Effects of Carbon/Nitrogen Sources in Media on Production of Hyaluronic Acid by Streptococcus zooepidemicus Submerged Fe

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ABSTRACT

Hyaluronic acid is a linear polysaccharide composed of repeating disaccharide units of D-glucuronic acid and N-acetylglucosamine linkaged by β(1-3) and β(1-4) glycosidic bond. The average molecular weight is typically in the range 106 to 108 dalton. It biodergradable material is significant water-holding and lubricant properties. Also using in medical applicanting such as treatment of joint disease, ophthalmic surgical derice, diagnostic marker and drug delivery. In this study, some of the hyaluronic acid-producting bacterial mutant which did not have property of hemolysis was selected from Streptococcus by the serial selection programs after UV and NTG treatment.Then culturing in the medium that has different carbon and nitrogen sources. The result shows that S. alactolyticus is 2.131 μm in average diameter which is biger than the others. S.equi has hemolysis reaction that hemolytic zone’s diameter was 6.2~6.4mm, and highest production of hyaluronic acid was 0.183g/L by S. zooepidimicus. It had been targeted S. zooepidimicus for mutation test because its’ high hyaluronic acid producing and no hemolysis reaction. The UV mutated species NO.7-7 had stable hyaluronic acid production between 0.356 to 0.386g/L and NTG mutation species NO.N9-17, NO.N17-14 was 0.343 to 0.391g/L. Capsular thickness and hyaluronic acid concentration of the mutated species increased at least 2.2 times that was batter than S. zooepidemicus. Glucose and yeast extract can provided best biomass and hyaluronic acid concentration then other carbon and nitrogen sources. Mutated species NO.7-7 and NO.N9-17 had 0.589g/L and 0.562g/L of maximum hyaluronic acid concentration and 1.17 and 1.16 of biomass by using C/N ratio 4:1 medium in shaken culture. Mutated species N17-14 had 0.584g/L of hyaluronic acid concentration and 1.25 of biomass by using C/N ratio 3:1 medium in shaken.

Keywords : Hyaluronic acid ; Streptococcus zooepidemicus ; Selection ; Mutation ; Fermentation

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REFERENCES

1.王盈錦和張淑貞。2001。高分子生醫材料。化工技術9(5):110-129。 2.王毅和鄭文艷。2005。玻璃酸製劑的臨床應用進展。食品與藥品7(8):11-16。 3.朱兆秀。2005。利用鏈球菌醱酵生產透明質酸。化工技術13(7):165-172。 4.李小迪。1997。透明質酸的保濕特性及其在化妝品中的應用。香料香精化妝品3:19-22。 5.李文任、黃偉韜和黃雄飛。2003。關節腔內注射透明質酸鈉治療膝關節骨性關節炎。現代臨床醫學生物工程學雜誌9(3):263。 6.姚敏杰、安海平和陳玉銘。1995。透明質酸發酵法製備研究。江蘇食品與發酵2:19-25。 7.倪杭生

、李潤、賀豔麗和羅敏。2001。透明質酸的離子交換層析純化。中國生化藥物雜誌32(11):485-487。 8.徐紅和陸志華。1998。透明質酸鈉在化妝品中的應用。中國生化藥物雜誌19(5):222-223。 9.高海軍、陳堅、管軼眾、堵國成和倫世儀。1999。獸疫鏈球菌搖瓶發酵法生產透明質酸。無錫輕工大學報18(3):17-22。 10.凌沛學、賀艷麗和張青。2005。透明質酸對骨關節炎的治療作用。食品與藥品。7(1):1-3。 11.唐上華。1984。工業菌株的改良:誘變和隨機篩選過程。工業微生物。46:24-34。 12.劉文斌、溫耀和孫思勤。2003。深層鞏膜切除聯合Healon GV注入治療開角型青光眼。眼科研究21(2):189-190。 13.陳樹人和黃煒智。2004。玻尿酸酶酵與分離純化技術。化工技術12(11):143- 152。 14.陳鵬、陸文雄、周勤夫和嚴雅靜。1999。透明質酸的應用及製備研究進展。上海大學學報5(1):69-73。 15.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。 16.郭學平、凌沛學、王春喜和張天民。2000。透明質酸的生產。藥物生物技術7(1):61-64。 17.馮建成、李潔、石衍君、金義鑫、袁琳和楊豔燕。2004。微生物發酵法生產透明?酸。現代商貿工業3:47-50。 18.馮建成、崔貞華、尹姣、曾潔莉和楊艷燕。2005。透明質酸產生菌的紫外線誘變及搖瓶條件的優化。湖北大學學報27(1):57-60。 19.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。 20.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。 21.張魯榕、殷蔚萭和孫淑潔。1991。血清透明質酸含量測定在肝病時的診斷意義。北京醫學13(2):119-122。 22.蔡曉雯。2001。應用於組織工程之天然高分子－透明質酸。生物產業12(4):276-282。 23.鄭曉龍、賀玲和楊新光。2002。透明質酸鈉在眼科的應用。實用醫藥雜誌19(5):387-388. 24.糜福龍、邱秀娟和陳俊瑜。2005。天然高分子生物醫用材料在化妝品領域之應用。化工技術13(7):155-164。 25.顧其勝、王文斌和吳萍。1998。醫用透明質酸鈉在臨床中的應用綜述。中國修復重建外科雜誌12(2):124-126。 26.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。 27.羅瑞明、郭美錦、儲炬、張嗣良。2003。高產大分子量透明質酸突變株NUF-036的選育。無錫輕工大學學報22(2):14-17。 28.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. 29.Andre, P. 2004. Hyaluronic acid and its use as a rejuvenation agent in cosmetic dermatology. Semin. Cutan. Med. Surg. 23(4): 218- 222. 30.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. 31.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: 2759-27648. 32.Aruffo, A., Stamenkovic, L., Melnick, M., Underhill, C. B. and Seed, B. 1990. CD44 is the principal cell surface receptor for hyaluronate. Cell. 61: 1303-1313. 33.Balaz, E. A. 1979. Ultrapure hyaluronic acid and the use therefore. United States Patent:4,141,973. 34.Balaz, E. A., Laurent, T. C. and Jeanloz, R. W. 1986. Nomenclature of hyaluronic acid. Biochem. J. 235: 903. 35.Barker, S. A. and Young, N. M. 1966. Isolation of hyaluronic acid by gel filtration on agarose. Carbohydr. Res. 2: 363-370. 36.Bergan, T. and Hovig, B. 1969. Hyaluronic acid capsule in a Streptococcus faecalis Var. zymogenes a comparison with related mucoid strains. Acta. Pathol. Microbiol. Scand. 75(1): 97-103. 37.Bitter, T. and Muir, H. M. 1962. A modified uronic acid carbazole reaction. Anal. Biochem. 4: 330-334. 38.Bracke, J. W., Minnetonka, M. N., Thacke, K. and Minneapolis, M. N. 1985. Hyaluronic acid from bacterial culture. United States Patent:4,517,295. 39.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. 40.Chung, J. H., Kim, H. J., Fagerholmb, P. and Cho, B. C. 1996. Effect of topically applied Na-hyaluronan on experimental corneal alkali wound healing. Korean J. Ophthalmol. 10(2): 68-75. 41.Cifonelli, J. A., Rebers, P. A. and Heddleston, K. H. 1970. The isolation and characterization of hyaluronic acid from Pasteurella multocida. Carbohydr Res.14: 272-276. 42.Cleary, P. P. and Larkin, A. 1979. Hyaluronic acid capsule strategy for oxygen resistance in group A streptococci. J. Bacteriol. 140(3): 1090-1097. 43.Courel, M. N., Maingonnat, C., Tranchepain, F., Deschrevel, B., Vincent, J. C., Bertrand, P. and Delpech, B. 2002. Importance of hyaluronan length in a hyaladherin based assay for hyaluronan. Anal. Biochem. 302: 285-290. 44.Day, R., Brooks, P., Conaghan, P.G. and Petersen, M. 2004. A double blind randomized multicenter parallel group study of the effectiveness and tolerance of intraarticular hyaluronan in osteoarthritis of the knee. J. Rheumatol. 31(4): 775-782. 45.Deangelis, P. L., Papaconstantinou, J. and Weigel, P. H. 1993. Isolation of a Streptococcus pyogenes gene locus that directs hyaluronan biosynthesis in acapsular mutants and in heterologous bacteria. J. Biol. Chem. 268(20): 14568-14571. 46.Dostal, G. H. and Gamelli, R. L. 1993. Fetal wound healing. Surg. Gynecol. Obstet. 176: 299-306. 47.Duranti, F., Salti, G., Bovani, B., Calandra, M. and Rosati, M. L. 1998. Injectable hyaluronic acid gel for soft tissue augmentation. Dermatol. Surg. 24(12): 1317-1325. 48.Entwistle, J., Zhang, S., Yang, B., Wong, C., Li, Q., Hall, C. L. Jingbo, A., Mowat, M., Greenberg, A. H., and Turley, E. A. 1995. Characterization of the murine gene encoding the hyaluronan receptor RHAMM. Gene. 163: 233-238. 49.Forsberg, N. and Gustafson, S. 1991. Characterization and purification of the hyaluronan receptor on liver endothelial cells. Biochim. Biophys. Acta. 1078: 12-18. 50.Fraser, J. R.E., Brown, T. J. and Lauent, T. C. 1998. Catabolism of hyaluronan. The Chemistry Biology and Medical Applications of Hyaluronan and its Derivatives. 85-92. Portland Press Ltd, London. 51.Giusti, P., Lazzeri, L. and Lelli, L. 1993. Bioartificial polymeric materials a new method to design biomaterials by using both biological and synthetic polymers. Trends. Polymer. Sci. 1: 261-266. 52.Goldbergn, R. L., Huff, J. P., Lenz, M. E., Glickman, P., Katz, R. and Thonar, E. J. M. 1991. Elevated plasma levels hyaluronate in patients with osteoarthritis and rheumatoid arthritis. Arthritis Rheum. 34(7): 799-807. 53.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). 54.Hascall, V. C. and Laurent, T. C. 1997. Hyaluronan: structure and physical properties. http://www.glycoforum.gr.jp. 55.Hascall, V. C. 1977. Interaction of cartilage proteoglycans with hyaluronic acid. J. Supramol. Struct. 7: 101-120. 56.Hasegawa, S., Nagatsuru, M., Shibutani, M., Yamamoto, S. and Hasebe, S. 1999. Productivity of concentrated hyaluronic acid using a maxbled fermentor. J. Biosci. Bioeng. 88(1): 68-71. 57.Hoffman, A. S. 2002. Hydrogels for biomedical application. Adv. Drug. Deliv. Rev. 43: 3-12. 58.Huang, L. L. 1994. Development of hyaluronan collagen fibrillar matrices. Chinese J. Med. Biol. Eng. 14(1): 53-56. 59.Johns, M. R., Goh, L. T. and Oeggerli, A. 1994. Effect of pH,

agitation and aeration on hyaluronic acid production by Streptococcus zooepidemicus. Biotech. Letters. 16(5): 507-512. 60.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. 61.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. 62.Kvam, C., Granese, D., Flaibani, A., Zanetti, F., and Paoletti, S. 1993. Purification and characterization of hyaluronan from synovial fluid. Anal. Biochem. 211: 44-49. 63.Laurent, T. C. 1987. Biochemistry of Hyaluronan. Acta. Otolaryngol. (Stockh) Suppl. 442: 7-24. 64.Laurent, T. C., Laurent, U. B. and Fraser, J. R. 1996. Serum hyaluronan as a disease marker. Ann Med. 28(3): 241-253. 65.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. 66.Laurent, T. C., and Fraser, J. R. E. 1992. Hyaluronan. FASEB J. 6: 2397-2404. 67.Lazzeri, L., and Barbani, N., Cascone, M. G. Lupinacci, D. and Giusti, P. 1994. Physicochemical and mechanical characterization of hydrogels of poly(vinyl achol) and hyaluronic acid. J. Mater. Sci. Mater. Med. 5: 862-867. 68.Liu, L. S., Ng, C. K., Thompson, A. Y., Poser, J. W. and Spiro, R. C. 2002. Hyaluronate heparin conjugate gels for the delivery of basic fibroblast growth factor (FGF-2). J. Biomed. Mater. Res. 62(1): 128-135. 69.Mccourt, P. A. G., Ek, B., Forsberg, N. and Gustafson, S. 1994. Intercellular adhesion molecule-1 is a cell surface receptor for hyaluronan. J. Biol. Chem. 269(48): 30081-30084. 70.Meyer, K., and Palmer, J., 1934. The Polysaccharide of the vitreous humor. J. Boil. Chem. 107: 629-634. 71.Miller, G. L. 1959. Use of dinitrosalicylic acid reagent for determination of reducing sugar. Anal. Chem. 31(3): 426-428. 72.Miller, D, and Stegmann, R. 1980. Use of Na-hyaluronate in anterior segment eye surgery. J. Am. Intraocul. Imp. Soc. 6: 13-15. 73.Morimoto, K., Metsugi, K., Katsumata, H., Iwanaga, K. and Kakemi., M. 2001. Effects of low viscosity sodium hyaluronate preparation on the pulmonary absorption of rhinsulin in rats. Drug. Dev. Ind. Pharm. 27(4): 365-371. 74.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. 75.Nimrod, A., Greenman, B., Kanner, D.and Landsberg, M. 1998b. High molecular weight sodium hyaluronate. United States Patent:4,784,990. 76.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. 77.Prehm, P. 1983. Synthesis of hyaluronate in differentiated teratocarcinoma cells characterization of the synthase. Biochem. J. 211: 181-189. 78.Prestwich, G. D., Marecak, D. M., Marecek, J. F., Vercruysse, K. P. and Ziebell, M. R. 1998. Controlled chemical modification of hyaluronic acid : synthesis, applications, and biodegradation of hydrazide derivatives. J. Control. Release. 53: 93-103. 79.Roseman, S., Frances, E. M., Julio, L. and Albert, D. 1953. The biosynthesis of hyaluronic acid by group a Streptococcus. J. Biol. Chem. 203: 213-225 80.Scott, J. E. 1998. Secondary and tertiary structures of hyaluronan in aqueous solution. http://www.glycoforum.gr.jp. 81.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. 82.Seastone, C.V. 1939. The virulence of group C hemolytic Streptococci of animal origin. J. Exp. Med. 70: 361-378. 83.Slevin, M., Kumar, S. and Gaffney, J. 2002. Angiogenic oligosaccharides of hyaluronan induce multiple signaling pathways affecting vascular endothelial cell mitogenic and wound healing responses. J. Biol. Chem. 277(43): 41046-41059. 84.SmedsrOd, B. 1991. Cellural events in the uptake and degradtion of hyaluronan . Adv. Drug. Deliv. Rev. 7: 265-278. 85.Sten, S. 2000. Method and means for the production of hyaluronic acid. United States Patent : 6,090,596 86.Stoolmiller, A. C. and Albert, D. 1969. The biosynthesis of hyaluronic acid by Streptococcus. J. Biol. Chem. 244(2): 236-246. 87.Sugahara, K., Schwartz, N. B. and Dorfman, A. 1979. Biosynthesis of hyaluronic acid by Streptococcus. J. Biol. Chem. 254(14): 6252-6261. 88.Thonard, J. C., Migliore, S. A. and Blustein, R. 1964. Isolation of hyaluronic acid from broth cultures of Streptococci. J. Biol. Chem. 239(3): 726-728. 89.Toole, B. P. 2001. Hyaluronan in morphogenesis. Semin. Cell Dev. Biol. 12: 79-87. 90.Toole, B. P. 1998. Hyaluronan in morphogenesis and tissue remodeling. http://www. glycoforum.gr.jp. 91.Underhill, C. B. 1992. CD44 the hyaluronan receptor. J Cell Sci. 103: 293-298. 92.Underhill C. B. and Toole B. P. 1979. Binding of hyaluronate to the surface of cultured cells. J. Cell Biol. 82: 475-484. 93.Warren, G. H and Gray, J. 1959. Isolation and purification of streptococcal hyaluronic acid. Proc. Soc. Exp. Biol. Med. 102: 125-127. 94.Weigel, P. H. 1998. Bacterial Hyaluronan Synthases. http://www. glycoforum.gr.jp. 95.Weissmann, B. and Meyer, K. 1954. The Structure of hyaluronic acid and of hyaluronic acid from umbilical cord. J. Am. Chem. Soc. 76: 1753-1757. 96.Yang, B., Zhang, L. and Turley, E. A. 1993. Identification of two hyaluronan binding domains in the hyaluronan receptor RHAMM. J. Biol. Chem. 268(12): 8617-8623.