

不同分子量的幾丁聚醣對綠膿桿菌之益菌作用研究

林宜泓、張耀南；吳建一

E-mail: 9606925@mail.dyu.edu.tw

摘要

本研究利用四種不同分子量之幾丁聚醣(水溶性W031、甲殼素SK10P8、食品級N96、工業級A72；其分子量分別為Mw 27.5 kDa、94.1 kDa、245.9 kDa、350.7 kDa)對綠膿桿菌 (*Pseudomonas aeruginosa*) 之抑菌作用探討。由實驗結果得知，當幾丁聚醣添加濃度為0.1%(w/v)或更高時，W031、SK10P8、N96等三種幾丁聚醣可完全抑制綠膿桿菌生長達到前48小時培養，其中水溶性W031幾丁聚醣之抑菌效果更可達到前72小時，但A72幾丁聚醣的抑菌效果卻僅有前32小時；幾丁聚醣之抑菌作用隨著其添加濃度增加而增加，且趨於不再改變。另外，當幾丁聚醣添加濃度在0.1%(w/v)以下時，幾丁聚醣對綠膿桿菌的抑菌作用似乎將隨其分子量增加而降低，但添加濃度高於0.1%(w/v)以上時，幾丁聚醣的抑菌效果似乎與其分子量無任何顯著關係，但仍以低分子量幾丁聚醣的抑菌效果為佳。

關鍵詞：幾丁聚醣、分子量、綠膿桿菌、抑菌作用

目錄

目錄 封面內頁 簽名頁 授權書.....	iii	中文摘要.....	iv	英文摘要.....	v
誌謝.....	v	目錄.....	vi	圖目錄.....	vii
表目錄.....	ix	1. 緒論.....	1	2. 文獻回顧.....	2
2.1 幾丁質與幾丁聚醣之發現及分佈.....	2	2.2 幾丁質與幾丁聚醣之結構.....	2	2.3 幾丁質與幾丁聚醣之物化特性.....	6
2.4 幾丁質與幾丁聚醣分子量的測定.....	6	2.5 幾丁質與幾丁聚醣去乙酰程度的測定.....	8	2.6 幾丁物質在生醫材料上的應用.....	13
2.7 幾丁物質在食品上的應用.....	17	2.8 幾丁物質在化工上的應用.....	18	2.9 幾丁物質在生物技術上的應用.....	19
2.10 幾丁物質在生理上的應用.....	19	2.11 幾丁物質在廢水處理方面的應用.....	20	2.12 幾丁聚醣的抑菌作用.....	20
2.13 綠膿桿菌.....	26	2.14 綠膿桿菌感染方式與症狀.....	27	2.15 綠膿桿菌的致病機制.....	30
3. 材料與方法.....	32	3.1 實驗材料.....	32	3.2 儀器設備.....	36
3.3 實驗材料之製備.....	37	3.4 實驗方法.....	37	4. 結果與討論.....	39
4.1 不同濃度的幾丁聚醣對綠膿桿菌之抑菌效果.....	39	4.2 不同分子量的幾丁聚醣對綠膿桿菌之抑菌效果.....	46	5. 結論.....	52
參考文獻.....	54				

參考文獻

- 1.丁名哲。1996。醫用微生物學。第333-339頁。合記圖書出版社。台北，台灣。
- 2.方紹威。1989。幾丁聚醣的抑制微生物作用及其在低糖金橘蜜餞之應用。國立台灣大學食品科技研究所碩士論文。台北。
- 3.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。
- 4.王三郎。1995。水產資源利用學。高立圖書出版社。台北，台灣。
- 5.王三郎。1998。應用微生物學。高立圖書出版社。台北，台灣。
- 6.王正一、林峰輝。2000。生醫材料概論。教育部醫學工程科技教育改近計劃。44-45。
- 7.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。
- 8.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。
- 9.吳襄、林坤偉。1994。生理學大綱。第44-45頁。藝軒出版社。台北，台灣。
- 10.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。
- 11.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。
- 12.阮進蕙、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。
- 13.林家奴、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。
- 14.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。
- 15.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。
- 16.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。
- 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.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。國立台灣大學食品科技研究所碩士論文。台北。
- 20.張根源。2001。智慧型材料之生物醫學應用。化工。48(2)。53-61。
- 21.彭仁信。2002。醋酸酐乙醯化反應之幾丁聚醣特性及幾丁聚醣酵素水解產物對微生物生長之影響。國立台灣海洋大學食品科學研究所碩士論文。基隆。
- 22.陽禎明、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。
- 23.蔡國珍、李晏雯。2000。利用蝦類多醣強化鯖魚漿煉製品品質。88年度

水產加工研究成果彙編, 食品工業發展研究所。新竹。98-121。24. 關山璋。1998。淺談骨科生醫材料之展望。工業材料。4(136)。81-84。

25. 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。26. Aiba, S. 1992. Studies on chitosan: 4. Lysozymic hydrolysis of partially N-acetylated chitosans. *International Journal of Biological Macromolecules*. 14(4):225-8

27. 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

28. 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*. 28:189-29

29. Austin, P.R., Brine, C.J., Castle, J.E., and Zikakis, J.P. 1981. Chitin: New facets of research. *Science*, 212:749-753

30. 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.* 238:11-31

31. Bough, W.A. 1975. Coagulation with chitosan-an aid to recovery of by-products from egg breaking wastes. *Poultry Sci.*, 54(6):1904-1912

32. Celis, R.A., Gatell, J.M., Alnela, M. 1988. Nosocomial pneumonia: a multivariate analysis of risk and prognosis. *Chest* 93: 318-325

33. 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 chimp chitin packed in small columns. *Environment International*. 22:463-468

34. Costerton, J.W. 1980. *Pseudomonas aeruginosa* in nature and disease. In: *Sabath LD, ed. Pseudomonas aeruginosa the organism, disease it causes, and their treatment*, 15-24

35. Curotto, E., and Aros, F. 1993. Quantitative determination of chitosan and the percentage of free amine groups. *Anal. Biochem.* 211:240-36

36. Davis, D.H., and Hayes, E.R. 1988. Determination of the degree of acetylation of chitin and chitosan. *Method in Enzymology*. 161: 442-37

37. Domard, A. 1987. Determination of N-acetyl content in chitosan samples by C.D. measurements. *Int. J. Biol. Macromol.* 9:333-38

38. Fang, S. W., Li, C. F. and Shih, D. Y. C. 1994. Antifungal activity of chitosan and its preservative effect on low-sugar candied kumquat. *J. Food Prot.* 56:136-140

39. Fick, J.R.B. 1993. *Pseudomonas aeruginosa* the opportunist: Pathogenesis and Disease. CRC Press, Inc., 1993

40. Finland, M. 1972. Changing patterns of susceptibility of common bacteriological pathogens to antimicrobial agents. *Ann. Inter. Med.* 76:1009-1036

41. Finlay, B.B. 1999. Bacterial disease in diverse hosts. *Cell*. 96:315-8

42. Gilardi, G.L. 1991. *Pseudomonas* and related genera. In: *Balows, A., Hausler, W.J., Herrmann, K.L., Isenberg, H.D., Shadomy, H.J., eds. Manual of clinical microbiology*, 5th ed. American Society for Microbiology, Washington, DC, 429-441

43. Hadwiger, L.A., Kendra, B., Fristensky, W., and Wagoner, W. 1986. Chitosan both activates genes in 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. Hirano, S. and Nagao, N. 1989. Effects of chitosan, pectic acid, lysozyme and chitinase on the growth of several phytopathogens. *Agric. Biol. Chem.* 53:3065-3066

46. 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*. 190:67-47

47. Jha, I.N., Iyengar, 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. Knorr, D. 1982. Functional properties of chitin and chitosan. *J. Food Sci.*, 47(2):593-595

50. Knorr, D. 1983. Dye binding properties of chitin and chitosan. *J. Food Sci.*, 48(1):36-37, 41-51

51. Knorr, D. 1984. Use of chitinous polymers in food-A challenge for food research and development. *Food Technol.*, January:85-97

52. Knorr, D., Wampler, T.P., and Teutonico, R.A. 1985. Formation of pyrazines by chitin pyrolysis. *J. Food Sci.*, 50(6):1762-1763

53. Kurita, K. 1998. *Chemistry and Application of chitin and chitosan*, *Polymer Degradation and Stability*, 59, 117-120

54. Lee, J.Y., Nam, S.H., Im, S.Y., Lee, Y.M., Seol, Y.J., Chung, C.P., and Lee, S.J. 2002. Enhanced bone formation by controlled growth factor delivery from chitosan-based biomaterials. *Journal of Controlled Release*, 78, 187-197

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. Lucy, M. Mutharia, Thalia I. Nicas, and Robert E.W. Hancock. 1982. Outer membrane proteins of *Pseudomonas aeruginosa* serotype strains. *J. Infect. Dis.* 146(6): 770-779

58. Majeti, N.V., and Kumar, R. 2000. The review of chitin and chitosan applications. *Reactive & Function Polymers*, 46, 1-27

59. Mi, F.L., Shyu, S.S., Wu, Y.B., Lee, S.T., Shyong, J.Y., and Huang, R.N. 2001. Fabrication and characterization of a sponge-like asymmetric chitosan membrane as a wound dressing. *Biomaterial*, 22, 165-173

60. 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

61. Mima, S., Miya, M., Iwamoto, R., and Yoshikawa, S. 1983. Highly deacetylated chitosan and its properties. *J. Applied Polymer Sci.* 28:1909-62

62. Mitani, T., Moriyama, A. and Ishii, H. 1992. Heavy metal uptake by swollen chitosan beads. *Bioscience Biotechnology Biochemistry*. 56:985-986

63. Mitsukawa, H., Shimizu, O., and Nishi, H. 1971. Colorimetric determination of α -amino nitrogen in urine and plasma with ninhydrin reaction. *Agr. Biol. Chem.* 35:272-64

64. Moore, G. K., and Roberts, G.A.F. 1980. Determination of the degree of N-acetylation of chitosan. *Int. J. Biol. Macromol.* 2:115-65

65. Muzzarelli, R.A., Mattioli-Belmonte, M., and Tietz, C. 1994. Stimulatory effect on bone formation exerted by modified chitosan. *Biomaterials*. (15):1075-1081

66. Muzzarelli, R.A.A., and Rocchetti, R. 1985. Determination of the degree of acetylation of chitosans by first derivative ultraviolet spectrophotometry. *Carbohydr. Polymers*. 5: 461-67

67. Nanjo, F., Katsumi, R., and Sakai, K. 1991. Enzymatic method for determination of the degree of deacetylation of chitosan. *Anal. Biochem.* 193:164-68

68. 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.* 238:1-69

69. Nishi, N., Noguchi, J., Tocura, S., and Shiota, H. 1979. Studies on chitin acetylation of chitin. *Polymer Journal*, 11(1):27-32

70. Nishimura, K., Ishihara, C.,

and Ukei, S. 1986. Stimulation of cytokine production in mice using deacetylated chitin. *Vaccine*. 5:136-140 71. Nishimura, K., Nishimura, S., Seo, H., Nishi, N., Tokura, S., and Azuma, I. 1987. Effect of multipoporous microspheres derived from chitin on the activation of mouse peritoneal macrophages. *Vaccine*. 5:136-140 72. Olsen, B., Weinstein, R.A., Nathan, C., and Cabins, S.A. 1984. Epidemiology of endemic *Pseudomonas aeruginosa*: why infection control efforts have failed. *J. Infect. Dis.* 150: 808-817 73. Park, Y.J., Lee, Y.M., Park, S.N., Sheen, S.Y., Chung, C.P., and Lee, S.J., 2000. Platelet derived growth factor releasing chitosan sponge for periodontal bone regeneration, *Biomaterials*, 21, 153-159 74. 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 75. Roberts, G.A.F., and Domszy, J.G. 1982. Determination of the viscometric constants for chitosan. *Int. J. Biol. Macromol.* 4:374 76. 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 77. 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 78. Sannan, T., Kurita, K., Ogura, K., and Iwakura, Y. 1978. Studies on chitin 7: I. R. Spectroscopic determination of degree of deacetylation. *Polymer*. 19:458 79. Schaberg D.R., Culver, D.H., Gaynes, R.P. 1991. Major trends in the microbial etiology of nosocomial infection. *Am. J. Med.* 91(Suppl): S72-S75 80. Seo, H., Mitsuhashi, K. and Tanibe, H. 1992. Antibacterial and antifungal fiber blended by chitosan. In *Advances in Chitin and Chitosan*, 34-40. Brine, C. J., Sandford, P. A., and Zikakis, J. P. (eds.), Elsevier Applied Science, N. Y. 12 81. 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 82. 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 83. 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 84. Sudarshan, N.R., Hoover, D.G., and Knorr, D. 1992. Antibacterial action of chitosan. *Food Biotechnology*. 6:257-272 85. 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 86. 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. Elsevier Applied Science, N. Y., 96-105 87. Tan, S.C., Khor, E., Tan, T.K., and Wong, S.M. 1998. The degree of deacetylation of chitosan: advocating the first derivative UV-spectrophotometry method of determination. *Talanta*. 45:713 88. Toei, K., and Kohara, T. 1976. A conductometric method for colloid titrations. *Analytica Chimica Acta*. 83:59 89. Tsai, G. J. and Su, W.H. 1999. Antibacterial activity of shimp chitosan against *Escherichia coli*. *J. Food Prot.* 62:239-243. 90. Tsai, G. J., Su, W. H. and Chen S. C. 2002. Antimicrobial activity of shrimp chitin and chitosan from different treatments and application to fish preservation. *Fisheries Sci.* 68:170-177. 91. Wang, G. H. 1992. Inhibition and inactivation of five species of food borne pathogens by chitosan. *J. Food Prot.* 55: 916-919. 92. 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, *Biomaterials*, 24, 3231-3220 93. 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