在發酵槽中探討 Agrobacterium sp. 菌株生產卡德蘭膠

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摘 要

卡德蘭膠(Curdlan)是一種不可溶性的細菌性胞外多醣體，由1,3-β-D-醣?鍵(1,3-β-D-glucosidic linkages)鍵結而成，大多為Agrobacterium sp.菌株和 Alcaligenes faecalis在限制氮源下合成。卡德蘭膠是一種溶鹼性的β-1,3-glucan多醣體且水溶液加熱至55°C後會形成凝膠狀，並在冷卻後形成熱可逆的膠體。當加熱至70°C以上時可以形成熱不可逆膠體。因此，卡德蘭膠可以應用在食品工業中作為食品添加劑。由於卡德蘭膠可以廣泛的應用，因此卡德蘭膠在未來的使用上會逐漸增加。所以，如何降低卡德蘭膠生產的成本很重要。本篇研究主要探討在發酵槽中以不同曝氣量及攪拌速率對Agrobacterium sp.菌株生產卡德蘭膠的影響。此外，探討不同純化方法對卡德蘭膠產量的影響以及卡德蘭膠溶液的流變學特性。最後，將發酵液純化出的樣品進行結構分析。本篇研究可分為四部份。

第一部份，本研究將Agrobacterium sp.菌株於5升發酵槽中進行批次培養，探討不同曝氣量及攪拌速率對卡德蘭膠生產的影響。以曝氣量0.0 vvm、0.5 vvm、1.0 vvm和1.5 vvm；攪拌速率150 rpm、300 rpm及600 rpm作為操作條件，進行菌株生長及卡德蘭膠生產的影響探討。實驗結果顯示，當曝氣量或攪拌速率提高時(曝氣量從0.0 vvm至1.5 vvm；攪拌速率從150 rpm至600 rpm)，菌體濃度和卡德蘭膠產量均會增加，顯示高的氧氣傳輸效率可以提高卡德蘭膠的產量。

第二部份針對卡德蘭膠的純化方法進行探討。發現鹼液濃度是影響卡德蘭膠純化的一個因素。當NaOH溶液濃度提高時(從0.2 N至2 N)，卡德蘭膠產量也會提高，且體積比(鹼液/發酵液)越大，卡德蘭膠濃度越高。此外，不同的酸液也會影響卡德蘭膠的純化產量。然而，NaOH的反應時間並不會影響卡德蘭膠的純化產量。

第三部份為不同卡德蘭膠濃度(1-6%)在不同溫度(10-50°C)和轉速(22-40 rpm)下的流變學特性探討。利用修飾後之power law model可合理解析卡德蘭膠溶液的流體行為，並可精確模擬流體的行為曲線。最後利用Arrhenius方程式計算出活化能，並發現到卡德蘭膠的活化能會隨著卡德蘭濃度的增加而減少。

第四部份則是對發酵液純化出的樣品進行結構分析。利用FT-IR (fourier transform infrared)及NMR (nuclear magnetic chromatography)進行分析。結果顯示，由發酵液中純化出之樣品確定為卡德蘭膠。

關鍵詞 : glucan、Agrobacterium sp.、發酵槽、純化條件、流變學

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參考文獻

1.丁懷謙。2000。食藥用菇多糖体之免疫生理活性。食品工業。32:28-422.尤新 編著。2001。機能性發酵製品。第199-1670頁。藝軒圖書出版社。台北，臺灣。3.水野卓和川何正允 編著。1922。菇類的化學生化學。第260-263頁。國立編譯館出版。台北，臺灣。4.余靜宜。2006。利用Agrobacterium sp.菌珠生產卡德蘭膠之研究。5.Allen, D. G. and Robinson, C. W. 1989. Hydrodynamics and mass transfer in Aspergillus niger fermentations in bubble coulumn and loop bioreactors. Biotechnology and Bioengineering 34: 731-740.6.Allen, D. G. and Robinson, C. W. 1990. Measurement of rheological properties of filamentous fermentation broths. Chemical Engineering Science 45: 37-48.7.Application of curdlan to controlled drug delivery III drug release from sustained release suppositories in vito. Biological and pharmaceutical bulletin 18: 1154-1158.8.Augustin, J. 1988. Glucans as modulating polysaccharide, their characteristics Banik, R.M., Kanari, B and Upadhyay, S.N. 2000. Exopolysaccharide of the gellan family prospects and potential. World Journal of Microbiology and Biotechnology 16:407-414.9.Bhattacharya, S., Bhat, K. K. and Raghuver, K. G. 1992. Rheology of Bengal gram cicer arictinum flour suspensions. Journal of Food Engineering 17: 83-96.10.Brito- De La Fuente, E., Choplin, L. and Tanguy, P. A. 1997. Mixing with helical ribbon impellers : effect of highly shear thinning behaviour and impeller geometry.11.Transactions of the Institution of Chemical Engineers A 75: 45-52.12.Broadfoot, R. and Miller, K. F. 1990. Rheological studies of massecuites and molasses. International Sugar Journal 92: 107-11213.Bueche, f. 1952. Viscosity, self-diffusion, and allied effects in solid polymers. The Journal of Chemical Physics 20(12): 1959-1964.14.Bueche, F. 1954. The viscoelastic properties of plastics. The Journal of Chemical Physics 22(4): 603-609.15.Bueche, F. 1968. Viscosity of entangled polymers; theory of variation with shear rate. The Journal of Chemical Physics 48(10): 4781-4784.16.Bueche, F. and Harding, S. W. 1958. A new absolute molecular weight method for linear polymers. Journal of Polymer Science 32(124): 177-186.17.Evans, S. G., Morrison, D., Kaneko, Y. and Havlik, I. 1998. The effect of curdlan sulfate on development in vitro of Plasmodium falciparum. Transactions of the Society of Instrument Technology 92: 87- 89.18.Flieger, M., Kantorova., M., Prezanka,A.,Rezanka, T. and Votruba, J. 2003. Bidegradable Plastics from Renewable Sources. Folia microbiologica. 48 :27-4419.Fraina, J., Sineriz, F.,Molina, O.E. and Perotti, N. I. 2001. Isolation and physico-chemical characterizeation of soluble scleraoglucan from scleraotiumrolfsii Rheological propertyes, molecular wight and conformational chrarcteristics. Carbohydrate research 44:41-5020.Geankoplis, C. J. 1983. Transport processes and unit operations (2en ed.). p. 54-58, 161-170. Allyn and Bacon, Boston.21.Giavasis, I. and Harvey, L. M.2000. Gellan gum.Critical Reviews in Biotechnlogy 20:177-211.22.Graessley., W. W. 1967. Viscosity of entangling polydisperse polymers. The Journal of Chemical Physics 47(6): 1942-1953.23.Graessley., W. W. 1971. Linear viscoelasticity in entangling polymer systems. The Journal of Chemical Physics 54(12): 5143-5157.24.Graessley., W. W. 1974. The entanglement concept in polymer rheology. Advances in Polymer Science 16: 1-179.25.Grigelmo, N. M., Ibarz, A. R. And Martin, O. B. 1999. Rheology of peach dietary fibre suspensions. Journal of Food Engineering 39: 91-99.26.Hangen, P. and Tung, M. A. 1967. Rheograms for power- law fluids using coaxial cylinder viscometers and a template method. Canadian Institute of Food Science and Technology 9: 98-104.27.Harada, T. 1977. Production properies and application of curdlan. Extracellular Microbial Polysaccarides. p. 265-283. American Chemical Society. Washington. DC.28.Hassan, B. H. and Hobani, A. I. 1998. Flow properties of roselle (Hibiscus sabdariffa L.) extract. Journal of Food Engineering 35: 495-47029.Haze, A., Yamamoto, Y., Miyanagi, K. and Uchida, S. 1994. Preparation of a segregation-reducing agent for hydraulic compositions. European Patent 588- 665.30.Hisamatsu, M., Amemura, A., Harada, T., Nakanishi, I. and Kimura, K.1977. Change in ability of Agrobacterium to produce water-soluble and water-insoluble beta-glucans. Journal of General Microbiology 103 : 375-379.31.Hisamatsu, M., Sano, K., Amemura, A. and Harada, T. 1978. Acid polysaccharides containing succinic acid in various strains of Agrobacterium. Carbohydrate Research 61: 89-96.32.Hisamatsu, M., Amemura, A., Harada, T.,Matsuo, T., Matsuda, H.,and Harada ,T. 1982. Cyclic (1β2)-β-D-glucan and the octasaccharide repating-unit of succinglycan produced by Agrobacterium. Journal of General Microbiology 128: 1973-1879.33.Holdsworth, S. D. 1971. Applicability of rheological models to the interpretation of flow and processing behaviour of fluid food products. Journal of Texture Studies 2: 393-418.34.Ibarz, A., Gonzalez, C. and Esplugas, S. 1994. Rheology of clarified fruit juices III: orange juices. Journal of Food Engineering 21: 485-494.35.Jeney, G and Anderson, D. P. 1993. Glucan injection or bath exposure given alone mechanism in rainbow trout (Oncorhychus mykiss) . Aquaculture 116:315-329.36.Jezequel, V. 1998. curdlan : A new Functional β-glucan. Cercal Foods World 43: 361- 364.37.Jong, S.C. and Birmingham, J.M. 1993. Medicinal and rherapeutic value of the shiitake mushroom. Advances in appleied microbiplogy 39: 153-184.38.Joris, K. and Vandamme, E. J. 1993. Improved production bacterial cellulose and its application potential. Microbiology 27-29.39.Kanke, M., Tanabe, E., Katayama, H., Koda, Y. And Yoshitomi, H. 1995.40.Kaur, S., Kaler, R. S. S. and Aamarpali, A. 2002. Effect of starch on the rheology of molasses. Journal of Food Engineering 55: 319-322.41.Kawase, Y. and Hashiguchi, N. 1996. Gas- liquid mass transfer in external- loop airlift columns with Newtonian and non-Newtonian fluids. The Chemical Engineering Journal 62: 35-42.42.Kawase, Y., Halard, B. and Moo-Young, M. 1992. Liquid phase mass transfer coefficients in bioreactors. Biotechnology and Bioengineering 39: 1133-1140.43.Kemblowski, Z. and Kristiansen, B. 1986. Rheometry of fermentation liquids. Biotechnology and Bioengineering 28: 1474-1483.44.Kemblowski, Z., Budzynski, P. and Owczarz, P. 1990. On- line measurements of the rheological properties of fermentation broth. Rheological Acta 29: 588-593.45.Khalil, K. E., Ramakrishna, P., Nanjundaswamy, A. M. and Patwardhan, M. V. 1989. Rheological behaviour of clarified banana Juice: effect of temperature and concentration. Journal of Food Engineering 10: 231-240.46.Lapasin,R. and Pricl, S. 1995. Rheology of industrial polysaccharides : Theory and Applications. Blackie Academic and professional.47.Lawford, H. G. and

Rousseau, J. D., 1992. Production of β -1,3-glucan exopolysaccharide in low shear systems. Applied Biochemistry and Biotechnology 34/35: 587-612.48.Lee, H. J. and Park, H. Y. 2001. Optimal production of curdlan by Agrobacterium sp. with feedback inferential control of optimal pH profile. Biotechnology Letters 23: 525-530.49.Lee, I. Y. 2002. Polysaccharides I Polysaccharides from Prokaryotes. Biopolymers 135-58.50.Lee, I. Y., 2002. Biology. Chemistry. Biotechnology. Application, Biopolmers.p.136-154.WILEY-VCH Verlag GmbH Weinheim, Germany.51.Lee, Y. I., Kim, K. M. Lee, H. J., Seo, T. W., Jung, K. J., Lee, W. H. And Y, H. Pa. 1999. Influence of agiation speed on production of curdlan by Agrobacterium species. Bioprocess Engineering 20: 283- 287.52.Lee, Y. I., Kim, K. M., Lee, H. J., Seo, T. W., Jung, K. J., Lee, W. H. and Y, H. Pa. 1999. Influence of agitation speed on production of curdlan by Agrobacterium species. Bioprocess Engineering 20: 283-287.53.Linton, J.D., Ash, S. G. and Huybrechts, L.1991. Microbial polysaccharides. In: Byron, D.(ed.). Bionaterials: nover materials from biological soures.StocktonPress.New York.54.Liu, T. and Yu, D. 1993. Morphological measurements on P. chrysogenum brothes by rheology and filtration methods. Biotechnology and Bioengineering 42: 777-784.55.Livesey, G. 1990. Energy values of unavailable carbohydrates and diets: An inquiry and analysis. American Journal of Clinical Nutrition. 51: 617- 621.56.Madar, Z. and Odes, H. s., 1990. Dietary fiber in metabolic diseases. In: Dietary fiber Research (Paoletri P. Ed.) Krager, Basel Interface Science 8: 396- 400.57.Meeullough, H. 1967. The determination of ammonia in whole bolld by a direct colorimetric method. Clinica Chimica Acta 17: 297-304.58.Metz, B., Kossen, N. W. F. And Suijdam, JC vam. 1979. The rheology of mould suspensions. Advances in Biochemical Engineering 11: 103-155.59.Misaki, A., Kishida, E., Kakuta, M. and Tabata, K1993. Carbohydratesand Carbohydrate Polymers: Antitumor funglal (1→3)- β -D-glucans: structural diversity and effcets of chemical modification.p. 116-129. Mount Prospect.IL.ATL press.60.Moon, C. J. and Lee, J. H. 2005. Use of curdlan and activated carbon composed adsorbents for heavy metal removal. Process Biochemistry 40: 1279- 1283.61.Moo-Young, M., Halard, B., Allen, D. C., Bureel, R. and Kawase, Y. 1987. Oxygen transfer to mycelial fermentation broths in an airlift fermenter. Beotechnology and Bioengineering 30: 746-753.62.Murooka, Y., Yamada, T. and Harada, T. 1977. Affinity chromatography of Klebsiella arylsulfatase on tyrosyl-hexame- thylenediamine- β -(1,3)-glucan and immunoadsorbent. Biochimica et biophysica acta 485: 134- 140.63.Nishinari, K., Zhang, H. and Ikeda, S. 2000. Hydrocolloid gels of polysaccharides and proteins. Current Opinion in Colloid and Interfae Science 5: 195-201.64.Nishinari, K.and Takahashi, R.2003. Interaction in polysaccharide solutions and gels. Current Opinion in Colloid and Interface Science 8:36-400.65.Osumi, M. S. 1998. The ultrastructure of yeast: cell wall structure and formation. Micron 29: 207-233.66.Pollock, T. J. 1993. Gellan-related polysaccharide and the genus Sphngomonas. Journal of General Microbiology 193: 1939-1945.67.Rao, M., Cooley, M. J. and Vitali, A. A. 1984. Flow properties of concentrated juices at low temperatures. Food Technology 38(3): 113-119.68.Roels, J. A., Van den Berg, J. And Voncken, R. M. 1974. The rheology of mycelial broths Biotechnology and Bioengineering 16: 181-2088.69.Ronen, M., Guterman, H. and Shabtai, Y. 2002. Monitoring and control of pullan peoduciton using vision sensor. Journal of biochemical and biophysical methods 51:243-249.70.Saravacos, G. D. 1970. Effect of temperature on viscosity of fruit juices and puress. Journal of Food Science 35: 122-125.71.Sausages, P. S. and Singal, R. S. 2004. Fermentative Production of curdlan. Applied Biochemistry and Biotechnology 118: 21-23.72.Schumpe, A and Deckwer, W. D. 1987. Viscous media in tower bioractors: Hydrodynamic characteristics and mass transfer properties. Bioprocess Engineering 2: 79-94.73.Statistica for Windows 5.0, 1995. Computer program manual. USA: StatSoft, Inc.74.Steffe, J. F. 1992. Rheological methods in food process engineering. P. 158-162. Freeman Press, East Lansing, Michign, USA.75.Stredansky, M., Conti, E., Bertocchi, C., Matulove, Maria. and Zanett,F. 1998. Succnoglycan production by Agrobacterium tumefaciens. Jouranl of fermentation and bioengineering 85: 398-403.76.Suterland, I. W. 1998. Novel and established application of microbial polysaccharides. Tibtech January 16:41-46.77.Suterland, I. W. 2001. Microbial polysaccharides from Gram-negative bacteria. International Dairy Journal 11:663-674.78.Wang, W. S. and Wang, D. H. 1997. Enhancement of the resistane of tilapia and grass carp to experimental Aeromonas hydrophila and Edwardila tarda infections by several polysaccharides. Comparative Immunology, Microbiology and Infectious Diseases 20: 261-270.79.Whitfield, C. 1988. Bacterial extraellular polysaccharides. Canadian Journal of Microbiology 34:415-420.80.Zeenhuizen, L. P. T. M. 1997. Succinoglycan and galactoglucan. Carbohydrate polymers 33: 139-144.81.Zulli, F., Suter, F., Biltz, H., Nissen, H. P. and Birman, M.1996. Carboxymethylated β -(1，3)-glucan, a beta glucan from baker?s yeast helps protect skin . Cosmetic and Toiletries III12:91-98