Journal of Entomology and Zoology Studies

Available online at www.entomoljournal.com

E-ISSN: 2320-7078
P-ISSN: 2349-6800
JEZS 2019; 7(1): 837-843

Received: 25-11-2018
Accepted: 29-12-2018

Nirja Thakur
Ph.D., Research Scholar, Department of Biotechnology, Dr. Y S Parmar, UHF, Nauni, Solan, Himachal Pradesh, India

Amarjit K Nath
Professor, Department of Biotechnology, Dr. Y S Parmar, UHF, Nauni, Solan, Himachal Pradesh, India

Anjali Chauhan
Assistant Professor, Department of Soil Science & Water Management, Dr. Y S Parmar, UHF, Nauni, Solan, Himachal Pradesh, India

Subhash Chand Parmar
Ph.D., Research Scholar, Department of Biotechnology, Dr. Y S Parmar, UHF, Nauni, Solan, Himachal Pradesh, India

Himanshu Pandey
Ph.D., Research Scholar, Department of Biotechnology, Dr. Y S Parmar, UHF, Nauni, Solan, Himachal Pradesh, India

Kanika Thakur
Ph.D., Research Scholar, Department of Biotechnology, Dr. Y S Parmar, UHF, Nauni, Solan, Himachal Pradesh, India

Correspondence
Nirja Thakur
Ph.D., Research Scholar, Department of Biotechnology, Dr. Y S Parmar, UHF, Nauni, Solan, Himachal Pradesh, India

Chitinases from microbial sources, their role as biocontrol agents and other potential applications

Nirja Thakur, Amarjit K Nath, Anjali Chauhan, Subhash Chand Parmar, Himanshu Pandey and Kanika Thakur

Abstract
Chitinases are extensively dispersed enzymes involved in the bioconversion process of wastes from crustacean chitin, chitin mineralization and in plant defense. Chitinases as biocontrol agents are of considerable importance. Plants, animals and microorganisms are the main sources of chitinase enzyme. Though, enzymes from microbial origin have tremendous applications in industrial sectors. Chitin and its derived compounds are of great concern not only as an under-utilized bio-resource but also as a new functional material of high potential in various fields. Utilization of chitinases for diverse aspects depends upon the supply of cost effective preparations. The biochemistry and genetics of these enzymes, their phylogenetic relationships and methods of estimation will make them more practical in a wide range of processes in the near future. This review focuses on production of chitinases, their classification, degradation, biocontrol attributes and their use in industrial applications.

Keywords: Chitinases, biocontrol, degradation, production, applications

Introduction
Enzymes are biocatalysts that carry out an array of chemical reactions and have a wide range of physiological, analytical and industrial applications. Although enzymes have been isolated, purified and studied from microbial, animal and plant sources but microorganisms are the most common sources of enzymes due to their broad biochemical diversity, feasibility of mass culture and ease of genetic manipulation. Chitin is a β (1-4) polymer of N-acetyl-D-glucosamine (GlcNAc) and is the most vital structural component of numerous biological systems such as mollusks, insects, crustaceans, fungi, algae and marine invertebrates [1]. Chitin and its derivatives are of marketable and biotechnological interest. Chitin is a Greek word signifying envelope and was discovered in 1811, as a substance occurring in mushrooms. It is the second most abundant natural biopolymer on earth after cellulose.
Chitinase (EC 3.2.1.14) is the main chitin degrading enzyme. The chitin catabolism usually takes place in two main steps which involves the initial cleavage of the chitin polymer by chitinase into chitin oligosaccharides and subsequently further cleavage to N-acetyl glucosamine monomers by chitobiases [2]. Chitinases produced by diverse microoraganisms have gained increased interest due to their broad array of biotechnological application, especially in the creation of chitooligosaccarides and N-acetyl D-glucosamine, biocontrol of pathogen and pests, production of sphaeroplasts and protoplasts from yeast and fungal species and bioconversion of chitin waste to single cell protein. Therefore, application of chitin-hydrolyzing enzymes (chitinases) is anticipated for efficient employment of this abundant biomass. Particularly, chitinases are used in agriculture to control plant pathogens. Chitinases and chitin oligomers produced by enzymatic hydrolysis of chitin can also be used in human health care. Chitin is also used as a sole source of carbon and nitrogen in the media to isolate chitinase producing organisms. The omnipresence of chitin in the environment makes it significant to realize the role and the underlying metabolic processes of chitin turnover in the environment.

Classification
Chitinases are classified on the basis of mode of action as projected by International Union of Biochemistry and Molecular Biology (IUBMB, 1992) and they categorized them into two broad categories, endochitinase (E.C. 3.2.1.14), which arbitrarily hydrolyses chitin polymer to produce soluble low molecular weight polymers.

The other is exochitinase, which consists of two categories, chitobiase (E.C. 3.2.1.29), which catalyze the progressive release of di-acetylchitobiose starting at the non-reducing end of the chitin and β-N-acetylglucosaminidase (E.C. 3.2.1.52), which sequentially removes NAG units from the non-reducing end of the products produced by endochitinases and chitobiases with higher affinity to the latter (3, 4, 5). Chitinases can also be grouped into glycosyl hydrolase families (GH) 18, 19 and 20 on the basis of amino acid sequence similarity which are structurally dissimilar [6]. Fungal chitinases chiefly belongs to GH family 18 which in addition consists of chitinases from bacteria [3, 5]. Family 19 chitinases have frequently been documented in plants. Family 20 includes the β-N-acetylhexosaminidases from bacteria like *Vibrio harveyi*, Streptomycetes and humans [7]. Chitinases family 18 involves substrate-assisted catalysis, which retains the anomeric configuration of the product. Family 18 chitinases are frequently subdued by allosaminidin [3, 8]. Fungal chitinases can also be separated into three major subgroups, namely, A, B and C, based on the amino acid sequences of their GH 18 modules. These subgroups usually differ in the architectures of their substrate-binding site and thus, their catalytic activities (exo vs. endo) and also have divergent carbohydrate-binding modules (CBM) [7, 9]. Subgroup A chitinases has a catalytic domain, but no CBMs and has a standard molecular mass of 40–50 kDa. Subgroup B chitinases have uneven sizes and domain structures with molecular mass ranging from 30–90 kDa. Subgroup C is a new subgroup of fungal chitinases ranging from 140–170 kDa in molecular mass and has a chitin-binding domain (CBM 18).

Structure of chitinases

The chitinases of the two dissimilar families do not contribute to amino acid sequence similarity and have totally different three-dimensional (3D) structures [10] and molecular mechanisms. Consequently, they are predicted to have evolved from varied ancestors. Family 18 have a number of conserved repeats of amino acids. It includes an enzyme core having eight strands of parallel β sheets, forming a barrel laid down α helices, which in turn forms a ring towards outside [11]. A multi domain arrangement including catalytic domains and both a cysteine rich chitin-binding domain and a serine/ threonine rich glycosylated domain have been recognized as one of the structural characteristics of chitinases in a variety of animals and microorganisms [12]. The resemblance revealed by bacterial and fungal chitinases suggests that the catalytic domains are comparable in all of these [13].

An extensive study of chitin binding domains in plant proteins discovered that the eight cysteines inside the chitin binding domain are significantly conserved. Additionally, plants also have chitin binding proteins (CBPs) having a cysteine rich chitin binding domain, lacking chitinase activity [14]. In bacteria the chitin binding domain is dissimilar than that found in plants which contain eight conserved cysteine residues [15]. The function of chitinases is also in the binding of a non-catalytic chitin binding protein to chitin [16]. Watanabe *et al.*, [17] reported only four amino acids in the catalytic domain that are conserved between bacterial and plant class III chitinases. The fungal chitinases connects to their substrate or cell wall with the aid of the chitin binding domain [18]. A six cysteine conserved region present in the chitin binding domain of CTS1 and K1Cts1p is most likely concerned in the protein protein interaction or tertiary structure through the disulphide bond formation [18, 19]. Viterbo *et al.*, [20] reported that the C-terminal chitin binding domain in insect chitinases binds to the substrate and has a typical six cysteine motif parallel to nematode chitinases.

Chitin degradation

It is an extremely synchronized procedure and the hydrolytic enzymes are induced by products of the chitin hydrolysis, GlcNAc [21], or soluble chitin oligomers [22], depending on the organism under study. As compared to $(GlcNAc)_2$, GlcNAc has also been reported to act as a suppressor of chitinase expression in a streptomyces strain [23] and this may be because its chief origin in natural systems could be from murein in cell walls instead of chitin [24]. Additional factors more usually regulating the expression of these and other hydrolytic enzymes are nutrient regime and accessibility of other, more readily accessible growth substrates. Total lysis of the insoluble chitin polymer characteristically consists of three main steps (1) cleaving the polymer into water soluble oligomers (2) splitting of these oligomers into dimers and (3) cleavage of the dimers into monomers. The first two steps are frequently catalyzed by chitinases. The occurrence of chitinases in bacteria is prevalent among phyla and the productions of multiple chitin lytic enzymes by individual bacterial strains come out to be a common trait [25, 26, 27, 28, 29]. Chitinases are usually grouped into family 18 and 19 glycoside hydrolases. The latter are uncommon in bacteria apart from for some members of the genus *Streptomyces* [30, 31, 27, 28, 29]. It has been hypothesized that family 18 and 19 glycoside hydrolases have evolved discretely, as genes belonging to these two parallel gene families show little or no sequence homology, nor do they share the same molecular level catalytic mechanism [32, 33, 34]. The existence of multiple genes in a single organism may be the consequence of gene duplication or attainment of genes from other organisms via lateral gene transfer [35]. In support of the previous mechanism, different chitinase gene sequences found within single organisms are often almost indistinguishable. There are evidences where chitinase genes simultaneously existing in a single organism are very dissimilar and come together with chitinase sequences from somewhat indistinctly related organisms [27, 36, 37].

Chitin degradation is also affected by more cryptic factors. For instance, a chitin-binding protein devoid of any catalytic domain has been revealed to facilitate the degradation of β-chitin by degrading the crystalline chitin polymer structure [38]. The protein showed important sequence similarity to a gene product in *Streptomyces olivaceoviridis* known to have high affinity to α-chitin [39]. It has been proposed that the capability to fabricate such proteins with high specific affinity to a certain crystalline chitin structure may be crucial for the capacity of bacteria to differentiate and react to specific crystalline chitin structures [40]. Such chitin-binding domains may also affect chitin degradation in some way by facilitating adhesion of cells to chitinous substrates, an attribute that is of particular significance in aquatic environments [41, 42].

Chitinase production

Microbial chitinase has been produced by liquid batch fermentation, continuous fermentation, and fed-batch fermentation. Additionally, solid-state fermentation and biphasic cell systems have also been used for the production of chitinase. Microbes in general produce inducible chitinases. Components of media, carbon sources, nitrogen

sources and agricultural residues viz., rice bran, wheat bran, etc. also affect extracellular chitinase production [43, 44]. An attractive effect of glucose on chitinase production was reported by Bhushan [43] when glucose was used with chitin in the production medium. On the other hand, a suppressing effect of glucose on chitinase production was reported by Miyashita *et al*. [45]. A number of other physical factors such as aeration, pH and incubation temperature also influence chitinase production. The addition of amino acids and their analogs such as tryptophan, tyrosine, glutamine, and arginine (0.1 mM) in the growth medium enhanced chitinase production from *Bacillus* sp. BG-11 [43].

Several other methods, such as cell immobilization [43], biphasic cell systems [46], solid-state fermentations, etc., have been used for improving chitinase production from different microorganisms [43]. Enhanced production of extracellular chitinase by *S. marcescens* in an aqueous two-phase system (ATP) of PEG and dextran was reported by some workers [46]. They reported maximum chitinase activity of 41.5 units in ATPs [2% (w/w) PEG 20,000 and 5.0% (w/w) dextran T 500] compared with 13.6 units in a polymer-free system.

As biocontrol agent

Chitinases as previously discussed have been detected in enormous variety of organisms, including those that contain chitin, such as insects, crustaceans, yeasts and fungi and also organisms that do not have chitin, such as bacteria, higher plants and vertebrates. In arthropods, chitinases are concerned with molting and digestion. Insects shed their old cuticles and rebuilt new ones and this is mediated by the release of chitinases in the molting fluid that accumulates between the old cuticle and the epidermis. The products of hydrolysis are recycled for the formation of the new cuticle as the larvae ingest the old cuticle. It seems that, chitinases found in the gut have a digestive purpose in addition to their role in breaking down the chitin present in the gut lining [47]. The model of fungal cell wall growth projected by Bartnicki-Garcia [48] described the job played by lytic enzymes in maintaining a balance between wall synthesis and wall lysis during hyphal apical growth.

Chitinases in insect control

Insect pathogenic fungi have a substantial role for the biological control of insect pests of plants. The greater part of these fungi occur in the Deuteromycotina and Zygomycotina. Many attempts have been made to make use of the Deuteromycotina fungi for insect control. In this regard, the peritrophic membrane and exoskeleton of insects act as physicochemical barriers to environmental hazards and predators. However, entomopathogenic fungi overcome these kinds of barriers by producing multiple extracellular enzymes, together with chitinolytic and proteolytic enzymes that help out to penetrate the cuticle and facilitate infection [49, 50]. Venom of some insects is reported to contain chitinolytic enzymes that might serve to aid the entry of venomous components into prey [51]. Likewise, the nematode *Brugia malayi* utilizes chitinase to break down defensive chitinous extracellular sheath and the peritrophic membrane to get entry into the mosquito host [52, 53]. Baculoviruses also contain genes for chitinases, but their specific role(s) in host infection is uncertain [54]. However, hydrolytic enzymes used by insects, fungi and other organisms for molting or barrier diffusion are potentially helpful in pest management because their physiological action is to wipe out vital structures such as the exoskeleton or peritrophic membrane of insects. Chitinases appear to be occupied in the penetration of host cuticle by entomopathogenic fungi [55-57]. Virulent isolates of *N. rileyi* exhibit considerably higher levels of chitinase activity than avirulent strains at the time of cuticle penetration [49]. Chitinase gene expression in entomopathogenic fungi is supposed to be controlled by a repressor-inducer system in which chitin or the oligomeric degradation products serve as inducers [50]. On the other hand, bacterial chitinases were unsuccessful in assays in which insects were fed a diet containing the enzymes. No mortality of the nymphal stages of the rice brown plant hopper, *Nilapawata lugens*, occurred when 0.09% w/v *Streptomyces griseus* chitinase was supplemented to an artificial diet [58].

Chitinases in the control of phytopathogenic fungi

Chitinase production has also been coupled with the biocontrol of various soil borne fungal diseases [59]. Chitinase producing bacteria and glucanases showed opposition or antagonism *in vitro* against fungi [60]. Fungal growth inhibition by plant chitinases and termination of fungal cell walls by a streptomycete chitinase and β-(1, 3)-glucanase have been long established [61]. Mutants of *Serratia marcescens* with inactivated chiA gene resulted in loss of bio control efficacy [62]. A recombinant *Escherichia coli* expressing chiA gene from *S. marcescens* was effective in reducing disease incidence caused by *Sclerotium rolfsii* and *Rhizoctonia solani* [63]. Chitinase genes from *S. marcescens* have been expressed in *Pseudomonas* sp. and the plant symbiont *Rhizobium meliloti*. The modified *Pseudomonas* strain was revealed to control the pathogens *F. oxysporum* and *Gaeumannomyces graminis* var. tritici [64]. The anti fungal activity of the transgenic *Rhizobium* during symbiosis on alfalfa roots was confirmed by lysis of *R. solani* hyphal tips treated with cell-free nodule extracts [65].

Application of chitinases

There are numerous applications of chitinases. Some of them are discussed below (Fig.1).

Cytochemical localization

Chitin and chitosan are ubiquitous polymers of fungal cell walls. Even though biochemical investigation can offer accurate information about their structures, cytochemical localization studies can disclose the functional specialization of these polymers. Wheat germ agglutinin–gold complex and chitinase gold complex have been used as probes for the discovery of GlcNAc residues in the secondary cell walls of plants and in pathogenic fungi.

Production of single-cell protein

Revah-Moiseev and Carrod [66] recommended the use of shellfish waste for the bioconversion of chitin to yeast single-cell protein (SCP) using chitinolytic enzymes. They used chitinase system of *S. marcescens* to hydrolyze the chitin and *Pichia kudriavazevii* to yield SCP (with 45% protein and 8–11% nucleic acids). The frequently used fungi as the source of SCP are *Hansenula polymorpha*, *Candida tropicalis*, *Saccharomyces cerevisiae* and *Myrothecium verrucaria*. Vyas and Deshpande [67] utilized chitinolytic enzymes of *M. verrucaria* and *S. cerevisiae* for the production of SCP from chitinous waste. The total protein content was reported to be 61%, with very low contents of nucleic acids (3.1%).

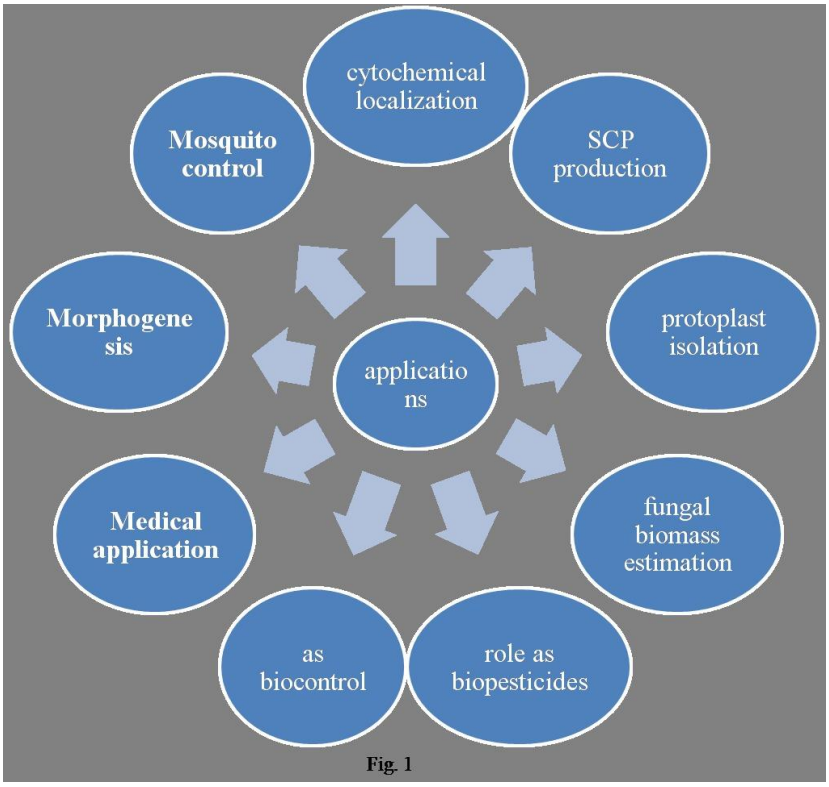

Fig 1: Application of chitinases

Isolation of protoplasts

Fungal protoplasts have been used as an efficient investigational apparatus in studying cell wall synthesis, enzyme synthesis and secretion, as well as in strain enhancement for biotechnological applications. As fungi have chitin in their cell walls, the chitinolytic enzyme seems to be indispensable along with other wall-degrading enzymes for protoplast development from fungi. Dahiya *et al*. [44] reported the efficiency of *Enterobacter* sp. NRG4 chitinase in the production of protoplasts from *Trichoderma reesei*, *Pleurotus florida*, *Agaricus bisporus*, and *A. niger*. Mizuno *et al*. [68] isolated protoplast from *Schizophyllum commune* using the culture filtrate of *B. circulans* KA-304. An enzyme complex from *B. circulans* WL-12 with elevated chitinase activity was efficient in generating protoplasts from *Phaffia rhodozyme* [69].

Production of chitooligosaccharides, glucosamine and GlcNAc

Chitooligosaccharides, glucosamines and GlcNAc have enormous pharmaceutical potential. Chitooligosaccharides are potentially helpful in human medicines. For instance, antitumor activity was shown by chitohexaose and chitoheptaose. *Vibrio alginolyticus* produced a chitinase that was used to prepare chitopentaose and chitotriose from colloidal chitin [70]. A chitinase preparation from *S. griseus* was used for the enzymatic hydrolysis of colloidal chitin. The chitobiose produced was subjected to chemical modifications to provide new disaccharide derivatives of 2-acetamido 2-deoxy d-allopyranose moieties that are possible intermediates for the synthesis of an enzyme inhibitor, *N*, *N*′-diacetyl-β-chitobiosyl allosamizoline [71].

Chitinase objective for biopesticides

The exoskeleton and gut lining of insects comprises of chitin. The molting chitinase enzyme has been discovered and described from *Bombyx mori* (silkworm), *Manduca sexta* (tobacco hawkmoth) and a number of other species. Likewise, chitinases have been caught up in different morphological events in fungi [72]. Allosamidin, a potent inhibitor of chitinase, was found to be inhibitory to the growth of mite (*Tetranychus urticae*) and a housefly larva (*Musca domestica*) after ingestion [73].

Fungal biomass estimation

A number of methods have been described to enumerate fungi in soil. This technique involves either direct microscopic surveillance or mining of fungus-specific indicator molecules such as glucosamine ergosterol. A strong connection has been reported between chitinase activity and fungal population in soils. Such connection was not found for bacteria and actinomycetes. Thus, chitinase activity is an appropriate indicator of actively growing fungi in soil. Miller *et al*. [74] revealed the association of chitinase activity with the content of fungus-specific indicator molecules using specific methylumbelliferyl substrates. Similarly, chitinase and chitin-binding proteins can be used for the discovery of fungal infections in humans [75].

Mosquito control

The universal socioeconomic aspects of diseases spread by mosquitoes made them possible targets for a variety of pest control agents. In case of mosquitoes, entomopathogenic fungus such as *Beauveria bassiana* could not infect the eggs of *Aedes aegypti*, a vector of yellow fever and dengue and other related species due to the aquatic environment. The scarabaeid eggs laid in the soil were found to be vulnerable to *B. bassiana* [76]. *M. verrucaria*, a saprophytic fungus, produces a total complex of an insect cuticle-degrading enzyme. It has been reported that both first and fourth instar larvae of mosquito *A. aegypti* could be killed within 48 h with the aid of the crude preparation from *M. verrucaria*.

Morphogenesis

Chitinases plays a significant role in yeast and insect morphogenesis. The role of chitinases in cell separation during growth in *S. cerevisiae*. The functional expression of chitinase and chitosanase and their effects on morphogenesis in the yeast *S. pombe*. When the *chiA* gene was expressed in *S. pombe*, yeast cells grew slowly and cells became elongated but when the *choA* gene was expressed, cells became swollen. However, expression of both *chiA* and *choA* genes resulted in elongated and fat cells.

Medical application

Chitinases can also be engaged in human health care, for making ophthalmic preparations with chitinases and microbiocides. A direct medical use has been recommended for chitinases in the therapy for fungal diseases in potentiating the activity of antifungal drugs [77]. They can also be used as possible additives in antifungal creams and lotions due to their broad applications.

Conclusion

Chitinase is an important enzyme that acts as biocontrol agents against insects and phytopathogenic fungi besides its other potential industrial applications. They are basically divided into two broad classes-exochitinases and endochitinases. The chitinases of two different families have completely different three-dimensional (3D) structures and molecular mechanisms. Chitin degradation by chitinase enzyme is a highly regulated process and is influenced by various factors. Commercially, the production of chitinase is carried out in the liquid batch fermentation, continuous fermentation, and fed-batch fermentation but solid-state fermentation can also be used. Several applications of chitinases have stimulated research in the study of biochemical, regulatory and molecular aspects of chitinolytic enzyme systems. Researchers are now interested in the discovery of novel enzymes that are more robust with respect to their kinetics and the identification of active site residues. In the future, protein engineering will offer the possibility of generating chitinases with entirely new functions.

References

1. Bhushan B. Production and characterization of a thermostable chitinase from a new alkalophilic Bacillus sp. BG-11. Journal of applied microbiology. 2000; 88(5):800-8.
2. Suginta W, Robertson PA, Austin B, Fry SC, Fothergill-Gilmore LA. Chitinases from Vibrio: activity screening and purification of chiA from Vibrio carchariae. Journal of applied microbiology. 2000; 89(1):76-84.
3. Matsumoto KS. Fungal chitinases. Advances in agricultural and food biotechnology. 2006; 6:289-304.
4. Hamid R, Khan MA, Ahmad M, Ahmad MM, Abdin MZ, Musarrat J *et al*. Chitinases: an update. Journal of pharmacy & bioallied sciences. 2013; 5(1):21.
5. Seidl V. Chitinases of filamentous fungi: a large group of diverse proteins with multiple physiological functions. Fungal Biology Reviews. 2008; 22(1):36-42.
6. Henrissat B, Davies G. Structural and sequence-based classification of glycoside hydrolases. Current opinion in structural biology. 1997; 7(5):637-44.
7. Dahiya N, Tewari R, Hoondal GS. Biotechnological aspects of chitinolytic enzymes: a review. Applied microbiology and biotechnology. 2006; 71(6):773-82.
8. Kasprzewska AN. Plant chitinases-regulation and function. Cellular and Molecular Biology Letters. 2003; 8(3):809-24.
9. Hartl L, Zach S, Seidl-Seiboth V. Fungal chitinases: diversity, mechanistic properties and biotechnological potential. Applied microbiology and biotechnology. 2012; 93(2):533-43.
10. Tews I, Terwisscha van Scheltinga AC, Perrakis A, Wilson KS, Dijkstra BW. Substrate-assisted catalysis unifies two families of chitinolytic enzymes. Journal of the American Chemical Society. 1997; 119(34):7954-9.
11. Di Giambattista R, Federici F, Petruccioli M, Fenice M. The chitinolytic activity of Penicillium janthinellum P9: purification, partial characterization and potential applications. Journal of applied microbiology. 2001; 91(3):498-505.
12. 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(12):1181-9.
13. Alam M, Nikaidou N, Tanaka H, Watanabe T. Cloning and sequencing of chiC gene of *Bacillus circulans* WL-12 and relationship of its product to some other chitinases and chitinase-like proteins. Journal of Fermentation and Bioengineering. 1995; 80(5):454-61.
14. Graham LS, Sticklen MB. Plant chitinases. Canadian Journal of Botany. 1994; 72(8):1057-83.
15. Poole DM, Hazlewood GP, Huskisson NS, Virden R, Gilbert HJ. The role of conserved tryptophan residues in the interaction of a bacterial cellulose binding domain with its ligand. FEMS microbiology letters. 1993; 106(1):77-83.
16. Seidl V, Huemer B, Seiboth B, Kubicek CP. A complete survey of Trichoderma chitinases reveals three distinct subgroups of family 18 chitinases. The FEBS journal. 2005; 272(22):5923-39.
17. Watanabe T, Kimura K, Sumiya T, Nikaidou N, Suzuki K, Suzuki M *et al*. Genetic analysis of the chitinase system of Serratia marcescens 2170. Journal of bacteriology. 1997; 179(22):7111-7.
18. Kuranda MJ, Robbins PW. Chitinase is required for cell separation during growth of Saccharomyces cerevisiae. Journal of Biological Chemistry. 1991; 266(29):19758-67.
19. Colussi PA, Taron CH. Kluyveromyces lactis LAC4 promoter variants that lack function in bacteria but retain full function in K. lactis. Applied and environmental microbiology. 2005; 71(11):7092-8.
20. Viterbo A, Montero M, Ramot O, Friesem D, Monte E, Llobell A *et al*. Expression regulation of the endochitinase chit36 from Trichoderma asperellum (T. harzianum T-203). Current Genetics. 2002; 42(2):114-22.
21. 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. Applied and environmental microbiology. 1997; 63(8):2989-96.
22. Keyhani NO, Roseman S. The Chitin Catabolic Cascade in the Marine Bacterium Vibrio furnissii molecular cloning, isolation, and characterization of a periplasmic chitodextrinase. Journal of Biological Chemistry. 1996; 271(52):33414-24.
23. Miyashita K, Shimizu N, Osanai S, Miyata S. Sequence

analysis and expression of the P450 aromatase and estrogen receptor genes in the Xenopus ovary. The Journal of steroid biochemistry and molecular biology. 2000; 75(2, 3):101-7
24. Benner R, Kaiser K, Abundance of amino sugars and peptidoglycan in marine particulate and dissolved organic matter. Limnology and Oceanography. 2003; 48(1):118-28.
25. Fuchs RL, McPherson SA, Drahos DJ. Cloning of a Serratia marcescens gene encoding chitinase. Applied and environmental microbiology. 1986; 51(3):504-9.
26. Romaguera A, Menge U, Breves R, Diekmann H. Chitinases of Streptomyces olivaceoviridis and significance of processing for multiplicity. Journal of bacteriology. 1992; 174(11):3450-4.
27. Saito A, Fujii T, Yoneyama T, Redenbach M, Ohno T, Watanabe T *et al*. High-multiplicity of chitinase genes in Streptomyces coelicolor A3 (2). Bioscience, biotechnology, and biochemistry. 1999; 63(4):710-8.
28. Shimosaka M, Fukumori Y, Narita T, Zhang XY, Kodaira R, Nogawa M *et al*. The bacterium Burkholderia gladioli strain CHB101 produces two different kinds of chitinases belonging to families 18 and 19 of the glycosyl hydrolases. Journal of bioscience and bioengineering. 2001; 91(1):103-5.
29. Tsujibo H, Kubota T, Yamamoto M, Miyamoto K, Inamori Y. Characterization of chitinase genes from an Alkaliphilic actinomycete, *Nocardiopsis prasina* OPC-131. Applied and environmental microbiology. 2003; 69(2):894-900.
30. Ohno T, Armand S, Hata T, Nikaidou N, Henrissat B, Mitsutomi M *et al*. A modular family 19 chitinase found in the prokaryotic organism Streptomyces griseus HUT 6037. Journal of bacteriology. 1996; 178(17):5065-70.
31. Watanabe T, Kanai R, Kawase T, Tanabe T, Mitsutomi M, Sakuda S *et al*. Family 19 chitinases of Streptomyces species: characterization and distribution. Microbiology. 1999; 145(12):3353-63.
32. Perrakis A, Tews I, Dauter Z, Oppenheim AB, Chet I, Wilson KS *et al*. Crystal structure of a bacterial chitinase at 2.3 Å resolution. Structure. 1994; 2(12):1169-80.
33. Davies G, Henrissat B. Structures and mechanisms of glycosyl hydrolases. Structure. 1995; 3(9):853-9.
34. Hart PJ, Monzingo AF, Ready MP, Ernst SR, Robertus JD. Crystal structure of an endochitinase from *Hordeum vulgare* L. seeds. Journal of molecular biology. 1993; 229(1):189-93.
35. Hunt DE, Gevers D, Vahora NM, Polz MF. Conservation of the chitin utilization pathway in the Vibrionaceae. Applied and environmental microbiology. 2008; 74(1):44-51.
36. Suzuki K, Taiyoji M, Sugawara N, Nikaidou N, Henrissat B, Watanabe T. The third chitinase gene (chiC) of Serratia marcescens 2170 and the relationship of its product to other bacterial chitinases. Biochemical Journal. 1999; 343(3):587-96.
37. Karlsson M, Stenlid J. Evolution of family 18 glycoside hydrolases: diversity, domain structures and phylogenetic relationships. Journal of molecular microbiology and biotechnology. 2009; 16(3-4):208-23.
38. Vaaje-Kolstad G, Horn SJ, van Aalten DM, Synstad B, Eijsink VG. The non-catalytic chitin-binding protein CBP21 from Serratia marcescens is essential for chitin degradation. Journal of Biological Chemistry, 2005.
39. Schnellmann J, Zeltins A, Blaak H, Schrempf H. The novel lectin-like protein CHB1 is encoded by a chitin-inducible Streptomyces olivaceoviridis gene and binds specifically to crystalline α-chitin of fungi and other organisms. Molecular microbiology. 1994; 13(5):807-19.
40. Svitil AL, Chadhain SM, Moore JA, Kirchman DL. Chitin Degradation Proteins Produced by the Marine Bacterium Vibrio harveyi Growing on Different Forms of Chitin. Applied and Environmental Microbiology. 1997; 63(2):408-13.
41. Montgomery MT, Kirchman DL. Induction of chitin-binding proteins during the specific attachment of the marine bacterium Vibrio harveyi to chitin. Applied and environmental microbiology. 1994; 60(12):4284-8.
42. Pruzzo C, Crippa A, Bertone S, Pane L, Carli A. Attachment of Vibrio alginolyticus to chitin mediated by chitin-binding proteins. Microbiology. 1996; 142(8):2181-6.
43. Bhushan B. Isolation, purification, characterization and scale-up production of a thermostable chitinase from an Alkalophilic microorganism. Panjab University, Chandigarh, 1998.
44. Dahiya N, Tewari R, Tiwari RP, Singh Hoondal G. Chitinase from Enterobacter sp. NRG4: Its purification, characterization and reaction pattern. Electronic Journal of Biotechnology. 2005; 8(2):14-25.
45. Miyashita K, Fujii T, Sawada Y. Molecular cloning and characterization of chitinase genes from Streptomyces lividans 66. Microbiology. 1991; 137(9):2065-72.
46. 46Chen JP, Lee MS. Enhanced production of Serratia marcescens chitinase in PEG/dextran aqueous two-phase systems. Enzyme and microbial technology. 1995; 17(11):1021-7.
47. Chen JP, Lee MS. Enhanced production of Serratia marcescens chitinase in PEG/dextran aqueous two-phase systems. Enzyme and microbial technology. 1995; 17(11):1021-7.
48. Bartnicki-Garcia S. Fundamental aspects of hyphal morphogenesis. In Microbe Differentiation 23rd Symposium of the Society of General Microbiology Cambridge University Press. 1973, 245-267.
49. El-Sayed GN, Coudron TA, Ignoffo CM, Riba G. Chitinolytic activity and virulence associated with native and mutant isolates of an entomopathogenic fungus, Nomuraea rileyi. Journal of Invertebrate Pathology. 1989; 54(3):394-403.
50. St Leger RJ, Cooper RM, Charnley AK. Cuticle-degrading enzymes of entomopathogenic fungi: regulation of production of chitinolytic enzymes. Microbiology. 1986; 132(6):1509-17.
51. Krishnan A, Nair PN, Jones D. Isolation cloning, and characterization of new chitinase stored in active form in chitin-lined venom reservoir. Journal of Biological Chemistry. 1994; 269(33):20971-6.
52. Huber M, Cabib E, Miller LH. Malaria parasite chitinase and penetration of the mosquito Peritrophic membrane. Proceedings of the National Academy of Sciences. 1991; 88(7):2807-10.
53. Shahabuddin M, Toyoshima T, Aikawa M, Kaslow DC. Transmission-blocking activity of a chitinase inhibitor and activation of malarial parasite chitinase by mosquito protease. Proceedings of the National Academy of Sciences. 1993; 90(9):4266-70.

54. Ayres MD, Howard SC, Kuzio J, Lopez-Ferber M, Possee RD. The complete DNA sequence of Autographa californica nuclear polyhedrosis virus. Virology. 1994; 202(2):586-605.
55. Leger RJ, Staples RC, Roberts DW. Entomopathogenic isolates of Metarhizium anisopliae, Beauveria bassiana, and Aspergillus flavus produce multiple extracellular chitinase isozymes. Journal of Invertebrate Pathology. 1993; 61(1):81-4.
56. Leger RS, Cooper RM, Charnley A. Characterization of chitinase and chitobiase produced by the entomopathogenic fungus Metarhizium anisopliae. Journal of Invertebrate Pathology. 1991; 58(3):415-26.
57. Coudron TA, Kroha MJ, Ignoffo CM. Levels of chitinolytic activity during development of three entomopathogenic fungi. Comparative biochemistry and physiology Part B: Comparative Biochemistry. 1984; 79(3):339-48.
58. Powell KS, Gatehouse AM, Hilder VA, Gatehouse JA. Antimetabolic effects of plant lectins and plant and fungal enzymes on the nymphal stages of two important rice pests, Nilaparvata lugens and Nephotettix cinciteps. Entomologia experimentalis et applicata. 1993; 66(2):119-26.
59. Buxton EW, Khalifa O, Ward V. Effect of soil amendment with chitin on pea wilt caused by Fusarium oxysporum f. pisi. Annals of Applied Biology. 1965; 55(1):83-8.
60. Schlumbaum A, Mauch F, Vögeli U, Boller T. Plant chitinases are potent inhibitors of fungal growth. Nature. 1986; 324(6095):365.
61. Oppenheim AB, Chet I. Cloned chitinases in fungal plant-pathogen control strategies. Trends in Biotechnology. 1992; 10:392-4.
62. Sundheim L. Biocontrol of Fusarium oxysporum with a chitinase encoding gene from Sermtia marcescens on a stable plasmid in Pseudomonas. J Cell Biochem. A. 1990; 13:171.
63. Sitrit Y, Barak Z, Kapulnik Y, Oppenheim AB, Chet I. Expression of Serratia marcescens chitinase gene in Rhizobium meliloti during symbiosis on alfalfa roots. Molecular plant-microbe interactions: MPMI (USA), 1993.
64. Revah-Moiseev S, Carroad PA. Conversion of the enzymatic hydrolysate of shellfish waste chitin to single-cell protein. Biotechnology and Bioengineering. 1981; 23(5):1067-78.
65. Vyas, PR, Deshpande MV. Enzymatic hydrolysis of chitin by *Myrothecium verrucaria*chitinase complex and its utilization to produce SCP Journal of General and Applied Microbiology. 1991; 37(3):267-75.
66. Mizuno K, Kimura O, Tachiki T. Protoplast formation from *Schizophyllum commune* by a culture filtrate of *Bacillus circulans* KA-304 grown on a cell-wall preparation of *S. commune* as a carbon source. Biosci. Biotechnol. Biochem. 1997; 61:852-857.
67. Johnson EA, Villa TG, Lewis MJ, Phaff HJ. Lysis of the cell wall of the yeast Phaffia rhodozyma by a lytic enzyme complex from Bacillus circulans WL-12 [feed supplement]. Journal of Applied Biochemistry, 1979.
68. Murao S, Kawada T, Itoh H, Oyama H, Shin T. Purification and characterization of a novel type of chitinase from Vibrio alginolyticus TK-22. Bioscience, biotechnology, and biochemistry. 1992; 56(2):368-9.
69. Terayama H, Takahashi S, Kuzuhara H. Large-scale preparation of N, N'-diacetylchitobiose by enzymic degradation of chitin and its chemical modifications. Journal of carbohydrate chemistry. 1993; 12(1):81-93.
70. Villagómez-Castro JC, López-Romero E. Identification and partial characterization of three chitinase forms in *Entamoeba invadens* with emphasis on their inhibition by allosamidin. Antonie Van Leeuwenhoek. 1996; 70(1):41-8.
71. Sakuda S, Isogai A, Matsumoto S, Suzuki A. Search for microbial insect growth regulators. The Journal of antibiotics. 1987; 40(3):296-300.
72. Miller M, Palojärvi A, Rangger A, Reeslev M, Kjøller A. The use of fluorogenic substrates to measure fungal presence and activity in soil. Applied and Environmental Microbiology. 1998; 64(2):613-7.
73. Laine RA, Lo WC. inventors; Louisiana State University, Mechanical College, assignee. Diagnosis of fungal infections with a chitinase. United States patent US. 1997; 5(587):292.
74. Ferron P. Fungal control. In: Kerkut GA, Gilbert LI (eds) Comprehensive insect biochemistry and pharmacology, Pergamon, Oxford, 1985; 12:313-346.
75. Kuranda MJ, Robbins PW. Chitinase is required for cell separation during growth of Saccharomyces cerevisiae. Journal of Biological Chemistry. 1991; 266(29):19758-67.
76. Shimono K, Matsuda H, Kawamukai M. Functional expression of chitinase and chitosanase, and their effects on morphologies in the yeast Schizosaccharomyces pombe. Bioscience, biotechnology, and biochemistry. 2002; 66(5):1143-7.
77. Pope AM, Davies DA. The influence of carbohydrases on the growth of fungal pathogens *in vitro* and *in vivo*. Postgraduate medical journal. 1979; 55(647):674-6.