

Isolation and Cellulase Characteristics for Bamboo Cellulolytic Microbes

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ABSTRACT

Results of the research showed that: (1) cellulolytic microbes strain ZQBC691 was isolated from bamboo shoots compost, can produce CMCase (endoglucanase) to hydrolyze carboxymethyl cellulose (CMC). Based on 16S rDNA sequence analysis, strain ZQBC691 was classified as *Streptomyces griseoaurantiacus*; (2) the optimal conditions in which the strain hydrolyzes CMC were initial pH of 5.3, the agitation rate is 150 rpm, culture temperature is 30 and CMC concentration is 15 g/L; (3) the strain showed higher hydrolytic ability for CMC and salicin, but lower degradation for avicel. Furthermore, it can hydrolyze bamboo fiber to produce glucose, lactic acid and acetic acid; (4) the optimal conditions for the strain's CMCase activity are pH of 5 (37.38 IU/L) and 50 (34.13 IU/L); (5) the pretreatment conditions for dilute sulfuric acid of bamboo powder are as follows: the pretreatment temperature at 100 for 60 min with 0.2 M H₂SO₄; and (6) the conversion rate using commercial cellulase (from *Trichoderma reesei*) for bamboo fiber is higher than for bamboo powder.

Keywords : cellulolytic microbes ; CMCase ; carboxymethyl cellulose ; *Streptomyces griseoaurantiacus* ZQBC691 ; bamboo fiber

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REFERENCES

尤智立, (2003), 嗜高溫纖維分解菌纖維分解酵素的探討, 國立中山大學生物科學研究所, 碩士論文。行政院農業委員會 http://www.coa.gov.tw/show_index.php 李建政、汪群慧, (2004), 廢棄物資源化與生物能源, 化學工業出版社, 北京。李鐵民、馬溪平、劉宏生, (2005), 環境微生物資源原理與應用, 化學工業出版社, 北京。李文龍, (2003), 澱粉葡萄糖化酵素固定在幾丁聚醣之研究, 國立雲林科技大學工業化學與災害防治研究所, 碩士論文。周柏伸, (2006), 利用酸前處理提高纖維酵素水解蔗渣效率之研究, 國立台

灣大學生物產業機電工程學研究所，碩士論文。周姿佑，(1999)，農業纖維素誘導嗜高溫菌生產纖維素之探討，國立台灣大學食品科技研究所，碩士論文。郁儀豪，(2003)，蔗渣堆肥中嗜高溫菌之分離與應用，國立臺灣大學環境工程學研究所，碩士論文。陳威廷，(2004)，纖維素水解菌之培養策略與纖維素水解酵素之鑑定，國立成功大學化學工程學系，碩士論文。張元銘，(2004)，快速熱裂解技術應用於生質燃料之製作，嘉南藥理科技大學環境工程與科學系，碩士論文。黃宜瑾，(2007)，介質研磨對纖維素之酵素水解動力學的影響，國立台灣大學生農學院食品科技研究所，碩士論文。葉丁源，(1997)，嗜高溫放線菌纖維素分解酵素之探討，國立台灣大學農業化學研究所，碩士論文。齊倍慶，(2001)，從堆肥中篩選纖維素分解酵素生產菌及其酵素性質研究，國立清華大學生命科學系，碩士論文。Alexander, M., (1977) "Introduction to Soil Microbiology, 2nd ed." John Wiley & Sons, New York, pp: 1-554. Beguin, P., (1987) "Cloning of cellulase gene" Critical Reviews in Biotechnology, 6: 129-162. Bhat, M.K., and S. Bhat, (1997) "Cellulose degrading enzymes and their potential industrial applications" Biotechnology Advances, 15: 583-520. Cosgrove, D.J., (1998) "Cell walls: structures, biogenesis, and expansion" In: Plant Physiology, Taiz L., Zeiger E., Eds., Sinauer Associates, Inc., Sunderland, pp: 409-443. Desvaux, M., (2005) "Clostridium cellulolyticum: model organism of mesophilic cellulolytic clostridia" FEMS Microbiology Reviews, 29: 741-764. Ghose, T.K., (1987) "Measurement of cellulase activities" Pure and Applied Chemistry, 59: 257-268. He, L., W. Li, Y. Huang, L. Wang, Z. Liu, B. Lanoot, M. Vancanneyt, and J. Swings, (2005) "Streptomyces jietaisiensis sp. nov., isolated from soil in northern China" International Journal of Systematic and Evolutionary Microbiology, 55: 1939-1944. Ilme'n M., A. Saloheimo, M.L. Onnela, and M.E. Penttila, (1997) "Regulation of cellulase gene expression in the filamentous fungus Trichoderma reesei" Applied and Environmental Microbiology, 63: 1298-1306. Kidby, D.K., and D.J. Davidson, (1973) "A convenient ferricyanide estimation of reducing sugars in the nanomole range" Analytical Biochemistry, 55: 321-325. Kim, Y.S., H.C. Jung, and J.G. Pan, (2000) "Bacterial cell surface display of an enzyme library for selective screening of improved cellulase variants" Applied and Environmental Microbiology, 66: 788-793. Lever, M., (1972) "A new reaction for colorimetric determination of carbohydrates next term" Analytical Biochemistry, 47: 273-279. Mandels, M., E.T. Reese, (1957) "Induction of cellulase in Trichoderma Viride as influenced by carbon sources and metals" Journal of Bacteriology, 73: 269-278. Matsumoto, N., I. Momose, M. Umekita, N. Kinoshita, M. Chino, H. Inuma, T. Sawa, M. Hamada, T. Takeuchi, (1998) "Diperamycin, a new antimicrobial antibiotic produced by Streptomyces griseourantiacus MK393-AF2. I. Taxonomy, fermentation, isolation, physico-chemical properties and biological activities" The Journal of Antibiotics, 51: 1087-1092. Miller, G.L., (1959) "Use of dinitrosalicylic acid reagent for determination of reducing sugar" Analytical Chemistry, 31: 426-428. Murai, T., M. Ueda, T. Kawaguchi, M. Arai, and A. Tanaka, (1998) "Assimilation of cellooligosaccharides by a cell surface-engineered yeast expressing α -glucosidase and carboxymethylcellulase from Aspergillus aculeatus" Applied and Environmental Microbiology, 64: 4857-4861. Ortega, N., M. D. Busto, and M. P. Mateos, (2001) "Kinetics of cellulose saccharification by Trichoderma reesei cellulases" International Biodeterioration & Biodegradation 47: 7-14. Ramos, L.P., (2003) "The chemistry involved in the steam treatment of lignocellulosic materials" Quimica Nova, 26: 863-871. Scurlock, J.M.O., D.C. Dayton, B. Hames, (2000) "Bamboo: an overlooked biomass resource" Biomass and Bioenergy, 19: 229-244. Singh, A., and K. Hayashi, (1995) "Microbial cellulase: protein architecture, molecular properties, and biosynthesis" Advances In Applied Microbiology, 40: 1-44. Somogyi, M., (1952) "Notes on sugar determination" Journal of Biological Chemistry, 195: 19-23. Teather, R.M., and P.J. Wood, (1982) "Use of Congo red- polysaccharide interactions in enumeration and characterization of cellulolytic bacteria from the bovine rumen" Applied and Environmental Microbiology, 43: 777-780. Wen, Z., W. Liao, and S. Chen, (2005) "Production of cellulase by Trichoderma reesei from dairy manure" Bioresource Technology, 96: 491-499. Wood, T.M., (1985) "Properties of cellulolytic enzyme systems" Biochemical Society Transactions, 13: 407-410. Zhang, L., D. Ruan, and S. Gao, (2002) "Dissolution and regeneration of cellulose in NaOH/thiourea aqueous solution" Journal of Polymer Science Part B: Polymer Physics, 40: 1521-1529. Zhang, Y.H.P., M.E. Himmel, and J.R. Mielenz, (2006) "Outlook for cellulase improvement: screening and selection strategies" Biotechnology Advances, 24: 452-481. Zhang, Y.H.P., and L.R. Lynd, (2005) "Determination of the number average degree of polymerization of cellodextrins and cellulose with application to enzymatic hydrolysis" Biomacromolecules, 6: 1510-1515.