D., Waterbury, J.B. and Webb, E.A. 2006. Phosphate utilization by the globally important marine diazotroph Trichodesmium. Nature. 439: 68 – 71. 26. Full, T.D., Jung, D.O. and Madigan, M.T., 2006. Production of poly-b- hydroxylalkanoates from soy molasses oligosaccharides by new, rapidly growing Bacillus species. Lett. Appl. Microbiol. 43: 377 – 384. 27. Gross, R.A. and Kalra, B. 2002. Biodegradable polymers for the environment. Sci. 297: 803 – 807. 28. Hahn, S.K., Chang, Y.K., Kim, B.S., Lee, K.M. and Chang, H.N. 1993. The recovery of poly(3-hydroxybutyrate) by using dispersions of sodium hypochlorite solution and chloroform. Biotechnol. Tech. 7: 209 – 212. 29. Hirota, R., Kuroda, A., Kato, J. and Ohtake, H. 2010. Bacterial phosphate metabolism and its application to phosphorus recovery and industrial bioprocesses. J. Biosci. Bioeng. 109: 423 – 432. 30. Hocking, P.J. and Marchessault, R.H. 1994. In: Griffin GJL, Editor, Chemistry and Technology of Biodegradable Polymers. p. 48 – 96. Blackie Academic, New York. 31. Holmes, P.A. 1985. Applications of PHB – a microbially produced biodegradable thermoplastic. Phys. Technol. 16: 32 – 36. 32. Hu, Y.J., Wei, X., Zhao, W., Liu, Y.S. and Chen, G.O. 2009. Biocompatibility of poly(3-hydroxybutyrate-co-3-hydroxyvalerate-co-3-hydroxyhexanoate) with bone marrow mesenchymal stem cells. Acta. Biomater. 5: 1115 – 1125. 33. Iwata, T. and Doi, Y. 2000. Morphology and crystal structure of solution-grown single crystals of poly[(R)-3-hydroxyvalerate]. Macromol. 33: 5559 – 5565. 34. Jacquel, N., Lo, C.W., Wei, Y.H., Wu, H.S. and Wang, S.S. 2008. Isolation and puri?cation of bacterial poly(3-hydroxyalkanoates). Biochem. Eng. J. 39: 15 – 27. 35. Jayasekara, R., Harding, I., Bowater, I. and Lornergan, G. 2005. Biodegradability of selected range of polymers and polymer blends and standard methods for assessment of biodegradation. J. Polym. Environ. 13: 231 – 51. 36. Kapritchkoff, F.M., Viotti, A.P., Alli, R.C.P., Zuccolo, M., Pradella, J.G.C., Maiorano, A.E., Miranda, E.A. and Bonomi, A. 2006. Enzymatic recovery and purification of polyhydroxybutyrate produced by Ralstonia eutropha. J. Biotechnol. 122: 453 – 462. 37. Kawai, F. 1995. Breakdown of plastics and polymers by microorganisms. Adv. Biochem. Eng. Biotechnol. 52: 151 – 94. 38. Kim, B.S., Lee, S.C., Lee, S.Y., Chang, H.N., Chang, Y.K. and Woo, S.I. 1994. Production of poly(3-hydroxybutyric-co-3-hydroxyvaleric acid) by fed-batch culture of Alcaligenes eutrophus with substrate control using on-line glucose analyzer. Enzyme Microb. Technol. 16: 556 – 565. 39. Koller, M., Bona, R., Chiellini, E., Fernandes, E.G., Horvat, P., Kutschera, C., Hesse, P. and Braunegg, G. 2008. Polyhydroxyalkanoate production from whey by Pseudomonas hydrogenovora. Bioresour. Technol. 99: 4854 – 4863. 40. Kornberg, A. 1995. Inorganic polyphosphate: toward making a forgotten polymer unforgettable. J. Bacteriol. 177: 491 – 496. 41. Koutinas, A.A., Xu, Y., Wang, R. and Webb, C. 2007. Polyhydroxybutyrate production from a novel feedstock derived from a wheat-based biorefinery. Enzyme Microb. Technol. 40: 1035 – 1044. 42. Kulaev, I.S. and Vagabov, V.M. 1983. Polyphosphate metabolism in microorganisms. Adv. Microbiol. Physiol. 24: 83 – 171. 43. Kulpreecha, S., Boonruangthavorn, A., Meksiriporn, B. and Thongchul, N. 2009. Inexpensive fed-batch cultivation for high poly(3-hydroxybutyrate) production by a new isolate of Bacillus megaterium. J. Biosci. Bioeng. 107: 240 – 245. 44. Lee, S.Y. 1996a. Review bacterial polyhydroxyalkanoates, Biotechnol. Bioeng. 49: 1 – 14. 45. Lee, S.Y. 1996b. Plastic bacteria? Progress and prospects for polyhydroxy- alkanoate production in bacteria. Trends Biotechnol. 14: 431 – 438. 46. Lee, W.H., Azizan, M.N.M. and Sudesh, K. 2004. E?ects of culture conditions on the composition of poly(3-hydroxybutyrate-co-4-hydroxybutyrate) synthesized by Comamonas acidovorans. Polym. Degrad. Stab. 84: 129 – 134. 47. Lee, W.H., Loo, C.Y., Nomura, C.T. and Sudesh, K. 2008. Biosynthesis of polyhydroxyalkanoate copolymers from mixtures of plant oils and 3-hydroxy- valerate precursors. Bioresour. Technol. 99: 6844 – 6851. 48. Liu, X.W., Wang, H.H., Chen, J.Y., Li, X.T. and Chen, G.Q. 2009. Biosynthesis of poly(3-hydroxybutyrate-co-3-hydroxyvalerate) by recombinant Escherichia coli harboring propionyl-CoA synthase gene (prpE) or propionate permease gene (prpP). Biochem. Eng. J. 43: 72 – 77. 49. Loo, C.Y. and Sudesh, K., 2007a. Biosynthesis and native granule characteristics of poly(3-hydroxybutyrate-co-3-hydroxyvalerate) in Delftia acidovorans. Int. J. Biol. Macromol. 40: 466 – 471. 50. Lopez, C., Pons, M.N. and Morgenroth, E. 2006. Endogenous processes during long-term starvation in activated sludge performing enhanced biological phosphorus removal. Water Res. 40: 1519 – 1530. 51. Lu, H., Keller, J. and Yuan, Z. 2007. Endogenous metabolism of Candidatus Accumulibacter phosphatis under various starvation conditions. Water Res. 41: 4646 – 4656. 52. Martin, H.G., Ivanova, N., Kunin, V., Warnecke, F., Barry, K.W., McHardy, A.C., Yeates, C., He, S., Salamov, A.A., Szeto, E., Dalin, E., Putnam, N.H., Shapiro, H.J., Pangilinan, J.L., Rigoutsos, I., Kyrpides, N.C., Blackall, L.L., McMahon, K.D. and Hugenholtz, P. 2006. Metagenomic analysis of two enhanced biological phosphorus removal (EBPR) sludge communities. Nat. Biotechnol. 24: 1263 – 1269. 53. Maurer, M., Gujer, W., Hany, R. and Bachmann, S. 1997. Intracellular carbon flow in phosphorus accumulating organisms from activated sludge systems. Water Res. 31: 907 – 917. 54. Meng, W., Kim, S.Y., Yuan, J., Kim, J.C., Kwon, O.H. and Kawazoe, N. 2007. Electrospun PHBV/collagen composite nano?brous sca?olds for tissue engineering. J. Biometer. Sci. Polymer Edn. 18: 81 – 94. 55. Metcalf, W.W. and Wanner, B.L. 1991. Involvement of the Escherichia coli phn (psiD) gene cluster in assimilation of phosphorus in the form of phosphonates, phosphite, Pi esters, and Pi. J. Bacteriol. 173: 587 – 600. 56. Mino, T., van Loosdrecht, M.C.M. and Heijnen, J.J. 1998. Microbiology and biochemistry of the enhanced biological phosphate removal process. Water Res. 32: 3193 – 3207. 57. Mueller, R.J. 2006. Biological degradation of synthetic polyesters—enzymes as potential catalysts for polyester recycling. Proc. Biochem. 41: 2124 – 2128. 58. Nath, A., Dixit, M., Bandiya, A., Chavda, S. and Desai, A. J. 2008. Enhanced PHB production and scale up studies using cheese whey in fed batch culture of Methylobacterium sp. ZP24. Bioresour. Technol. 99: 5749 – 5755. 59. Nikel, P.I., Almeida, A., Melillo, E.C., Galvagno, M.A. and Pettinari, M.J. 2006. New recombinant Escherichia coli strain tailored for the production of poly(3-hydroxybutyrate) from agro-industrial by-products. Appl. Environ. Microbiol. 72: 3949 – 3954. 60. Nonato, R.V., Mantelatto, P.E. and Rossell, C.E.V. 2001. Integrated production of biodegradable plastic, sugar and ethanol. Appl. Microbiol. Biotechnol. 57: 1 – 5. 61. Pavia, D.L., Lampman, G.M. and Kriz, G.S. 1988. Introduction to Organic Laboratory Techniques, 3rd Ed. p. 286-291. Saunders, Fort Worth, TX, USA. 62.

Quillaguam?鴨, J., Doan-Van, T., Guzman, H., Guzman, D., Martin, J., Everest, A. and Hatti-Kaul, R. 2008. Poly(3-hydroxybutyrate) production by Halomonas boliviensis in fed-batch culture. Appl. Microbiol. Biotechnol. 78: 227 – 232. 63. Qiu, Y.Z., Han, J. and Chen, G.Q. 2006. Metabolic engineering of Aeromonas hydrophila for the enhanced production of poly(3-hydroxybutyrate-co-3- hydroxyhexanoate). Appl. Microbiol. Biotechnol. 69: 537 – 542. 64. Rivard, C., Moens, L., Roberts, K., Brigham, J. and Kelley, S. 1995. Starch esters asbiodegradable plastics: Effects of ester group chain length and degree of substitution on anaerobic biodegradation. Enz. Microbial. Tech. 17: 848 – 852. 65. Seymour, R.B. 1989. Polymer science before&after 1899: notable developments during the lifetime of Maurtis Dekker. J. Macromol. Sci. Chem. 26: 1023 – 1032. 66. Shah, A.A., Hasan, F., Hameed, A. and Ahmed, S. 2008. Biological degradation of plastics: A comprehensive review. Biotechnol. Adv. 26: 246 – 265. 67. Sheu, D.S., Chen, W.M., Yang, J.Y. and Chang, R.C. 2009. Thermophilic bacterium Caldimonas taiwanensis produces poly(3-hydroxybutyrate-co-3- hydroxyvalerate) from starch and valerate as carbon sources. Enzyme Microb. Technol. 44: 289 – 294. 68. Shimamura, E., Kasuya, K., Kobayashi, G., Shiotani, T., Shima, Y. and Doi, Y. 1994. Physical properties and biodegradability of microbial poly(3-hydroxyl- butyrate-co-3-hydroxyhexanoate). Macromol. 27: 878 – 880. 69. Snell, K.D. and Peoples, O.P. 2002. Polyhydroxyalkanoate polymers and their production in transgenic plants. Metab. Eng. 4: 29 – 40. 70. Steinb?佟hel, A. and Fuchtenbusch, B. 1998. Bacteria and other biological systems for polyester production. TIPTECH. 16: 419 – 427. 71. Steinb?佟hel, A., Debzi, E.M., Marchessault, R.H. and Timm, A. 1993. Synthesis and production of poly (3-hydroxyvaleric acid) homopolyester by Chromabacterium violaceum. Appl. Microbiol. Biotechnol. 39: 443 – 9. 72. Steinb?佟hel, A, Valentin, H.E. and Sch?民ebaum, A. 1994. Application of recombinant gene technology for production of polyhydroxyalkanoic acids: biosynthesis of poly(4-hydroxybutyric acid) homopolyester. J. Environ. Polym. Degradation. 2: 67 – 74. 73. Suriyamongkol, P., Weselake, R., Narine, S., Moloney, M. and Shah, S. 2007. Biotechnological approaches for the production of polyhydroxyalkanoates in microorganisms and plants — A review. Biotechnol. Adv. 25: 148 – 175. 74. Tzeng, C.M. and Kornberg, A. 2000. The multiple activities of polyphosphate kinase of Escherichia coli and their subunit structure determined by radiation target analysis. J. Biol. Chem. 275: 3977 – 3983. 75. Tokiwa, Y. and Calabia, B.P. 2004. Degradation of microbial polyesters. Biotechnol. Lett. 26: 1181 – 1189. 76. Van der Walle, G.A.M., De Koning, G.J.M., Weusthuis, R.A. and Eggink, G. 2001. Properties, modifications and applications of biopolyesters. Adv. Biochem. Eng. Biotechnol. 71: 263 – 291. 77. Wackett, L.P., Shames, S.L., Venditti, C.P. and Walsh, C.T. 1987. Bacterial carbon – phosphorus lyase: products, rates, and regulation of phosphonic and phosphinic acid metabolism. J. Bacteriol. 169: 710 – 717. 78. Wang, D.B., Li, X.M., Yang, Q., Zheng, W., Liu, Z.Y., Liu, Y.L., Cao, J.B., Yue, X., Shen, T.T., Zeng, G.M. and Deng, J.H. 2009. The probable metabolic relation between phosphate uptake and energy storages formations under single-stage oxic condition. Bioresour. Technol. 100: 4005 – 4011. 79. Wang, F. and Lee, S.Y. 1997. Poly(3-hydroxybutyrate) production with high productivity and high polymer content by a fed-batch culture of Alcaligenes latus under nitrogen limitation. Appl. Environ. Microbiol. 63: 3703 – 3706. 80. Wanner, B.L. 1994. Molecular genetics of carbon – phosphorus bond cleavage in Bacteria. Biodegradation. 5: 175 – 184. 81. Williams, S.F. and Peoples O.P. 1997. Making plastics green. Chem. Br. 33: 29 – 32. 82. Witt, U., Muller, R.J. and Deckwer, W.D. 1997. Biodegradation behavior and material properties of aliphatic/aromatic polyesters of commercial importance. J. Environ. Poly. Degrad. l5: 81 – 89. 83. Yamane, T., Chen, X.F. and Ueda, S. 1996. Growth-associated production of poly(3-hydroxyvalerate) from n-pentanol by a methylotrophic bacterium, Paracoccus denitri?cans. Appl. Environ. Microbiol. 62: 380 – 384. 84. Yamane, T. 1993. Yield of poly-D(—)-3-hydroxybutyrate from various carbon sources: a theoretical study. Biotechnol. Bioeng. 41: 165 – 170. 85. Yu, S.T., Lin, C.C. and Too, J.R. 2005. PHBV production by Ralstonia eutropha in a continuous stirred tank reactor. Process. Biochem. 40: 2729 – 2734. 86. Zhang, Y., Xin, J., Chen, L., Song, H. and Xia, C. 2008. Biosynthesis of poly-3-hydroxybutyrate with a high molecular weight by methanotroph from methane and methanol. J. Nat. Gas Chem.17: 103 – 109.