

Enhanced Production of Hyaluronic Acid by Streptococcus Fermentation with Control Strategy of Metabolism and Physiology

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ABSTRACT

Hyaluronic acid is a linear polysaccharide composed of repeating subunits of β -1,4-linked disaccharide of glucuronic acid β -1,3-N-acetylglucosamine. It has been existed in extracellular matrix in animal tissue. There are close relations in capsule and hyaluronidas. The studies have demonstrated the role played by reactive oxygen-derived species in depolymerization of hyaluronic acid (HA), that antioxidants would protect HA from oxidative degradation. The effect of various cultures on temperature, pH, agitation rate and added various antioxidants concentration of hyaluronic acid production by *Streptococcus zooepidemicus* was studied. And the effects of extracellular hyaluronic acid and capsule hyaluronic acid were investigated. Morphological details of the sample were evaluated using an image analyzer. The result exhibited that extracellular hyaluronic acid concentration of 37 (1.45 g/L), pH 7.5 (1.74 g/L), 150 rpm (1.57 g/L), 50 mg/kg of gallic acid (1.58 g/L), 250 mg/kg of ascorbic acid (1.54 g/L), 250 mg/kg of tocopherol (1.49 g/L) had the maximum yield. The capsule hyaluronic acid had the maximum yield in 37 (0.08 g/L), pH 7.5 (0.08 g/L), 150 rpm (0.09 g/L). On the other hand, the capsule were significantly increased in 6h by culture time.

Keywords : Hyaluronic acid ; *Streptococcus zooepidemicus* ; culture temperature ; antioxidant

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REFERENCES

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發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發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. Prisell, 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.; Konttinen, 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.