以發酵槽高密度培養PENICILLIUM CHRYSOGENUM 生產PENICILLIN V之研究

陳惠婷、

E-mail: 9015655@mail.dyu.edu.tw

摘 要

青黴素屬於微生物的二次代謝產物，具有抑菌的作用，依所結合側鏈的不同分為天然型青黴素、半合成青黴素及生合成青黴素，各有不同的構造與特性。生合成青黴素中的青黴素V具有對酸安定的特性，適用於口服藥劑之調製。本實驗以PENICILLIUM CHRYSOGENUM (CCRC 31619 OR ATCC 28089)為菌株生產青黴素V，首先以振盪培養探討起始PH值與不同碳源對培養過程之影響，之後將振盪培養最適的起始PH值與碳源用於發酵槽批次培養，於發酵槽培養中所改變的變因有二：⑴酵母萃取物的添加，⑵發酵槽的攪拌速率。 振盪培養結果顯示：孢子濃度為7.3*106 SPORES/ML時，培養基起始PH值為6.0、6.5及7.0之下，菌體濃度以起始PH 6.5時為最多，而青黴素的產量也最高，可達0.15 G/L。葡萄糖與硫酸銨消耗情形也以起始PH值為6.5時最為顯著。其次，將起始PH值固定在6.5，探討不同碳源培養之關係時發現：葡萄糖做為碳源時，青黴素V產量最多；而蔗糖做為碳源時的青黴素V最終產量與葡萄糖為碳源時相差不多；以乳糖做為碳源時，會因為乳糖的消耗速率較慢而使青黴素V產量較差。所以由實驗結果得知，振盪培養P. CHRYSOGENUM生產青黴素V的最適起始PH值為6.5，最佳碳源為葡萄糖。 發酵槽批次培養中，添加酵母萃取物做為氮源，結果發現，可能是因菌體細胞發生自溶而使菌體濃度與青黴素V產量稍微下降，但因為酵母萃取物成分複雜，可能對之後的研究條件有所影響，所以探討發酵槽攪拌速率時仍以硫酸銨做為唯一的氮源。 在發酵槽的攪拌速率對培養之影響方面，當攪拌速率為350 RPM時，菌體濃度最高，達到8.7 G/L，此時青黴素V的產量也最高，為0.40 G/L。溶氧量在攪拌速率為350 RPM時最高，可提供較充足的氧氣使菌體生長。雖然攪拌速率愈高產生的剪應力會愈大，理應對菌體的生長有所影響，但在此可能是因為發酵槽攪拌速率在150 ~ 350 RPM時之間，剪應力對於菌體細胞的影響並不顯著之因素所致。 由於P. CHRYSOGENUM屬於好氧菌，培養時應維持較高的溶氧量。以發酵槽培養時，因為能不斷通入空氣提高發酵液之溶氧量，所以青黴素V產量比以振盪培養的產量高出許多，其中最大的因素應該是，振盪培養時，氧氣供應不足，以致菌體生長受到限制，而使得青黴素V產量比以發酵槽培養時為低。

關鍵詞 : Penicillium chrysogenum ; 青黴素V ; 振盪培養 ; 批次培養 ; 起始pH值 ; 碳源 ; 酵母萃取物 ; 攪拌速率

目錄

參考文獻

1.王三郎:應用微生物學，二版。 P. 421 - 432，高立圖書，台北(1999)。 2.田蔚城:生物技術導論。生物技術的發展與應用，一版，P. 1 - 18，田蔚城主編。九州圖書，台北 (1999)。 3.向明:微生物釀酵的放大。生物技術的發展與應用，一版，P. 151 - 163，田蔚城主編。九州

圖書，台北 (1999)。 4.吳欣達:聚- B -羥丁酸及其衍生聚合物生化製程開發。大葉大學食品工程研究所碩士論文，彰化 (1999)。 5.江淑芬:生技醫藥品素描，化工資訊，13(4):77 - 89 (1999)。 6.李秀、賴滋漢:食品分析與檢驗。P. 171 - 181，精華出版社，台中 (1992)。 7.段盛秀、楊海明:食品化學實驗。P. 20 - 26，藝軒出版社，台北 (1998)。 8.翁正榮:添加油相對以青黴菌發酵生產盤尼西林之影響。成功大學化學工程研究所碩士論文，台南 (1995)。 9.張嘉倫:屠宰場廢棄豬血之利用。大葉大學食品工程研究所碩士論文，彰化 (1994)。 10.彭兆賢:添加正十六烷對發酵生產盤尼西林之影響。成功大學化學工程研究所碩士論文，台南 (1994)。 11.黃翰賢:利用SCHIZOCHYTRIUM屬海洋真菌生產二十二碳六烯酸。大葉大學食品工程研究所碩士論文，彰化 (1999)。 12.邱健人編譯:食品工業微生物學。復文書局，台南 (1986)。 13.楊慶昌、施純榮:特化產業回顧與展望，化工資訊，13(3):24 -32 (1999)。 14.劉英俊:最新微生物應用工業。P. 353 - 459，中央圖書出版社，台北 (1996)。 15.陳思豐:淺談內醯抗生素 - 盤尼西林及頭孢子菌素，化工技術，1(1):53 - 59 (1993)。 16.陳智偉:添加增稠劑對青黴菌發酵生產盤尼西林之影響。成功大學化學工程研究所碩士論文，台南 (1996)。 17.賴滋漢、賴業超主編:食品科技辭典，增訂版。P. 123，富林出版社，台中 (1994)。 18.ALVAREZ, E., J. M. CANTORL, J. L. BARREDO, B. DIEZ AND J. F. MARTIN, PURIFICATION TO HOMOGENEITY AND CHARACTERIZAION OF ACYL COENZYME A:6 - AMINOPENICILLANIC ACID AND ACYL -TRANSFERASE OF PENICILLIUM CHRYSOGENUM, ANTIMICROB. AGENTS CHEM., 31:1675 - 1682 (1987). 19.ALVAREZ, E., B. MEESSCHAERT, E. MONTENEGRO, S. GUTIERREZ, B. DIEZ, J. L. BARREDO AND J. F. MARTIN, THE ISOPENICILLIN - N ACYLTRANSFERASE, OF PENICILLIUM CHRYSOGENUM HAS ISOPEN -ICILLIN - N AMINODEHYDROLASE, 6 - AMINOPENICILLANIC ACYLTRANSFERASE AND PENICILLIN AMI -DASE, ALL OF WHICH ARE ENCODED BY THE SINGLE PENDE GENE, EUR. J. BIOCHEM., 215:323 - 332 (1993). 20.AMANULLAH, A., P. J?STEN, A. DAVIES, G. C. PAUL, A.W. NIENOW AND C. R. THOMAS, AGITATIO -N INDUCED MYCELIAL FRAGMENTATION OF ASPERGILLUS ORYZAE AND PENICILLIUM CHRYSOGENUM, J. BIOCHEM. ENGINEER., 5:109 - 114 (2000). 21.AMANULLAH, A., P. J?STEN, A. DAVIES, G. C. PAUL, A.W. NIENOW AND C. R. THOMAS, EFFECTS OF AGITATION INTENSITY ON MYCELIAL MORPHOLOGY AND PROTEIN PRODUCTION IN CHEMOSTAT CULTU -RES OF RECOMBINANT ASPERGILLUS ORYZAE, BIOTECHNOL. BIOENG., 62:434 - 446 (1999). 22.ARIYO, B., C. BUCKE AND T. KESHAVARZ, ALGINATE OLIGOSACCHARIDES AS ENHANCERS OF PENICIL -LIN PRODUCTION IN CULTURES OF PENICILLIUM CHRYSOGENUM, BIOTECHNOL. BIOENG., 53:17 - 20 (1997). 23.ARIYO, B., C. TAMERLER, C. BUCKE AND T. KESHAVARZ, ENCHANCED PENICILLIN PRODUCTION BY OLIGOSACCHARIDES FROM BATCH CULTURES OF PENICILLIUM CHRYSOGENUM IN STIRRED - TANK REAC -TORS, FEMS MICROBIOL. LETT., 166:165 - 170 (1998). 24.BANKO, G., S. L. DEMAIN AND S. WOLFE, Δ- (L - A - AMINOADIPYL) - L - CYSTEINYL - D - VALINE SYNTHETASE (ACV - SYNTHETASE) : A MULTIFUNCTIONAL ENZYME WITH BOARD SUBSTRACT SPECIFICITY FOR THE SYNTHESIS OF PENICILLIN AND CEPHALOSPORIN PRECURSORS, J. AM. CHEM. SOC., 109:2858 - 2860 (1987). 25.BRUNDIDGE, S. P., F. C. A. GATEA, D. J. HOOK, C. SAPINO, R. P. ELANDER AND R. P. MORIN, ASSOCIATION OF 6 - OXO - PIPERIDINE - 2 - CARBOXYLIC ACID WITH PENICILLIN V PRODUCTION IN PENICILLIUM CHRYSOGENUM FERMENTATION, J. ANTIBIOT., (TOKYO) 23:1348 - 1351 (1980). 26.CRUEGER, W. AND A. CRUEGER, ANTIBIOTICS, IN "BIOTECHNOLOGY: A TEXTBOOK OF INDUSTRIAL MI -CROBIOLOGY", EDITED BY T. D. BROCK, PP.197 - 237, SCIENCE TECH., USA (1984). 27.FENCL, Z., CELL AGEING AND AUTOLYSIS, IN "THE FILAMENTOUS FUNGI III", EDITED BY J. E. SMITH AND D. R. BARRY, PP. 389 - 405, EDWARD ARNOLD, N.Y. (1974). 28.GUERRANT, G., ANALYSIS OF SHORT- CHAIN ACIDS FROM ANAEROBIC BACTERIA BY HIGH - PERFORMA -NCE LIQUID CHROMATOGRAPHY, J. CLIN. BIOCHEM., 16:355 - 360 (1998). 29.HARVEY, L. M., B. MCNEIL, D. R. BERRY AND S. WHITE, AUTOLYSIS IN BATCH CULTURES OF PENI -CILLIUM CHRYSOGENUM AT VARYING AGITATION RATES, ENZYME MICROB. TECHNOL., 22:446 - 458 (1998). 30.HENRIKSEN, C. M., J. NIELSEN AND J. VILLADSEN, HIGH EXOGENOUS CONCENTRATIONS OF PHENOXY -ACETIC ACID ARE CRUCIAL FOR A HIGH PENICILLIN V PRODUCTIVITY IN PENICILLIUM CHRYSOGENU -M, MICROBIOL., 144:2001 - 2006 (1998). 31.HERNAWAN, T. AND G. FLEET, CHEMICAL AND CYTOLOGICAL CHANGES DURING THE AUTOLYSIS OF YEA -ST, J. IND. MICROBIOL., 14:440 - 450 (1995). 32.HOTOP, S., J. MOLLER, J. NIEHOFF AND K. SCHUGERL, INFLUENCE OF THE PRECULTURE CONDITIO -NS ON THE PELLET SIZE DISTRIBUTION OF PENICILLIUM CHRYSOGENUM CULTIVATIONS, BIOCHEM., 28:99 - 104 (1993). 33.JARVIS, F. G. AND M. J. JOHNSON, THE ROLE OF THE CONSTITUENTS OF SYNTHETIC MEDIA FOR PENICILLIN PRODUCTION, AM. CHEM. SOC. J., 69:3010 - 3017 (1947). 34.JORGENSEN, H. S., METABOLIC FLUXS IN PENICILLIUM CHRYSOGENUM PH. D. DISERTATION, TECHNI -CAL UNIVERSITY OF DENMARK, LYNGBY DENMARK (1993). 35.JORGENSEN, H. S., J. NIELSEN, J. VILLADSEN AND H. MOLLGAARD, ANALYSIS OF THE PENICILLI -N V BIOSYNTHESIS DURING FED - BATCH CULTIVATION WITH A HIGH YIELDING STRAIN OF PENICIL -LIUM CHRYSOGENUM, APPL. MICROBIOL BIOTECHNOL., 43:123 - 130 (1995). 36.J?STEN, P., G. C. PAUL, A. W. NIENOW AND C. R. THOMAS, DEPENDENCE OF PENICILLIUM CHRYSO -GENUM GROWTH, MORPHOLOGY, VACUOLATION AND PRODUCTIVITY IN FED - BATCH FERMENTATION ON IMPELLER TYPE AND AGITATION INTENSITY, BIOTECHNOL. BIOENG,, 59:762 - 775 (1998). 37.J?STEN, P., G. C. PAUL, A. W. NIENOW AND C. R. THOMAS, DEPENDENCE OF MYCELIAL MORPHOLOG -Y ON IMPELLER TYPE AND AGITATION INTENSITY, BIOTECHNOL. BIOENG,, 52:634 - 648 (1996). 38.LEONHARTSBERGER, S., R. M. LAFFERTY AND L. KORNETI, USE OF COLLAGEN HYDROLYSATE AS A CO -MPLEX NITROGEN SOURCE FOR THE SYNTHESIS OF PENICILLIN BY PENICILLIUM CHRYSOGENUM, J. BACTERIOL., 30:299 - 313 (1993). 39.LIGGETT, R. W. AND H. KOFFLER, CORN STEEP LIQUOR IN

MICROBIOLOGY, BACTERIOL. REV., 12: 297 - 311 (1948). 40.MCNEIL, B., D. R. BERRY, L. M. HARVEY, A. GRANT AND S. WHITE, MEASUREMENT OF AUTOLYSIS IN SUBMERGED BATCH CULTURES OF PENICILLIUM CHRYSOGENUM, BIOTECHNOL. BIOENG,, 57:297 - 305 (1998). 41.METZ, B. AND N. W. F. KOSSEN, THE GROWTH OF MOLDS IN THE FORM OF PELLETS : A LITERATURE REVIEW, BIOTECHNOL. BIOENG., 19:781 - 799 (1977). 42.NIELSEN, J., L.L. JOHANSEN, M. JACOBSEN, P. KRABBEN AND J. VILLADSEN, PELLET FORMATION AND FRAGMENTATION IN SUBMERGED CULTURES OF PENICILLIUM CHRYSOGENUM AND ITS RELATION TO PENICILLIN PRODUCTION, BIOTECHNOL. PROG., 11:93 - 98 (1995). 43.NIELSEN, J., A SIMPLE MORPHOLOGICALLY STRUCTURE MODEL DESCRIBING THE GROWTH OF FILAMENT -OUS MICROORGANISMS, BIOTECHNOL. BIOENG., 41:715 - 727 (1993). 44.PACKER, H. L. AND C. R. THOMAS, MORPHOLOGICAL MEASUREMENTS ON FILAMENTOUS MICROORGANISI -MS BY FULLY AUTOMATIC IMAGE ANALYSIS, BIOTECHNOL. BIOENG. 35:870 - 881 (1990). 45.PAN, C. H., L. HEPLER AND R. P. ELANDER, THE EFFECT OF ION ON A HIGH-YIELDING INDUSTRIA -L STRAIN OF PENICILLIUM CHRYSOGENUM AND PRODUCTION LEVELS OF PENICILLIN G, J. FERMENT. TECHNOL., 53:854 - 861 (1975). 46.PAUL, G. C., C. A. KENT AND C. R. THOMAS, HYPHAL VACUOLATION AND FRAGMENTATION IN PENIC -ILLIUM CHRYSOGENUM, BIOTECH. BIOENG., 44:655 - 660 (1994). 47.PECINA, R., HIGH - PERFORMANCE LIQUID CHROMATOGRAPHY ELUTION BEHAVIOR OF ALCHOLS, ALDEH -YDES, KETONES, ORGANIC ACIDS AND CARBOHYDRATES ON A STRONG CATIONEXCHANGE STATIONARY PHASE, J. CHROM., 287:245 - 258 (1984). 48.PERLMAN, D., "THE HISTORY OF PENICILLIN PRODUCTION", CHEM. ENGINEER. PROG. SYMP. SERIES - 100, 66:24 - 30 (1970). 49.PHILLIPS, D. H., OXYGEN TRANSFER INTO MYCELIAL PELLETS, BIOTECHNOL. BIOENG. 8:456 - 460 (1966). 50.PIRT, S. J. AND D. S. CALLOW, CONTINUOUS- FLOW CULTURE OF THE FILAMENTOUS MOULD PENICIL -LIUM CHRYSOGENUM AND THE CONTROL OF ITS MORPHOLOGY, NATURE., 184:307 - 310 (1959). 51.PRESCOTT, L. M., J. P. HARLEY AND D. A. KLEIN, MICROBIOLOGY, WM. C. BROWN PUBLISHERS, USA (1990). 52.PUSZTAHELYI, T., I. POCSI, J. KOZMA AND A. SZENTIRMAI, AGEING OF PENICILLIUM CHRYSOGENU -M CULTURES UNDER CARBON STARVATION:I MORPHOLOGICAL CHANGES AND SECONDARY METABOLITE PR -ODUCTION. BIOTECHNOL. APPL. BIOCHEM., 25:81 - 86 (1997A). 53.PUSZTAHELYI, T., I. POCSI, J. KOZMA AND A. SZENTIRMAI, AGEING OF PENICILLIUM CHRYSOGENU -M CULTURES UNDER CARBON STARVATION:II PROTEASE AND N - ACETYL - B - D - HEXOSAMINIDASE PRODUCTION. BIOTECHNOL. APPL. BIOCHEM., 25:87 - 93 (1997B). 54.RIGHELATO, R. C., A. P. J. TRINCI AND S. J. PIRT, THE INFLUENCE OF MAINTENANCE ENERGY AND GROWTH RATE ON THE METABOLIC ACTIVITY, MORPHOLOGY AND CONIDATION OF PENICILLIUM CHRYSOGENUM, J. GEN. MICROBIOL., 50:399 - 412 (1968). 55.SCHUGLER, K. AND G. SEIDEL, MONITORING OF THE CONCENTRATION OF B - LACTAM ANTIBIOTICS AND THEIR PRECURSORS IN COMPLEX CULTIVATION MEDIA BY HIGH - PERFORMANCE LIQUID CHROMAT -OGRAPHY, J. CHROM. A., 812:179 - 189 (1998). 56.SMITH, J. J., M.D. LILLY AND R. I. FOX, THE EFFECT OF AGITATION ON THE MORPHOLOGY AND PENICILLIN PRODUCTION OF PENICILLIUM CHRYSOGENUM, BIOTECHNOL. BIOENG. 35:1011 - 1023 (1990). 57.SWARTZ, R. W., THE USE OF ECONOMIC ANALYSIS OF PENICILLIN G MANUFACTURING COSTS IN ESTABLISHING PRIORITIES FOR FERMENTATION PROCESS IMPROVEMENT, IN "ANNUAL REPORT ON FERMENTATION PROCESS" VOL. 3. EDITED BY D. PERLMAN AND G. T. TSAO, PP. 75 - 110, ACADE -MIC, NEW YORK (1979). 58.SYLVESTER J. C. AND R. D. COGHILL, THE PENICILLIN FERMENTATION, IN "INDUSTRIAL FERMENT -ATION" VOL. 2, EDITED BY L. A. UNDERKOFLER AND R. J. HICKEY, PP. 219 - 263, CHEMICAL PUBLISHING, NEW YORK (1954). 59.TRINCI, A. P. J. AND R. C. RIGHELAO, CHANGES IN CONSTITUENTS AND ULTRASTRUCTURE OF HYP -HAL COMPARTMENTS DURING AUTOLYSIS OF GLUCOSE STARVED PENICILLIUM CHRYSOGENUM, J. GEN. MICROBIOL., 60:239 - 249 (1970). 60.VAN SUIJDAM, J. C. V., N. W. F. KOSSEN AND P. G. PAUL, AN INOCULUM TECHNIQUE FOR THE PRODUCTION OF FUNGAL PELLETS, APPL. MICROBIOL. BIOTECHNOL., 10:211 - 221 (1980). 61.WHITE, R. L., E. JOHN, J. E. BALDWIN AND E. P. ABRAHAM, STOICHIOMETRY OF OXYGEN CONSUMP -TION IN THE BIOSYNTHESIS OF ISOPENICILLIN FROM A TRIPEPTIDE, BIOCHEM. J., 203:791 - 7 93 (1982).