

# Study on production of bacterial cellulose by isolated gluconacetobacter sp. WU2 and WU3 strains

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## ABSTRACT

Bacterial cellulose (BC), which is synthesized and secreted by the gram negative bacterium, *Gluconacetobacter* sp.WU2、*Gluconacetobacter* sp.WU3, displays unique physical, chemical, and mechanical properties including a high crystallinity, a high water holding capacity, a well-developed surface area comprised of nanofibers, elasticity, mechanical strength, and biocompatibility. Static batch fermentations for bacterial cellulose production were studied in carbon sources, nitrogen sources, pH, temperature, organic acid (citric acid, succinic acid and acetic acid), ethanol concentration (0-15%) in flask under 30 by isolated. *Gluconacetobacter* sp. WU2 and *Gluconacetobacter* sp. WU3. These results showed that *Gluconacetobacter* sp.WU3 was the best BC producer without pH controlled, the WU3 strain could produce BC at a yield exceeding 1.92 g/L of glucose/peptone mixed medium, which was comparable to the yields by WU2 at 30. Structural changes in never-dried, disintegrated bacterial cellulose by various drying process were examined. The pretreated/ treated bacterial cellulose were characterized by X-ray diffractometry (XRD), Fourier Transform Infrared spectroscopy (FTIR) and Scanning electron microscope (SEM). In additionally, Hunter lab colour parameters were determined to assess the effect of different alkali treatments on the colour characteristics of the bacterial cellulose. The overall quality of the freeze dried membranes had higher 'L' values.

Keywords : *Gluconacetobacter* sp.WU2、*Gluconacetobacter* sp.WU3、bacterial cellulose

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- 1.田蕙萍。2009。以*Acetobacter xylinum* WU1生產細菌纖維素之最適培養條件及其抗菌應用之研究。大葉大學生物產業科技學系碩士論文。彰化。
- 2.沈玫?。2007。細菌纖維素生產菌之篩選及其作培養條件之研究。國立中興大學食品暨應用生物科技學系。碩士論文。
- 3.許啟南。細菌纖維素之生產條件與物理性質。國立台灣海洋大學食品科學系碩士論文。基隆。
- 4.張建成。2000。含親水基聚矽氧水膠薄膜之製備研究。雲林科技大學工業化學與災害防治研究所碩士論文。雲林。
- 5.Backdahl, H., Helenius, G., Bodin, A., Nannmark, U., Johansson, B. R., Risberg, B. and Gatenholm, P. 2006. Mechanical properties of bacterial cellulose and interactions with smooth muscle cells. *Biomaterials*. 27: 2141-2149.
- 6.Bae, S. and Shoda, M. 2004. Bacterial cellulose production by fed-batch fermentation in molasses medium. *Biotechnology progress*. 20 (5) : 1366-1371.
- 7.Bae, S. and Shoda, M. 2005. Statistical optimization of culture conditions for bacterial cellulose production using Box-Behnken design. *Biotechnol. Bioeng.* 90: 20 – 28.
- 8.Bellamy, W. D. 1974. Single cell proteins from cellulosic wastes. *Biotechnol. Bioeng.* 16: 869 – 880.
- 9.Benziman, M., Haigler, C. H., Brown Jr., R. M., White, A. R. and Cooper, K. M. 1980. Cellulose biogenesis: Polymerization and crystallization are coupled processes in *Acetobacter xylinum*. *Proc. Natl. Acad. Sci. U. S. A.* 77: 6678 – 6682.
- 10.Bielecki, S., Krystynowicz, A., Turkiewicz, M. and Kalinowska, H. 2005. Bacterial Cellulose. In: *Polysaccharides and Polyamides in the Food Industry*, Steinbu"chel, A. and Rhee, S. K. (Eds.), Wiley- VCH Verlag, Weinheim, Germany. pp. 31 – 85.
- 11.Borzani, W. and De Souza, S. J. 1998. A simple method to control the bacterial production of cellulosic films in order to obtain dried pellicles presenting a desired average thickness. *World J. Microbiol. Biotechnol.* 14: 59 – 61.
- 12.Brown Jr., R. M. 1987. The biosynthesis of cellulose. *Food Hydrocoll.* 1: 345 – 351.
- 13.Brown Jr., R. M. and Saxena, I. M. 2000. Cellulose biosynthesis: A model for understanding the assembly of biopolymers. *Plant Physiol. Biochem.* 38: 57 – 67.
- 14.Brown Jr., R. M., Willison, J. H. M. and Richardson, C. L. 1976. Cellulose biosynthesis in *Acetobacter xylinum*: Visualization of the site of synthesis and direct measurement of the in vivo process. *Proc. Natl. Acad. Sci. U. S. A.* 73: 4565 – 4569.
- 15.Brown, A. J. 1886. On an acetic ferment which forms cellulose. *J. Chem. Soc. Trans.* 49: 432 – 439.
- 16.Brown, E. E. and Laborie, M. P. G. 2007. Bioengineering bacterial cellulose/poly(ethylene oxide) nanocomposites, *Biomacromolecules*. 8 : 3074.
- 17.Brown, R. M. 1989. Microbial cellulose as a building block resource for specialty products and processes therefore, *PCT Int. Appl. WO 8912107 A1*, 37.
- 18.Brown, R. M., Haigler, C. H., Suttie, J., White, A. R., Roberts, E., Smith, C., Itoh, T. and Cooper, K. 1983. The biosynthesis and degradation of cellulose. *J. Appl. Polym. Sci.* 37: 33 – 78.
- 19.Brown, R.M. Jr. 1989. Cellulose structure and function aspects. *Ellis Howoodide*. 144-151.
- 20.Budhiono, A., Rosidi, B., Taher, H. and Iguchi, M. 1999. Kinetic aspects of bacterial cellulose formation in nata-de-coco culture system. *Carbohydr. Polym.* 40: 137 – 143.
- 21.Canale-Parol, E. and Wolfe, R. S. 1960. Studies on *Sarcina Ventricula* I. stock culture. *J. bacteriol.* 79: 857-862.
- 22.Chao, Y., Ishida, T., Sugano, Y. and Shoda, M. 2000. Bacterial cellulose production by *Acetobacter xylinum* in a 50L internal-loop airlift reactor. *Biotechnol. Bioeng.* 68: 345 – 352.
- 23.Chao, Y., Mitarai, M., Sugann, Y. and Shoda, M. 2001. Effect of assition of water- soluble polysaccharides on bacterial cellulose production in a 50-L airlift reactor. *Biotechnology progress*. 17 (4) : 781-785.
- 24.Chao, Y., Sugano, Y. and Shoda, M. 2001. Bacterial cellulose production under oxygen-enriched air at different fructose concentrations in a 50-liter, internal-loop airlift reactor. *Appl. Microbiol. Biotechnol.* 55: 673 – 679.
- 25.Charpentier, P. A., Maguire, A. and Wan, W. 2006. Surface modification of polyester to produce a bacterial cellulose-based vascular prosthetic device. *Appl. surf. sci.* 252: 6360-6367.
- 26.Choi, Y., Ahn, Y., Kang, M., Jun, H., Kim, I. S. and Moon, S. 2004. Preparation and characterization of acrylic acid-treated bacterial cellulose cation-exchange membrane. *J. chem. technol. biotechnol.* 79: 79-84.
- 27.Cienchanska, D. 2004. Multifunctional bacterial cellulose/chitosan composite materials for medical applications. *Fibres and Textiles in Eastern Europe*, 12: 69-72.
- 28.Cousins, S. K. and Brown Jr., R. M. 1997. X-ray diffraction and ultrastructural analyses of dye-altered celluloses support van der Waals forces as the initial step in cellulose crystallization. *Polymer*. 38: 897 – 902.
- 29.Czaja, W., Krystynowicz, A., Bielecki S. and Brown, R. M. Jr. 2006. Microbial cellulose-the natural power to heal wounds. *Biomaterials*. 27, 145-51.
- 30.Czaja, W., Krystynowicz, A., Bielecki, S. and Brown Jr., R. M. 2006. Microbial cellulose – The natural power to heal wounds. *Biomaterials*. 27: 145 – 151.
- 31.Czaja, W., Romanovicz, D. and Brown, R. M. 2004. Structural investigations of microbial cellulose produced in stationary and agitated culture. *Cellulose*. 11: 403 – 411.
- 32.Czaja, W., Young, D. J., Kawechi, M. and Brown, R. M. Jr. 2007. The future prospects of microbial cellulose in biomedical applications. *Biomacromolecules*. 8: 1-12.
- 33.De Faveri, D., Torre, P., Molinari, F., Perego, P. and Converti, A. 2003. Carbon material balances and bioenergetics of 2,3-butanediol bio-oxidation by *Acetobacter hansenii*. *Enzyme and Microbial Technology*. 33, 708 – 719.
- 34.De Iannino, N. I., Couso, R. O. and Dankert, M. A. 1988. Lipid-linked intermediates and the synthesis of acetan in *Acetobacter xylinum*. *J. Gen. Microbiol.* 134: 1731 – 1736.
- 35.De Wulf, P., Joris, K. and Vandamme, E. J. 1996. Improved cellulose formation by an *Acetobacter xylinum* mutant limited in (keto)gluconate synthesis. *J. Chem. Technol. Biotechnol.* 67: 376 – 380.
- 36.Delmer, D. P. 1987. Cellulose biosynthesis, *Ann. Rev. Plant Physiol.* 38: 259 – 290.
- 37.Delmer, D. P. and Amor, Y. 1995. Cellulose biosynthesis. *Plant Cell*. 7: 987 – 1000.
- 38.Doyle, P. S., Bibette, J. Bancaud, A. J. L. 2002. Self-assembled magnetic matrices for DNA separation chips. *Science*. 295: 2237.
- 39.Dubey, V., Saxena, C., Singh, L., Ramana, K.V. and Chauhan R. S. 2002. Pervaporation of binary water-ethanol mixtures through bacterial cellulose membrane. *Sep. Purif. Technol.* 27: 163 – 171.
- 40.Einfeldt, L., Klemm, D. and Schmauder, H. P. 1993. Acetylated carbohydrate derivatives as C-sources for *Acetobacter xylinum*. *Nat. Prod. Res.* 2: 263 – 269.
- 41.Elvie E. Brown, M. S. 2007. Bacterial Cellulose/Thermoplastic Polymer Nanocomposites. Washington State University Department of Chemical Engineering. U. S. A.
- 42.Evans, B. R., O'Neill H. M., Malyvanh, V. P., Lee, I. and Woodward, J. 2003. alladium-bacterial cellulose membranes

for fuel cells. *Biosens. Bioelectron.* 18: 917 – 943. Farah, L. F. X. 1990. Process of the preparation of cellulose film, cellulose film produced thereby, artificial skin graft and its use. US patent 4912049. 44. George, J., Ramana, K. V., Sabapathy, S. N. and Bawa, A. S. 2005a. Physico-mechanical properties of chemically treated bacterial (*Acetobacter xylinum*) cellulose membrane. *World J. Microbiol. Biotechnol.* 21: 1323 – 1327. 45. George, J., Ramana, K. V., Sabapathy, S. N., Jambur, H. J. and Bawa, A. S. 2005b. Characterization of chemically treated bacterial (*Acetobacter xylinum*) biopolymer: Some thermo-mechanical properties. *Int. J. Biol. Macromol.* 37: 189 – 194. 46. Geyer, U., Heinze, T., Stein, A., Klemm, D., Marsch, S., Schumann, D. and Schmauder, H. P. 1994a. Formation, derivatization and applications of bacterial cellulose, *Int. J. Biol. Macromol.* 16: 343 – 347. 47. Geyer, U., Klemm, D. and Schmauder, H. P. 1994b. Kinetics of the utilization of different C sources and the cellulose formation by *Acetobacter xylinum*. *Acta. Biotechnol.* 14: 261 – 266. 48. Gromet-Elhanan, Z. and Hestrin, S. 1963. Synthesis of cellulose by *Acetobacter xylinum*. VI. growth on citric acid-cycle intermediates. *J. Bacteriol.* 85: 284 – 292. 49. Grunert, M., Winter, W. T. 2000. Progress in the development of cellulose-reinforced nanocomposites. *Abstr. Pap. Am. Chem. Soc.*, 219: 126-PMSE Part 2. 50. Ha, J.H., Shehzad, O., Khan, S., Lee, S.Y., Park, J.W., Khan, T. and Park, J.K. 2008. Production of bacterial cellulose by a static cultivation using the waste from beer culture broth. *Korean Journal of Chemical Engineering.* 25 (4) : 812-815. 51. Hamlyn, P. F., Crighton, J., Dobb, M. G. and Tasker, A. 1997. Cellulose product. UK Patent Application GB 2314856 A No. 9713991.9. 52. Han J. and Craighead H. G. 2002. Separation of long DNA molecules in a microfabricated entropic trap array. *Science.* 288: 1026. 53. Hestrin, S. and Schramm, M. 1954. Synthesis of cellulose by *Acetobacter Xylinum* 2. Preparation of freeze-dried cells capable of polymerizing glucose to cellulose. *Biochem. J.* 58: 345-352. 54. Hong, F. and Qiu, K. 2008. An alternative carbon source from konjac powder for enhancing production of bacterial cellulose in static cultures by a model strain *Acetobacter aceti* subsp. *xylinus* ATCC 23770. *Carbohydrate Polymers.* 72: 545 – 549. 55. Hornung, M., Ludwig, M. and Schmauder, H. P. 2007. Optimizing the production of bacterial cellulose in surface culture: A novel aerosol bioreactor working on a fed batch principle (Part 3). *Eng. Life Sci.* 7: 35 – 41. 56. Hornung, M., Ludwig, M., Gerrard, A. M. and Schmauder, H. P. 2006. Optimizing the production of bacterial cellulose in surface culture: Evaluation of substrate mass transfer influences on the bioreaction (Part 1). *Eng. Life Sci.* 6: 537 – 545. 57. Hornung, M., Ludwig, M., Gerrard, A. M. and Schmauder, H. P. 2006. Optimizing the production of bacterial cellulose in surface culture: Evaluation of product movement influences on the bioreaction (Part 2). *Eng. Life Sci.* 6: 546 – 551. 58. Hung L. R., Tegenfeldt J. O., Kraeft J. J., Strum J. C., Austin R. H. and Cox E. C. 2002. A DNA prism for high-speed continuous fractionation of large DNA molecules. *Nat. Biotechnol.* 20: 1048. 59. Hwang, J. W., Yang, Y. K., Hwang, J. K., Pyun, Y. R. and Kim, Y. S. 1999. Effects of pH and dissolved oxygen on cellulose production by *Acetobacter xylinum* BRC5 in agitated culture. *J. Biosci. Bioeng.* 88: 183 – 188. 60. Iguchi, M., Mitsuhashi, S., Ichimura, K., Nishi, Y., Uryu, M., Yamanaka, S. and Watanabe, K. 1988. Bacterial cellulose-containing molding material having high dynamic strength. US patent 4742164. 61. Iguchi, M., Yamanaka, S. and Budhiono, A. 2000. Bacterial cellulose – A masterpiece of nature 's arts. *J. Mater. Sci.* 35: 261 – 270. 62. Ishida, T., Sugano, Y., Nakai, T. and Shoda, M. 2002. Effects of acetan on production of bacterial cellulose by *Acetobacter xylinum*. *Biosci. Biotechnol. Biochem.* 66: 1677 – 1681. 63. Ishihara, M., Matsunaga, M., Hayashi, N. and Tisler, V. 2002. Utilization of D-xylose as carbon source for production of bacterial cellulose. *Enzyme Microb. Technol.* 31: 986 – 991. 64. Ishikawa, A., Matsuoka, M., Tsuchida, T. and Yoshinaga, F. 1995. Increasing of bacterial cellulose production by sulfoguanidine-resistant mutants derived from *Acetobacter xylinum* subsp. *Sucofermentans* BPR2001. *Bioscience, Biotechnology, and Biochemistry.* 59: 2259-2263. 65. Jesus, E. G., Andres, R. M. and Magno, E. T. 1971. A study on the isolation and screening of microorganisms for production of diverse textured Nata, *Philipp. J. Sci.* 100: 41 – 52. 66. Johnson, D. C. and Neogi, A. N. 1989. Sheeted products formed from reticulated microbial cellulose. US patent 4863565. 67. Jonas, R. and Farah, L. F. 1998. Production and application of microbial cellulose. *Polym. Degrad. Stabil.* 59: 101 – 106. 68. Joo S.H., Goo, K.H., Ki, K.K., Soo, K.H., Gyun, K.Y. and Joon, L.S. 2003. Increased production of bacterial cellulose by *Acetobacter* sp. V6 in synthetic media under shaking culture conditions. *Bioresource technology.* 86 (3) : 215-219. 69. Jung, J. Y., Park, J. K. and Chang, H. N. 2005. Bacterial cellulose production by *Gluconoacetobacter hansenii* in an agitated culture without living non-cellulose producing cells. *Enzyme Microb. Technol.* 37: 347 – 354. 70. Kai, A. 1984. The structure of the nascent fibril produced by *Acetobacter xylinum*: The lattice spacing of cellulose produced in the presence of a fluorescent brightener. *Macromol. Rapid Commun.* 5: 653 – 655. 71. Kawano, S., Tajima, K., Kono, H., Erata, T., Munekata, M. and Takai, M. 2002. Effects of endogenous endo- $\alpha$ -1,4-glucanase on cellulose biosynthesis in *Acetobacter xylinum* ATCC23769. *J. Biosci. Bioeng.* 94: 275 – 281. 72. Kawano, S., Tajima, K., Kono, H., Numata, Y., Yamashita, H., Satoh, Y. and Munekata, M. 2008. Regulation of endoglucanase gene (cmcaX) expression in *Acetobacter xylinum*. *J. Biosci. Bioeng.* 106: 88 – 94. 73. Kawano, S., Tajima, K., Uemori, Y., Yamashita, H., Erata, T., Munekata, M. and Takai, M. 2002. Cloning of cellulose synthesis related genes from *Acetobacter xylinum* ATCC23769 and ATCC53582: Comparison of cellulose synthetic ability between strains. *DNA Res.* 9:149 – 156. 74. Kawano, S., Yasutake, Y., Tajima, K., Satoh, Y., Yao, M., Tanaka, I. and Munekata, M. 2005. Crystallization and preliminary crystallographic analysis of the cellulose biosynthesis- related protein CMCAx from *Acetobacter xylinum*, *Acta Crystallogr. F. Struct. Biol. Cryst. Commun.* 61: 252 – 254. 75. Keshk, S. and Sameshima, K. 2006. Influence of lignosulfonate on crystal structure and productivity of bacterial cellulose in a static culture. *Enzyme Microb. Technol.* 40: 4 – 8. 76. Keshk, S. M. A. S. and Sameshima, K. 2005. Evaluation of different carbon sources for bacterial cellulose production. *Afr. J. Biotechnol.* 4: 478 – 482. 77. Khan, T., Salman and Park, J. P. 2008. Simple Fed-batch Cultivation Strategy for the Enhanced Production of a Single-sugar Glucuronic Acid-based Oligosaccharides by a Cellulose-producing *Gluconoacetobacter hansenii* Strain. *Biotechnology and Bioprocess Engineering.* 13: 240-247. 78. Kim, J. Y., Kim, J. N., Wee, Y. J., Park, D. H. and Ryu, H. W. 2007. Bacterial cellulose production by *Gluconoacetobacter* sp. RKY5 in a rotary biofilm contactor. *Appl. Biochem. Biotechnol.* 137: 529 – 537. 79. Kim, S. Y., Kim, J. N., Wee, Y. J., Park, D. H. and Ryu, H. W. 2006. Production of bacterial cellulose by *Gluconoacetobacter* sp. RKY5 isolated from persimmon vinegar.

Appl. Biochem. Biotechnol. 131: 705 – 715. 80.Kim, S.Y., Kim, J.N., Wee, Y.J., Park, D.H. and Ryu, H.W. 2006. Production of bacterial cellulose by *Gluconacetobacter* sp. RKY5 isolated from persimmon vinegar. Applied Biochemistry and Biotechnology. 131 (1-3) :705-715. 81.Klemm, D., Heublein, B., Fink, H. P. and Bohn, A. 2001. Cellulose: Fascinating biopolymer and sustainable raw material. Angew. Chem. Int. Ed. 44: 3358. 82.Klemm, D., Heublein, B., Fink, H. P. and Bohn, A. 2005. Cellulose: Fascinating biopolymer and sustainable raw material. Angew. Chem. Int. Edit. 44: 3358 – 3393. 83.Klemm, D., Schumann, D., Udhardt, U. and Marsch, S. 2001. Bacterial synthesized cellulose - artificial blood vessels for microsurgery. Prog. Polym. Sci. 26: 1561-1603. 84.Kongruang, S. 2008. Bacterial cellulose production by *Acetobacter xylinum* strains from agricultural waste products. Appl. Biochem. Biotechnol. 148: 245 – 256. 85.Kouda, T., Yano, H. and Yoshinaga, F. 1997. Effect of agitator configuration on bacterial cellulose productivity in aerated and agitated culture. J. Ferment. Bioeng. 83: 371 – 376. 86.Krystynowicz, A., Czaja, W., Wiktorowska-Jeziarska, A., Goncalves-Mioekiewicz, M., Turkiewicz, M. and Bielecki, S. 2002. Factors affecting the yield and properties of bacterial cellulose. J. Ind. Microbiol. Biotechnol. 29:189 – 195. 87.Kurita, K., Kojima, T., Nishiyama, Y. and Shimojoh, M. 2000. Synthesis and some properties of nonnatural amino polysaccharides: Branched chitin and chitosan. Macromolecules. 33: 4711-4716. 88.Łaskiewicz, B. 1998. Solubility of bacterial cellulose and its structural properties. J. Appl. Polym. Sci. 67: 1871 – 1876. 89.Legeza, V. I., Galenko-Yaroshevskii, V. P., Zinov'ev, E. V., Paramonov, B. A., Kreichman, G. S., Turkovskii, I. I., Gumenyuk, E. S., Karnovich, A. G. and Khripunov, A. K. 2004. Effects of new wound dressings on healing of thermal burns of the skin in acute radiation disease. Bulletin of Experimental Biology and Medicine. 138: 311-315. 90.Lin, F. C. and Brown Jr, R. M. 1989. Purification of Cellulose Synthase from *Acetobacter xylinum*. In: Cellulose and Wood: Chemistry and Technology, C. Schuerch (Ed.), John Wiley & Sons, Inc. pp. 473 – 492. New York, USA. 91.Lin, F. C., Brown Jr. R. M., Cooper, J. B. and Delmer, D. P. 1985. Synthesis of fibrils in vitro by a solubilized cellulose synthase from *Acetobacter xylinum*. Science. 230: 822 – 825. 92.Masaoka, S., Ohe, T. and Sakota, N. 1993. Production of cellulose from glucose by *Acetobacter xylinum*. J. Ferment. Bioeng. 75: 18 – 22. 93.Matsuoka, M., Tsuchida, T., Matsushita, K., Adachi, O. and Yoshinaga, F. 1996. A synthetic medium for bacterial cellulose production by *Acetobacter xylinum* subsp. *Sucrofermentans*. Biosci. Biotechnol. Biochem. 60: 575 – 579. 94.Matthysse, A. G., Thomas, D. and White, A. R. 1995. Mechanisms of cellulose synthesis in *Agrobacterium tumefaciens*. J. Bacteriol. 177: 1076-1081. 95.Miranda, B. T., Miranda, S. R., Chan, L. P. and Saqueton, E. R. 1965. Some studies on nata. Nat. Appl. Sci. Bull. 19: 67-79. 96.Mu"hlethaler, K. 1949. The structure of bacterial cellulose. Biochim. Biophys. Acta. 3: 527 – 535. 97.Nakagaito, A. N., Iwamoto, S. and Yano, H. 2005. Bacterial cellulose: The ultimate nano-scalar cellulose morphology for the production of high-strength composites. Appl. Phys. A: Mater. Sci. Process. 80: 93 – 97. 98.Nakai, T., Moriya, A., Tonouchi, N., Tsuchida, T., Yoshinaga, F., Horinouchi, S., Sone, Y., Mori, H., Sakai, F. and Hayashi, T. 1998. Control of expression by the cellulose synthase (*bcsA*) promoter region from *Acetobacter xylinum* BPR 2001. Gene. 213:93 – 100. 99.Nakai, T., Nishiyama, Y., Kuga, S., Sugano, Y. and Shoda, M. 2002. ORF2 gene involves in the construction of high-order structure of bacterial cellulose, Biochem. Biophys. Res. Commun. 295:458 – 462. 100.Napoli, C., Dazzo, F. and Hubbell, D. 1975. Production of cellulose microfibrils by *Rhizobium*. Appl. Microbiol. 30: 123-131. 101.Naritomi, T., Kouda, T., Yano, H. and Yoshinaga, F. 1998a. Effect of lactate on i.bacterial cellulose production from fructose in continuous culture. J. Ferment. Bioeng. 85: 89 – 95. 102.Naritomi, T., Kouda, T., Yano, H. and Yoshinaga, F. 1998b. Effect of ethanol on bacterial cellulose production from fructose in continuous culture. Journal of Fermentation and Bioengineering. 85(6) : 598-603. 103.Ng, C. C. and Shyu, Y. T. 2004. Development and production of cholesterol-lowering *Monascus-nata* complex. World J. Microbiol. Biotechnol. 20: 875 – 879. 104.Nguyen, V. Y., Flanagan, B., Gidley, M. J. and Dykes, G. A. 2008. Characterization of cellulose production by a *Gluconacetobacter xylinus* strain from kombucha, Curr. Microbiol. 57: 449 – 453. 105.Nobles Jr, D. R. and Brown Jr, R. M. 2008. Transgenic expression of *Gluconacetobacter xylinus* strain ATCC 53582 cellulose synthase genes in the cyanobacterium *Synechococcus leopoliensis* strain UTCC 100. Cellulose. 15: 691 – 701. 106.Nogi, M., Handa, K., Nakagaito, A. N. and Yano, H. 2005. Optically transparent bionanofiber composites with low sensitivity to refractive index of the polymer matrix. Appl. Phys. Lett. 87: 243110. 107.Nogi, M., Handa, K., Nakagaito, A. N. and Yano, H. 2005. Optically transparent bionanofiber composites with low sensitivity to refractive index of the polymer matrix. Appl. Phys. Lett. 87: 1-3. 108.Noro, N., Sugano, Y. and Shoda, M. 2004. Utilization of the buffering capacity of corn steep liquor in bacterial cellulose production by *Acetobacter xylinum*. Appl. Microbiol. Biotechnol. 64: 199 – 205. 109.Orts, W. J., Shey, J., Imam, S. H., Glenn, G. M., Guttman, M. E. and Revol, J. F. 2005. Application of cellulose microfibrils in polymer nanocomposites. J. Polym. Environ. 13: 301 – 306. 110.Park, J. K., Jung, J. Y. and Park, Y.H. 2003. Cellulose production by *Gluconacetobacter hansenii* in a medium containing ethanol. Biotechnol. Lett. 25: 2055 – 2059. 111.Pecoraro, E', Manzani, D., Messaddeq, Y and Ribeiro, S. J. L. 2008. Chap.17: Bacterial Cellulose from *Gluconacetobacter xylinus*: Preparation, Properties and Applications. In Belgacem, M. N. (Eds. ), Monomers, Polymers and Composites from Renewable Resources. p.369-382. E'cole Francaise de Papeterie et des Industries Graphiques (INPG), Grenoble, France. 112.Premjet, S., Premjet, D. and Ohtani, Y. 2007. The effect of ingredients of sugar cane molasses on bacterial cellulose production by *Acetobacter xylinum* ATCC 10245, Sen ' i Gakkaishi. 63: 193 – 199. 113.Puri, V. P. 1984. Effect of crystallinity and degree of polymerization of cellulose on enzymatic saccharification. Biotechnol. Bioeng. 26: 1219 – 1222. 114.Ramana, K. V., Tomar, A. and Singh, L. 2000. Effect of various carbon and nitrogen sources on cellulose synthesis by *Acetobacter xylinum*. World J. Microbiol. Biotechnol. 16: 245 – 248. 115.Richmond, P. A. 1991. Occurrence and Functions of Native Cellulose. In: Biosynthesis and Biodegradation of Cellulose. Haigler, C.H. and Weimer, P. J. (Eds.), Marcel Dekker, Inc. pp. 5 – 23. New York, USA. 116.Ross, P., Mayer, R. and Benziman, M. 1991. Cellulose biosynthesis and function in bacteria. Microbiol. Rev. 55: 35 – 58. 117.Ross, P., Mayer, R. and Benziman, M. 1991. Cellulose biosynthesis and function in bacteria. Microbiological Reviews. 55 : 35-58. 118.Sakairi, N., Asano, H., Ogawa, M., Nishi, N. and Tokura, S. 1998. A method for direct harvest of bacterial cellulose filaments during continuous cultivation of *Acetobacter xylinum*, Carbohydr. Polym. 35: 233 – 237. 119.Saxena, I. M., Kudlicka,

K., Okuda, K. and Brown Jr. R. M. 1994. Characterization of genes in the cellulose-synthesizing operon (acs operon) of *Acetobacter xylinum*: Implications for cellulose crystallization. *J. Bacteriol.* 176: 5735 – 5752. 120.Schrecker, S. and Gostomski, P. 2005. Determining the water holding capacity of microbial cellulose. *Biotechnol. Lett.* 27: 1435 – 1438. 121.Seráfica, G. Mormino, R. and Bungay, H. 2002. Inclusion of solid particles in bacterial cellulose. *Applied Microbiology and Biotechnology.* 58 (6) : 756-760. 122.Seto, A., Saito, Y., Matsushige, M., Kobayashi, H., Sasaki, Y., Tonouchi, N., Tsuchida, T., Yoshinaga, F., Ueda, K. and Beppu, T. 2006. Effective cellulose production by a coculture of *Gluconacetobacter xylinus* and *Lactobacillus mali*. *Appl. Microbiol. Biotechnol.* 73:915 – 921. 123.Shah, J. and Brown, R. M. Jr. 2005. Towards electronic paper displays made from microbial cellulose. *Appl. Microbiol. Biotechnol.* 66: 352-355. 124.Sherif, M.A.S., Keshk and Kazuhiko Sameshima.(2005)Evaluation of different carbon sources for bacterial cellulose production.*Enzyme and Microbial Technology* 40 (2006) 4 – 8. 125.Shibazaki, H., Kuga, S., Onabe, F. and Usuda, M. 1993. Bacterial cellulose membrane as separation medium. *J. Appl. Polym. Sci.* 50: 965 – 969. 126.Shigematsu, T., Takamine, K., Kitazato, M., Morita, T., Naritomi, T., Morimura, S. and Kida, K. 2005. Cellulose production from glucose using a glucose dehydrogenase gene (gdh)-deficient mutant of *Gluconacetobacter xylinus* and its use for bioconversion of sweet potato pulp. *J. Biosci. Bioeng.* 99: 415 – 422. 127.Shirai, A., Takahashi, M., Kaneko, H., Nishimura, S., Ogawa, M., Nishi, N. and Tokura, S. 1994. Biosynthesis of a novel polysaccharide by *Acetobacter xylinum*. *Int. J. Biol. Macromol.* 16 : 297 – 300. 128.Shoda, M. and Sugano, Y. 2005. Recent advances in bacterial cellulose production. *Biotechnology and Bioprocess Engineering.* 10:1-8. 129.Sokolnicki, A. M., Fisher, R. J., Harrah, T. P. and Kaplan, D. L. 2006. Permeability of bacterial cellulose membranes. *J. Membr. Sci.* 272: 15-27. 130.Son, H. J., Kim, H. G., Kim, K. K., Kim, H. S., Kim, Y. G. and Lee, S. J. 2003. Increased production of bacterial cellulose by *Acetobacter* sp. V6 in synthetic media under shaking culture conditions. *Bioresour. Technol.* 86: 215 – 219. 131.Son, H. J., Moon-Su Heo, M. S., Kim, Y. G. and Lee, S. J. 2001 . Optimization of fermentation conditions for the production of bacterial cellulose by a newly isolated *Acetobacter* sp. A9 in shaking cultures. *Biotechnol. Appl. Biochem.* 33:1 – 5 (Printed in Great Britain). 132.Spiers, A. J., Bohannon, J., Gehrig, S. M. and Rainey, P. B. 2003. Biofilm formation at the air-liquid interface by the *Pseudomonas fluorescens* SBW25 wrinkly spreader requires an acetylated form of cellulose. *Mol. Microbiol.* 50: 15-27. 133.Standal, R., Iversen, T. G., Coucheron, D. H., Fjaervik, E., Blatny, J. M. And Valla, S.1994. A new gene required for cellulose production and a gene encoding cellulolytic activity in *Acetobacter xylinum* are colocalized with the bcs operon. *J. Bacteriol.* 176: 665 – 672. 134.Stephens, S. R., Westland, J. A. and Neogi, A. N. 1990. Method of using bacterial cellulose as a dietary fiber component. US patent 4960763. 135.Sun, D. P., Zhang, J. D., Zhou, L. L., Zhu, M. Y., Wu, Q. H. and Xu, C. Y. 2005. Production of bacterial cellulose with *Acetobacter xylinum* 1.1812 fermentation. *J. Nanjing Univ. Sci. Technol.* 29: 601 – 604. 136.Sutherland, I. W. 1998. Novel and established applications of microbial polysaccharides. *Trends Biotechnol.* 16: 41 – 46. 137.Tabuchi, M. and Baba, Y. 2005. Design for DNA separation medium using bacterial cellulose fibrils. *Anal. Chem.*77: 7090 . 138.Tabuchi, M., Ueda, M., Kaji, N., Yamasaki, Y., Nagasaki, Y., Yoshikawa, K., Kataoka, K. and Baba, Y. 2004. Nanospheres for DNA separation chips. *Nat. Biotechnol.* 22: 337 . 139.Tal, R., Wong, H. C., Calhoon, R., Gelfand, D., Fear, A. L., Volman, G., Mayer, R., Ross, P., Amikam, D., Weinhouse, H., Cohen, A., Sapir, S., Ohana, P. and Benziman, M. 1998. Three cdg operons control cellular turnover of cyclic di-GMP in *Acetobacter xylinum*: Genetic organization and occurrence of conserved domains in isoenzymes. *J. Bacteriol.* 180: 4416 – 4425. 140.Tantratian, S., Tammarate, P., Krusong, W., Bhattarakosol, P. and Phunsri. 2005. Effect of dissolved oxygen on cellulose production by *Acetobacter* sp.. *J. Sci. Res. Chula Univ.* 30: 179 – 186. 141.Tantratian, S., Tammarate, P., Krusong, W., Bhattarakosol, P. And Phunsri, A. 2005. Effect of dissolved oxygen on cellulose production by *Acetobacter* sp. *J. Sci. Res. Chula. Univ.* 30 (2) : 179-186. 142.Toda, K., Asakura, T., Fukaya, M., Entani, E. and Kawamura, Y. 1997. Cellulose production by acetic acid-resistant *Acetobacter xylinum*. *J. Ferment. Bioeng.* 84: 228 – 231. 143.Toda, K., Asakura, T., Fukaya, M., Entani, E. and Kawamura, Y. 1997. Cellulose production by acetic acid-resistant *Acetobacter xylinum*. *Journal of Fermentation and Bioengineering.* 84(3): 228-231. 144.Tokoh, C., Takabe, K. J. and Fujita, M. 2002. Cellulose synthesized by *Acetobacter xylinum* in the presence of plant cell wall polysaccharides. *Cellulose.* 9:65 – 74. 145.Tokoh, K., Takabe, M., Fujita, H. and Saiki. 1998. Cellulose synthesized by *Acetobacter xylinum* in the presence of acetyl glucomannan. *Cellulose.* 5: 249 – 261. 146.Tonouchi, N., Tsuchida, T., Yoshinaga, F., Beppu, T. and Horinouchi, S. 1996. Characterization of the biosynthetic pathway of cellulose from glucose and fructose in *Acetobacter xylinum*, *Biosci. Biotechnol. Biochem.* 60: 1377 – 1379. 147.Valla, S. and Kjosbakken, J. 1982. Cellulose negative mutants of *Acetobacter xylinum*. *J. Gen. Microbiol.* 128: 1401 – 1408. 148.Vandamme, E. J., De Baets, S., Vanbaelen, A., Joris, K. and De Wulf, P. 1998. Improved production of bacterial cellulose and its application potential. *Polym. Degradation Stab.* 59: 93-99. 149.Volkmoth W. D., Austin R. H. 1992. DNA electrophoresis in microlithographic arrays. *Nature.* 358: 600 . 150.Wan, W.K. and Millon, L.E. 2005. Poly(vinyl alcohol)-bacterial cellulose nanocomposite. *U.S. Pat.Appl., Publ. US 2005037082 A1:16.* 151.Wan, W.K., Hutter, J.L., Millon, L. and Guhadós, G. 2006. Bacterial cellulose and its nanocomposites for biomedical applications. *ACS Symp. Ser.* 938: 221-241. 152.Wanichapichart, P., Kaewnopparat, S., Buaking, K. and Puthai, W. 2002. Characterization of cellulose membranes produced by *Acetobacter xylinum*, *J. Sci. Technol.* 24: 855 – 862. 153.Watanabe K., Eto Y., Takano S., Nakamori S., Shibai H. and Yamanaka S. 1993. A new bacterial cellulose substrate for mammalian cell culture. *Cytotechnology.* 13: 107-114. 154.Watanabe, K., Tabuchi, M., Morinaga, Y. and Yoshinaga, F. 1998. Structural features and properties of bacterial cellulose produced in agitated culture. *Cellulose.* 5: 187 – 200. 155.Westland, J. A., Penny, G. S., Stephens, R. S. and Winslow, A. R. 1994. Method of supporting fractures in geologic formations and hydraulic fluid composition for same. US patent 5350528. 156.White, A. R. and Brown Jr., R. M. 1981. Enzymatic hydrolysis of cellulose: Visual characterization of the process. *Proc. Natl. Acad. Sci. U. S. A.* 78: 1047 – 1051. 157.William, W. S. and Cannon, R. E. 1989. Alternative environmental roles for cellulose produced by *Acetobacter xylinum*. *Appl. Environ. Microbiol.* 55: 2448 – 2452. 158.Wong, H. C., Fear, A. L., Calhoon, R. D., Eichinger, G. H., Mayer, R., Amikam, D., Benziman, M., Gelfand, D.

H., Meade, J. H., Emerick, A. W., Bruner, R., Ben-Bassat, A. and Tal, R. 1990. Genetic organization of the cellulose synthase operon in *Acetobacter xylinum*. Proc. Natl. Acad. Sci. U. S. A, 87: 8130 – 8134. 159. Yang, Y. K., Park, S. H., Hwang, J. W., Pyun, Y. R. and Kim, Y. S. 1998. Cellulose production by *Acetobacter xylinum* BRC5 under agitated condition. J. Ferment. Bioeng. 85: 312 – 317. 160. Yoshino, T., Asakura, T. and Toda, K. 1996. Cellulose production by *Acetobacter pasteurianus* on silicone membrane. J. Ferment. Bioeng. 81: 32 – 36. 161. Zaar, K. 1979. Visualization of pores (export sites) correlated with cellulose production in the envelope of the Gram-negative bacterium *Acetobacter xylinum*. J. Cell Biol. 80: 773 – 777.