

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-糖?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 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 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)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。食藥用菇多糖體之免疫生理活性。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. 菌株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 column 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 vivo. *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 Raghuvver, 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 masseccutes and molasses. *International Sugar Journal* 92: 107-112.
- 13.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 :27-44.
- 19.Fraina, J., Sineriz, F.,Molina, O.E. and Perotti, N. I. 2001. Isolation and physico-chemical characterization of soluble scleraoglucan from scleraotiumrolfsii Rheological propertyes, molecular wight and conformational chararacteristics. *Carbohydrate research* 44:41-50.
- 20.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 Biotechnology* 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: 495-470.
- 29.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 (1 2)- -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 (*Oncorhynchus mykiss*) . *Aquaculture* 116:315-329.
- 36.Jezequel, V. 1998. curdlan : A new Functional -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 appleeid microbiology* 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 Owczar, 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 α -1,3-glucan exopolysaccharide in low shear systems. *Applied Biochemistry and Biotechnology* 34/35: 587-612.

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.

Lee, I. Y. 2002. Polysaccharides I Polysaccharides from Prokaryotes. *Biopolymers* 135-58.

Lee, I. Y., 2002. Biology. Chemistry. Biotechnology. Application, *Biopolymers*. p.136-154. WILEY-VCH Verlag GmbH Weinheim, Germany.

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.

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.

Linton, J.D., Ash, S. G. and Huybrechts, L.1991. Microbial polysaccharides. In: Byron, D.(ed.). *Bionaterials: novel materials from biological sources*. Stockton Press. New York.

Liu, T. and Yu, D. 1993. Morphological measurements on *P. chrysogenum* broths by rheology and filtration methods. *Biotechnology and Bioengineering* 42: 777-784.

Livesey, G. 1990. Energy values of unavailable carbohydrates and diets: An inquiry and analysis. *American Journal of Clinical Nutrition*. 51: 617- 621.

Madar, Z. and Odes, H. s., 1990. Dietary fiber in metabolic diseases. In: *Dietary fiber Research* (Paoletti P. Ed.) Krager, Basel *Interface Science* 8: 396- 400.

Meeullough, H. 1967. The determination of ammonia in whole blood by a direct colorimetric method. *Clinica Chimica Acta* 17: 297-304.

Metz, B., Kossen, N. W. F. And Suijdam, J.C. van. 1979. The rheology of mould suspensions. *Advances in Biochemical Engineering* 11: 103-155.

Misaki, A., Kishida, E., Kakuta, M. and Tabata, K.1993. Carbohydrates and Carbohydrate Polymers: Antitumor fungal (1-3)- β -D-glucans: structural diversity and effects of chemical modification. p. 116-129. Mount Prospect. IL. ATL press.

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.

Moo-Young, M., Halard, B., Allen, D. C., Bureel, R. and Kawase, Y. 1987. Oxygen transfer to mycelial fermentation broths in an airlift fermenter. *Biotechnology and Bioengineering* 30: 746-753.

Murooka, Y., Yamada, T. and Harada, T. 1977. Affinity chromatography of *Klebsiella* arylsulfatase on tyrosyl-hexamethylenediamine-(1,3)-glucan and immuno-adsorbent. *Biochimica et biophysica acta* 485: 134- 140.

Nishinari, K., Zhang, H. and Ikeda, S. 2000. Hydrocolloid gels of polysaccharides and proteins. *Current Opinion in Colloid and Interface Science* 5: 195-201.

Nishinari, K. and Takahashi, R.2003. Interaction in polysaccharide solutions and gels. *Current Opinion in Colloid and Interface Science* 8:36-400.

Osumi, M. S. 1998. The ultrastructure of yeast: cell wall structure and formation. *Micron* 29: 207-233.

Pollock, T. J. 1993. Gellan-related polysaccharide and the genus *Sphingomonas*. *Journal of General Microbiology* 193: 1939-1945.

Rao, M., Cooley, M. J. and Vitali, A. A. 1984. Flow properties of concentrated juices at low temperatures. *Food Technology* 38(3): 113-119.

Roels, J. A., Van den Berg, J. And Voncken, R. M. 1974. The rheology of mycelial broths *Biotechnology and Bioengineering* 16: 181-208.

Ronen, M., Guterman, H. and Shabtai, Y. 2002. Monitoring and control of pullan production using vision sensor. *Journal of biochemical and biophysical methods* 51:243-249.

Saravacos, G. D. 1970. Effect of temperature on viscosity of fruit juices and purees. *Journal of Food Science* 35: 122-125.

Sausages, P. S. and Singal, R. S. 2004. Fermentative Production of curdlan. *Applied Biochemistry and Biotechnology* 118: 21-23.

Schumpe, A and Deckwer, W. D. 1987. Viscous media in tower bioreactors: Hydrodynamic characteristics and mass transfer properties. *Bioprocess Engineering* 2: 79-94.

Statistica for Windows 5.0, 1995. Computer program manual. USA: StatSoft, Inc.

Steffe, J. F. 1992. Rheological methods in food process engineering. P. 158-162. Freeman Press, East Lansing, Michigan, USA.

Stredansky, M., Conti, E., Bertocchi, C., Matulove, Maria. and Zanetti, F. 1998. Succinoglycan production by *Agrobacterium tumefaciens*. *Journal of fermentation and bioengineering* 85: 398-403.

Suterland, I. W. 1998. Novel and established application of microbial polysaccharides. *Tibtech* January 16:41-46.

Suterland, I. W. 2001. Microbial polysaccharides from Gram-negative bacteria. *International Dairy Journal* 11:663-674.

Wang, W. S. and Wang, D. H. 1997. Enhancement of the resistance of tilapia and grass carp to experimental *Aeromonas hydrophila* and *Edwardsiella ictaluri* infections by several polysaccharides. *Comparative Immunology, Microbiology and Infectious Diseases* 20: 261-270.

Whitfield, C. 1988. Bacterial extracellular polysaccharides. *Canadian Journal of Microbiology* 34:415-420.

Zeenhuizen, L. P. T. M. 1997. Succinoglycan and galactoglucan. *Carbohydrate polymers* 33: 139-144.

Zulli, F., Suter, F., Biltz, H., Nissen, H. P. and Birman, M.1996. Carboxymethylated α -(1,3)-glucan, a beta glucan from baker's yeast helps protect skin. *Cosmetic and Toiletries* 12:91-98