

Production of N-acetylchitooligosaccharides from Chitin by Indigenous Strains

連德昇、涂瑞澤；余世宗

E-mail: 9125207@mail.dyu.edu.tw

ABSTRACT

Abstract Recently, studies on physiological functions of N-acetylchito- oligosaccharides have brought attention from researchers in the fields of food science and medicine. N-acetylchitooligosaccharides are important for their general immune system enhancement, including infection prevention, anti-tumor, anti-fungi and anti- bacteria capabilities, etc. The main purposes of this study are (1) to screen some strains producing acetylchitooligosaccharides with higher degrees of polymerization from local soil, and (2) to produce N-acetyl- chitooligosaccharides in a large quantity by a fermenter. Meanwhile, an optimal operating condition for bacterial growth and the production of N-acetylchitooligosaccharides was explored. Eight indigenous strains named sequentially from “ Too 1 ” to “ Too 8 ” were isolated from soil and ocean sand to produce N-acetylchitooligosaccharides. Batches of each of these eight strains were grown in flasks to compare their chitinase activities. Strain “ Too 3 ” has been shown to yield the most active chitinase. While N-acetylchito-hexaose (GlcNAC)6 can be obtained from the hydrolysis of chitin with chitinases produced by “ Too 7 ” and “ Too 8 ” , only (GlcNAC)2 was found in the hydrolysates of chitin from strain “ Too 2 ” . “ Too 7 ” would reach an exponential phase in 8 h and a stationary phase within around 16 h if cultivated in a colloidal chitin broth in a rotary shaker. The pH level was raised during the microbial growth period, possibly caused by the ammonia produced by the N-acetylchitooligosaccharide metabolism. Furthermore, “ Too 7 ” was cultivated in CB (chitin broth), CCB (colloidal chitin broth), and GB (glucose broth) separately to compare their chitinase activities. The chitinase produced in CB has been shown to be the most active (192.56 U/g), that in CCB (184.21 U/g) being the second, and GB (80 U/g) the least. The experimental results showed that “ Too 7 ” needed chitin to induce the production of chitinase. “ Too 7 ” could produce more chitinase, when it cultivated in basic medium with extra inorganic nitrogen. Though “ Too 7 ” could directly use ammonia, only inorganic nitrogen source seems not sufficient for good growth of this strain. “ Too 7 ” was cultivated in a fermenter using either CB or LPB as a medium. The experimental results showed that in either CB or LPB, the hydrolysates of chitin consisted mainly of N-acetylglucosamine through the exponential phase, but N-acetylchitooligosaccharides with higher degrees were also detected. Perhaps the N-acetylchitooligosaccharides are the intermediate products of hydrolysis in the chitin metabolism. In addition, the amount of N-acetylchito-biose increases as the increase of the incubation time in both CB and LPB. N-acetylchitobiose was produced more in LPB (0.27 g/L) and only 0.16 g/L in CB. It is possible that N-acetylchito oligosaccharides with higher degrees can be produced by less biomass and less chitinase, and consequently, the hydrolysis of chitin decreases. Therefore, if cultivation conditions such as incubation time, hydrolysis rate, biomass and substrate concentrations are properly controlled, N-acetylchitooligosaccharides with higher degrees would be obtained.

Keywords: chitin, chitinase, N-acetylchitooligosaccharides

Keywords : chitin ; chitinase ; N-acetylchitooligosaccharides

Table of Contents

目 錄 封面內頁 頁次 簽名頁 授權書.....	iii 中文摘要.....
.....iv 英文摘要.....	vi 誌謝.....
.....ix 目錄.....	x 圖目錄.....
.....xv 表目錄.....	xviii 第一章.....
緒論.....	1 第二章 文獻回顧.....
幾丁質.....	4 2.1.1 簡介.....
.....4 2.1.2 理化性質.....	9 2.1.3 幾丁質的酵素性分解.....
乙醯幾丁寡醣.....	9 2.2 N-乙醯幾丁寡醣.....
幾丁寡醣之製備.....	10 2.2.1 簡介.....
.....11 2.2.3 N-乙醯幾丁寡醣的分離與純化.....	13 2.3 幾丁質酵素.....
.....14 2.4 幾丁質酵素之天然分布.....	19 2.4.1 動物之幾丁質酵素.....
.....19 2.4.2 植物之幾丁質酵素.....	19 2.4.3 微生物之幾丁質酵素.....
.....20 2.5 幾丁質酵素活性測定.....	21 2.5.1 傳統方法.....
.....21 2.5.2 快速檢測法.....	21 2.6 幾丁質酵素之應用.....
.....21 2.6.1 菌體生產之應用.....	21 2.6.2 微生物生理及生化上之應用.....
	22

2.6.3 N-乙醯幾丁寡醣之製備.....	22	2.7 幾丁質酵素之生產現況.....	22	2.8 發
酵槽簡介.....	25	第三章 材料與方法.....	30	3.1
實驗材料.....	30	3.2 器材.....	31	3.3 實
驗方法.....	33	3.3.1 培養基配製.....	33	3.3.2 製備膠
態幾丁質.....	35	3.3.3 採樣地點.....	35	3.3.4 篩選菌株.....
	35	3.3.5 菌株鑑定.....	36	3.3.6 酵素活性測定.....
	36	3.3.7 還原糖含量.....	37	3.3.8 SDS-聚丙烯醯胺膠體電泳分
分析 (SDS-PAGE)	37	3.3.8.1 定義.....	37	3.3.8.2 操作步驟.....
37 3.3.9 水解產物前處理.....	40	3.3.10 高效能液相層析儀分析.....	40	
3.3.11 酵素之特性探討.....	40	3.3.11.1 粗酵素液之最適基質濃度.....	40	3.3.11.2 粗酵
酵素液之最適反應時間.....	41	3.3.11.3 粗酵素液之最適溫度.....	41	3.3.11.4 粗酵素液之最適pH值
41 3.3.12 發酵槽之發酵試驗.....	41	41 3.3.12.1 發酵培養條件.....	41	
41 3.3.12.2 操作步驟.....	42	第四章 結果與討論.....	42	
43 4.1 前言.....	43	4.2 幾丁質分解菌之篩選、鑑定.....	43	
43 4.2.1 土壤中幾丁質分解菌之篩選.....	43	4.2.2 菌株鑑定.....	46	
4.2.3 各菌株之幾丁質分解酵素活性比較.....	46	4.2.4 各菌株之N-乙醯幾丁寡糖分析.....	55	4.3 Too 7
菌株之特性分析.....	55	4.3.1 Too 7菌株之生長趨勢.....	55	4.3.2 粗酵素液
分解幾丁質之最適條件探討.....	57	57 4.3.2.1 最適反應時間.....	57	分解幾丁質之最適條件探討.....
57 4.3.2.3 最適溫度.....	57	57 4.3.2.2 最適基質濃度.....	57	
61 4.3.3 Too 7生長過程幾丁質分解酵素之活性變化.....	65	57 4.3.2.3 最適溫度.....	57	
還原糖及幾丁質...分解酵素之影響.....	70	61 4.3.2.4 最適pH值.....	61	
70 4.3.4.1 菌體量之變化.....	70	61 4.3.3 Too 7生長過程幾丁質分解酵素之活性變化.....	65	
70 4.3.4.2 還原糖之變化.....	70	65 4.3.4 碳源對Too 7菌體生長、還原糖及幾丁質...分解酵素之影響.....	65	
70 4.3.4.3 幾丁質分解酵素之活性變化.....	72	70 4.3.4.1 菌體量之變化.....	70	
73 4.3.5 氮源對Too 7菌體生長、還原醣及幾丁質...分解酵素之影響.....	76	70 4.3.4.2 還原糖之變化.....	70	
76 4.3.5.2 還原糖之變化.....	79	72 4.3.4.3 幾丁質分解酵素之活性變化.....	72	
79 4.3.5.4 pH值之變化.....	82	72 4.3.4.4 pH值之變化.....	72	
82 4.4 發酵槽之發酵試驗.....	85	76 4.3.5.1 菌體量之變化.....	76	
4.4.2 還原糖之變化.....	87	76 4.3.5.2 幾丁質分解酵素之活性變化.....	79	
9.4.4.4 幾丁質含量之變化.....	90	79 4.3.5.3 幾丁質分解酵素之活性變化.....	79	
93 4.4.5 CB與限磷培養基之N-乙醯幾丁寡醣分析.....	93	82 4.3.6 Too 7 與 Too 8之N-乙醯幾丁寡糖分析.....	82	
98 5.1 結論.....	98	82 4.4.1 菌體量之變化.....	85	
98 5.2 未來展望....	98	87 4.4.3 幾丁質分解酵素之活性變化.....	90	
99 參考文獻.....	100	90 4.4.4 幾	90	
109 附錄一 還原 醣標準曲線.....	109	93 4.4.5 CB與限磷培養基之N-乙醯幾丁寡醣分析.....	93	
N-乙醯幾丁二醣標準曲線.....	111	93 第五章 結論與未 來展望.....	93	
112 附 錄五 N-乙醯幾丁四醣標準曲線.....	113	98 5.1 結論.....	98	
114 附錄七 N-乙醯幾丁六醣標準曲線.....	115	98 5.2 未來展望....	98	
之應用型式.....	7	100 參考文獻.....	100	
圖2.2 幾丁質、幾丁聚醣、纖維素之化學構造.....	8	110 附錄三 N-乙醯幾丁三醣標準曲線.....	110	
17 圖2.4 幾丁質的水解物.....	18	111 附錄四 N-乙醯幾丁五醣標準曲線.....	112	
27 圖2.6 氣舉式發酵槽.....	28	113 附錄六 N-乙醯幾丁六醣標準曲線.....	112	
丁質產生透明環之形態.....	44	115 圖 目 錄 圖2.1 幾丁質、幾丁聚醣之製備及其在食品 之應用型式.....	115	
44 圖4.2 菌株純化之結果.....	44	圖2.2 幾丁質酵素之作用機制.....	8	
還原糖對時間曲線圖.....	48	圖2.3 幾丁質酵素之作用機制.....	8	
48 圖4.4 Too 2之酵素活性與還原糖對時間曲線圖.....	48	17 圖2.4 幾丁質的水解物.....	18	
與還原糖對時間曲線圖.....	49	27 圖2.6 氣舉式發酵槽.....	28	
49 圖4.6 Too 4之酵素活性與還原糖對時間曲線圖.....	49	27 圖4.1 欲篩選的菌株於膠態幾	28	
性與還原糖對時間曲線圖.....	50	44 圖4.3 Too 1之酵素活性與 還原糖對時間曲線圖.....	44	
50 圖4.8 Too 6之酵素活性與還原糖對時間曲線圖.....	50	44 圖4.5 Too 3之酵素活性 與還原糖對時間曲線圖.....	48	
50 圖4.9 Too 7之酵素 活性與還原糖對時間曲線圖.....	50	44 圖4.7 Too 5之酵素活性 與還原糖對時間曲線圖.....	49	
51 圖4.10 Too 8之酵素活性與還原糖對時間曲線圖.....	51	44 圖4.11 各菌株之 酵素活性對時間關係圖.....	51	
51 圖4.12 各菌株以CCB培養48 h 後於SDS-PAGE中之	53	53 圖4.12 各菌株以CCB培養48 h 後於SDS-PAGE中之	53	
特性.....	54	53 圖4.12 各菌株以CCB培養48 h 後於SDS-PAGE中之	53	
54 圖4.13 Too 7菌株於CCB中培養之生長曲線.....	58	53 圖4.12 各菌株以CCB培養48 h 後於SDS-PAGE中之	53	
58 圖4.14 Too 7菌株 於CCB中培養之生長曲線.....	58	53 圖4.12 各菌株以CCB培養48 h 後於SDS-PAGE中之	53	
59 圖4.15 Too 7之酵素所產生還原糖量對時間之關係圖.....	60	53 圖4.12 各菌株以CCB培養48 h 後於SDS-PAGE中之	53	
60 圖4.16 Too 7之 酵素所產生還原糖量對基質濃度之關係圖.....	60	53 圖4.12 各菌株以CCB培養48 h 後於SDS-PAGE中之	53	
62 圖4.17 Too 7之酵素活性對溫度之關係圖.....	63	53 圖4.12 各菌株以CCB培養48 h 後於SDS-PAGE中之	53	
64 圖4.19 以CB培養基培養Too 7菌株，培養液中幾丁質	64	53 圖4.12 各菌株以CCB培養48 h 後於SDS-PAGE中之	53	
圖4.20 以CCB培養基培養Too 7菌株培養液中幾丁質	66	53 圖4.12 各菌株以CCB培養48 h 後於SDS-PAGE中之	53	
活性與還原糖之變化.....	66	53 圖4.12 各菌株以CCB培養48 h 後於SDS-PAGE中之	53	
67 圖4.21 Too 7菌株於CB中之幾丁質分解酵素活性變化.....	68	53 圖4.12 各菌株以CCB培養48 h 後於SDS-PAGE中之	53	
68 圖4.22 Too 7菌株於CCB 中之幾丁質分解酵素活性變化.....	68	53 圖4.12 各菌株以CCB培養48 h 後於SDS-PAGE中之	53	
69 圖4.23 Too 7菌株培養於CCB與CB中之菌體量變化情形.....	71	53 圖4.12 各菌株以CCB培養48 h 後於SDS-PAGE中之	53	
71 圖4.24 Too 7菌株培養 於CCB與CB中之還原糖與酵素產量	72	53 圖4.12 各菌株以CCB培養48 h 後於SDS-PAGE中之	53	
72 圖4.25 Too 7菌株培養 於CB中之幾丁質酵素產量變化情形.....	75	53 圖4.12 各菌株以CCB培養48 h 後於SDS-PAGE中之	53	
75 圖4.27 Too 7菌株培 養於CCB與CB中之pH變化情形.....	75	53 圖4.12 各菌株以CCB培養48 h 後於SDS-PAGE中之	53	
77 圖4.28 以不同氮源組合培養Too 7菌株之菌體量	77	53 圖4.12 各菌株以CCB培養48 h 後於SDS-PAGE中之	53	
圖4.27 變化情形.....	77	53 圖4.12 各菌株以CCB培養48 h 後於SDS-PAGE中之	53	

.....78 圖4.29 以不同氮源組合培養Too 7菌株之還原糖	圖4.27 變化情形.....
.....80 圖4.30 以不同氮源組合培養Too 7菌株之幾丁質	圖4.31 分解酵素產量變化情形.....
....81 圖4.31 以不同氮源組合培養Too 7菌株之pH	圖4.27 變化情形.....83 圖4.32
Too 7菌株培養於CB中之水解產物.....84 圖4.33 Too 8菌株培養於CB中之水解產物.....86 圖4.34	
以發酵槽分別培養Too7菌株於CB與限磷 圖4.35 培養基中之菌體量變化情形.....88 圖4.35 以發酵槽分	
別培養Too7菌株於CB與限磷 圖4.35 培養基中之還原糖變化情形.....89 圖4.36 以發酵槽分別培養Too7	
菌株於CB與限磷 圖4.35 培養基中之幾丁質分解酵素產量變化情形.....91 圖4.37 以發酵槽分別培養Too7菌株於CB與	
限磷 圖4.35 培養基中單位菌體之酵素產量變化情形.....92 圖4.38 以發酵槽分別培養Too7菌株於CB與限磷 圖4.35	
培養基中之幾丁質變化情形.....94 圖4.39 以發酵槽分別培養Too7菌株於CB中之水解 圖4.35 產物變化	
情形.....95 圖4.40 以發酵槽分別培養Too7菌株於限磷培養基中之 水解產物變化情形...	
.....96 表 目 錄 表2.1 幾丁質、幾丁聚醣及其衍生物在食品工業之應用.....6 表2.2 不同菌株	
所得幾丁質酵素產量之比較.....23 表4.1 採集樣品之時間、地點及代號.....45 表4.2 篩得	
菌株之簡單鑑定試驗.....47 表4.3 各菌株之幾丁質分解酵素水解幾丁質之產物.....56	

REFERENCES

參考文獻 王三郎，水產資源利用學。高立圖書出版社，台北(1996)。李建武、蕭能庚、余瑞元、陳麗蓉、陳雅蕙、陳來同、袁明秀，生物化學實驗原理和方法。藝軒圖書出版社，台北(1999)。阮進惠、林翰良、羅淑珍，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(6):596-611 (1997)。邱少華，利用綠膿桿菌K-187發酵蝦蟹殼廢棄物生產幾丁質酶之應用及量產條件之研究。大葉大學食品工程研究所碩士文，彰化(1997)。呂明洲，*Pseudomonas aeruginosa* K-181 所生產幾丁質酵素之探討，大葉大學食品工程研究所碩士論文，彰化(1994)。林欣榜，幾丁類物質在食品加工上之應用，食品工業，26-37 (1999)。吳豐智、曾如玲，神奇的物質-幾丁質和幾丁聚醣。化工技術，5(7):196-201 (1997)。洪啟章，*Bacillus cereus* NTU-FC-4 幾丁質酵素之研究。台灣大學農業化學研究所碩士論文，台北(1994)。袁國芳，幾丁與幾丁聚醣在食品工業上之應用。食品工業，19-25 (1999)。梁舜欣，N-乙醯幾丁寡醣製備。台灣大學農業化學研究所碩士論文，台北(1990)。陳惠婷，以發酵槽高密度培養*Pencillium chrysogenum*生產penicillin V之研究。大葉大學食品工程研究所碩士論文，彰化(2000)。康建智，細菌幾丁質酵素基因的誘導表現及生產N-乙醯幾丁寡醣最適化條件的研究。國立台灣海洋大學食品科學系碩士論文，基隆(2001)。黃安德，利用部分純化之*Amycolatopsis orientalis*細胞外N-乙醯葡萄糖胺酵素製備N-乙醯幾丁寡醣。國立台灣海洋大學水產食品科學系碩士學位論文，基隆(1998)。黃昭仁，微生物生產幾丁質酵素之研究，大葉大學食品工程研究所碩士論文，彰化(1998)。歐宜書，*Pseudomonas aeruginosa* K-187生產蛋白質分解酵素之研究。大葉大學食品工程研究所碩士論文，彰化(1998)。陳幸臣、許嘉珍，以微生物分解蝦殼製取幾丁質與其部分去乙醯化。中國農業化學會誌，35(3):342-353 (1997)。陳幸臣，幾丁質酵素生產與應用。食品生物技術研討會專輯，34-41 (2000)。陳坤上、黃佩芬、陳聰松、陳幸臣，幾丁寡醣製備條件之探討。食品科學，23(6):874-883 (1996)。陳美惠、莊淑惠、吳志津，幾丁聚醣的物化特性，食品工業，1-7 (1999)。蘇南維、李敏雄，*Listonella damsela* NTU-FC-6 幾丁質酵素之生產與基本性質之探討。中國農業化學會誌，36(1):65-76 (1998)。蘇南維，*Listonella damsela* NTU-FC-6 幾丁質酵素之研究。台灣大學農業化學研究所碩士論文，台北(1995)。蘇遠志、黃世佑，微生物化學工程學。華香園出版社，台北(1999)。劉瓊淑，幾丁質、幾丁聚醣及其相關酵素之特性與用。食品工業，26(1) : 26-36 (1994)。A/Banat, B.M.A., Y. Kameyama, T. Yoshioka, D. Koga, Purification and characterization of a 54kDa chitinase from *Bombyx mori*, Insect Biochem. Molec. Biol., 29:537-547 (1999). Aiba S. I., Preparation of N-acetylchitooligosaccharides by hydrolysis of chitosan with chitinase followed by N-acetylation, Carbohydr. Res., 265:323-328 (1994). Amy, L. S., S. M. N. Chadha, J. A. Moore and D. L. Kirchman, Chitin degradation proteins produced by the marine bacterium *Vibrio harveyi* growing on different from of chitin, Appl. Environ. Microbiol., 63:408-413 (1997). Angel, Z. H., A. B. Angel, , Chitinolytic activity from *Neurospora crassa*, J. Gen. Microbiol., 129:3319-3321 (1983). Araki, Y., E. Ito, A pathway of chitosan formation in *Mucor rouxii*:enzymatic deacetylation of chitin, Biochem. Biophys. Res. Commun., 56:669-674 (1974). Bassler, B. L., C. Yu, A. M. Lee, and S. Roseman, Chitin utilization by marine bacteria, degradation and catabolism of chitin oligosaccharides by *Vibrio funissii*, J. Bio. Chem., 266:24276-24281 (1991). Bhushan, B., Production and characterization of a thermostable chitinase from a new alkalophilic *Bacillus* sp. BG-11, J. Appl. Microbiol., 88:800-808 (2000). Bokmaa; E., T. Barendsb, A. C. Terwisscha van Scheltingab, B. W. Dijkstrab, and J. J. Beintemaa, Enzyme kinetics of hevamine, a chitinase from the rubber tree *Hevea brasiliensis*, FEBS Lett., 478 : 119-122 (2000) Byrnea, N. D., M. Duxburya and N. Sharpeb, The determination of chitinase activity of grapes: an introductory enzyme assay, Biochem. Molec. Biol. Educ., 29:144—146 (2001). Carroad, D. A. and R. A. Tom, Bioconversion of shellfish chitin waste:process conception and selection of microorganism, J. Food Sci, 43:1158-1164 (1978). Chang, K. B., J. Lee and W. R. Fu, HPLC analysis of N-acetyl- chito-oligosaccharides during the acid hydrolysis of chitin, J. Food Drug Anal., 8:75-83 (2000). Felle, H. H. E. Kondorosi, A. Kondorosi, M. Schultze, How alfalfa root hairs discriminate between Nod factors and oligochitin elicitors, Plant Physiol., 124 : 1373-1380 (2000). Felse, P. A. and T. Panda, Production of microbial chitinase - A revisit, Bioproc. Eng., 23:127-134 (2000). Felse, P. A. and T. Panda, Submerged culture production of chitinase by *Trichoderma harzianum* in stirred, Biochem. Eng. J., 4:115—120 (2000). Hsu, S. C. and J. L. Lockwood, Powdered chitin agar as a selective medium for enumeration of actinomycetes in water and soil, Appl. Microbiol., 29:422-426 (1975). Huang, I. J., W. L. Cheng, C. M. Chiang and M. Y. Lue, Identification and purification of chitinases from bacterium TSC-Ch105, Rept. Taiwan Sugar Res. Inst., 166:67-79 (1999). Imoto, I. and K. Yagishita, Simple activity measurement of lysozyme, Agric. Biol. Chem., 72: 11154-11156

(1971). Kapat, A., T. Panda, pH and thermal stability studies of chitinase from *Trichoderma harzianum* : A thermodynamic consideration, Bioproc. Eng., 16:269-272 (1997). Knorr, D., Use of chitinous polymer in food, Food Technol., 1:85-89 (1984). Koga, D., N. Sueshige, K. Orikono, T. Utsumi, S. Tanaka, Y. Yamada and A. Ide, Efficiency of chitinolytic enzyme in the formation of *Trichoderma matsutake* protoplasts, Agric. Biol. Chem., 52:2091-2094 (1988). Koga, D., Y. Sasaki, Y. Uchiumi, N. Hiria, Y. Arakane and Y. Nagamatsu, Purification and characterization of *Bombyx mori* chitinase, Insect Biochem. Molec. Biol., 27:757-767 (1997). Koga, K., Y. Iwamoto, H. Sakamoto, K. Hatano, M. Sano and I. Kato, Purification and characterization of beta-N-acetylhexosaminidase from *Trichoderma harzianum*, Agric Biol Chem., 55:2817-2823 (1991). Kurita, K., Chemistry and application of chitin and chitosan, Polym. degradation stab., 59:117-120 (1998). Lee, H.S., D.S. Han, S. J. Choi, S. W. Choi, D. S. Kim, D. H. Bai and J. H. Yu, Purification and characterization, and primary structure of a chitinase from *Pseudomonas* sp. YHS-A2, Appl. Microbiol Biotechnol., 54:397-405 (2000). Lonhienne, T., E. Baise, G. Feller, V. Bouriotis and C. Gerday, Enzyme activity determination on macromolecular substrates by isothermal titration calorimetry : application to mesophilic and psychrophilic chitinases, Biochim. Biophys. Acta., 1545:349-356 (2001). Mabuchi, N., I. Hashizume and Y. Araki, Characterization of chitinase excreted by *Bacillus cereus* CH, Can. J. Microbiol., 46:370-375 (2000). Mitsutomi, M., T. Hata and T. Kuwahara, Purification and characteristics of Novel chitinases from *Streptomyces griseus* HUT 6037, J. Ferment. Bioeng., 80:153-158 (1995). Muraki, E., F. Yaku and H. Kojima, Preparation and crystallization of D-glucosamine oligosaccharides with dp 6-8, Carbohydr. Res., 239:227-237 (1993). Muzzarelli, R. A. A., Chitin, Appl. Environ. Microbiol. , 53 : 1718-1724 (1997). Nielsen , M. N., J. Sorensen, Chitinolytic activity of *Pseudomonas* *E* uorescens isolates from barley and sugar beet rhizosphere, FEMS Microbiol. Ecol., 30:217-227 (1999). Ohishi K., M. Yamagishi, T. Ohta, M. Suzuki, H. Izumida, H. Sano, M. Nishuima and T. Miwa, Purification and characteristics of two chitinases from *Vibrio alginolyticus* H-8, J. Ferment. Bioeng., 82:598-600 (1996). Sakai, K., A. Yokota, H. Kurokawa, M. Wakayama and M. Moriguchi, Purification and Characterization of Three Thermostable Endochitinases of a Noble *Bacillus* Strain, MH-1,Isolated from Chitin-Containing Compost, Appl. Environ. Microbiol., 64:3397—3402 (1998). Sakai K., M. Narihara, Y. Kasama, M. Wakayama and M. Moriguchi, Purification and characterization of thermostable beta-N-acetyl- hexosaminidase of *Bacillus stearothermophilus* CH-4 isolated from chitin-containing compost, Appl. Environ. Microbiol., 60:2911-2915 (1994). Sakurada M., D. P. Morgavi, K. Komatani, Y. Tomita and R. Onodera, Purification and characteristics of an autolytic chitinase of *Piromyces communis* OTS1 from culture medium, Cur. Microbiol., 35:48-51 (1997). Shahidi, F., Vidana Arachchi, J.K., and Jeon, Y.J., Food applications of chitin and chitosan, Trends Food Sci. Technol., 10:37-51 (1999). Shimoda, K., K. Nakajima, Y. Hiratsuka, S. I. Nishimura and K. Kurita, Efficient preparation of -(1 6)-(GlcNAc)2 by enzymatic conversion of chitin and chito-oligosaccharides, Carbohydr. Polym., 29:149-154 (1996). Suginta, W., P.A.W. Robertson, B. Austin, S.C. Fry and L.A. Fothergill-Gilmore, Chitinases from *Vibrio*:activity screenig and purification of chA from *Vibrio carchariae*, J. Appl. Microbiol., 89:76-84 (2000). Suresh, P.V., M. Chandrasekaran, Impact of process parameters on chitinase production by an alkalophilic marine *Beauveria bassiana* in solid state fermentation, Proc. Biochem., 34:257—267 (1999). Svitil, A. L., S. M. N. Chadhain, J. A. Moore and D. L. Kirchman, Chitin degradation proteins produced by the marine bacterium *Vibrio harveyi* growing on different forms of chitin, Appl. Environ. Microbiol. 63:408-413 (1997). Tang, S. C., M. C. Chang and C. Y. Cheng, Use of colloid chitin and diatomaceous earth in continuous cake-filtration fermentation to produce creatinase, Proc. Biochem., 33:519-526 (1998). Thamthiankul, S., S. Suan-Ngay, S. Tantimavanich and W. Panbangred, Chitinase from *Bacillus thuringiensis* subsp. Pakistani, Appl. Microbiol. Biotechnol., 56:395—401 (2001) Tokuyasu, K., H.Ono, Mayumi O.K., Kiyoshi H. and Yutaka M., Deacetylation of chitin oligosaccharides of dp 2-4 by chitin deacetylase from *Colletotrichum lindemuthianum*, Carbohydr. Rse., 303:353-358 (1997) Tsigos, I., N. Zydowicz, A. Martinou, A. Domard and V. Bouriotis, Mode of action of chitin deacetylase from *Mucor rouxii* on N-acetylchitooligosaccharides, Eur. J. Biochem., 261:698-705 (1999). Tsutomu, T., A. Kasumi, T. Yasuyaki and S. Venzo, Isolation and characterization of themostable chitinase from *Bacillus licheniformis*, Biochim. Biophys. Acta, 1078:404-411 (1991). Wang , S. L., J. R. Hwang, Microbial reclamation of shellfish wastes for the production of chitinases, Enzyme Microb. Technol., 28:376—382 (2001). Wang, S. Y., A. L. Moyne, G. Thottappilly, S. J. Wu, R. D. Locy and N. K. Singh, Purification and characterization of a *Bacillus cereus* exochitinase, Enzyme Microb. Technol., 28:492-498 (2001). Wiwat, C., P. Siwayaprahm and A. Bhumiratana, Purification and characterization of chitinase from *Bacillus circulans* No.4.1, Cur. Microbiol., 39:134-140 (1999). Yamamoto, Y., Y. Fukunaga, H. Aoyagi and H. Tanaka, Purification and characteristics of chitinase secreted by cultured *Wasabia japonica* cells, J. Ferment. Bioeng., 80:148-152 (1995). Usami, Y., Y. Okamoto, T. Takayama, Y. Shigemasa and S. Minami, Effect of N-acetyl-D-glucosamine and D-glucosamine oligomer on canine polymorphonuclear cells in in vitro, Carbohydr. Polym., 36:137-141 (1998).