Production and Gene Cloning of Chitinases

連德昇、?瑞澤 ; 余世宗

E-mail: 9607424@mail.dyu.edu.tw

ABSTRACT

In this study, Aeromonas sp. DYU-Too7 was stimulated to produce extra- cellular chitinases using various substrates, and the chitinases were then purified with chromatographic methods. Results indicated that the chitinases could be produced, when DYU-Too7 was cultivated in a medium containing either chitin or glucosamine (GlcN) as the sole carbon source. In the chitin medium, maximal chitinase production occurred when using a medium containing 2% chitin. Maximal glucosamine production occurred in the medium containing 0.1% GlcN. The content of this experimentally obtained 36 kDa chitinase (denoted as Chi36) was highest among the chitinases. The Chi36 chitinases produced using both media were chromatographically purified separately, and their properties were compared. There were no significant differences in the enzymatic properties of Chi36 produced with the chitin medium versus the glucosamine medium. For instance, both of the Chi36s had an optimal reacting pH of 5.0 and showed high thermal stability between 10 and 60°C and high pH stability in the range of pH 5.0 - 8.0. Using either Chi36 in the hydrolysis of colloidal chitin produces chitobiose, making the chitinase of Aeromonas sp. DYU-Too7 an exo-type enzyme. However, the Chi36 produced using a chitin versus glucosamine medium showed differing levels of activity in certain environments. For instance, after reacting the two Chi36s with 10 mM Hg2+ for 1 h, the Chi36 produced in a chitin medium retained 55% of its original activity, while the Chi36 produced in a GlcN medium retained 67% of its original activity. The feasibility study of protein collection and purification was performed through adsorption using modified nano-diamond as the absorber. The Chi36 purified in the previous study was used as the target protein to explore the optimal operating conditions. Results indicated the following optimal conditions: the ratio between the protein and modified nano-diamond was 75, the pH environment was 4.1, and the efficiency of protein adsorption was 86%. The enzymatic properties had no significant change after the chitinase was adsorbed. The efficiency of protein desorption from modified nano-diamond was almost 100% in a Tri-HCl buffer of pH 8.0 or 9.0, however, the desorption efficiency decreased to 76% in a buffer of pH 7.0. Next, modified nano-diamond was used to collect protein from a culture broth, replacing the often used ammonium sulfate precipitation procedure. Results showed that 87% of the crude protein was recovered after the adsorption step. The efficiency of protein desorption from modified nano-diamond was 88% in a Tri-HCl buffer of pH 8.0, and the properties of crude protein were not signify- cantly altered through adsorption of modified nano-diamond and desorption from nano-diamond. The gene encoding Chi36 produced by Aeromonas sp. DYU-Too7 was amplified using a polymerase chain reaction (PCR). Results indicated that the open reading frame of the gene, chi36, is 1,080 bp long encoding the protein of Chi36 with 360 amino acids, of which 27 amino acids compose the N-terminal signal peptide. In addition, the sequence 137FDGIDIDLE145 of Chi36 is homologous with the consensus catalytic sequence of family 18 of glycosyl hydrolases. Furthermore, the chi36 genes with and without the signal peptide were inserted into E. coli to produce recombinant proteins. Results showed that the recombinant protein produced from the gene, including a signal sequence to produce a signal peptide, showed chitinolytic activity, and produced chitobiose as the main hydrolysate in the hydrolysis of colloidal chitin. However, when the recombinant gene did not include the signal sequence, the recombinant protein produced thereafter did not show chitinase activity.

Keywords : chitin ; GlcN (glucosamine) ; chitobiose ; chitinase ; nano-diamond ; PCR (polymerase chain reaction)

Table of Contents

目錄 封面內頁 簽名頁 授權書iii 中文摘要iv 英文摘要vi 誌謝viii 目錄x 圖目錄xiii 表目錄xxiii 1. 緒論1 2. 文獻回顧3 2.1 幾丁質分解酵素3 2.1.1 幾丁質分解酵素之分佈3 2.1.2 幾丁質分解酵素之分類5 2.1.3 幾丁質分解酵素之應用8 2.2 幾丁質分解酵素之純化與特性分析11 2.2.1 幾丁質分解酵素之純化11 2.2.2 幾丁質分解酵素之特性分析12 2.3 工業鑽石14 2.3.1 鑽石之特性14 2.3.2 工業鑽石於生物醫學之應用23 2.3.3 工業鑽石於蛋白質吸附之應用24 2.4 幾丁質分解酵素之基因選殖25 2.4.1 幾丁質分解酵素之基因選殖技術25 2.4.2 基因重組酵素之特性與應用31 3. 不同基質對Aeromonas sp. DYU-Too7生合成幾丁質分解酵素之影響37 3.1 前言38 3.2 材料與方法39 3.3 結果與討論48 3.4 結論74 4. Aeromonas sp. DYU-Too7生合成幾丁質分解酵素之純化與特性分析75 4.1 前言76 4.2 材料與方法77 4.3 結果與討論83 4.4 結論126 5. 奈米化之工業鑽石於幾丁質分解酵素之應用127 5.1 前言128 5.2 材料與方法129 5.3 結果與討論133 5.4 結論155 6.Aeromonas sp. DYU-Too7之幾丁質分解酵素的基因選殖156 6.1 前言157 6.2 材料與方法158 6.3 結果與討論169 6.4 結論201 7. 總結論202 參考文獻205 附錄221 圖目錄 圖2.1 幾丁質分解酵素之作用機制7 圖3.1 Aeromonas sp. DYU-Too7於不同基質中生長之pH變化49 圖3.2 Aeromonas sp. DYU-Too7於

不同基質中生長之生質量變化50 圖3.3 Aeromonas sp. DYU-Too7於不同基質培養16 h之胞外蛋白質的SDS-PAGE分析。Con：控制組、C：幾丁質、G：葡萄糖、F：果糖、NAc：N-乙醯葡萄糖胺、N：葡萄糖胺、S：蔗糖、L：乳糖51 圖3.4 Aeromonas sp. DYU-Too7於不同基質培養40h之胞外蛋白質SDS-PAGE分析。Con：控制組、C：幾丁質、G：葡萄糖、F：果糖、NAc：N-乙醯葡萄糖胺、N：葡萄糖胺、S：蔗糖、L：乳糖52 圖3.5 Aeromonas sp. DYU-Too7於不同基質培養16 h之胞外蛋白質的活性電泳染色分析。Con：控制組、C：幾丁質、G：葡萄糖、F：果糖、NAc：N-乙醯葡萄糖胺、N：葡萄糖胺、S：蔗糖、L：乳糖54 圖3.6 Aeromonas sp. DYU-Too7於不同基質培養40 h之胞外蛋白質的活性電泳染色分析。Con：控制組、C：幾丁質、G：葡萄糖、F：果糖、NAc：N-乙醯葡萄糖胺、N：葡萄糖胺、S：蔗糖、L：乳糖55 圖3.7 幾丁質濃度對Aeromonas sp. DYU-Too7之發酵液pH的響56 圖3.8 幾丁質濃度對Aeromonas sp. DYU-Too7之生質量的影響58 圖3.9 幾丁質濃度對Aeromonas sp. DYU-Too7之發酵液中還原醣的影響59 圖3.10 幾丁質濃度對Aeromonas sp. DYU-Too7之幾丁質分解酵素活性的影響(以還原醣法測定)60 圖3.11 幾丁質濃度對Aeromonas sp. DYU-Too7之蛋白質量的影響62 圖3.12 葡萄糖胺濃度對Aeromonas sp. DYU-Too7之發酵液pH值的影響64 圖3.13 葡萄糖胺濃度對Aeromonas sp. DYU-Too7之生質量的影響66 圖3.14 葡萄糖胺濃度對Aeromonas sp. DYU-Too7之幾丁質分解酵素活性的影響(以染料結合法測定)67 圖3.15 葡萄糖胺濃度對Aeromonas sp. DYU-Too7之蛋白質量的影響68 圖3.16 葡萄糖胺濃度對Aeromonas sp. DYU-Too7之發酵液中殘餘葡萄糖胺的影響69 圖3.17 Aeromonas sp. DYU-Too7培養於含幾丁質2.0%之基礎培養基中幾丁質分解酵素、生質量、蛋白質、幾丁質殘餘量、還原醣量及pH之變化71 圖3.18 Aeromonas sp. DYU-Too7培養於含葡萄糖胺0.1%之基礎培養基中幾丁質分解酵素、生質量、蛋白質、葡萄糖胺殘餘量及pH之變化73 圖4.1 Aeromonas sp. DYU-Too7培養於幾丁質之蛋白質經DEAE-sepharose CL-6B分離之結果84 圖4.2 Aeromonas sp. DYU-Too7培養於幾丁質之蛋白質經DEAE-sepharose CL-6B分離之第1-7波峰(如圖4.1)的SDS-PAGE分析圖。第i行表示第i個波峰之層析圖(i = 1, 2, …, 7)85 圖4.3 Aeromonas sp. DYU-Too7培養於幾丁質之蛋白質DEAE-sepharose CL-6B分離之波峰(如圖4.1)的活性電泳染色分析圖。Lane 1為第2波峰、Lane 2為第3波峰、Lane 3為第5波峰、Lane 4為第6波峰87 圖4.4 活性波峰Ie (如圖4.1所示之波峰1-3)以Sephacryl S-100HR分離之結果88 圖4.5 以SDS-PAGE分析Ie (如圖4.1所示之波峰1-3)經Sephacryl S-100HR分離之第30-40管89 圖4.6 以活性電泳染色法分析Ie (如圖4.1所示之波峰1-3)經Sephacryl S-100HR分離之第30-40管90 圖4.7 pH值對Aeromonas sp. DYU-Too7培養於幾丁質所分泌之36 kDa幾丁質分解酵素的影響與其pH穩定性。(●) : McIlvaine buffer (pH 3.0-9.0) ; (△) : Tris-HCl buffer (pH 8.0-11.0)92 圖4.8 溫度對Aeromonas sp. DYU-Too7培養於幾丁質所分泌之36 kDa幾丁質分解酵素的影響與其溫度穩定性94 圖4.9 Aeromonas sp. DYU-Too7培養於幾丁質所分泌之36 kDa幾丁質分解酵素的Km 與 Vmax。圖中為Lineweaver-Burk雙倒數圖形，R2 = 0.998 96 圖4.10 Aeromonas sp. DYU-Too7培養於幾丁質所分泌之36 kDa幾丁質分解酵素水解膠態幾丁質的產物分析99 圖4.11 以ESI-Q-TOF MS分析Ie (如圖4.1所示之波峰1-3)經Sephacryl S-100HR分離之第30-40管101 圖4.12 經Mascot資料庫比對，Bacillus cereus之chitinase A胺基酸序列，與Aeromonas sp. DYU-Too7培養於幾丁質所分泌之36 kDa幾丁質分解酵素的相符性 (底部黑線為相符處)102 圖4.13 Aeromonas sp. DYU-Too7培養於葡萄糖胺之酵素液經DEAE-sepharose CL-6B分離之結果104 圖4.14 Aeromonas sp. DYU-Too7培養於葡萄糖胺之酵素液DEAE-sepharose CL-6B分離之第13-23管的SDS-PAGE分析圖。第i行表示第i個波峰之層析圖(i = 13, 14, …, 23)105 圖4.15 Aeromonas sp. DYU-Too7培養於葡萄糖胺之酵素液DEAE-sepharose CL-6B分離之第13-23管的活性電泳染色法分析圖。第i行表示第i個波峰之層析圖(i = 13, 14, …,23) 106 圖4.16 Aeromonas sp. DYU-Too7培養於葡萄糖胺之酵素液DEAE-sepharose CL-6B分離之第37-40管的SDS-PAGE分析圖。第i行表示第i個波峰之層析圖(i = 37, 38, …, 40)107 圖4.17 Aeromonas sp. DYU-Too7培養於葡萄糖胺之酵素液DEAE-sepharose CL-6B分離之第37-40管的活性電泳染色法分析圖。第i行表示第i個波峰之層析圖(i = 37, 38, …, 40)108 圖4.18活性波峰E1(如圖4.13所示之第13-23管)以Sephacryl S-100HR分離之結果109 圖4.19活性波峰E2 (如圖4.13所示之第37-40管)以Sephacryl S-100HR分離之結果110 圖4.20 以SDS-PAGE分析E1 (如圖4.13所示之第13-23管)經Sephacryl S-100HR分離之第19-21管111 圖4.21 以活性電泳染色法分析E1 (如圖4.13所示之第13-23管)經Sephacryl S-100HR分離之第19-21管112 圖4.22 以SDS-PAGE分析E2 (如圖4.13所示之第37-40管)經Sephacryl S-100HR分離之第17-18管114 圖4.23 以活性電泳染色法分析E2 (如圖4.13所示之第37-40管)經Sephacryl S-100HR分離之第17-18管115 圖4.24 pH值對Aeromonas sp. DYU-Too7培養於葡萄糖胺所分泌之36 kDa幾丁質分解酵素的影響與其pH穩定性。(●) : McIlvaine buffer (pH 3.0-8.0) ; (△) : Tris-HCl buffer (pH 7.0-10.0)117 圖4.25 溫度對Aeromonas sp. DYU-Too7培養於葡萄糖胺所分泌之36 kDa幾丁質分解酵素的影響與其溫度穩定性119 圖4.26 Aeromonas sp. DYU-Too7培養於葡萄糖胺所分泌之36 kDa幾丁質分解酵素水解膠態幾丁質的產物分析122 圖4.27 以ESI-Q-TOF MS分析E1(如圖4.13所示之第13-23管)經Sephacryl S-100HR分離之第20管123 圖4.28 經Mascot資料庫比對，Bacillus cereus之chitinase A胺基酸序列，與Aeromonas sp. DYU-Too7培養於葡萄糖胺所分泌之36 kDa幾丁質分解酵素的相符性(底部黑線為相符處)124 圖5.1 工業鑽石含量(不含甲酸)對蛋白質吸附效率之影響134 圖5.2 工業鑽石含量(含甲酸0.1%)對蛋白質吸附效率之影響135 圖5.3 甲酸含量(0-0.5%)對工業鑽石吸附蛋白質之影響136 圖5.4 以SDS-PAGE分析甲酸對奈米化之工業鑽石吸附蛋白質的影響。A與D：固定化酵素之沉澱物；B與E：吸附酵素之上清液；C：未吸附之酵素。A與B不含甲酸；D與E含甲酸(0.067%, pH4.10)138 圖5.5 工業鑽石含量(含甲酸0.067%)對蛋白質吸附效率之影響139 圖5.6 pH值對被工業鑽石吸附的酵素之影響。(●) : McI- lvaine buffer (pH 3.0-8.0) ; (△) : Tris-HCl buffer (pH 7.0-10.0)141 圖5.7 溫度對被工業鑽石吸附的酵素之影響142 圖5.8 被工業鑽石吸附的酵素之pH穩定性試驗。(●) : McI- lvaine buffer (pH 3.0-8.0) ; (△) : Tris-HCl buffer (pH 7.0-10.0)143 圖5.9 被工業鑽石吸附的酵素之溫度穩定性試驗145 圖5.10 以不同pH緩衝液脫附被工業鑽石吸附的酵素。(●) : McIlvaine buffer (pH 3.0-8.0) ; (△) : Tris-HCl buffer (pH 7.0-10.0)146 圖5.11 利用SDS-PAGE檢視不同pH緩衝液所

脫附之吸附酵素。A與B：pH 8.0緩衝液所脫附之上清液與沉澱物；C與D：pH 5.0緩衝液所脫附之上清液與沉澱物；E與F：未脫附之酵素的上清液與沉澱物147 圖5.12 利用活性電泳染色法檢視不同pH緩衝液所脫附之吸附酵素。A與B: pH 8.0緩衝液所脫附之上清液與沉澱物；C與D: pH 5.0緩衝液所脫附之上清液與沉澱物；E與F: 未脫附之酵素的上清液與沉澱物149 圖5.13 以不同pH緩衝液脫附奈米化之工業鑽石上Aeromonas sp. DYU-Too7所分泌的胞外蛋白質150 圖5.14 利用SDS-PAGE檢視奈米化之工業鑽石吸附與脫附蛋白質情況。A：粗酵素；B與C：未脫附之酵素的沉澱物與上清液；D與E：緩衝液pH 8.0所脫附之上清液與沉澱物153 圖5.15 利用活性電泳染色法檢視奈米化之工業鑽石吸附與脫附蛋白質情況。A：粗酵素；B與C：未脫附之酵素的上清液與沉澱物；D與E：緩衝液pH 8.0所脫附之上清液與沉澱物154 圖6.1 Aeromonas sp. DYU-Too7之chi36基因，以chi36-F與chi36-R進行PCR增幅(50-58°C)後之洋菜膠體電泳圖170 圖6.2 重組質體pGEM-chi36之洋菜膠體電泳圖，其中C、F、J及K可能含有欲選殖DNA片段171 圖6.3 重組質體pGEM-chi36以限制性內切?(BstZI)切割之洋菜膠體電泳173 圖6.4 chi36之核?酸序列與Chi36胺基酸序列。方框為訊息胜?，星號『*』為轉譯終止密碼，繪實線處為共有催化區域及灰色背景為酵素活性位175 圖6.5 Aeromonas sp. DYU-Too7之chi36基因的限切酵素圖譜176 圖6.6 Aeromonas sp. DYU-Too7之chi36與Bacillus cereus之chiA核?酸序列的比較178 圖6.7 利用SignalP 3.0分析Chi36之訊息胜?181 圖6.8 利用DNAstar分析chi36(P)之核?酸與Chi36(P)胺基酸序列組成182 圖6.9 利用DNAstar分析chi36(M)之核?酸與Chi36(M)胺基酸序列組成183 圖6.10 Aeromonas sp. DYU-Too7之chi36基因(含能產生訊息胜?)，以chi36(P)-F與chi36(P)-R進行PCR增幅(50-55°C)後之洋菜膠體電泳圖185 圖6.11 Aeromonas sp. DYU-Too7之chi36基因(缺少能產生訊息胜?)，以chi36(M)-F與chi36(M)-R進行PCR增幅(50-55°C)後之洋菜膠體電泳圖186 圖6.12 純化chi36(P)與chi36(M)之PCR產物。Lane 1：chi36(M)、 Lane 2：chi36(P) 187 圖6.13 重組質體pGEM-chi36(P)之洋菜膠體電泳圖，其中C含有欲選殖DNA片段188 圖6.14 重組質體pGEM-chi36(M)之洋菜膠體電泳圖，其中D含有欲選殖DNA片段189 圖6.15 重組質體pUC18-chi36(P)之洋菜膠體電泳圖，其中A-J含有欲選殖DNA片段190 圖6.16 重組質體pUC18-chi36(M)之洋菜膠體電泳圖，其中D、F及G含有欲選殖DNA片段191 圖6.17 IPTG濃度對E. coli JM109 (pUC18-chi36(P))與E. coli JM109 (pUC18-chi36(M))生產胞內幾丁質分解酵素之影響193 圖6.18 IPTG濃度對E. coli JM109 (pUC18-chi36(P))與E. coli JM109 (pUC18-chi36(M))生產胞外幾丁質分解酵素之影響194 圖6.19 pH值對E. coli JM109 (pUC18-chi36(P))所生合成之胞內幾丁質分解酵素的影響與其pH穩定性。(●) : McIlvaine buffer (pH 3.0-8.0) ; (△) : Tris-HCl buffer (pH 7.0-10.0)196 圖6.20 溫度對E. coli JM109 (pUC18-chi36(P))所生合成之胞內幾丁質分解酵素的影響與其溫度穩定性198 圖6.21 以(a) E. coli JM109 (pUC18-chi36(P))與(b) E. coli JM109 (pUC18-chi36(M))所生合成之胞內幾丁質分解酵素水解膠態幾丁質之產物分析199 表目錄 表2.1 各種幾丁質分解酵素之純化與特性15 表2.2 奈米鑽石的應用22 表2.3 各種幾丁質分解酵素之基因選殖32 表3.1 基礎培養基組成41 表3.2 蛋白質電泳膠片組成45 表3.3 幾丁質濃度對Aeromonas sp. DYU-Too 7之發酵液中幾丁質殘餘量的影響63 表4.1 Aeromonas sp. DYU-Too7培養於幾丁質之酵素液的純化總表91 表4.2 金屬離子或EDTA對Aeromonas sp. DYU- Too7培養於幾丁質所分泌之36 kDa幾丁質分解酵素活性的影響98 表4.3 Aeromonas sp. DYU-Too7培養於葡萄糖胺之酵素液的純化總表116 表4.4 金屬離子或EDTA對Aeromonas sp. DYU-Too7培養於葡萄糖胺所分泌之36 kDa幾丁質分解酵素活性的影響120 表5.1 奈米化之工業鑽石吸附與脫附Aeromonas sp. DYU-Too7的蛋白質與幾丁質分解酵素之效率151 表6.1 大腸桿菌之培養基組成160

REFERENCES

1.王三郎。1997。水產資源利用學。高立出版社，台北。 2.宋健民。2005。奈米鑽石的大千世界。工業材料雜誌，217: 158-170。 3.宋健民。2006。鑽氟超滑面與鑽石感測器。物理雙月刊，28(6): 967-977。 4.江文彥。1998。鑽石其特性與用途。化工技術，6(6): 224-229。 5.汪建民。1999。陶瓷技術手冊。中華民國粉末冶金協會，新竹。 6.余樹楨。2007。人造鑽石。科學發展，409: 66-71。 7.姚少凌。2000。Pseudomonas fluorescens生產脂肪水解酵素固定於聚甲基丙烯醯胺以生產S-AMPA的應用。國立清華大學化學工程研究所碩士學位論文，新竹。 8.陳坤上、黃佩芬、陳聰松、陳幸臣。1996。幾丁寡醣製備條件之探討。食品科學，23(6): 874-883。 9.曾士齊、劉永賢、宣仲華。2005。奈米鑽石合成之研究。環球技術學院科技人文學刊，2: 33-43。 10.黃安德。1998。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研究所碩士學位論文，基隆。 11.連德昇。2002。以本土菌株分解幾丁質生產N-乙醯幾丁寡醣之研究。大葉大學食品工程研究所碩士論文，彰化。 12.韓肇中、張煥正。2006。奈米鑽石新生命。中央研究院週報，1064。 13.Alam, M. M., Mizutani, T., Isono, M., Nikaidou, N. and Watanabe, T. 1996. Three chitinase (ChiA, ChiB, ChiC) comprise the chitinase system of Bacillus circulans WL-12. J. Ferment. Bioeng., 82: 28-36. 14.Alam, M. M., Nikaidou, N., Tanaka, H. and Watanabe, T. 1995. Cloning and sequencing of chiC gene of Bacillus circulans WL-12 and relationship of its product to some other chitinase and chitinase-like proteins. J. Ferment. Bioeng., 80: 454-461. 11.Armard, S., Tomuta, H., Heyraud, A., Gey, C., Watanabe, T. and Henrissat, B. 1994. Sterochemical course of hydrolysis reaction catalyzed by chitinase A1 and D from Bacillus circulans WL-12. FEBS Lett., 343: 177-180. 12.Arora, N., Ahmad, T., Rajagopal, R. and Bhatnagar, R. K. 2003. A constitu- tively expressed 36 kDa exochitinase from Bacillus thuringiensis HD-1. Biochem. Biophys. Res. Commun., 307: 620-625. 13.Bendt, A., Huller, H., Kammel, U., Helmke, E. and Schweder, T. 2001. Clon- ing, expression, and characterization of a chitinase gene from the Antarctic psychrotolerant bacterium Vibrio sp. strain Fi:7. Extremophiles., 5(2): 119-26. 14.Berkeley, R. C. W., Brewer S. J., Ortiz J. M. and Gillespie, J. B. 1973. An exo-β-N-acetylglucosaminidase from Bacillus subtilis B: characterization. Biochim. Biophys. Acta., 399: 157-168. 15.Bernard, N. 1911. Sur la fonction fungicides des bulbes d'Ophydes. Ann. Sci. Nat. Bot., 14: 221-234. 16.Bhushan, B. 2000. Production and characterization of a thermostable chitinase from a new alkalophilic Bacillus sp. BG-11. J.

Appl. Microbiol., 88: 800-808. 17.Binod, P., Pusztahelyi, T., Nagy, V., Sandhya, C., Szakacs, G., Pocsi, I. and Pandey, A. 2005. Production and purification of extracellular chitinases from Penicillium aculeatum NRRL 2129 under solid-state fermentation. Enzyme Microb. Technol., 36: 880-887. 18.Boller, T., Gehri, A., Mauch, F. and Vogeli, U. 1983. Chitinase in bean Leaves: Induction by ethylene, purification, properties, and possible function. Planta., 157: 22-31. 19.Boot, R. G., Renkema, G. H., Strijland, A. V., Zonneveld, A. J. and Aerts, J. M. 1995. Cloning of a cDNA encoding chitotriosidase, a human chitinase pro- duced by macrophages. J. Biol. Chem., 270(44): 26252-26256. 20.Boot, R. G., Blommaart, E. F. and Swart, E. 2001. Identification of a novel acidic mammalian chitinase distinct from chitotriosidase. J. Biol. Chem., 276: 6770-6778. 21.Bradford, M. M. 1976. A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. Anal. Biochem., 72: 248-254. 22.Carroad, D. A. and Tom, R. A. 1978. Bioconversion of shellfish chitin waste: process conception and selection of microorganism. J. Food Sci., 43: 1158-1164. 23.Chang, K. B., Lee, J. and Fu, W. R. 2000. HPLC analysis of N-acetylchitooligosaccharides during the acid hydrolysis of chitin. J. Food Drug Anal., 8: 75-83. 24.Chen, J. P. and Chang, K. C. 1994. Immobilization of chitinase on a reversibly soluble-insoluble polymer for chitin hydrolysis. J. Chem. Technol. Biotechnol., 60(2): 133-140. 25.Chen, J. P., Narayana, F. and Chang, M. C. 1991. Clong and expression of chitinase gene from Aeromonas hydrophlia in E. coli. Appl. Environ. Micro- biol., 57: 2426-2428. 26.Chernin, L. S., De-la, F. L., Sobolev, V., Haran, S., Vorgias, C. E., Oppenheim, A. B. and Chet, I. 1997. Molecular cloning, structural analysis, and expression in Escherichia coli of a chitinase gene from Enterobacter agglomerans. Appl. Environ Microbiol., 63(3): 834-839. 27.Chet, I. and Inbar, J. 1994. Biological control of fungal pathogens. Appl. Biochem. Biotechnol., 48(1): 37-43. 28.Clarke, V. A., Platt, N. and Butters, T. D. 1995. Cloning and expression of the beta-N-acetylglucosaminidase gene from Streptococcus pneumoniae. J. Biol. Chem., 270(15): 8805-8814. 29.Collinge, D. B., Kragh, K. M., Mikkslesen, J. D., Nielsen, K. K., Rasmussen, U. and Vad, K. 1993. Plant chitinases. J. Plant., 3: 31-40. 30.Cruz, M. A. L., Gomes, V. M., Fernandes, K. V. S., Machado, O. L. T. and Xavier-Filho, J. 2002. Identification and partial characterization of a chitinase and a β-1,3-glucanase from Copernicia cerifera wax. Plant Physiol. Bioch., 40: 11-16. 31.Duo-Chuan, L. I., Chen, S. and Jing, L. U. 2005. Purification and partial characterization of two chitinases from the mycoparasitic fungus Talaromyces flavus. Mycopathologia., 159(2): 223-229. 32.El-Katatny, M. H., Somitsch, W., Robra, K. H., El-Katatny, M. S. and Gubitz, G. M. 2000. Production of chitinase and β-1,3-glucanase by Trichoderma harzianum for control of the phytopathogenic fungus Sclerotium rolfsii. Food Technol Biotechnol., 38(3): 173-180. 33.Fang, W., Leng, B., Xiao, Y., Jin, K., Ma, J., Fan, Y., Feng, J., Yang, X., Zhang, Y. and Pei, Y. 2005. Cloning of Beauveria bassiana chitinase gene Bbchit1 and its application to improve fungal strain virulence. Appl. Environ. Microbiol., 71(1): 363-370. 34.Frandberg, E. and J. Schnurer (1994) Evaluation of a chromogenic chitooligo- saccharide analogue, p-nitrophenyl-b-D-N,N'-diacetylchitobiose, for the measurement of the chitinolytic activity of bacteria. J. Appl. Bacteriol. 76: 259-263. 35.Gal, S. W., Choi, J. Y., Kim, C. Y., Cheong, Y. H., Choi, Y. J., Bahk, J. D., Lee, S. Y. and Cho, M. J. 1997. Isolation and characterization of the 54-kDa and 22-kDa chitinase genes of Serratia marcescens KCTC2172. FEMS Micro. Biol. Lett., 151(2): 197-204. 36.Garcia, I., Lora, J. M., de la Cruz, J., Benitez, T., Llobell, A. and Pintor-Toro, J. A. 1994. Cloning and characterization of a chitinase (chit42) cDNA from the mycoparasitic fungus Trichoderma harzianum. Curr. Genet., 27(1): 83-89 37.Glasgow, L. R., Paulson, J. C. and Hill, R. L. 1977. Systematic purification of five glycosidases from Streptococcus (Diplococcus) pneumoniae. J. Biol. Chem., 252(23): 8615-8623. 38.Guo, S. H., Chen, J. K. and Lee, W. C. 2004. Purification and characterization of extracellular chitinase from Aeromonas schubertii. Enzyme Microb. Tech- nol., 35(6-7): 550-556. 39.Guo, R. F., Li, D. C. and Wang, R. 2005. Purification and properties of a thermostable chitinase from thermophilic fungus Thermomyces lanuginosus. Wei Sheng Wu Xue Bao., 45(2): 270-274. 40.Henrissat, B. 1991. A classification of glycosyl hydrolases based on amino-acid sequence similarities. Biochem. J., 280: 309-316. 41.Howie, W., Joe, L., Newbigin, E., Suslow, T. and Dunsmuir, P. 1994. Transgenic tobacco plants which express the chiA gene from Serratia marcescens have enhanced tolerance to Rhizoctonia solani. Trans. Res., 3: 90-98. 42.Hsu, S. C. and Lockwood, J. L. 1975. Powdered chitin agar as a selective medium for enumeration of actinomycetes in water and soil. Appl. Microbiol., 29: 422-426. 43.Huang, C. J. and Chen. C. Y. 2005. High-level expression and characterization of two chitinases, ChiCH and ChiCW, of Bacillus cereus 28-9 in Escherichia coli. Biochem. Biophys. Res. Commun., 327(1): 8-17. 44.Huang, L. C. L. and Chang H. C. 2004. Adsorption and immobilization of cytochrome c on nanodiamonds. Langmuir., 20: 5879-5884. 45.Ilyina, A. V., Tikhonov, V. E., Albulov, A. I. and Varlamov, V. P. 2000. Enzymic preparation of acid-free-water-soluble chitosan. Process Biochem., 35: 563-568. 46.Imoto, I. and Yagishita, K. 1971. Simple activity measurement of lysozyme. Agric. Biol. Chem., 72: 11154-1156. 47.Iranzo, M., Aguado, C., Pallotti, C., Canizares, J. V. and Mormeneo, S. 2002. The use of trypsin to solubilize wall protein from Candida albicans let to the identification of chitinase 2 as an enzyme covalently linked to the yeast wall structure. Res. in Microbiol., 153: 227-232. 48.Iseli, B., Armand, S., Boller, T., Neuhaus, J. M. and Henrissat, B. 1996. Plant chitinases use two different hydrolytic mechanisms. FEBS Lett., 382(1-2): 186-188. 49.Jaques, A. K., Fukamizo, T., Hall, D., Barton, R. C., Escott, G. M., Parkinson, T., Hitchcock, C. A. and Adams, D. J. 2003. Disruption of the gene encoding the ChiB1 chitinase of Aspergillus fumigatus and characterization of a recombi- nant gene product. Microbiol., 149(10): 2931-2939. 50.Jeuniaux, C. 1961. Chitinase: an addition to the list of hydrolases in the digestive tract of vertebrates. Nature., 192: 135-136. 51.Jeuniaux, C. 1966. Chitinases. Methods Enzymol., 8: 644-650. 52.Jolles, P. and Muzzarelli, R. A. A. 1999. Chitin and Chitinases. Birkhauser Verlarg, Basel. 53.Jones, J. D. G., Grady, K. L., Suslow, T. V. and Bedbrook, J. R. 1986. Isolation and characterization of genes encoding two chitinase enzymes from Serratia marcescens. The EMBO Journal, 5: 476-473 54.Joshi, S., Kozlowski, M., Selvaraj, G., Iyer, V. N. and Davies, R. W. 1988. Cloning of the gene of the chitin utilization regulon of Serratia liquefacients. J. Bacteriol., 170: 2984-2988. 55.Kang, S. C., Park, S. and Lee, D. G. 1998. Isolation and characterization of a chitinase cDNA from the entomopathogenic fungus, Metarhizium anisopliae. FEMS Microbiol. Lett., 165(2): 267-271. 56.Karrer, P and Hofmann, A. 1929. Uber den enzymatischen abbau von chitin

und chitosan. Helv. Chim. Acta., 12: 616-637. 57.Kawachi, I., Fujieda, T., Ujita, M., Ishii, Y., Yamagishi, K., Sato, H., Funagu- ma, T. and Hara, A. 2001. Purification and properties of extracellular chitinases from the parasitic fungus Isaria japonica. J. Ferment. Technol., 92(6): 544-549. 58.Kelkar, H. S., Shankar, V. and Deshpande, M. V. 1990. Rapid isolation and regeneration of Sclerotium rolfsii protoplasts and their potential application for starch hydrolysis. Enzyme Microb. Technol., 12: 510-514. 59.Kim, K. J., Yang, Y. J. and Kim, J. G. 2003. Purification and characterization of chitinase from Streptomyces sp. M-20. J. Biochem. Mol. Biol., 36(2): 185-189. 60.Koga, D., Sasaki, Y., Uchiumi, Y., Hirai, N., Arakane, Y. and Nagamatsu, Y. 1997. Purification and characterization of Bombyx mori chitinases. Insect Biochem. Mol. Biol., 27(8-9): 757-767. 61.Koga, K, Iwamoto, Y., Sakamoto, H., Hatano, K., Sano, M. and Kato, I. 1991. Purification and characterization of beta-N-acetylhexosaminidase from Trichoderma harzianum. Agric. Biol. Chem., 55: 2817-2823. 62.Koga, D., Tsukamoto, T., Sueshige, N., Utsumi, T. and Ide, A. 1989. Kinetics of chitinase from yam, Dioscorea opposita Thunb. Agric. Biol. Chem., 53(12): 3121-3126. 63.Kong, X. L., Huang, L. C. L., Hsu, C. M., Chen, W. H., Han, C. C. and Chang, H. C. 2005. High-affinity capture of proteins by diamond nanoparticles for mass spectrometric analysis. Anal. Chem., 77: 259-265. 64.Krishnaveni, S., Liang, G. H., Muthukrishnan S. and Manickam, A. 1999. Purification and partial characterization of chitinases from sorghum seeds. Plant Sci., 144: 1-7. 65.Kupiec, C. R. and Chet, I. 1998. The molecular biology of chitin digestion. Curr. Opin in Biotechnol., 9: 270-277. 66.Lan, X., Ozawa, N., Nishiwaki, N., Kodaira, R., Okazaki, M. and Shimosaka, M. 2004. Purification, cloning, and sequence analysis of beta-N-acetylgluco- saminidase from the chitinolytic bacterium Aeromonas hydrophila strain SUWA-9. Biosci. Biotechnol. Biochem., 68(5): 1082-1090. 67.Lin, C. S., Chen, H. C. and Lin, F. P. 1997. Expression and characterization of the recombinant gene encoding chitinase from Aeromonas caviae. Enzyme Microb. Technol., 21: 472-478. 68.Lonhienne, T., Mavromatis, K., Vorgias, C. E., Buchon, L., Gerday, C. and Bouriotis, V. 2001. Cloning, sequences, and characterization of two chitinase genes from the Antarctic Arthrobacter sp. strain TAD20: isolation and partial characterization of the enzymes. J. Bacteriol., 183(5): 1773-1779. 69.Lundblad, G., Hederstedt, B., Lind, J. and Steby, M. 1974. Chitinase in goat serum. Preliminary purification and characterization. Eur. J. Biochem., 46: 367-376. 70.Malik, A., Wenuganen, S., Suwanto, A. and Tjahjono, B. 2003. Cloning, DNA sequence, and expression of Aeromonas caviae WS7b chitinase gene. Mol. Biotechnol., 23(1): 1-10. 71.Micha, J. C., Dandrifosse, G. and Jeuniaux, C. 1973. Distribution and tissue localization of chitinase synthesis in low vertebrates. Arch. Int. Physiol. Biochim., 81: 439-376. 72.Mitsutomi, M., Hata, T. and Kuwahara, T. 1995. Purification and characteri- zation of novel chitinases from Streptomyces griseus HUT6037. J. Ferment. Bioeng., 80: 153-158. 73.Miyashita, K. and Fujii, T. 1993. Nucleotide sequence and analysis of a gene (chiA) for a chitinase from Streptomyces lividans 66. Biosci. Biotech. Biochem., 57: 1691-1698. 74.Miyashita, K., Fujii, T. and Saito, A. 2000. Induction and repression of a Streptomyces lividans chitinase gene promoter in response to various carbon sources. Biosci. Biotech. Biochem., 64(1): 39-43. 75.Monreal, J. and Reese, E. T. 1969. The chitinase of Serratia marcescens. Can. J. Microbiol., 15(7): 689-96. 76.Murao, S., Kawada, T., Itoh, H., Oyama, H. and Shin, T. 1992. Purification and characterization of a novel type of chitinase from Vibrio alginolyticus TK-22. Biosci. Biotech. Biochem., 56: 368-369. 77.Nanjo, F., Ishikawa, M., Katsumi, R. and Sakai, K. 1990. Purification, pro- perties and transglycosylation reaction of a β-N-acetylhexosaminidase from Nocardia orientalis. Agric. Biol. Chem., 54: 899-906. 78.Nawani, N. N., Kapadnis, B. P., Das, A. D., Rao, A. S and Mahajan, S. K. 2002. Purification and characterization of a thermophilic and acidophilic chitinase from Microbispora sp. V2. J. Appl. Microbiol., 93: 965-975. 79.Ogawa, K., Yoshida, N., Kariya, K., Ohnishi, C. and Ikeda, R. 2002. Purification and characterization of a novel chitinase from Burkholderia cepacia strain KH2 isolated from the bed log of Lentinus edodes, Shiitake mushroom. J. Gen. Appl. Microbiol., 48(1): 25-33. 80.Ohishi, K., Yamagishi, M., Ohta, T., Suzuki, M., Izumida, H., Sano, H., Nishijima, M. and Miwa, T. 1996. Purification and properties of two chitinases from Vibrio alginilyticus H-8. J. Ferment. Technol., 82(6): 598-600. 81.Okazaki, K., Yamashita, Y., Noda, M., Sueyoshi, N., Kameshita, I. and Hayakawa, S. 2004. Molecular cloning and expression of the gene encoding family 19 chitinase from Streptomyces sp. J-13-3. Biosci. Biotechnol. Biochem., 68(2): 341-351. 82.Ordentlich, A., Elad, Y. and Chet, J. 1988. The role of chitinase of Serratia marcescens in biocontrol of Sclerotium rolfsii. J. Phytopathol., 78: 84-88. 83.Orikoshi, H., Baba, N., Nakayama, S., Kashu, H., Miyamoto, K., Yasuda, M., Inamori, Y. and Tsujibo, H. 2003. Molecular analysis of the gene encoding a novel cold-adapted chitinase (ChiB) from a marine bacterium, Alteromonas sp. Strain O-7. J. Bacteriol., 185(4): 1153-1160. 84.Oshima, H., Miyazaki, R., Ohe, Y., Hayashi, H., Kawamura, K. and Kikuyama, S. 2002. Isolation and sequence of a novel amphibian pancreatic chitinase. Comp. Biochem. Physiol. B Biochem. Mol. Biol., 132(2): 381-388. 85.Overdijk, B. and Steijn, G. J. V. 1994. Human serum contains a chitinase: identification of an enzyme, formerly described as 4-methylumbelliferyl-tetra- N-acetylchitotetraoside hydrolase (MU-TACT hydrolase). Glycobiology, 4(6): 797-803. 86.Patil, R. S., Ghormade, V. and Deshpande, M. V. 2000. Chitinolytic enzymes: an exploration. Enzyme Microb. Technol., 26(7): 473-483. 87.Punja, Z, K. and Zhang, Y. Y. 1993. Plant chitinase and their roles in resistance to fungal diseases. J. Nematol., 25(4): 526-540. 88.Righetti, P. G., Gianazza, E., Gelfi, C. and Chiari, M. 1990. In: Gel Electro- phoresis of Proteins: A Practical Approach. Hames, B. D. and Richwood, D. (Eds.), 2nd Ed. p.149-216. Oxford University Press, New York, USA. 89.Revah-Moiseev, S. and Carroad, A. 1981. Conversion of the enzymatic hydro- lysate of shellfish waste chitin to single-cell protein. Biotechnol. Bioeng., 23: 1067-1078. 90.Robbins, P. W., Albright, C. and Benfield, B. 1988. Cloning and expression of a streptomyces plicatus chitinase (chitinase-63) in Escherichia coli. J. Biol. Chem., 263(1): 443-447. 91.Roberts, W. K. and Selitrennikoff, C. P. 1988. Plant and bacterial chitinases differ in antifungal activity. J. Gen. Microbiol., 134: 169-176. 92.Roe, S. 2001. Protein Purification Applications. New York: Oxford. 93.Roffey, P. E. and Pemberton, J. M. 1990. Cloning and expression of an Aeromonas hydrophila chitinase gene in Escherichia coli. Curr Microbiol., 21: 329-337. 94.Sakurada, M., Morgavi, D. P., Komatani, K., Tomita, Y. and Onodera, R. 1997. Purification and characteristics of an autolytic chitinase of Piromyces communis OTS1 from culture medium. Cur. Microbiol., 35: 48-51. 95.Scopes, B. K. 1987. Protein Purification: Principles and Practice. New York: Springer-Verlag.

96.Shapira, R., Ordentlich, A., Chet, I. and Oppenheim, A. B. 1989. Control of plant disease by chitinase expressed from cloned DNA in Escherichia coli. Phytopathology., 79: 1246-1249. 97.Shiro, M., Ueda, M., Kawaguchi, T. and Arai, M. 1996. Cloning of a cluster of chitinases genes from Aeromonas sp. No. 10S-24. Biochim. Biophy. Acta., 1350: 44-48. 98.Sitrit, Y., Vorgias, C. E., Chet, I. and Oppenheim, A. B. 1995. Cloning and primary structure of the ChiA gene from Aeromonas caviae. J. Bacteriol., 177: 4178-4189. 99.Skujins, J. J., Potgieter, H. J. and Alexander, M. 1965. Dissolution of fungal cell walls by a Streptomycete chitinase and beta-(1-3) glucanase. Arch. Biochem. Biophys., 111(2): 358-364. 100.Smith, R. J. and Grula, E. F. 1983. Chitinase is an inducible enzyme in Beauveria bassiana. J. Invert Pathol., 42: 319-326. 101.Souza, R. F., Gomes, R. C., Coelho, R. R., Alviano, C. S. and Soares, R. M. 2003. Purification and characterization of an endochitinase produced by Colletotrichum gloeosporioides. FEMS Microbiol. Lett., 222(1): 45-50. 102.Suginta, W., Vongsuwan, A., Songsiriritthigul, C., Prinz, H., Estibeiro, P., Duncan, R. R., Svasti, J. and Fothergill-Gilmore, L. A. 2004. An endochitinase A from Vibrio carchariae: cloning, expression, mass and sequence analyses, and chitin hydrolysis. Arch. Biochem. Biophys., 424(2): 171-80. 103.Sutrisno, A., Ueda, M., Inui, H., Kawaguchi, T., Nakano, Y., Arai, M. and Miyatake, K. 2001. Expression of a gene encoding chitinase (pCA 8 ORF) from Aeromonas sp. no. 10S-24 in Escherichia coli and enzyme characterization. J. Biosci.Bioeng., 91(6): 599-602. 104.Suzuki, K., Tokoro, A., Suzuki, S. and Suzuki, M. 1985. Enhancing effects of N-acetyl-chito-oligo-saccharides on the active oxygen-generating and micro- bicidal activities of peritoneal exudates cells in mice. Chem. Pharm. Bull., 33: 886-888. 105.Taira, T., Toma, N. and Ishihara, M. 2005. Purification, characterization, and antifungal activity of chitinases from pineapple (Ananas comosus) leaf. Biosci. Biotechnol. Biochem., 69(1): 189-196. 106.Takayanagi, T., Ajisaka, K., Takiguchi, Y. and Shimahara, K. 1991. Isolation and characterization of thermostable chitinases from Bacillus licheniformis X-7u. Biochim. Biophys. Acta., 1078(3): 404-410. 107.Takegawa, K., Mikami, B., Iwahara, S., Morita, Y., Yamamoto, K. and Tochikura, T. 1991. Complete amino acid sequence of endo-β-N-acetyl-glucosaminidase from Flavobacterium sp. Eur. J. Biochem., 202: 175-180. 108.Tang, S. C., Chang M. C. and Cheng, C. Y. 1998. Use of colloid chitin and diatomaceous earth in continuous cake-filtration fermentation to produce creatinase. Proc. Biochem., 33: 519-526. 109.Terwisscha van Scheltingz, A. C., Hennig, M. and Dijkstra, B. W. 1996. The 1.8 A resolution structure of hevamine, a plant chitinase/lysozyme, and analysis of the conserved sequence and structure motifs of glycosyl hydrolase family 18. J. Mol. Biol., 262: 243-257 110.Tews, I., Vincentelli, R. and Vorgias, C. E. 1996. N-acetyl-glucosaminidase (chitobiase) from Serratia marcescens: gene sequence, and protein production and purification in Escherichia coli. Gene., 1170(1): 63-67. 111.Tokoro, A., Kobayashi, M., Tatewaki, N., Suzuki, K., Okawa, Y., Mikami, T., and Suzuki, M. 1989. Protective effect of N-acetylchitohexaose on Listeria monocytogenes infection in mice. Microbiol. Immunol., 33: 357-367. 112.Trudel, J. and Asselin, A. 1989. Detection of chitinase activity after polyacryl-amide gel electrophoresis. Anal. Biochem., 178: 362-366. 113.Tsujibo, H., Fujimoto, K., Tanno, H., Miyamoto, K., Imada, C., Okami, Y. and Inamori, Y. 1994. Gene, purification and characterization of N-acetyl- β - glucosaminisase from a marine bacterium. Alteromonas sp. strain O-7. Gene, 146: 111-115. 114.Tsujibo, H., Hatano, N., Endo, H., Miyamoto, K. and Inamori, Y. 2000. Purification and characterization of a thermostable chitinase from Streptomyces thermoviolaceus OPC-520 and cloning of the encoding gene. Biosci. Biotechnol. Biochem., 64(1): 96-102. 115.Tsujibo, H., Miyamoto, K., Yoshimura, M., Takata, M., Miyamoto, J. and Inamori, Y. 2002. Molecular cloning of the gene encoding a novel beta-N- acetylhexosaminidase from a marine bacterium, Alteromonas sp. strain O-7, and characterization of the cloned enzyme. Biosci. Biotechnol. Biochem., 66(2): 471-475. 116.Tsujibo, H., Yoshida, Y., Miyamoto, K., Imada, C., Okami, Y. and Inamori, Y. 1992. Purification, properties, and partial amino acid sequence of chitinase from a marine Alteromonas sp. strain O-7. Can. J. Microbiol., 38(9): 891-897. 117.Ueda, M., Fujiwara, A., Kawaguchi, T. and Arai, M. 1995. Purification and some properties of six chitinases from Aeromonas sp. no. 10S-24. Biosci. Biotechnol. Biochem., 59(11): 2162-2164. 118.Ueda, M., Kawaguchi, T. and Arai, M. 1994. Molecualr cloning and nucleotide sequence of the gene encoding chitinase II from Aeromonas sp. 10S-24. J. Ferment. Bioeng., 78(3): 205-211. 119.Ueda, M., Fujta, Y., Kawaguchi, T. and Arai, M. 2000. Cloning, ncleotide sequence and expression of the β-N-acetylglucominidase gene from Aeromonas sp. No. 10S-24. J. Biosci. Bioeng., 89(2): 164-169. 120.Ueda, M., Kotani, Y., Sutrisno, A., Nakazawa, M. and Miyatake, K. 2005. Purification and characterization of chitinase B from moderately thermophilic bacterium Ralstonia sp. A-471. Biosci Biotechnol. Biochem., 69(4): 842-844. 121.Ulhoa, C. J. and Peberdy, J. F. 1991. Regulation of chitinase synthesis in Trichoderma harzianum. J. Gen Microbiol., 137(9): 2163-2169. 122.Usui, T., Hayashi, Y., Nanjo, F., Sakai, K. and Ishido, Y. 1987. Transglycolsy- lation reaction of a chitinase purified from Nocardia orientalis. Biochem. Biophys. Acta., 923(2): 302-309. 123.Vaidya, R., Roy, S., Macmil, S., Gandhi, S., Vyas, P. and Chhatpar, H. S. 2003. Purification and characterization of chitinase from Alcaligenes xylosoxydans. Biotechnol. Lett., 25(9): 715-717. 124.Vasseur, V., Arigoni, F., Andersen, H., Defago, G., Bompeix, G. and Seng, J. 1990. Isolation and characterization of Aphanocladium album chitinase-overproducing mutants. J. Gen. Microbiol., 136: 2561-2567. 125.Vyas, P. R. and Deshpande, M. V. 1989. Chitinase production by Myrothecium verrucaria and its significance for fungal mycelia degradation. J. Appl. Gen. Microbiol., 35: 343-350. 126.Vyas, P. R and Deshpande, M. V. 1991. Enzymatic hydrolysis of chitin by Myrothecium verrucaria chitinase complex and its utilization to produce SCP. J. Appl. Gen. Microbiol., 37: 267-275. 127.Wadsworth, S. A. and Zikakis, J. P. 1984. Chitinase from soybean seeds: purification and some properties of the enzyme system. J. Agric. Food Chem., 32: 1284-1288. 128.Wang, J. and Carlisle, J. A. 2006. Covalent immobilization of glucose oxidase on conducting ultrananocrystalline diamond thin films. Diamond & Relat. Mater., 15: 279-284. 129.Wang, S. L. and Chio, S. H. 1998a. Deproteinization of shrimp and crab shell with the protease of Pseudomonas aeruginosa K-187. Enzyme Microb. Technol., 22: 629-633. 130.Wang, S. L. and Chio, S. H. 1998b. Reversible immobilization of chitinase via coupling to reversibly-soluble polymer. Enzyme Microb. Technol., 22: 634-640. 131.Wang, S. L. and Hwang, J. R. 2001. Microbial reclamation of shellfish wastes for the production of chitinases. Enzyme Microb. Technol., 28(4-5): 376-382. 132.Wang, S., Moyne, A., Thottappilly, G., Wu, S., Locy, R. D. and Singh, N. K. 2001. Purification

and characterization of a Bacillus cereus exochitinase. Enzyme Microb. Technol., 28(6): 492-498. 133.Watanabe T, Kobori, K., Miyashita, K., Fujii, T., Sakai, H., Uchida, M. and Tanaka, H. (1993) Identification of glutamic acid 204 and aspartic acid 200 in chitinase A1 of Bacillus circulans WL-12 as essential residues for chitinase activity. J. Biol. Chem., 268: 18567-18572. 134.Wiwat, C., Siwayaprahm, P. and Bhumiratana, A. 1999. Purification and characterization of chitinase from Bacillus circulans No. 4.1. Curr. Microbiol., 39: 134-140. 135.Xia, G., Jin, C., Zhou, J., Yang, S., Zhang, S. and Jin, C. 2001. A novel chitinase having a unique mode of action from Aspergillus fumigatus YJ-407. Eur. J. Biochem., 268(14): 4079-4085. 136.Yamamoto, Y., Fukunaga, Y., Aoyagi, H. and Tanaka, H. 1995. Purification and characteristics of chitinase secreted by cultured Wasabia japonica cells. J. Ferment. Technol., 80(2): 148-152. 137.Yano, S., Rattanakit, N., Wakayama, M. and Tachiki, T. 2005. Cloning and expression of a Bacillus circulans KA-304 gene encoding chitinase I, which participates in protoplast formation of Schizophyllum commune. Biosci. Biotechnol. Biochem., 69(3): 602-609. 138.Young, M. E. and Carroad, P. A. 1981. Dependence of extracellular chitinase activity of Serratia marcescens QMB1466 on continuous culture dilution rate. Can. J. Microbiol. 27(1):142-144. 139.Yuli, P. E., Suhartono, M. T., Rukayadi, Y., Hwang, J. K. and Pyun, Y. R. 2004. Characteristics of thermostable chitinase enzymes from the Indonesian Bacillus sp. 13.26. Enzyme Microb. Technol., 35(2-3): 147-153. 140.Zhu, B. C. R., Lo, J. Y., Li, Y. T., Li, S. C., Jaynes, J. M., Gildemeister, O. S., Laine, R. A. and Ou, C. Y. 1992. Thermostable, salt tolerant, wide pH range novel chitobiase from Vibrio parahemolyticus: isolation, characterization, molecular cloning, and expression. J. Biochem., 112: 163-167.