The molecular biology of chitin digestion

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Chitinases catalyze the hydrolysis of chitin, an unbranched polymer of β-1,4-N-acetylglucosamine. In recent years, soil-borne microorganisms that produce chitinases are considered as potential biocontrol agents against fungi and nematodes which cause diseases of agricultural crops. Chitinases also play an important physiological and ecological role in ecosystems as recyclers of chitin, by generating carbon and nitrogen sources. Many chitinases of varied organisms have been isolated and their corresponding genes cloned.

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Abbreviations

BEC	barley endochitinase
GlcNAc	N-acetylglucosamine
MUF	4-methylumbelliferone,7-hydroxy-4-methylcoumarin
PNP	p-nitrophenol

Introduction

Chitin, a poly-β-1,4-N-acetylglucosamine (GlcNAc), is the second most abundant polymer in nature, after cellulose. Chitin is present in insects, crustaceans and in most fungi; plants, vertebrates and prokaryotes do not contain chitin. Chitinases, however, are synthesized by a vast array of organisms, including those who are not composed of chitin.

Chitinases can be classified in two major categories. Endochitinases (EC 3.2.1.14) cleave chitin randomly at internal sites, generating soluble low molecular mass multimers of GlcNAc, such as chitotetraose, chitotriose and the dimer di-acetylchitobiose [1]. Exo-chitinases can be divided into two subcategories: chitobiosidases (EC 3.2.1.29) [2], which catalyze the progressive release of di-acetylchitobiose starting at the nonreducing end of the chitin microfibril; and 1-4-β-N-acetylglucosaminidases (EC 3.2.1.30), which cleave the oligomeric products of endochitinases and chitibiosidases generating monomers of GlcNAc [1].

Based on amino acid sequence similarity (which is indicative of folding similaritity, if sequence similarity is high and dispersed over the entire sequence) of chitinases from various organisms, five classes of chitinases have been proposed. These classes can be grouped into two families of glycosyl hydrolases, family 18 and 19 [3,4]. Chitinases from classes I, II and IV are of plant origin and make up the family 19 glycosyl hydrolases [1,5••]. These chitinases share a homologous catalytic domain in addition to the signal peptide found in all of them. Class I chitinases consist of a cysteine-rich amino-terminal domain linked by a short glycine/proline-rich region (signal peptide) to the catalytic domain [6]. The cysteine-rich domain (also referred to as the wheat-germ agglutinin domain) is important for binding of chitin but not for catalytic activity [7]. Most of the class I chitinases contain a carboxy-terminal signal peptide that is essential for targeting into the plant cell vacuole [8]. Class II chitinases, found mainly in dicotyledons, lack the cysteine-rich domain and the carboxy-terminal vacuolar targeting signal, indicating that these chitinases do not bind chitin and are secreted to the apoplast [9]. Class IV chitinases, also identified mainly in dicotyledons, comprise a group of extracellular chitinases that share 41–47% sequence identity with class I chitinases in the catalytic domain and also contain cysteine-rich regions resembling chitin-binding domains; however, class IV chitinases are smaller because of deletions in both domains [10]. Phylogenetic analysis of chitinases from classes I, II and IV suggests a larger evolutionary distance between chitinases of class IV and those of classes I and II, suggesting a remote divergence between these classes [5••].

Class III chitinases are mainly plant and fungal in origin [5••]. Together with class V chitinases they make up the family 18 glycosyl hydrolases [3,4], which are structurally unrelated to family 19 [11]. Class III includes the bifunctional lysozyme/chitinase enzyme of *Havea brasiliensis* [12]. Class V is mainly comprised of bacterial chitinases; however, two class V proteins with endochitinase activity, resembling bacterial chitinases, have been isolated from tobacco [13].

This review will cover papers published in recent years that deal with the molecular biology of chitin digestion (i.e. the genes encoding chitinases), their regulation and structure.

Plant chitinases

The proposed role of plant chitinases is a defense mechanism against chitin-containing organisms. It has been observed that purified barley chitinases inhibit the growth of fungal hyphae [14]. Heterologous chitinase gene expression is used in various plants to enhance their defense mechanisms against fungal pathogens [15].

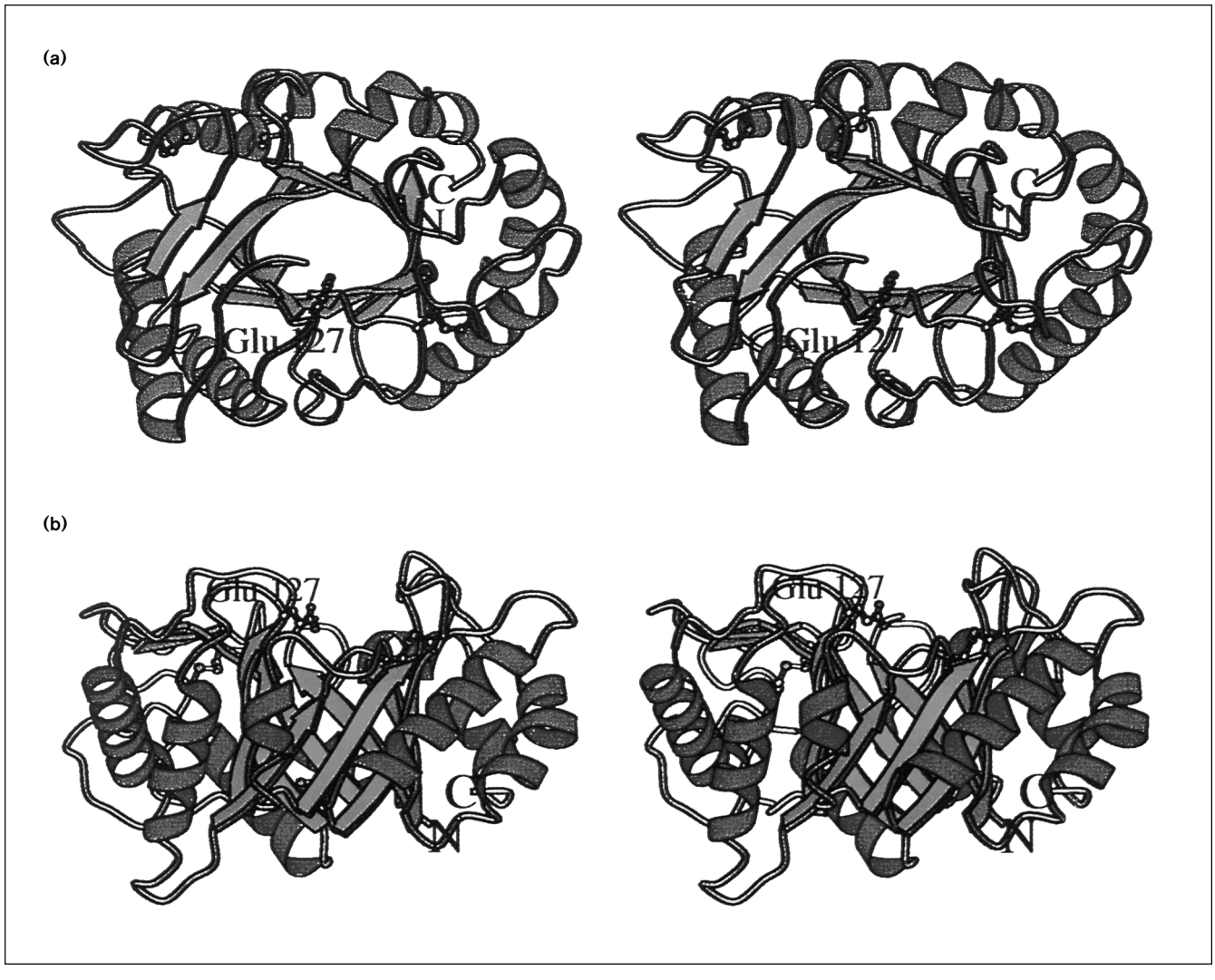
Plant chitinases use two different hydrolytic mechanisms. When the chitinase hydrolyze a β-1,4 linkage, the GlcNAc at the reducing end contains an asymmetric carbon 1, which exists as two different stereoisomers: configuration

α (hydrogen atom above the sugar ring plane) or β (OH group above the sugar ring plane). In chitin oligomers the β configuration is normally dominant. Chitinases of family 19, such as the 32 kDa bean chitinase (class I), invert the anomeric configuration of the hydrolysed GlcNAc residue. Chitinases of family 18, such as the 27 kDa cucumber chitinase (class III), retain the anomeric configuration of the hydrolysed residue [16].

In order to study the structure-function relationships of chitinases, the crystal structures of two prototypical plant chitinases, the 26 kDa endochitinase (class II) from *Horde volgare* L. (barley) [17] and the 29 kDa hevamine (class III) from *Hevea brasiliensis* (rubber tree) [18], have been determined (Figure 1). Hevamine, a chitinase/lysozyme with a $(\beta\alpha)_8$-barrel structure, belongs to family 18 of glycosyl hydrolases and retains the anomeric configuration of the hydrolysed GlcNAc residue [19]. It contains a substrate-binding cleft located at the carboxy-terminal end of the β-barrel, near Glu127, a conserved residue of the catalytic site [18] (Figure 1).

The barley endochitinase (BEC) is rich in α-helices and contains an elongated cleft that runs through the enzyme (Figure 2). The cleft is proposed to be the substrate-binding region [17]. A chitinase–substrate model was proposed in which a GlcNAc hexamer forms hydrogen bonds with several highly conserved residues stretching into the proposed active site cleft of BEC. Using lysozyme as a paradigm, Andersen *et al.* [20•] performed site directed mutagenesis of two glutamic acid residues suspected to constitute the catalytic site of BEC. These mutations

Figure 1



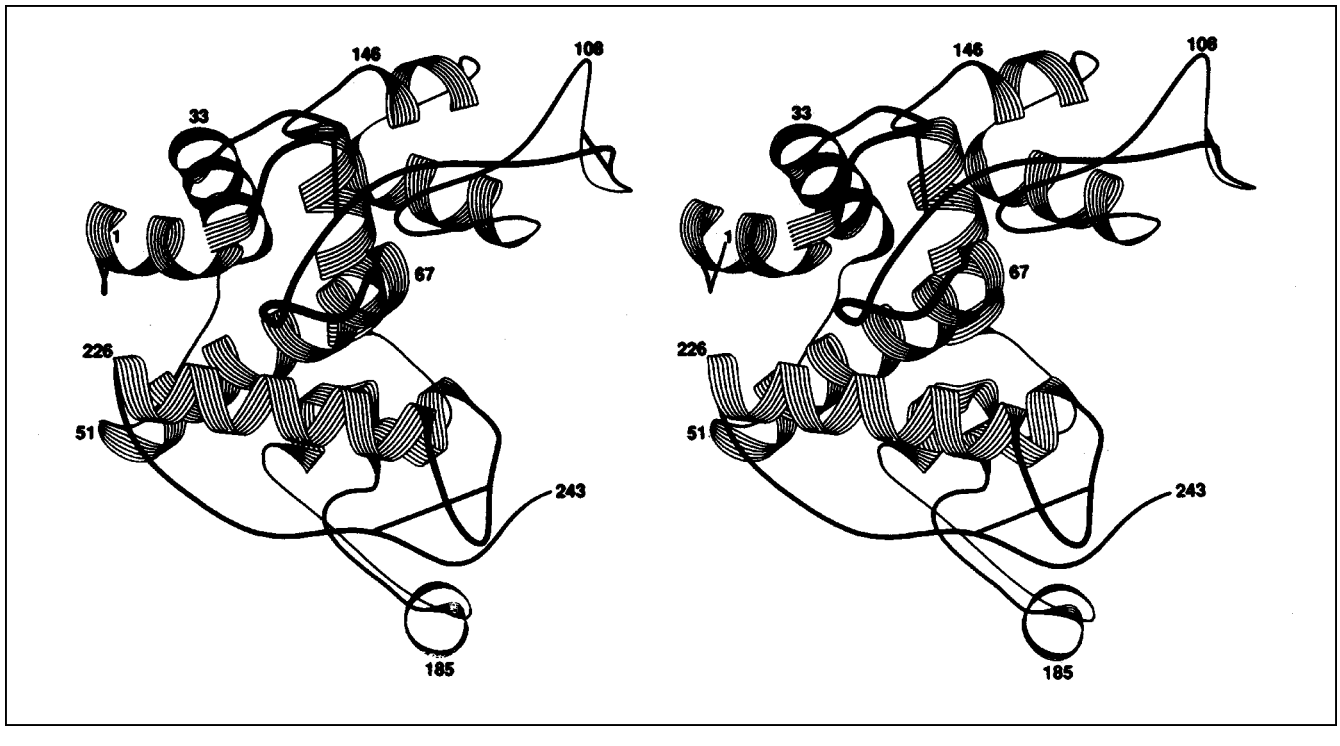
A stereoview ribbon diagram of the structure of hevamine (class III). The conserved Glu127 in the catalytic site and cysteine residues are shown in ball-and-stick representation. The amino and carboxyl termini are indicated. **(a)** Top view of the barrel. **(b)** Side view of the barrel, showing the cleft formed by the conserved loops at the carboxyl termini of the barrel strands. Adapted with permission from [18].

completely abolished or dramatically reduced the chitinase activity, indicating that the two glutamic acid residues, 22 amino acids apart, are essential for the catalytic activity of BEC. The authors suggested that one of the glutamic acid residues probably acts as the general acid catalyst, by analogy with other glycosyl hydrolases, while the other glutamic acid residue may function as a base, promoting a nucleophilic attack by water on the anomeric carbon of the chitinous substrate. This classifies BEC as an inverting glycosyl hydrolase (which justifies it belonging to family 19), where one of the two catalytic residues acts as a general acid and the other as a general base.

Most of the studies on plant chitinases have focused on their role as pathogenesis-related (PR) proteins. In other cases, however, chitinases and other enzymes produced by plants as part of a hypersensitive response are for the purpose of generating mycorrhizal associations with symbiotic fungi. Spruce (*Picea abies*) cells respond to elicitors from ectomycorrhizal fungi with an array of physiological phenomena. Salzer *et al.* [21••] demonstrated that two class I exochitinases of 28 and 36 kDa, which are produced by spruce cells, can inactivate fungal elicitors. The enzymes had no inhibitory effect on growth of the mycorrhizal fungi *Amanita muscaria*, *Hebeloma crustuliniforme* and *Suillis variegatus*. The inactivation of the elicitors by chitinases is most probably caused by cleavage of elicitor-active GlcNAc oligomers to inactive monomers. This indicates that constitutively expressed chitinases, which are localized in the apoplastic space of the host root, could degrade part of the chitinous elicitors (produced by mycorrhizal fungi) on their way across the plant cell wall before they reach their receptors in the plant plasma membrane. Thus, inactivation of fungal chitin-derived elicitors might be one of many instrumental preconditions required to create a compatible interaction between plant and fungus in the mycorrhiza.

Chitinases are expressed by plants in response to assorted environmental stimuli, such as fungal challenge [22•] and osmotic pressure [23], or developmental stage, such as fruit ripening [24•,25], a process induced by ethylene. The expression of chitinases under different environmental and morphological conditions is driven by specific promoter elements. In a study by Ficker *et al.* [26], the promoter of a gene encoding a pistil-specific endochitinase of *Solanum tuberosum* L. (potato) was cloned (SK2). The 1 kb promoter fragment was fused to a *GUS* reporter gene and two deletions were created (the first deletion was of 600 bp from the 5′ end of the promoter region [which was 1 kb long] and the second was of 770 bp from the 5′ end of the promoter region) in order to find the region that regulates the specific expression of the SK2 chitinase. A 370 bp region located 230 bp upstream of the translation start codon was found to contain the regulatory sequence sufficient for the high

Figure 2



A steroview ribbon drawing of the barley seed endochitinase (class II) backbone. The heavy lines represent three disulfide bonds, formed between cysteine residues. Adapted with permission from [17].

developmentally controlled expression of SK2 in the pistils; however, no unique sequences that could explain the pistil-specific expression were detected in this study.

In a promoter analysis of the gene encoding chitinase RC24 from rice (*Oryza sativa* L.), several elements were found that share high homology to sequences that bind an elicitor-inducible factor in the promoter of phenylalanine ammonia-lyase from parsley [27]. Indeed, expression of RC24 was up-regulated following treatment with fungal elicitors and wounding stimuli. It will be interesting to study whether these specific *cis*-acting elements can bind rice or parsley stress-inducible factors. If they do, it will indicate that there is a general elicitor-responsive element that regulates the expression of stress-responsive genes in plants.

Bacterial chitinases

Bacteria produce chitinases to meet nutritional needs. They usually produce several chitinases, probably to hydrolyze the diversity of chitins found in nature [28]. Bacterial chitinases belong to family 18 of glycosyl hydrolases. These enzymes operate by a mechanism leading to overall retention of the anomeric configuration of the hydrolysed residue [29]. It has been demonstrated that conserved glutamic acid and aspartic acid residues (four amino acids apart) of chitinase A1 of *Bacillus circulans* WL-12 were essential for the hydrolysis of chitin [30]. These residues are also conserved in ChiA of *Serratia marcescens*; however, the determination of the crystal structure of ChiA [31] demonstrated that the same glutamic acid residue but a more distant aspartic acid residue (76 amino acids distant) are the most likely amino acid residues to constitute the active site of the enzyme (Figure 3).

The chitin-binding domain in bacterial chitinases can be located either in the amino-terminal or carboxy-terminal domains of the enzymes [32].

A summary of the domain structure of chitinases from *Streptomyces* identified two major classes, the members of each class share homology in the signal peptide, chitin-binding and catalytic domains [33•]. Chitinase C of *Streptomyces lividans*, and chitinase 63 of *Streptomyces plicatus* share 26–29% identity with *Nicotiana tobaccum* class V chitinase and the same homology to ChiA of *S. marcescens* and chitinase A of *B. circulans*, suggesting that all these enzymes can be classified as belonging to class V chitinases. Alignment of promoter regions of chitinase genes isolated so far from *Streptomyces* showed that all the promoters contain a conserved sequence (TGGTCCAGACC) indicative of a binding site for a regulatory protein, and in some of the promoters this sequence is doubled, forming a direct repeat, indicative of two sites for the regulatory protein, which might bind as a dimer.

A comprehensive approach to elucidate the chitinolytic system of *S. marcescens* strain 7120 was carried-out using transposon mutagenesis [34•]. Mutants that produced aberrant clearing-zones on agar plates containing colloidal chitin were selected. This approach enabled the authors to isolate mutants that were defective in different genes necessary for the proper and full digestion of chitin. A preliminary screen distinguished mutants that were either damaged in the production of chitinases, impaired in the regulation of chitinase expression, or defective in chitinase excretion.

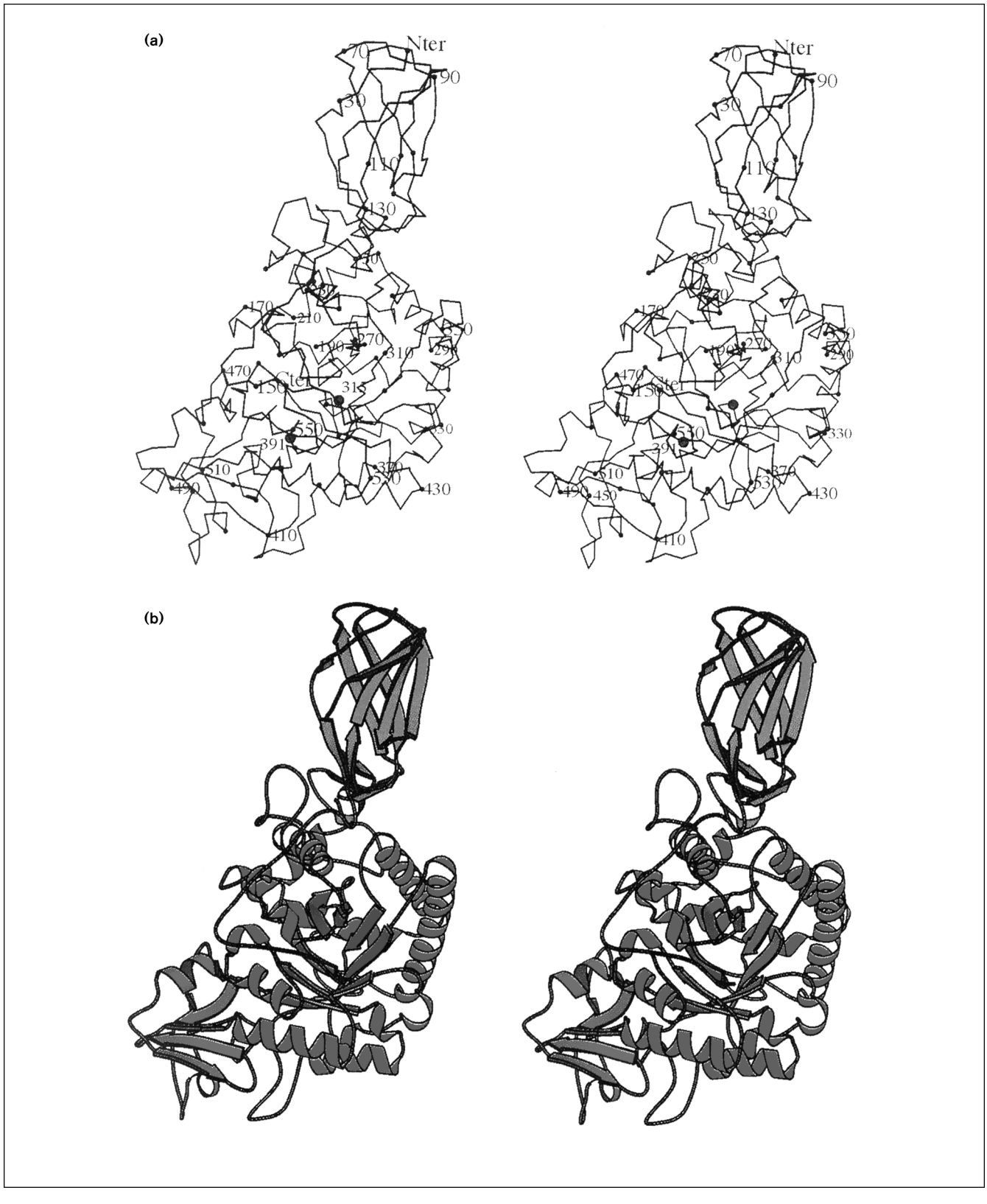
Most of the bacterial chitinases isolated and sequenced so far are included in family 18 of glycosyl hydrolases; however, there is one report of a chitinase (C-1) isolated from *Streptomyces griseus* HUT 6037 that belongs to family 19 of glycosyl hydrolases [35]. Until the isolation of this chitinase, family 19 contained exclusively chitinases of plant origin. Chitinase C-1 of *S. griseus* HUT 6037 can hydrolyze glucosamine–GlcNAc and GlcNAc–GlcNAc linkages unlike bacterial chitinases, which can hydrolyze only GlcNAc–GlcNAc and GlcNAc–glucosamine linkages (the residue at the non-reducing side of the linkage must be GlcNAc to be hydrolyzed). This observation suggested to the authors that the catalytic site of chitinase C-1 is different from that of other microbial chitinases. Indeed, a region from amino acid 82 to 294 of chitinase C-1 showed significant similarity to the catalytic domain of plant chitinases of classes I, II and IV, forming family 19 of glycosyl hydrolases. The amino-terminal region of chitinase C-1 was found to share sequence similarity with non-catalytic domains of other bacterial lytic enzymes such as chitinases, cellulases and proteases — it is postulated that this domain serves for chitin-binding [35]. Ohno *et al.* [35] also verified the belonging of C-1 to family 19 glycosyl hydrolases by demonstrating that it hydrolyzed chitotetraose ($[GlcNAc]_4$) causing inversion of the anomeric configuration, a mechanism that is distinctive of family 19 hydrolases.

So far, bifunctional chitinases that also have lysozyme activity have only been described in plants and are classified as class III chitinases. Two enzymes with bifunctional chitinase/lysozyme activity (designated chitinase I and II), however, were purified from *Pseudomonas aeruginosa* K-187 [36]. These chitinases had antibacterial activity towards both Gram-negative and Gram-positive bacteria. This is an interesting phenomenon because chitinases isolated so far from bacteria had antifungal activity, whereas these enzymes can antagonize bacteria as well.

Fungal chitinases

Fungal chitinases have multiple functions. Similar to the bacterial chitinases, they play a role in nutrition, but they are also active in fungal developmental processes and in morphogenesis, because chitin is a major cell wall component in fungi (reviewed in [1]). Chitinases also play a key role in the mycoparasitic activity of *Trichoderma* species against several plant pathogenic fungi, making *Trichoderma* a biocontrol agent [37–39].

Figure 3



Stereo representation of ChiA of *S. marcescens* (class V). **(a)** Cα trace, every 20th residue is numbered. The proposed active site residues, Glu315 and Asp391, are numbered and indicated by large dots. **(b)** Ribbon diagram of the structure in the same orientation as (a). Adapted with permission from [31].

Fungal chitinases share high amino acid homology with class III plant chitinases [5••,40]; however, *ech*42, encoding an endochitinase of *Trichoderma harzianum*, shares homology with bacterial chitinases [41] and with a tobacco class V chitinase, suggesting that it should be included in class V chitinases. Most fungal enzymes contain a signal peptide, some contain a chitin binding domain [42], whereas others do not [41].

Chitinases cloned from the mycoparasitic filamentous fungus *T. harzianum* are generally induced by chitin and repressed by glucose [40,43,44], suggesting that these chitinases are subjected to catabolite repression by glucose. The binding of Cre1, the carbon catabolite repressor, to promoter sequences of the endochitinase gene *ech*42 of *T. harzianum* was studied in the interaction of *Trichoderma* with the fungus *Botrytis cinerea*, a potent plant pathogen. The Cre1 repressor bound the promoter of *ech*42 when the two fungi were not in contact; however, when contact occurred the binding of Cre1 was relieved and a different mycoparasitic regulator bound the *ech*42 promoter, demonstrating that the *ech*42 gene is repressed in growth with glucose and positively regulated upon contact with host fungi [45].

Chitinases of marine organisms

Chitin is probably the most abundant biopolymer in the marine environment. Chitinases of marine organisms isolated so far share only little homology with other chitinases.

A thorough study of the chitinolytic system of the marine bacterium *Vibrio furnissii* was described in 1996 [46–49]. This bacterium can utilize chitin as a sole source of carbon and nitrogen. Three chitinases, designated ExoI, EndoI and ExoII, have been cloned from this bacterium. ExoII, a 36 kDa protein, was classified as an exo-chitinase capable of hydrolyzing p-nitrophenol (PNP)-β-GlcNAc and 4-methylumbelliferone,7-hydroxy-4-methylcoumarin (MUF)-β-GlcNAc. The substrate specificity of the enzyme shows that it is a unique β-N-acetylglucosaminidase. ExoII hydrolases $(GlcNAc)_n$ poorly compared to PNP-β-GlcNAc. Analysis of sequences in the data bank showed only one hexosaminidase homologue to ExoII, encoded by the *cht*60 gene from the marine bacterium *Alteromonas* sp. strain 0-7. The proposed function of ExoII is to hydrolyze phenolic-β-GlcNAc so that the phenol derivatives can serve as a signal to *V. furnissii* to invade its host. The other two chitinases of *V. furnissii* EndoI and ExoI, convert chitin oligosaccharides to GlcNAc and $(GlcNAc)_2$.

A marine bacterium, capable of degrading flake-chitin (as opposed to colloidal chitin), was isolated and identified as *Aeromonas hydrophila* [50]. This bacterium secretes five chitinases and one β-N-acetylglucosaminidase. Chi-A, the most active enzyme in flake-chitin degradation, was purified and characterized. The amino-terminal sequence of Chi-A showed extensive homology with chitinases from Gram-negative bacteria such as *Aeromonas caviae* and *Serratia marcescens*.

An interesting approach to the study of chitinase regulation as a result of environmental changes was carried by Techkarnjanaruk *et al.* [51•] with the marine bacterium *Pseudoalteromonas* sp. strain S9. The authors created a chitinase-negative mutant of S9 by use of transposon insertion, with a *lac*Z reporter gene (encoding β-galactosidase). The mutant expressed *lac*Z and showed no clearing zone around colonies on chitin-containing plates (which is indicative of a mutant, as chitinase-producing colonies cause a clearing zone to be produced on the opaque chitin-containing plates). The *lac*Z expression was derived from the interrupted chitinase promoter. The interrupted gene was cloned and sequenced and showed homology to bacterial chitinases such as chitinase A of *Clostridium thermocellum*, chitinase A precursor of *Bacillus circulans*, and a chitinase of *Kurthia zopfii*. The chitinase promoter was induced by chitin and GlcNAc. Expression was also induced in growth with 10% CO_2 or in cultures at stationary phase, where CO_2 levels were high. The chitinase expression was not induced as a function of other environmental stress conditions, such as exposure to UV, heat or cold. Catabolite repression was not observed when glucose was added as a carbon source in minimal medium.

The first chitinase sequence from crustaceans was described by Watanabe *et al.* [52]. They isolated a cDNA encoding a chitinase from the kuruma prawn *Penaeus japonicus* by PCR amplification of a hepatopancreas cDNA library using degenerate oligonucleotide primers derived from two conserved regions of known chitinases. The enzyme, designated Pjchi-1, showed limited homology to other eukaryotic chitinases, such as the chitinase precursor of the tobacco hornworm *Manduca sexta* and the endochitinase precursor in the nematode *Brugia malayi*.

Conclusions

Chitinases are produced by a large number of organisms. In all of those investigated so far, multiple different genes have been detected, probably to meet the need to efficiently digest the variable chitinous products in nature. This can explain why most of the molecular biology research carried out on chitin digestion so far concentrated on isolating chitinase-encoding genes and classifying them. Chitin digestion, however, is a regulated process and many questions regarding the regulation of this complex process still need to be elucidated. Chitinases play an important role in the defense mechanisms of plants against pathogens and in the mycoparasitic processes of fungi. Both plants and fungi can be engineered to express their own chitinases in a more effective manner. Chitinases can also be expressed in heterologous systems and confer better resistance against chitin-containing pathogens, or aid in the recycling of chitin in chitin-rich environments. In order to successfully achieve these goals, more information is needed on chitinases from various

sources but special efforts should be made to study specific regulatory elements and regulatory proteins that influence the expression of chitinases. We assume that this will be the future trend in the study of chitin digestion.

References and recommended reading

Papers of particular interest, published within the annual period of review, have been highlighted as:

- • of special interest
- •• of outstanding interest

1. Sahai AS, Manocha MS: **Chitinases of fungi and plants: their involvement in morphogenesis and host–parasite interaction.** *FEMS Microbiol Rev* 1993, **11**:317-338.
2. Harman GE, Hayes CK, Lorito M, Broadway RM, Di Pietro A, Peterbauer C, Tronsmo A: **Chitinolytic enzymes of *Trichoderma harzianum*: purification of chitobiosidase and endochitinase.** *Phytopathology* 1993, **83**:313-318.
3. Henrissat B: **Classification of glycosyl hydrolases based on amino acid sequence similarities.** *Biochem J* 1991, **280**:309-316.
4. Henrissat B, Bairoch A: **New families in the classification of glycosyl hydrolases based on amino acid sequence similarities.** *Biochem J* 1993, **293**:781-788.
5. •• Hamel F, Boivin R, Temblay C, Bellemare G: **Structural and evolutionary relationships among chitinases of flowering plants.** *J Mol Evol* 1997, **44**:614-624.

This paper presents analysis of the catalytic domains of chitinase sequences of flowering plants and investigates the evolutionary relationships between chitinase classes.

6. Perrakis A, Wilson KS, Chet I, Oppenheim AB, Vorgias CE: **Phylogenetic relationships of chitinases.** In *Chitin Enzymology*. Edited by Muzzarelli RAA. Ancona: European Chitin Society; 1993:217-232.
7. Iseli B, Boller T, Neuhaus J-M: **The N-terminal cysteine-rich domain of tobacco class I chitinase is essential for chitin binding but not for catalytic or antifungal activity.** *Plant Physiol* 1993, **103**:221-226.
8. Neuhaus J-M, Sticher L, Meins F Jr, Boller T: **A short C-terminal sequence is necessary and sufficient for the targeting of chitinases to the plant vacuole.** *Proc Natl Acad Sci USA* 1991, **88**:10362-10366.
9. Shinshi H, Neuhaus J-M, Ryals J, Meins F Jr: **Structure of a tobacco endochitinase gene: evidence that different chitinase genes can arise by transposition of sequences encoding a cysteine-rich domain.** *Plant Mol Biol* 1990, **14**:357-368.
10. Collinge DB, Kragh KM, Mikkelsen JD, Nielsen KK, Rasmussen U, Vad K: **Plant chitinases.** *Plant J* 1993, **3**:31-40.
11. Meins F Jr, Neuhaus J-M, Sperisen C, Ryals J: **The primary structure of plant pathogenesis- related glucanohydrolases and their genes.** In *Genes Involved in Plant Defence*. Edited by Boller T, Meins F Jr. New York: Springer Verlag; 1992:245-282.
12. Jekel PA, Hartmann JBH, Beintema JJ: **The primary structure of hevamine, an enzyme with lysozyme/chitinase activity from *Havea brasiliensis* latex.** *Eur J Biochem* 1991, **200**:123-130.
13. Melchers LS, Apotheker-de Groot M, van der Knaap JA, Ponstein AS, Sela-buurlage MB, Bol JF, Cornelissen BJC, van den Elzen PJM, Linthorst HJM: **A new class of tobacco chitinases homologous to bacterial exo-chitinases displays antifungal activity.** *Plant J* 1994, **5**:569-480.
14. Leah R, Tommerup H, Svendsen I, Mundy J: **Biochemical and molecular characterization of three barley seed proteins with anti-fungal properties.** *J Biol Chem* 1991, **266**:1564-1573.
15. Schickler H, Chet I: **Heterologous chitinase gene expression to improve plant defense against phytopathogenic fungi.** *J Ind Microbiol Biotechnol* 1997, **19**:196-201.
16. Iseli B, Armand S, Boller T, Neuhaus J-M, Henrissat B: **Plant chitinases use two different hydrolytic mechanisms.** *FEBS Lett* 1996, **382**:186-188.
17. Hart PJ, Monzingo AF, Ready MP, Ernst SR, Robertus JD: **Crystal structure of an endochitinase from *Hordeum vulgare* L seeds.** *J Mol Biol* 1993, **229**:189-193.
18. Terwisscha van Scheltinga AC, Kalk KH, Beintema JJ, Dijkstra BW: **Crystal structures of hevamine, a plant defence protein with chitinase and lysozyme activity, and its complex with an inhibitor.** *Structure* 1994, **2**:1181-1189.
19. Terwisscha VS, Armand CS, Kalk KH, Isogai A, Henrissat B, Dijkstra BW: **Stereochemistry of chitin hydrolysis by a plant chitinase/lysozyme and x-ray structure of a complex with allosamidin: evidence for substrate assisted catalysis.** *Biochemistry* 1995, **34**:15619-15623.
20. Andersen MD, Jensen A, Robertus JD, Leah R, Skriver K: • **Heterologous expression and characterization of wild-type and mutant forms of a 26 kDa endochitinase from barley (*Hordeum vulgare* L).** *Biochem J* 1997, **322**:815-822.

This paper describes the expression of a wild-type and site directed mutagenized chitinase gene in *E. coli* and the determination of the essential amino acids for the catalytic activity of the enzyme.

21. Salzer P, Hübner B, Sirrenberg A, Hager A: •• **Differential effect of purified spruce chitinases and β-1,3-glucanases on the activity of elicitors from ectomycorrhizal fungi.** *Plant Physiol* 1997, **114**:957-968.

This paper describes the purification of lytic enzymes from spruce cells and their role in the interaction with ectomycorrhizal fungi. The results are very interesting: only chitinases are involved in destroying the elicitors, which means that the elicitors are oligomers of chitin and not glucan.

22. Busam G, Kassemeyer H-H, Matern U: • **Differential expression of chitinases in *Vitis vinifera* L responding to systemic acquired resistance activators or fungal challlenge.** *Plant Physiol* 1997, **115**:1029-1038.

This paper describes the cloning of two chitinase genes and their use to probe the induction response of grapevine cells to salicylic acid and yeast elicitors.

23. Yun D-J, D'Urzo MP, Abad L, Takeda S, Salzman R, Chen Z, Lee H, Hasegawa PM, Bressan RA: **Novel osmotically induced antifungal chitinases and bacterial expression of an active recombinant isoform.** *Plant Physiol* 1996, **111**:1219-1225.
24. Clendennen SK, May GD: • **Differential gene expression in ripening banana fruit.** *Plant Physiol* 1997, **115**:463-469.

This paper describes the differential screening of cDNA libraries of banana pulp from different ripening stages and the isolation of differentially expressed mRNAs.

25. Robinson SP, Jacobs AK, Dry IB: **A class IV chitinase is highly expressed in grape berries during ripening.** *Plant Physiol* 1997, **114**:771-778.
26. Ficker M, Wemmer T, Thompson RD: **A promoter directing high level expression in pistils of transgenic plants.** *Plant Mol Biol* 1997, **35**:425-431.
27. Xu Y, Zhu Q, Panbangred W, Shirasu K, Lamb C: **Regulation, expression and function of a new basic chitinase gene in rice (*oryza sativa* L).** *Plant Mol Biol* 1996, **30**:387-401.
28. Svitil AL, Chadhain SMN, Moore JA, Kirchman DL: **Chitin degradation proteins produced by the marine bacterium *Vibrio harveyi* growing on different forms of chitin.** *Appl Environ Microbiol* 1997, **63**:408-413.
29. Armand S, Tomita H, Heyraud A, Gey C, Watanabe T, Henrissat B: **Stereochemical course of the hydrolysis reaction catalyzed by chitinases A1 and D from *Bacillus circulans* WL-12.** *FEBS Lett* 1994, **343**:177-180.
30. Watanabe T, Kobori K, Kiyotaka Miyashita K, Fujii T, Shakai H, Uchida M, Tanaka H: **Identification of glutamic acid 204 and aspartic acid 200 in chitinase A1 of *Bacilus circulans* WL-12 as essential residues for chitinase activity.** *J Biol Chem* 1993, **268**:18567-18572.
31. Perrakis A, Tews I, Dauter Z, Oppenheim AB, Chet I, Wilson KS, Vorgias CE: **Crystal structure of a bacterial chitinase at 23 Å resolution.** *Structure* 1994, **2**:1169-1180.
32. Watanabe T, Ito Y, Yamada T, Hashimoto M, Sekine S, Tanaka H: **The roles of the C-terminal domain and type III domains of chitinase A1 from *Bacillus circulans* WL-12 in chitin degradation.** *J Bacteriol* 1994, **176**:4465-4472.
33. Miyashita K, Fujii T, Watanabe A, Ueno H: • **Nucleotide sequence and expression of a gene (*chiB*) for a chitinase from *Streptomyces lividans*.** *J Ferment Bioeng* 1997, **83**:26-31.

This paper contains a schematic representation of the domain structures of chitinases of *Streptomyces* and an alignment of promoter sequences of these genes.

34. • Watanabe T, Kimura K, Sumiya T, Nikaidou N, Suzuki K, Suzuki M, Taiyoji M, Ferrer S, Regue M**: Genetic analysis of the chitinase system of *Serratia marcescens* 2170.** *J Bacteriol* 1997, **179**:7111-7117.
In this paper, a wider approach to studying a chitinolytic system was taken. The authors describe the analysis and classification of Tn5 mutants defective in several aspects of chitin digestion. This approach can detect not only the chitinases but also the genes that are important for the regulation of chitinase expression, rather than focusing on one gene, as is usually the case.

35. Ohno T, Armand S, Hata T, Nikaidou N, Henrissat B, Mitsutomi M, Watanabe T: **A modular family 19 chitinase found in the prokaryotic organism *Streptomyces griseus* HUT 6037.** *J Bacteriol* 1996, **178**:5065-5070.

36. Wang S-L, Chang W-T: **Purification and characterization of two bifunctional chitinases/lysozymes extracellularly produced by *Pseudomonas aeruginosa* K-187 in a shrimp and crab shell powder medium.** *App Environ Microbiol* 1997, **63**:380-386.

37. Elad Y, Chet I, Henis Y: **Degradation of plant pathogenic fungi by *Trichoderma harzianum.*** *Can J Microbiol* 1982, **28**:719-725.

38. Chet I: ***Trichoderma:* application, mode of action, and potential as biocontrol agent of soilborne plant pathogenic fungi.** In *Innovative Approaches to Plant Disease Control.* Edited by Chet I. New York: John Wiley & Sons; 1987:137-160.

39. Haran S, Schickler H, Chet I: **Molecular mechanisms of lytic enzymes involved in the biocontrol activity of *Trichoderma harzianum.*** *Microbiol* 1996, **142**:2321-2331.

40. Limn MC, Lora JM, Garcia I, de la Cruz J, Llobell A, Bentez T, Pintor-Toro JA: **Primary structure and expression pattern of the 33-kDa chitinase gene from the mycoparasitic fungus *Trichoderma harzianum.*** *Curr Genet* 1995, **28**:478-483.

41. Hayes CK, Klemsdal S, Lorito M, Di Pietro A, Peterbauer C, Nakas JP, Tronsmo A, Harman GE: **Isolation and sequence of an endochitinase-encoding gene from a cDNA library of *Trichoderma harzianum.*** *Gene* 1994, **138**:143-148.

42. Kuranda MJ, Robbins PW: **Chitinase is required for cell separation during the growth of *Saccharomyces cerevisiae.*** *J Biol Chem* 1991, **266**:19758-19767.

43. Carsolio C, Gutiérrez A, Jiménez B, Montagu MV, Herrera-Estrella A**: Characterization of *ech-42*, a *Trichoderma harzianum* endochitinase gene expressed during mycoparasitism.** *Proc Natl Acad Sci USA* 1994, **91**:10903-10907.

44. Peterbauer CK, Lorita M, Hayes CK, Harman GE, Kubicek CP: **Molecular cloning and expression of the *nag1* gene (N-acetyl-β-D-glucosaminidase-encoding gene) from *Trichoderma harzianum P1.*** *Curr Genet* 1996, **30**:325-331.

45. Lorito M, Mach RL, Sposato P, Strauss J, Peterbauer CK, Kubicek CP: **Mycoparasitic interaction relieves binding of the Cre1 carbon catabolite repressor protein to promoter sequences of the *ech42* (endochitinase-encoding) gene in *Trichoderma harzianum.*** *Proc Natl Acad Sci USA* 1996, **93**:14868-14872.

46. Keyhani NO, Roseman S: **The chitin catabolic cascade in the marine bacterium*Vibrio furnissii.* Molecular cloning, isolation and characterization of a periplasmic chitodextrinase.** *J Biochem* 1996, **271**:33414-33424.

47. Keyhani NO, Roseman S: **The chitin catabolic cascade in the marine bacterium *Vibrio furnissii.* Molecular cloning, isolation, and characterization of a periplasmic β-N-acetylglucosaminidase.** *J Biol Chem* 1996, **271**:33425-33432.

48. Bouma CL, Roseman S**: Sugar transport by the marine chitinolytic bacterium *Vibrio furnissii.* Molecular cloning and analysis of the glucose and N-acetylglucosamine permeases.** *J Biol Chem* 1996, **271**:33457-33467.

49. Chitlaru E, Roseman S: **Molecular cloning and characterization of a novel β-N-acetyl-D-glucosaminidase from *Vibrio furnissii.*** *J Biol Chem* 1996, **271**:33433-33439.

50. Hiraga K, Shou L, Kitazawa M, Takahashi S, Shimada M, Sato R, Oda K**: Isolation and characterization of chitinase from a flake-chitin degrading marine bacterium, *Aeromonas hydrophila* H-2330.** *Biosc Biotech Biochem* 1997, **61**:174-176.

51. • Techkarnjanaruk S, Pongpattanakitshote S, Goodman AE: **Use of a promoterless *lacZ* gene insertion to investigate chitinase gene expression in the marine bacterium *Pseudoalteromonas* sp. strain S9.** *Appl Environ Microbiol* 1997, **63**:2989-2996.
This paper describes the re-classification of a marine bacterium and the characterization of a chitinase gene from this bacterium by means of transposon-interrupted chitinase-negative *lac*Z+ mutant.

52. Watanabe T, Kono M, Aida K, Nagasawa H: **Isolation of a cDNA encoding a putative chitinase precursor in the kuruma prawn *Penaeus japonicus.*** *Mol Marine Biol Biotechnol* 1996, **5**:299-303.