

促進鏈球菌透明質酸醱酵產程生理代謝控制之策略

蘇伊伶、徐泰浩

E-mail: 9607756@mail.dyu.edu.tw

摘要

透明質酸 (hyaluronic acid) 又名玻尿酸或透明多醣體 (hyaluronan)，由D-葡萄糖醛酸與N-乙醯胺基葡萄糖相互交替連接而成的直鏈多糖體，廣泛存在於動物的組織細胞間質和某些細菌的莢膜。莢膜有無及其大小與菌株所產生之透明質酸?活性有密切之關係。研究指出活性氧衍生物與自由基能分解透明質酸，而抗氧化劑可抑制氧化壓力所造成之透明質酸分解。本研究探討獸疫鏈球菌 (Streptococcus zooepidemicus) 醱酵時不同因子如：溫度、起始pH值、攪拌速率及抗氧化劑之添加對生產游離性透明質酸、莢膜性透明質酸及莢膜大小分析之影響，實驗利用影像系統分析菌體莢膜之有無及大小，並分析各因子莢膜性與游離性透明質酸生成之相關性，結果顯示，游離性透明質酸之生成以37 產量為1.45 g/L、起始pH 7.5產量1.74 g/L、150 rpm產量1.57 g/L、50 mg/L沒食子酸產量1.58 g/L、250 mg/L抗壞血酸產量1.54 g/L、250 mg/L生育醇產量1.49 g/L培養下最高。莢膜性透明質酸的產量上則是在37 產量0.08 g/L、起始pH 7.5產量0.08 g/L，150 rpm產量0.09 g/L時最好。此外，莢膜大小均在培養6小時最大，此外則隨著培養時間之增加而有減少之情況，而溶血環的作用則與此結果相反。

關鍵詞：透明質酸；獸疫鏈球菌；培養溫度；抗氧化劑

目錄

中文摘要.....	iv	英文摘要.....	v	誌謝.....	vi	目錄.....	vii
圖目錄.....	x	1. 前言.....	1	2. 文獻回顧.....	1	3.1 透明質酸之特性.....	3
3.2.1 透明質酸之由來.....	3	3.2.2 透明質酸的分佈.....	3	3.2.3 透明質酸的結構.....	4	4.2 透明質酸之製備與純化.....	7
4.2.1 利用動物直接萃取.....	7	4.2.2 利用微生物直接萃取.....	7	4.2.3 透明質酸之生化合成結構.....	7	4.2.4 透明質酸之性質.....	11
4.2.4.1 透明質酸之黏彈性.....	11	4.2.4.2 透明質酸之分子量.....	12	4.2.4.3 透明質酸之降解反應.....	12	4.2.5 影響透明質酸之因子.....	15
4.2.5.1 溫度影響.....	15	4.2.5.2 pH值影響.....	15	4.2.5.3 攪拌速率影響.....	16	4.2.5.4 抗氧化劑影響.....	16
4.2.6 透明質酸之應用.....	17	4.2.6.1 透明質酸於醫藥方面之應用.....	17	4.2.6.2 透明質酸於化妝品方面之應用.....	19	4.2.6.3 透明質酸於保健食品方面之應用.....	20
3. 材料與方法.....	21	3.1 實驗材料.....	21	3.1.1 實驗藥品.....	21	3.1.2 儀器設備.....	22
3.2 培養基.....	22	3.2.1 種菌培養基.....	22	3.2.2 基礎培養基.....	23	3.3 實驗方法.....	23
3.3.1 試驗菌株.....	23	3.3.2 種菌培養.....	23	3.3.3 種菌保存.....	23	3.3.4 溶血環試驗.....	24
3.4 不同溫度之搖瓶培養試驗.....	24	3.5 不同起始pH值之搖瓶培養試驗.....	24	3.6 不同攪拌速率之搖瓶培養試驗.....	25	3.7 添加不同抗氧化劑之搖瓶培養試驗.....	25
3.8 游離性透明質酸之試驗.....	26	3.9 莢膜性透明質酸之試驗.....	26	3.10 分析方法.....	26	3.10.1 菌體生質量之測定.....	26
3.10.2 透明質酸含量之測定.....	27	3.10.3 菌體莢膜染色之分析.....	28	4. 結果與討論.....	29	4.1 菌體生質量與游離性透明質酸產量之比較.....	29
4.1.1 溫度之影響.....	29	4.1.2 起始pH值之影響.....	32	4.1.3 不同轉速之影響.....	36	4.1.4 抗氧化劑之影響.....	39
4.2 莢膜性透明質酸產量之比較.....	47	4.3 溶血性試驗.....	51	4.4 莢膜大小之分析.....	53	5. 結論.....	67
參考文獻.....	68						

參考文獻

1. 三古、倂田薰和須見哲郎。1988。透明質酸的分子量保濕能力。香妝會志12(1):50-59。
2. 王雲萍。1994。生物科技的寵兒— 玻尿酸。化工資訊。5: 44-49
3. 吳鎮宇。2004。以微生物生產透明質酸製程參數之探討。南台科技大學論文。台南。
4. 沈慧彥。2006。培養基中碳氮源對獸疫鏈球菌醱酵產程生成透明質酸之影響。大葉大學論文。彰化。
5. 郭學平、王春喜、凌沛學和張天民。1998。透明質酸及其發酵生產概述。中國生化藥物雜誌19(4):209-212。
6. 黃定國。2001。透明質酸之開發與應用。菌種保存及研究簡訊14(3):1-9。
7. 黃怡倩。2006。環境因子對獸疫鏈球菌醱酵產程生成透明質酸之影響。大葉大學論文。彰化。
8. 張效良、劉隆躍和吳功柱。1999。人臍帶透明質酸製備及理化性質分析。中國藥房10(1):10-11。
9. 羅曼和蔣立科。1999。牛眼透明質酸的分離及性質測定。生物化學與生物物理

進展26(6):596-600. 10. Akasaka, J., Seto, S., Yanagi, M., Fukushima, S. and Mitsui, T. 1988. Industrial production of hyaluronic acid by *Streptococcus zooepidemicus*. *Journal of the Society Cosmetic of Chemists Japan*. 22 : 35-42 11. Allen, A. G., Lindsay, H., Seilly, D., Bolitho, S., Peters, S. E. and Maskell, D. J. 2004. Identification and characterisation of hyaluronate lyase from *Streptococcus suis*. *Microb. Pathog.* 36(6): 327-335 12. Armstrong, D. C., Cooney, M. J. and Johns, M. R. 1997. Growth and amino acid requirements of hyaluronic acid producing *Streptococcus zooepidemicus*. *Appl. Microbiol. Biotechnol.* 47: 309-312. 13. Armstrong, D. C., and Johns, M. R. 1997. Culture conditions affect the molecular weight properties of hyaluronic acid produced by *Streptococcus zooepidemicus*. *Appl. environ. microbiol.* 63: 2759-2764. 14. Balaz, E. A. 1979. Ultrapure hyaluronic acid and the use therefore. United States Patent:4,141,973. 15. Balaz, E. A., Laurent, T. C. and Jeanloz, R. W. 1986. Nomenclature of hyaluronic acid. *Biochem. J.* 235: 903. 16. Balazs, E., 1990. " The physical properties of synovial fluid and the specific role of hyaluronic acid, " *Curr. Ther. Res.*, 47: 437 – 443 17. Balaze, E. A., 1990. Medical applications of hyaluronic acid its derivatives : cosmetic and pharmaceutical application of polymers. Plenum press, New York. 18. Bentley, J. P., Dunphy, J.E., Van Winkle, W. Jr., eds.1968. Mucopolysaccharide synthesis in healing wounds. Repair and regeneration. New York : McGraw-Hill: 151~160. 19. Bitter, T. and Muir, H. M. 1962. A modified uronic acid carbazole reaction. *Anal. Biochem.* 4: 330-334. 20. Bothner, L. M. and Wilk, O., 1987. " Rheology of hyaluronate, " *Acta Otolaryngol.*, 44: 25~30. 21. Bracke, J. W. and Thacker, K. 1985. Hyaluronic acid from bacterial culture. U.S. patent. 4, 517, 295. 22. Carbera, R. C. 1995. The in vivo effect of hyaluronan associated protein-collagen complex on wound healing. *Biochem. And Molecular Biol. Int.* 37: 151-158. 23. Carlin, G.; Djursater, R.; Smedegard, G.; Gerdin, B. 1985. Effect of anti-inflammatory drugs on xanthine-oxidase induced depolymerization of hyaluronic acid. *Agents Action.* 16 : 377-384. 24. Chong, B. F. and Nielsen, L. K. 2003. Aerobic cultivation of *Streptococcus zooepidemicus* and the role of NADH oxidase. *Biochem. Eng. J.* 16: 153-162. 25. Chong, B. F. and Nielsen, L. K. 2003. Amplifying the cellular reduction potential of *Streptococcus Zooepidemicus*. *Journal of biotechnology.* 100 : 33-41 26. Cleary, P. P. and Larkin, A. 1979. Hyaluronic acid capsule strategy for oxygen resistance in group A streptococci. *J. Bacteriol.* 140(3): 1090-1097. 27. Dostal, G. H. and Gamelli, R. L. 1993. Fetal wound healing. *Surg. Gynecol. Obstet.* 176: 299-306. 28. Fessler JH, Fessler LI. 1966. Electron microscopic visualization of the polysaccharide hyaluronic acid. *Proc Natl Acad Sci USA.* 56 : 141-147. 29. Goa, K. L. and Benfield, P. 1994. Hyaluronic acid. A review of its pharmacology and use as a surgical aid in ophthalmology and its therapeutic potential in joint disease and wound healing. *Drugs.* 47 : 536-566. 30. Goh, L. T. 1998. Effect of culture conditions on rates of intrinsic hyaluronic acid production by *Streptococcus equi* subsp. *zooepidemicus*. *Chemical Engineering.* (Brisbane: University of Queensland). 31. Greenwald, R. A.; Moy, W. W. 1980. Effect of oxygen-derived free radicals on hyaluronic acid. *Arthritis Rheum.* 23 : 455-463 32. Greenwald, R. A. 1981. Effect of oxygen-derived free radicals on connective tissue macromolecules : inhibition by copper-penicillamine complex. *J. Rheumatol. Suppl.* 7 : 9-13. 33. Hascall, V. C. and Laurent, T. C. 1997. Hyaluronan: structure and physical properties. <http://www.glycoforum.gr.jp>. 34. Hawkins, C. L.; Davies, M. J. 1996. Direct detection and identification of radicals generated during the hydroxyl radical-induced degradation of hyaluronic acid and related materials. *Free Radic. Biol. Med.* 21 : 275-290 35. Johns, M. R., Goh, L. T., and Oeggri, A. 1994. Effect of pH, agitation and aeration on hyaluronic acid production by *Streptococcus zooepidemicus*. *Biotechnology letters.* 16 : 507-512. 36. Kass, E. H. and Seastone, C. V. 1944. The role of the mucoid polysaccharide hyaluronic acid in the virulence of group A hemolytic *Streptococci*. *The Journal of Experimental Medicine.* 70 : 319-330. 37. Kendall, F. E., Heidelberger, M. and Dawson, M. H. 1937. A serologically inactive polysaccharide elaborated by mucoid strains of group a hemolytic *Streptococcus*. *J. Biol. Chem.* 118: 61-69. 38. Kim, J. H., Yoo, S. J., Oh, D. K., Kweon, Y. G., Park, D. W., Lee, C. H. and Gil, G. H. 1996. Selection of a *Streptococcus equi* mutant and optimization of culture conditions for the production of high molecular weight hyaluronic acid. *Enzyme Microb. Technol.* 19: 440-445. 39. Laurent, T. C., and Fraser, J. R. E. 1986. The properties and turnover of hyaluronan. In: function of the proteoglycans (Evered D, Whelan J, Eds) ciba foundation symposium 124, pp9-29, New York. Wiley. 40. Laurent, T. C., 1987. " Biochemistry of hyaluronan, " *Acta Otolaryngol.*, 42: 7~24 41. Laurent, T. C., and Fraser, J. R. E. 1992. Hyaluronan. *FASEB J.* 6: 2397-2404. 42. Lindvall, S.; Rydell, G. 1994. Influence of various compounds on the degradation of hyaluronic acid by a myeloperoxidase system. *Chem. Biol. Interact.* 90 : 1-12. 43. Lurie, Ziva., Offer, Tal., Russo, Angelo., Samuni, Amram., Nitzan, Dorrit. 2003. Do stable nitroxide radicals catalyze or inhibit the degradation of hyaluronic acid ? 35 : 169-178. 44. McCord, J. M. 1974. Free radicals and inflammation : protection of synovial fluid by superoxide dismutase. *Science* 185 : 529-531. 45. Meyer, K., 1947. " The biological significance of hyaluronic acid and hyaluronidase, " *Physiol. Rev.*, 27: 335-359 46. Meyer, K., and Palmer, J., 1934. The Polysaccharide of the vitreous humor. *J. Boil. Chem.* 107: 629-634. 47. Nimrod, A., Greenman, B., Kanner, D., Landsberg, M. and Beak, Y. 1988a. Method of producing high molecular weight sodium hyaluronate by fermentation of *Streptococcus*. United States Patent:4,780,414. 48. Oksala, O., Salo, T., Tammi, R., Hakkinen, L., Jalkanen, M., Inki, P. and Larjava, H., " Expression of proteoglycans and hyaluronan during wound healing. " *J. Histochem. Cytochem.*, 43: 125-135 (1995). 49. O' Regan, M., Martini, I., Crescenzi, F., Luca, C., and Lansing, M. 1994. Molecular mechanisms and genetics of hyaluronan biosynthesis. *Int. J. Biol. Macromol.* 16: 283-286. 50. Pace, G. W. and Righelato, R. C. 1980. Production of extracellular microbial polysaccharides. *Advances in Biochemical Engineering.* 15 : 41-70. 51. Prisel, P. T., Camber, O., Hiselius, J. and Norstedt, G., 1992. " Evaluation hyaluronan as a vehicle for peptide growth-factors. " *Int. J. Pharm.*, 85: 51-56 52. Sarri, H. 1991. Oxygen derived free-radicals and synovial-fluid hyaluronate. *Ann. Rheum. Dis.* 50 : 389-392 53. Sarri, H.; Kontinen, Y. T.; Friman, C.; Soras, T. 1993. Differential effects of reactive oxygen species on native synovial-fluid and purified human umbilical-cord hyaluronate. *Inflammation* 17 : 403-415. 54. Schmut, O.; Hofmann, H. 1975. Studies on the generation of hydrogen peroxide during some non-enzymic reactions changing the hyaluronic acid molecule. *Biochim. Biophys. Acta* 411 :231-235. 55. Scott, J. E., Cummings, C., Brass, A. and Chen, Y. 1991. Secondary and tertiary structures of hyaluronan in aqueous solution, investigated by rotary shadowing-electron microscopy and computer simulation. *Biochem. J.* 274:

699-705. 56. Scott, J., Heatley, F., and Hull, W., 1984. " Secondary Structure of Hyaluronate in Solution, " *Biochem. J.*, 220:197-205. 57. Tokita, Y. and Okamoto, A.. 1995. " Hydrolytic degradation of hyaluronic acid, " *Polym. Degrad. Stab.*, 48:269-273 58 Van de Rijn, I. 1983. Streptococcal hyaluronic acid proposed mechanisms of degradation and loss of synthesis during stationary phase. *Journal of Bacteriology.* 156 : 1059-1065. 59. West, D. C., I. N. Hampson, F. Arnold and S. Kumar. 1985. Angiogenesis induced by degradation products of hyaluronic acid. *Science* 28 : 1324. 60. Wessels, M. R., Moses, A. E., Goldberg, J.B. and DiCesare, T. J. 1991. Hyaluronic acid capsule is a virulence factor for mucoid group A Streptococci. *Proceeding of the National Academy of Sciences of the United States of America.* 88 : 8317-8321. 61. Whitnack, E., Bison, A. L. and Beachy, E. H. 1981. Hyaluronate capsule prevents attachment of group A Streptococci to mouse peritoneal macrophages. *Infection and Immunity.* 31 : 985-991.