Antimicrobial Activity of Different Molecular Weight Chitosan on Bacillus subtilis

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ABSTRACT

The antimicrobial effects of different molecular weight (MW) chitosan (water-soluble-grade W031, chitosan-grade SK10P8, food-grade N96, industry-grade A72 with 27.5、94.1、245.9、350.7 kDa, respectively) in 1% acetic acid solutions on the growth of Bacillus subtilis were investigated in this study.

The results showed the industry-grade A72 (350.7 kDa) could inhibit Bacillus subtilis growth before 4 hour. After 4 hour incubation, the inhibition was not obtained clear. The Bacillus subtilis has inhibited before 8 hour incubation with food-grade N96 (245.9 kDa), and incubation with soluble-grade W031, chitosan-grade SK10P8 have inhibited before 12 hour. In this, the lower molecular weight of chitosan could effective to inhibit Bacillus subtilis growth. The 50~60% inhibited of Bacillus subtilis growth in 0.02% (w/v) of chitosan concentration. When chitosan concentration was 0.04% (w/v), the Bacillus subtilis was not growth. In this study, we found the optimal inhibition in 0.04% (w/v) soluble-grade W031 (27.5 kDa). In health food, we suggest use food-grade N96 (0.02% (w/v)) because the Bacillus subtilis DYU1002 will not be inhibited in health food.

Keywords : chitosan、Bacillus subtilis DYU1002、molecular weight、antimicrobial activity

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圖目錄

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REFERENCES

1.王三郎。1995。水產資源利用學。高立圖書出版社。台北，台灣。2.方紹威。1990。幾丁質與幾丁聚醣在廢水處理、生化、食品和醫藥上之研究發展現況。藥物食品檢驗局調查研究年報。8: 20-30。3.行政院衛生署。2006。台北。台灣。4.江晃榮。1998。生物高分子(幾丁質、膠原蛋白)在食品工業上的應用。原料應用。150(6):19-25。5.江晃榮。2000。幾丁質生技產品-在醫療、食品及環保上之應用。經濟部技術處。6.李安榮，鄒台黎。2000。新編藥物學。第38頁。永大書局有限公司。台北，台灣。7.阮勝威。1996。由靈芝子實體經萃取後之廢渣所製成之薄膜對於天竺鼠傷口及組織纖維母細胞之影響。臺北醫學院醫學研究所碩士論文。台北。8.阮進惠、林翰良、羅淑珍。1997。幾丁聚醣水解物之連續式生產及其抑菌作用。中國農業化學會誌。35:596-611。9.邱子玲。2004。自然環境及市售納豆產品中Bacillus分離株之特性分析及種類鑑定。輔仁大學生命科學系碩士論文。10.林睿哲，莊文喜。2000。血液相容性高分子生醫材料。化工技術。8(10)。230-240。11.林家妏、張曉婷、吳柏昇、林睿哲。2001。幾丁聚醣於生醫材料之應用與特性。化工。48(2)。84-91。12.林漢釗。2005。益生性枯草桿菌Bacillus subtilis WG6-14在水稻栽培與病害管理上之應用性。中興大學植物病理學系所碩士論文。13.卓貴美。2000。圖解生理學。五南圖書出版公司，台北，台灣。14.洪敏元、劉良慧、林育娟、何明聰、賴明華。2000。當代生理學。第41-42頁。華杏出版股份有限公司。台北，台灣。15.孫啟書。1996。人工皮膚之可能材質論靈芝薄膜對傷口癒合之影響。臺北醫學院醫學研究所碩士論文。台北。16.許元勳。2003。現代生物科技的新寵兒-神奇納豆菌。生物產業14(1):53-59。17.莊仲揚。2002。幾丁聚醣於生醫產業上的應用。化工資訊。16(4)。46-50。18.陳美惠、莊淑惠、吳志津。1999。幾丁聚醣的物化特性。食品工業月刊。31(10)。1-6。19.張壽昌。2000。大豆蛋白健康機能解讀。食品資訊178:36-38。20.張根源。2001。智慧型材料之生物醫學應用。化工。48(2)。53-61。21.郭建良。1995。低分子量幾丁聚醣對雙叉桿菌及其他細菌之影響。國立臺灣大學食品科技研究所碩士論文。台北。22.黃群昇。2002。超音波技術探討血液濃度、血流、與血栓形成的關係。中原大學醫學工程學系碩士論文。23.黃宗慶。1999。攝食枯草菌發酵大豆、黑豆及薏仁對老鼠血液血纖維蛋白水解活性及凝血作用之影響。靜宜大學食品營養學系碩士論文。24.須見洋行。1993。納豆治百病。培琳出版社。台北，台灣。25.楊禎明，林浩慈。2001。含肝素的改值熱可塑性在血液相容性的研究。化工。48(2)。78-83。26.楊惠如。2005。枯草桿菌處理對苦瓜種子發芽及植株生長之影響。中興大學園藝學系所碩士論文。27.廖曉玲。2002。新世紀醫學-納豆天然的藥用食品。安立出版社。台北，台灣。28.盧義發、湯政宜。1995。大豆蛋白的降血膽固醇作用。輔仁民生學誌1(1):131-144。29.鍾青萍、余世望、梁牲媛。2001。納豆菌的抗菌作用及其培養基的優化。食品工業科技5:20-2230.蘇遠志。2001。幾丁質與幾丁聚醣之機能及其有效利用。生物資源生物技術。3(2)。6-19。31.蘇遠志。2003。納豆菌代謝產物的開發與應用。生物產業14(2):117-130。32.Aiba, S. 1992. Studies on chitosan: 4. Lysozymic hydrolysis of partially N-acetylated chitosans. International Journal of Biological Macromolecules. 14(4): 225-228.33.Allan, G.G., Fox, J.R., and Kong, N. 1979. A critical evaluation of the potential sources of chitin and chitosan. In:Chitin and chitosan, Proceedings of the First International Conference on Chitin and Chitosan. ed. by Muzzarelli, R.A.A. and Pariser, E.R. MIT Sea Grant Program, Cambridge, Mass, 64-78.34.Alonso, I.G., Peniche-Covas, C., and Nieto, J.M. 1983. Determination of the degree of acetylation of chitin and chitosan by thermal analysis. J. Thermal Analysis. 28: 189.35.Austin, P.R., Brine, C.J., Castle, J.E., and Zikakis, J.P. 1981. Chitin:New facets of research.Science. 212: 749-753.36.Beri, R.G., Walker, J., Reese, E.T., and Rollings, J.E. 1993. Characterization of chitosans via coupled size-exclusion chromatography and multiple-angle laser light-scattering technique. Carbohydrate. Res. 238: 11.37.Bough, W.A. 1975. Coagulation with chitosan-aid to recovery of by-products from egg breaking wastes.Poultry Sci. 54(6): 1904-1912.38.Chui, V.W.D., Mok, K.W., Ng, C.Y., Luong, B.P. and Ma, K.K. 1996. Removal and recovery cooper(II), chromium(III), and nickel(II) from solution using crude chrimp chitin packed in small columns. Environment International. 22: 463-468.39.Curotto, E., and Aros, F. 1993. Quantitative determination of chitosan and the percentage of free amine groups. Anal. Biochem. 211: 240.40.Davis, D.H., and Hayes, E.R. 1988. Determination of the degree of acetylation of chitin and chitosan. Method in Enzymology. 161: 442.41.Domard, A. 1987. Determination of N-acetyl content in chitosan samples by C.D. measurements. Int. J. Biol. Macromol. 9: 333.42.Esaki H, Onozaki H, Osawa T. 1994. Antioxidative activity of fermented soybeen products. Symposium Series. 546: 353-360.43.Hadwiger, L.A., Kendra, B., Fristensky, W., and Wagoner, W. 1986. Chitosan both activates genesin fungi. In: Chitin in Nature and Technology, ed. by Muzzarelli, R.A.A., Jeuniaux, C. and Gooday, G.W. Plenum Press, New York, 209-214.44.Hirano, S. 1997. Application of Chitin and Chitosan. ed. Goosen, M. F. A. Technomic Publishers. Tottori. 31-54.45.Holan, Z., and Voyruba, J. 1980. New method of chitin determination based on deacetylation and gas-liquid chromatographic assay of liberated acetic acid. J. Chromatography. 190: 67.46. http://www.protech-tio2.com/Organism(1)/qr9.htm47.Jha, I.N., Iyenganr, L., and Prabhakara Rao, A.V.S. 1988. Removal of cadmium using chitosan. Journal of Environmental Engineering. 11: 962-974.48.Khor, E., and Lee, Y.L. 2003. Implantable application of chitin and chitosan. Biomaterial. 24: 2339-2349.49.Kiers J.L., A.E.A. Van laeken, F.M. Rbouts, M.J.R. Nout, 2000. In vitro digestibity of Bacillus fermented

soya bean. International Journal of Food Microbiology. 60: 163-169.50.Knorr, D. 1982. Functional properties of chitin and chitosan. J. Food Sci. 47(2): 593-595.51.Knorr, D. 1983. Dye binding properties of chitin and chitosan. J. Food Sci. 48(1): 36-41.52.Knorr, D. 1984. Use of chitinous polymers in food-A challenge for food research and development. Food Technol. 85-97.53.Knorr, D., Wampler, T.P., and Teutonico, R.A. 1985. Formation of pyrazines by chitin pyrolysis. J.Food Sci. 50(6): 1762-1763.54.Kurita, K. 1998. Chemistry and Application of chitin and chitosan. Polymer Degradation and Stability. 59: 117-120.55.Lepri, L. and Desideri, P.G. 1978. Separation and identification of water-soluble food dyes by ion-exchange and soap thin-layer chromatography. Journal of Chromatography. 161: 279-286.56.Leuba, J.L. and Stossel, P. 1986. Chitosan and other polamines: antifungal activity and interaction with biological membranes. In: Chitin in Nature and Technology. ed. By Muzarelli, R.A.A., Jeuniaux, C. and Gooday, G.W. Plenum Press, New York, 215-222.57.Majeti, N.V., and Kumar, R. 2000. The review of chitin and chitosan applications. Reactive&Function Polymers. 46: 1-27.58.Maruyama M, Sumi H. 1998. Effect of natto diet on blood pressure. Basic and Clinical Aspects of Japanese Traditional Food Natto II. 1-3.59.Mi, F.L., Wu, Y.B., Shyu, S.S., Chao, A.C., Lai, J.Y., and Su, C.C. 2002. Asymmetric chitosan membrane prepared by dry/wet phase separation:a new type of wound dressing for controlled antibacterial release. Journal of Membrane Science. 212: 237-254.60.Mima, S., Miya, M., Iwamoto, R., and Yoshikawa, S. 1983. Highly deacetylated chitosan and its properties. J. Applied Polymer Sci. 28: 1909.61.Mitani, T., Moriyama, A. and Ishii, H. 1992. Heavy metal uptake by swollen chitosan beads. Bioscience Biotechnology Biochemistry. 56: 985-986.62.Mitsukawa, H., Shimizu, O., and Nishi, H. 1971. Colorimetric determination of α-amino nitrogen in urine and plasma with ninhydrin reaction. Agr. Biol. Chem. 35: 272.63.Muzzarelli, R.A., Mattioli-Belmonte, M., and Tietz, C. 1994. Stimulatory effect on bone formation exerted by modified chitosan. Biomaterials. (15): 1075-1081.64.Nanjo, F., Katsumi, R., and Sakai, K. 1991. Enzymatic method for determination of the degree of deacetylation of chitosan. Anal. Biochem. 193: 164.65.Niola, F., Basora, N., Chornet, E., and Vidal, P.F. 1993. A rapid method for the determination of the degree of N-acetylation of chitin-chitosan samples by acid hydrolysis and HPLC. Carbohydr. Res. 238: 1.66.Nishi, N., Noguchi, J., Tocura, S., and Shiota, H. 1979. Studies on chitin Ⅰ. Acetylation of chitin. Polymer Journal. 11(1): 27-32.67.Nishimura, K., Ishihara, C., and Ukei, S. 1986. Stimulation of cytokine production in mice using deacetylated chitin. Vaccine. 5: 136-140.68.Nishimura, K., Nishimura, S., Seo, H., Nishi, N., Tokura, S., and Azuma, I.1987. Effect of multipoprous microspheres derived from chitin on the activation of mouse peritoneal macrophages. Vaccine. 5: 136-140.69.Ramanathan, S., and Block, L.H. 2001. The use of chitosan gels as matrices for electrically-modulated drug delivery. Journal of Controlled Release. 70: 109-123.70.Roberts, G.A.F., and Domszy, J.G. 1982. Determination of the viscometric constants for chitosan. Int. J. Biol. Macromol. 4: 374.71.Roller, S. and Covill, N. 1999. The antifungal properties of chitosan in laboratory media and apple juice. International Journal of Food Microbiology. 47(1-2): 67-77.72.Saito, M., Tabeta, R., and Ogawa, K. 1987. High-resolution solid-state 13C NMR study of chitosan and its salts with acid:conformation characterization of polymorph and helical structures as viewed from the conformation-dependent 13C chemical shifts. Macromolecules. 20: 2424.73.Sato T, Yamada Y, Ohtani Y, Mitsui N, Murasawa H, Araki S. 2001. Production of Menaquinone (Vitamin K2)-7 by Bacillus subtilis. Journal of Bioscience and Bioengineering. 91(1):16-20.74.Soto-Peralta, N.V., Muller, H. and Konner, D. 1989. Effect of chitosan treatments on the clarity and color of apple juice. Journal of Food Science. 54: 495-496.75.Stanley, W.L., Watters, G.G., Chan, B., and Mercer, J.M. 1975. Lactase and enzymes bound to chitin with glutaraldehyde. Biotechnology and Bioengineering. 17: 315-326.76.Su, C.H., Sun, C.S., Wei, J.S., Ho, H.O., Hu, C.H., and Sheu, M.T. 1999. Development of fungal mycelia as skin substitutes: Effects on wound healing and fibroblast. Biomaterials. 20: 61-68.77.Sudarshan, N.R., Hoover, D.G., and Knorr, D. 1992. Antibacterial action of chitosan. Food Biotechnology. 6: 257-272.78.Sumi H. 1990. Nattokinase properties and recent application for heathy food. Biology Industrial. 7: 724-730.79.Sumi H, Hamada H, Nakanishi K, Hiratani H. 1980. Enhancement of the fibrinolytic activity in plasma by oral administration of Nattokinase I. Acta Haematol. 84: 139-143.80.Sumi H, Hamada H, Tsushima H, Mihara H, Muraki H. 1987. A novel fibrinolytic enzyme (Nattokinase) in the vegetable cheese Natto; a typical and popular soybean food in the Japanese diet. Experientia. 43: 1110-1111.81.Sumi H, Hamada H, Nakanishi K, Hiratani H. 1990. Enhancement of the fibrinolytic activity in plasma by oral administration of Nattokinase I. Acta Haematol. 84: 139-143.82.Sumi H, Hamada H. 1990. A novel strong fibrinolytic enzyme (Nattokinase) in the vegetable cheese- Natto. Fibrinolysis. 2: 67.83.Suzuki, S., Okawa, Y., Okura, Y., Hashimoto, K. and Suzuki, M. 1982. Proceedings of the second international conference on chitin and chitosan. Sapporp, Japan., 210-212.84.Suzuki, S., Watanabe, T., Mikami, T., Matsumoto, T. and Suzuki, M. 1992. Immuno-enhancing effects of N-acetyl-chitohexanose. In advance in chitin and chitosan, ed. by Brine, C. J., Sandford, P. A., and Zikakis, J. P. Elservier Applied Science, N. Y., 96-105.85.Toei, K., and Kohara, T. 1976. A conductometric method for colloid titrations. Analytica Chimica Acta. 83: 59.86.Wang, X.H., Wang, W.J., Feng, Q.L., Cui, F.Z., Xu, Y.X., Song, X.H., and Mark, V.D.W. 2003. Crosslinked collagen/chitosan matrix for artificial livers,Biomaetrials. 24: 3231-3220.87.Wu, A.C.M., and Bough, W.A. 1976. Determination of molecular-weight distribution of chitosan by high-performance liquid chromatography. J.Chromatography. 128: 87.