利用重組大腸桿菌生產玻尿酸 = The production of hyaluronan by recombinant escherichia coli

潘建名、李泰林

E-mail: 9708032@mail.dyu.edu.tw

摘 要

玻尿酸(hyaluronan或hyaluronic acid；HA)又稱為透明質酸，由D-葡萄糖醛酸(D-glucurnic acid；GlcA)與N-乙醯葡萄糖胺(N-acetylglucosamine；GlcNAc)為雙糖單位，重複聚合而成的直鏈式多糖體。具有特殊生物相容性、保濕能力、潤滑等特性，已廣泛被應用於生物醫學、化妝品工業等領域。其傳統生產方式由動物組織萃取，產量低且價格昂貴；現今則利用微生物發酵生產，主要生產菌種為A型與C型鏈球菌(streptococci)。野生型的鏈球菌雖具有豐富的玻尿酸莢膜(capsule)，但也會產生?珀誚撖壑峔鋊早P病性的外毒素，所以應用於工業化生產時會篩選不具致病力的突變株為生產菌種，但突變株往往合成玻尿酸的能力也大為降低。在A型鏈球菌玻尿酸生合成是由一組玻尿酸合成?操縱組 (hyaluronic acid synthase operon; has operon) 所調控。因此本研究使用安全性較高且長期被用來生產重組蛋白的大腸桿菌 (Escherichia coli；E. coli) DH5?恁A送入構築化膿性鏈球菌(Streptococcus pyogenes；S. pyogenes) 的玻尿酸合成?(hyaluronic acid synthase; Has) 基因操縱組及持續性表現的啟動子(constitutive promoter) ace的表現質體，做為生產玻尿酸的菌株。經不同的構築、培養基成份及轉形處理，探討對玻尿酸產量之影響。實驗結果得知經pACE-spHasABC轉形後之菌株，並未能於菌體外產生玻尿酸莢膜；經pACE-spHasBC轉形後，於胞內的glucuronic acid產量為27.22 mg/L遠高於pACE-spHasABC的17.74 mg/L。在培養基中添加輔因子MgCl2與受質glucosamine對於玻尿酸的產量與未添加並無顯著差異。在轉形作用時，添加小片段玻尿酸或共轉染具小片段玻尿酸合成?活性之質體，玻尿酸產量比未添加的高出30%。由以上資料推測，欲利用大腸桿菌生產玻尿酸，除了has operon外，尚須要短鏈的玻尿酸作為引子及其它有利於輸送玻尿酸到胞外之蛋白質。

關鍵詞 : 玻尿酸 ; 化膿性鏈球菌 ; 操縱組 ; 持續性表現啟動子

目錄

參考文獻

1. 王盈錦和張淑貞。2001。高分子生醫材料。化工技術。9:110- 129。 2. 王毅和鄭文艷。2005。玻璃酸製劑的臨床應用發展。食品與藥品。7: 11-16。 3. 朱兆秀。2005。利用鏈球菌發酵生產透明質酸。化工技術。13 : 165-172。 4. 李小迪。1997。透明質酸的保溼特性以其在化妝品的應用。香料香精化妝品。3: 19-22。 5. 李文任、黃偉蹈和黃雄飛。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: 263 6. 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。大葉大學生物產業科技碩士班。碩士論文。 7. 姚敏傑、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。 8. 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: 485-487。 9. 徐紅和陸至華。1998。透明質酸鈉在化妝品中的應用。中國生化藥物雜誌。19: 222-223。 10.高海軍、陳堅、堵國城、章燕芳、陳金春和陳國強。2003。攪拌與混合對獸疫鏈球菌發酵生產透明質酸的影響。化工學報。54: 350-356。 11.高海軍、陳監、管失眾、堵國城和倫世儀。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: 17-22。 12.凌沛學、賀豔麗、和張青。2005。透明質酸對骨關節炎的治療作用。食品與藥品。7: 1-3 13.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發展。上海大學學報。5: 69-73。 14.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: 209-212。 15.郭學平、王春喜、凌沛學和張天民。2000。透明質酸的生產。藥物生物技術。7: 61-64。 16.郭學平。2002。微生物發酵法生產透明質酸。精細與專用化學品。3: 21-22。 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質酸。現代商貿工業。3:47-50。 18.黃定國。2001。透明質酸之開發與應用。菌種保存及研究簡訊。14:1-9。 19.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。大葉大學生物產業科技碩士班。碩士論文。 20.張魯榕、殷蔚黃和孫淑潔。1991。血清透明質酸含量測定在肝病時的診斷意義。北京醫學。13:119-122。 21.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。10:10-11。 22.楊芳鏘和陳啟倫。2004。生物高分子之發酵生產技術及其應用。化工技術。12:129-141。 23.趙惠明。2003。透明質酸發酵條件的研究。嘉興學院學報。15: 38-40。 24.劉文斌、溫耀和孫思勤。2003。深層鞏膜切除聯合Healon GV注入治療開角形青光眼。眼科研究。21:189-190。 25.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。12:276-282。 26.鄭雅雯。2004。在大腸桿菌表現系統表現Streptococcus zooepidemicus HAS 蛋白質。國立交通大學生化工程研究所。碩士論文。 27.鄭曉龍、賀玲和楊新光。2002。透明質酸鈉在眼科的應用。實用醫學雜誌。19:387-388。 28.蕭至君。2003。利用大腸桿菌生產透明質酸之研究。國立陽明大學醫學工程研究所。碩士論文。 29.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。13:155-164。 30.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。26:596-600。 31.羅瑞明、郭美錦、儲炬和張嗣良。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。22:14- 17。 32.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。12:124-126。 33.Allen, A.G., Lindsay, H., Seilly, D., Bolitho, S., Peters, S. E. and Maskell, D. J. 2004. Identification and characterization of hyaluronate lyase form Streptococcus suis. Microb. Pathog. 36: 327-335. 34.Armstrong, D. C., Cooney, M. J. and John, M. R. 1997. Growth and amino acid requirements of hyaluronic acid producing Streptococcus zooepidemicus. Appl Microbiol Biotechnol. 47: 309-312. 35.Asari, A. and Miyauchi, S. 2000. Medical applications of hyaluronan. http://www.glycoforum.gr.jp/science/hyaluronan/HA13/HA13E.html. 36.Ashbaugh, C. D., Sebastian A. 1998. Molecular analysis of the capsule gene region of group A Streptococcus: the hasAB genes are sufficient for capsule expression. J. Bacteriol. Sept: 4955-4959. 37.Balazs, E. A. 1979. Ultrapure hyaluronic acid and the use therefore. United States Patent : 4,141,973. 38.Balazs, E. A., Laurent, T. C. and Jeanloz, R. W. 1986. Nomenclature of hyaluronic acid. Biochem. J. 235: 903. 39.Balazs, E. A. and Denlinger, J. L. 1989. Clinical uses of hyaluronan. Ciba Foundation symposium. 143: 265-275. 40.Barker, S. A. and Young, N. M. 1966. Isolation of hyaluronic acid by gel filtration on agarose. Carbohydr. Res. 2: 363-370. 41.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: 97-103. 42.Bliss, J. M., Garon, C. F. and Silver, R. P. 1996. Polysialic acid export in Escherichia coli K1 the role of KpsT, the ATP-binding component of an ABC transporter, in chain translocation. Glycobiology. 6: 445-452. 43.Bracke, J. W., Minnetonka, M. N., Thacke, K. and Minneapolis, M. N. 1985. Hyaluonic acid form bacterial culture. United States Patent: 4,517,295. 44.Cappuccino, J. G.. and Sherman, N. 2005. Microbiology a laboratory manual. seventh edition. p.85. 45.Cheung, W. F., cruz, T. F. and Turley, E. A. 1999. Receptor for hyaluronan- mediated motility (EHAMM), a hyaladherin that regulates cell responses to growth fectors. Biochem Soc Trans. 27: 135-142. 47.Chong, B. F. and Nielsen, L. K. 2003.Acerobic cultivation of Streptococcus zooepidemicus and the role of NADH oxidase. J. Biochem. Eng. 16: 153-162. 48.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: 68-75. 49.Cifonelli, J. A., Rebers, P. A. and Heddleston, K. H. 1970. The isolation and characterization oh hyaluronic acid form Pasteurella multocida. Carbohydra. Res. 14: 272-276. 50.Claudio, D. L., Manfred, L., Irene, M. 1995. Enzymatic synthesis of hyaluronic acid with regeneration of sugar nucleotides ., J. Am. Chem.Soc.117:5869-5870. 51.Cleary, P. P. and Larkin, A. 1979. Hyaluronic acid capsule strategy for oxygen resistance in group A Streptococci. J. Bacteriol. 140 : 1090-1097. 52.Collis, L., Hall, C., Lange, L., Ziebell, M. R., Prestwich, G. D. and Turley, E. A. 1998. Rapid hyaluronan uptake is associated with enhanced motility: implications for an intracellular mode of action. FEBS Letter. 440: 444-449. 53.Crater, D. L., Dougherty, B. A. and Van de Rijn, I. 1995. Molecular characterization of hasC form an operon required for hyaluronic acid synthesis in group A Streptococci. J. Biol. Chem. 270: 28676-28680. 54.Crater, D. L. and Van de Rijn, I. 1995. Hyaluronic acid synthesis operon (has) Expression in group A Streptococci. J. Biol. Chem. 270: 18452-18458. 55.Day, R., Brooks, P., Conaghan, P. G. and Petersen, M. 2004. A double blind randomized multicenter parallel group study of the effectiveness and toleramce of intraarticular hyaluronan in osteoarthritis of the knee. J. Rheumatol. 31: 775 -782. 56.DeAngelis, P. L., Papaconstantinou, J. and Weigel, P. H. 1993. Isolation of a

Streptococcus pyogenes gnen locus that directs hyaluronan biosynthesis in acapsular mutants and in heterologous bacteria. J. Biol. Chem. 268 : 14568-14571. 57.Dostal, G. H. and Gamelli, R. L. 1993. Fetal wound healing. Surg. Gynecol. Obstes. 176 : 299-306. 58.Dougherty, B. A., Van de Rijn, I. 1993. Molecular characterization of hasB form an operon required for hyaluronic acid synthesis in group A Streptococci. J. Biol. Chem. 268 : 7118-7124. 59.Dougherty, B. A., Van de Rijn, I. 1994. Molecular characterization of hasA form an operon required for hyaluronic acid synthesis in group A Streptococci. J. Biol. Chem. 269 : 169-175. 60.Dowthwaite, G. P., Edward, J. C. W. and Pitsillides, A. A. 1998. An essential role for the interation between hyaluronan and hyaluronan binding proteins during joint development. J. Histo Cytochem. 46: 641-651. 61.Fagerholm, P., Koul, S. and Trocme, S. 1987. Corneal endothelial protection by heparin and sodium hyaluronate surface coating of PMMA intraocular lenses. Acta Ophthalmo. 65: 110-114. 62.Fouissace, E., Milas, M. and Rinaudo, M. 1993. Shear rate, concentration, molecular wight, and temperature viscosity dependencies of hyaluronate, a wooormlike polyelectrolyte. Macromolecules. 26: 6945-6951. 63.Fraser, J. R. E., Laurent, Y. C. and Laurent, U. B. G. 1997. Hyaluronan: its nature, distribution, functions and turnover. J Intern Med. 24 : 227-233. 64.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. p.85-92. Portland Press Ltd, London. 65.Furnus, C. C., Matos de, D. G. and Martinez, A. G. 1998. Effect of hyaluronic acid on development of in vitro produced bovine embryos. Theriogenology. 49: 1489-1499. 66.Gerdin, B. and Hallgren, R. 1997. Dynamic role of hyaluronan (HYA) in connective tissue activation and inflammation. J. Intern. Med. 242: 49-55. 67.Giusti, P., Lazzeri, L. and Lelli, L. 1993. Bioartificial polymeric materials a new method to design biomaterials by using both biological and syuthetic polymers. Trends. Polymer. Sci. 1: 261-266. 68.Glasser, D. B., Mastuda, M. and Edlhauser, H. F. 1986. A comparison of the efficacy and toxicity of and intraocular pressure response to viscous solutions in the anterior chamber. Arch Ophthalmol. 104: 1819-1824. 69.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. 70.Hardwick, C., Hoare, K., Owens, R., Hohn, P., Hook, M. and Moore, D. 1992. Molecular cloning of novel hyaluronan receptor that mediates tumor cell motility. J. Cell Biol. 117: 1343-1350. 71.Harley, C. B. and Reynolds, R. P. 1987. Analysis of E.coli promoter sequences. Nucleic Acids Res. 15 : 2343-2357. 72.Hascall, V. C. and Laurent, T. C. 1997. Hyaluronan: structure and physical properties. http://www.glycoforum.gr.jp/science/hyaluronan/HA01/HA01E.html. 73.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: 68-71. 74.Haxaire, K., Braccini, I., Milas, M., Rinaudo, M. and Perez, S. 2000 Conformational behavior of hyaluronan in relation to its physical properties as probed by molecular modeling., Glycobiology. 10 : 587-594. 75.Heldin, P. and Laurent, T. C. 2000. Biosynthesis of hyaluronan. In: carbohydrates in chemistry and biology. (Ernst B, Hart G, Sinay P, Eds.) 3, 363-372, Wiley / WCH, Weinheim. 76.Hoffmal, A. S. 2002. Hydrogels for biomedical application. Adv. Drog. Deliv. Rev. 43: 3-12. 77.Holmstrom, B. and ?i?ica, J. 1967. Production of hyaluronic acid by Streptococcal strain in bacth culture. Appl Microbiol. 15: 1409-1413. 78.Hoshi, H., Nakagawa, H., Nishiguchi, S., Iwata, K., Niikura, K., Monde, K., and Nishimura, S. 2004. An engineered hyaluronan synthase characterization of recombinant human hyaluronan synthase 2 expressed in Escherichia coli. J. Biol. Chem. 279: 2341 – 2349. 79.Huang, L. L. 1994 Development of hyaluronan collagen fibrillar matrices. Chinese J. Med. Biol. Eng. 14(1): 53-56. 80.Johns, M. R., Goh, L. T., and Oeggrli, A. 1994. Effect of pH, agitation and aeration on hyaluronic acid production by Streptococcus zooepidemicus. Biotec. letters. 16: 507-512. 81.Kemblowski, Z. and Kristiansen, B. 1986. Rheometry of Fermentation Liquids. Biotec and Bioeng. 28: 1474-1483. 82.Kendall, F. E., Heidelberger, M. And Dawson, M. H. 1937. A serologically inactive polysaccharide elaborated by mucoid strain of group A hemolytic Streptococcus. J. Biol Chem. 118: 61-69. 83.Kim, J. H., Yoo, S. J., Oh, D. K., Kweon, Y. G., Park, D. W., Lee, C. H. and Gill, 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. 84.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) cida foundation symposium 124, p. 9-29, New York. Wiley. 85.Laurent, T. C. 1987. Biochemistry of Hyaluronan. Acta. Otolaryngol. (Stockn) Suppl. 442: 7-24. 86.Laurent, T. C. and Fraser, J. R. E. 1992. Hyaluronan. FASEB J. 6: 2397-2404. 87.Laurent, T. C., Laurent, U. B. and Fraser, J. R. 1996. Serum hyaluronan as a disease marker. Ann Med. 28 : 241-253. 88.Lazzeri, L., Barbani, N., Cascone, M. G., Lupinacci, D. and Giusti, P. 1994. Physicochemical and mechanical characterization of hydrogels of poly (vinylachol) and hyaluronic acid. J. Mater. Sci. Mater. Med. 5: 862-867. 89.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. Master. Res. 62 : 128-135. 90.MacLennan, A. P. 1956. The production of capsules, hyaluronic acid and hyaluronidase by 25 strain of group C Streptococci. J. Gen. Microbiol. 15: 485-491. 91.Matrasso, S. L. 2004. Understanding and using hyaluronic acid . Aesthetic Surg. J. 24: 361-364. 92.Meyer, K. and Palmer, J. 1934. The polysaccharide of the vitreous humor. J. Biol. Chem. 107: 629-634. 93.Miller, D. and Stegman, R. 1980. Usa of sodium hyaluronate in anterior segment eye surgery. J. Am. Intraocul. Implant. Soc. 6: 13-15. 94.Miller, G. L. 1959. Use of dinitrosalicylic acid reagent for determination of reducing suger. Anal. Chem. 31(3): 426-428. 95.Miyauchi, S. and Iwatw, S. 1984. Biochemical studies on the use of sodium hyaluronate in the anterior eye segment. IV. The protective efficacy of the corneal endothelium. Current eye research. 3: 1063-1067. 96.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 : 365-371. 97.Nimrod, A., Greenman, B., Kanner, D. and Landsberg, M. 1998. High molecular weight sodium hyaluronate. United States Patent : 4,784,990. 98.Ouskova, G., Spellerberg, B. and Prehm, P. 2004. Hyaluronan release from Streptococcus pyogenes export by an ABC transporter. Glycobiology. 14 : 931-938. 99.Prehm, P. 1983. Synthesis of hyaluronate in differentiated teratocarcinoma cells characterization of the synthase. Biochem. J. 211: 181-189. 100.Prehm, P. 2002. Hyaluronan. In: Chapter15,

Biopolymers. (Vandamme, E. J., DeBaest, S. and Steinbucel, A. eds.) Wiley- Vch, KGaA, Weinheim. p. 379-406. 101.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. 102.Pummill, P. E., Achyuthan, A. M. and DeAngelis, P. L. 1998. Enzymological characterization of recombinant Xenopus DG42, a vertebrate hyaluronan synthase. J. Biol. Chem.. 273 : 4976-4981. 103.Roseman , S., Mose, F. E., Ludowieg, J. and Dorfman, A. 1953. The biosynthesisof hyaluronic acid by group A Streptococcus. I. utilization of 1-C14-Glucose. J. Biol. Chem. 203: 213-225. 104.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. Hyaluronan is a very efficient network-forming polymer. Biochem. J. 274: 699-705. 105.Scott, J. E. 1998. Secondary and tertiary structures of hyaluronan in aqueous solution. http://www.glycoforum.gr.jp/science/hyaluronan/HA02/HA02E.html 106.Shah, C. B. and Barnett, S.M., 1992. Swelling behavior of hyaluronic acid gels., J. Appl. Polym. Sci . 45:293-298. 107.Shimada, E. and Matsumura, G. 1975. Viscosity and molecular weight of hyaluronic acids. Biochem. J.78: 513-517. 109.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: 41046-41059. 110.Sloma, A., Behr, R., Widner, W., Tang, M.,Sternberg, D. and Brown, S. 2003. Methods for producing hyaluronan in a recombinant host cell. World patent application WO03/054163. 111.Stanbury, P. F., Whitaker, A. and Hall, S.J. 1995. Principles of Fermentation Technology, 2 nd ed., Pergamon Publications, N. Y. 112.Sten, S. 2000. Method and means for the production of hyaluronic acid. United States Patent: 6,090,596. 113.Sugahara, K., Schwartz, N. B. and Dorfman, A. 1979. A biosynthesis of hyaluronic acid by Streptococcus. J. Biol. Chem. 254: 6252-6261. 114.Thonard, J. C., Migliore, S. A. and Blustein, R. 1964. Isolation of hyaluronic acid form broth cultures of Streptococci. J. Bio. Chem. 239(3): 726-728. 115.Toole, B. P. 1997. hyaluronan in morphogenesis. J. Intern. Med. 242: 35-40. 116.Toole, B. P. 1998. Hyaluronan in morphogenesis and tissue remodeling. http://www.glycoforum.gr.jp/science/hyaluronan/HA08/HA08E.html. 117.Toole, B. P. 2001. Hyaluronan in morphogenesis. Semin. Cell Dev.Biol. 12: 79-87. 118.Underhill, C. B. and Toole, B. P. 1979. Binding of hyaluronate to the surface of cultured cells. J. Cell Biol. 82: 475-484. 119.Warren, G. H. and Gray, J. 1959. Isolation and purification of streptococcal hyaluronic acid. Proc. Soc. Exp. Biol. Med. 102: 125-127. 120.Weigel, P. H. 1998. Bacterial Hyaluronan Synthases. http://www.glycoforum.gr. jp/science/hyaluronan/HA06/HA06E.html. 121.Weissmann, B. and Meyer, K. 1954. The structure of hyaluronic acid and hyaluronic acid form umbilical cord. J. Am. Chem. Soc. 76: 1753-1757. 122.Wessels, M. R., Goldberg, J. B., Moses, A. E. and DiCesare, T. J. 1994. Effects on virulence of mutations in a locus essential for hyaluronic acid capsule expression in group A Streptococci. Infect Immun. 62: 433-441. 123.Whistler, R. L. 1965. Chapter 5. Hyaluronic acid. In: Carbohydrate Chemistry. Academic Press, London, UK, p.43-63. 124.Whitnack, E., Bison, A. L. and Beachy, E. H. 1981. Hyaluronate capsule prevents attachment of group A Streptococci to mouse peritioneal macrophages. Infect Immun. 31: 985-991. 125.Widner, B., Behr, R., Von Dollen, R., Tang, M., Heu, T., Sloma, A., Sternberg, D., DeAngelis, P. L., Weigel, P. H. and Brown, S. 2005. Hyaluronic acid production in Bacillus subtilis. Appl Environ Microbiol. 71: 3747-3752. 126.Williams, D. C., Van, F. R. M., Muth, W. L., Burnett, J. P. 1982. Cytoplasmic inclusion bodies in Escherichia coli producing biosynthetic human insulin proteins. Science. 215 : 687-689.