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Diversity of chitinases and their industrial potential

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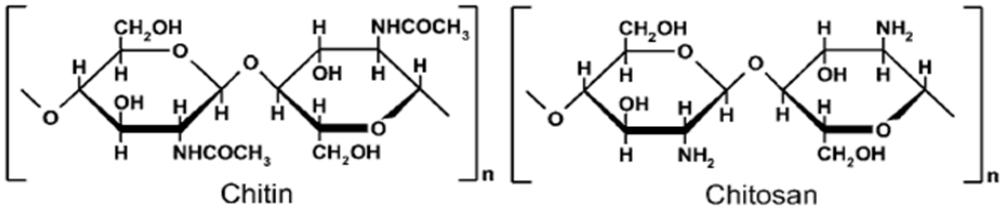
Abstract
Chitinases are of immense importance for their diversity and a variety of potential uses. They are found in almost all life forms including bacteria, fungi, plants, insects, mammals and viruses. The substrate of chitinases is primarily chitin which is a complex biopolymer resistant to degradation. Hydrolysis of chitin by chitinolytic enzymes leads to the production of monomers which are of tremendous industrial uses. Biologically active monomers of chitinase degradation are potentially useful as medicine. Different organisms produce a wide variety of chitinase enzymes that exhibit different substrate specificities and other properties useful for various functions. In bacteria, chitinases play roles in nutrition and parasitism whereas in fungi, protozoa, and invertebrates they are also involved in morphogenesis. Baculoviruses, which are used for biological control of insect pests, also produce chitinases for pathogenesis. Chitinases have been exploited for production of single cell proteins, preparation of bioactive oligosaccharides, pathogen inhibition and reinforcement of plant defense. Chitinase activity in human serum has recently been described. The possible role suggested is a defense against fungal pathogens. Chitinase production is markedly influence by time and pH in microorganisms.

Keywords: Chitinase, chitin, hydrolysis, chito-oligosaccharide, plant defense, single sell protein etc.

Introduction
Chitinase is present in chitin-containing microorganisms, bacteria and plants with diversity of roles such as chitin metabolism in growing hyphae, defense mechanisms in response to pathogens and abiotic stresses, in nutrition and parasitism. Apart from application of chitinases as inhibitors and biopesticides, the chitinases have been used for production of single cell proteins (SCPs) for animal and aquaculture feed, isolation of fungal protoplasts, preparation of bioactive chito-oligosaccharides, phytopathogen inhibition and strengthening plant defense responses.



The chitinases have not set biotechnological uses at commercial scale due to their high cost; however there is research in diverse fields like application of chitinases as biopesticides and for better understanding about antagonistic mechanisms. Chitinase genes have been reported, cloned and characterized from bacteria, yeasts, plants and fungi. Isolation of these chitinase genes from microorganisms have their uses as plant defense reinforcement in tobacco, rice, wheat etc. Chitinase belong to glycosyl hydrolase families 18 and 19 according to classification made by Henrissat and Bairoch. The activity and stability of enzyme preparation and its costs are the most important factors to be considered for chitinase applications, hence the necessity to study potential producers and inexpensive inducers.

Like cellulose, chitin is an abundant biopolymer that is relatively resistant to degradation. Chitin is an abundant renewable natural resource obtained from marine invertebrates, insects, fungi and algae. The
complete enzymatic hydrolysis of chitin to free *N*-acetylglucosamine (GlcNAc) is performed by a chitinolytic system, the action of which is known to be synergistic and consecutive. The studies of chitnolytic enzymes from plants, insects, and microorganisms with respect to their role and applications have been extensively reviewed. The present review brings together different aspects of the current knowledge on chitinolytic enzymes and their applications.

Chitinase substrate description

The substrate of chitinase is primarily chitin which is a crystalline biopolymer widely spread in nature with three forms viz. α, β and γ- chitins. Chitin is a polysaccharide composed of 1, 4 N- α acetyl- D glucosamine units. It is highly distributed in nature as a constituent of insect exoskeletons, shells of crustaceans and fungal cell walls. The main commercial sources for chitin production are crustacean wastes due to their abundance and disponibility. Among the limitations when using crustacean wastes, it is encountered that its perish ability and seasonal production and decomposition generally occurs before any possible use. The conventional methods of chitin production involve the use of strong alkalis and acids, making the process ecologically aggressive and a source of pollution. It also promoted certain degree of depolymerization, thus reducing the chitin quality. However yeilds can be low with the use of enzymes. Papain, pepsin, trypsin and pronase have been reported for deproteinisation. Also for this purpose, the use of proteolytic microorganisms such as *Pseudomonas maltophila, Bacillus subtilis*, *Streptococcus faecium, Aspergillus oryzae* etc.

α – chitin is the most abundant isomorphous form. It is tightly compacted due to its crystalline structure where the chains are in antiparallel fashion favoring strong hydrogen bonding. β–chitin has an arrangement in parallel with weaker intermolecular forces that leads to less stable molecule than the α-chitin. The third polymorphic form γ-chitin is a mixture of both α and β-chitins. α–chitins are not soluble and do not swell in common solvent, whereas β-chitins can be swollen in water as well as dissolved in formic acid.

Degrading enzymes: Types and classification

The chitinases of the family 18 have been found to possess a common $(\alpha/\beta)_8$– barrel domain consisting of 8 α- helices and 8 β- strands. They are distributed in wide range of organisms including bacteria, fungi, plants, insects, mammals and viruses. The catalytic reaction of the family 18 enzymes takes place through a retaining mechanism in which β- anomers are produced by hydrolysis of β-1,4-glycosidic linkages. The chitinases of the family 19 have been reported as similar to lysozyme and chitosanase. Their catalytic domains have high α- helical content. These chitinases have been found in plants and some *Streptomyces* strains. Substrate assisted catalysis is the most widely accepted model for catalytic mechanism of family 18 chitinases, whereas a general acid and base mechanism has been suggested to be the catalytic mechanism for family 19 chitinases. The family 18 chitinases are sensitive to allosamidin, a potent chitinase inhibitor but a family 19 chitinase from higher plants has been shown to be insensitive. Family 18 chitinases hydrolyse GlcNAc-GlcNAc and GlcNAc-Glc linkages, whereas family 19 chitinases hydrolyse GlcNAc-GlcNAc and GlcN-GlcNAc.

Chitinases are also classified according to the N-terminal sequence, localization of the enzymes, isoelectric pH, signal peptide and inducers. The class I chitinases are found in plants, whereas the class II enzymes are found in plants, fungi and bacteria. Class III chitinases do not show any sequence similarity to enzymes of either class I and class II. Class IV chitinases have similar properties to class I chitinases including immunological properties, but they are significantly smaller than the class I chitinases. Class V has been detected in studies on symbiotic plant-microbe interactions.

The enzymatic hydrolysis of chitin is carried out by a chitinolytic system classified into: endo-chitinases, exo-chitinases, chitibiase and βN-acetyl hexosaminidases. Endochitinases randomly cleave along the internal chain of chitin, producing low molecular oloigomers of N-acetyl glucosamine such as chitotetraose and chitotriose eventually giving diacetylchitobiose as predominant. On the other hand exo-chitinases release diacetylchitobiose without producing N-acetylglucosamine or oligomers. B N-acetyl hexosaminidases split diacetylchitobiose as well as chitotriose and chitotetraose into N-acetyl glucosamine monomers. Since βN-acetyl hexosaminidases also act on diacetylchitobiose, it is also called chitobiase. Some chitinases show lysozyme activity corresponding to the cleavage of a glycosidic bond between the C1 of N-acetyl muramic acid and the C4 of N-acetyl hexosamine in the bacterial peptidoglycan.

Chitinase sources

Plant chitinases: Chitinases are constitutively present in seeds, stems, tubers and flowers which are tissue specific as well as developmentally regulated. Plant chitinases are induced by the attacks of phytopathogens as pathogenesis related proteins (PRPs) in plant self defense or by contact with elicitors such as chitooligosaccharides or growth regulators such as ethylene. Isolation of genes of chitinases from *Trichoderma* spp. were reported and transferred to the plants in order to increase the resistance against phytopathogens. The chitinases of the plants can be detected in the early stage of growth during their development. This supports their role not only as mechanism of defense since they are also found in seeds. The chitinases of plants are generally endochitinases of smaller mol. wt. than the chitinases present in the insects. The chitinases inhibit the growth of the fungi synergistically with other enzymes like β-1, 3- glucanases which are present in potatoes, tobacco, citrus fruits, beans, tomatoes, yam, peas etc.

Insect chitinases: The chitinases present in the insects have been described from *Bombyx mori, Manduca sexta*. These enzymes play important roles in degradative enzymes during ecdysis where endochitinases randomly break down cuticle to chitooligosaccharides that afterwards are hydrolysed by exoenzymes to N-acetylglucosamine. The insect chitinases also have defensive roles against their own parasites. The production of enzymes in insects is

regulated by hormone during transformation of the larvae. Chitinases are also found in crustaceans such as prawn, shrimps and krills. Chitinases in crustaceans are induced before molting process in the integument. Chitinases are also expressed in the hepatopancreas for digestion of chitin containing foods. Koga *et al.* reported properties of purified chitinases from liver of prawn and these chitinases were identified as endochitinases.

Microbial chitinases: Chitinases are widely distributed in bacteria such as *Serratia, Chromobacterium, Klebsiella, Pseudomonas, Clostridium, Vibrio, Arthrobacter, Aeromonas, Streptomyces.* They are also found in fungi *Trichoderma, Bacillus, Penicillium, Neurospora, Mucor, Lycoperdon, Aspergillus, Beauveria, Myrothecium, Conidiobolus, Metharhizium, Stachybotrys* and *Agaricus.* Due to insolubility, size, molecular complexity and heterogeneous composition, the chitin is not degraded inside the cell, but microorganisms secrete enzymes with different specificity to transform or hydrolyse chitin. Microorganisms produce chitinases in higher amounts than animals and plants, generally as inducible extracellular that are of two types viz. endo- and exo-chitinases. Chitinolytic strains were screened and amongst the isolates, a bacterial isolate later identified as Bacillus licheniformis was found to produce maximum chitinase. Baculoviruses, whichare used for biological control of insect pests, also produce chitinases for pathogenesis. Chitinase activity in human serum has recently been described. The possible role suggested is a defense against fungal pathogens. Human chitinases may be related to allergies and asthma has been linked to enhanced chitinase expression levels.

Cell wall chitinases of fungi

The fungal cell wall is composed of chitin, 1,3-β- and 1,6-β glucan, mannan and proteins. During growth and morphogenesis there is breakage within and between polymers, conducted by the action of chitinases and glucanases. For instance, during budding of *Saccharomyces cerevisiae*, the degradation of chitin deposits at the bud site leads to the separation and involves the activity if of extensively glycosylated endochitinase with an apparent molecular mass of approximately 130 Kd. cst1 gene encoding this enzyme and most recently a second chitinases encoding gene cst2 were identified in *S. cerevisiae*. Disruption of cts1 and cts2 genes promotes psedohyphal growth and abnormal spore wall biosynthesis leading to the failure to form a matureasci respectively. The opportunistic fungal pathogen *Aspergillus fumigatus* contains atleast 11 conserved active site domains for chitinases of glycosyl hydrolase family 18. These enzymes may be divided into 'fungal/bacterial' chitinases which are similar to chitinases of bacteria and 'fungal/plant chitinases which resemble the chitinases from plants. 'Fungal/plant' chitinases are larger than 'fungal/bacterial chitinases. Disruption of genes encoding the chitinases of *A. fumigatus* ChiB1 which is differentially expressed during the growth, or the cst1 of *Coccidioides immitis* may contribute to the digestion and utilization of exogenous chitin as a sourse of organic nutrients for energy and biosynthesis. Many of the multiple chitinase-encoding genes of filamentous fungi may therefore encode secreted enzymes with nutritional roles rather than cell wall hydrolases for growth and morphogenesis. Mycoparasitic activity is related to the production of cell wall degrading enzymes (CWDE) such as β-1,3- glucanases, proteases and chitinases. The majority of known mycoparasitechitinase genes are from *Trichoderma harzianum, T. atroviride and T. virens*. Chitinase encoding genes have been cloned and characterized from *Trichoderma* spp. such as chit33, ech42, chit42 and nag1. The endochitinase gene ech42 and the protease encoding gene prb1 were highly expressed when *Trichoderma* strains were cultivated in chitin rich medium or in dual culture with a host. Furthermore transgenic plants containing ech42 and transformed *Trichoderma* trains carrying multiple copies of prb1 showed a higher resistance to several fungi. These results suggest that these genes are directly involved in mycoparasitism. Mrissette *et al* characterized a gene encoding endochitinase of 44 Kd sechi44 from *Stachybotrys elegans* which showed similarities with extracellular endochitinase for mycoparasitism*T. atroviride* ech42. The association of plants roots with mycorrhizal fungi *Hebeloma syrjense* secretes enzymes such as chitinases, phosphatases, phytases and proteases for conversion of organic residues to nutrients such as N and P. An endophytic fungi *Neotyphodium* infects grass *Poa ampla*, a fungal endochitinase was detected evidencing that might have a role in nutrition, growth or defense of nematodes.

Chitinase occurs naturally in many common foods. This is at least one cause of the cross-reaction phenomenon in latex-fruit syndrome. Bananas, chestnuts, kiwis, avocados, papaya, and tomatoes, for example, all contain significant levels of chitinase.

Production of fungal chitinases

Microbial chitinases are produced constitutively during growth and these enzymes are detected in low levels. Addition of chitin to the culture media induces ample production of chitinases. Besides chitin, several carbon sources act as inducers like glucosamine, N-acetylglucosamine, chitobiose, chitooligosaccharides depending on the chitinases producers. For example, with *Serratia marcescence*, chitobiose has been reported as inducer, however when it used N- acetylglucosamine the enzyme production is repressed. Oranusi and Trinci produced bacterial chitinases from *Vibrio alginolyticus, Streptomyces griseus, Arthrobacter, Bacillus and Cytophaga spp.* using colloidal chitin and fungal cell wall as carbon sources. Similar results were reported by Frandberg and Schnurer in *Bacillus pabuli.* In fungi, the chitinases expresssion follows a similar pattern as that of bacteria in terms of induction by chitin and repression by glucose in *Beauveria brassiana. Trichoderma harzianum, Aspergillus fumigates, Lecanicillium lecanii, Metharhizium anisopltae and Fusarium*. St. Leger *et al.* obtained higher levels of chitinases using chitin as sole carbon source and it decreased down to 86% with alanin as substrate. With cellulose the chitinolytic activity was not detected. Bidochka and Khachatourians found that high chitinolytic activity was detected when *B. brassiana* was cultivaterd with cuticle of insects which release N-acetylglucosamine to provide enough amounts of carbon and nitrogen sources for fungal growth. Fang *et al.* purified and characterized an endochitinase Bbchit1 from submerged culture of *B. brassiana* in a medium supplemented with chitin. *Lecanicillium* is another genus extensively studied in which mycoparasitic and entomopathogenic species are gathered

and they produce extracellular enzymes such as chitinases in their antagonistic mechanism. Different culture conditions were established in order to improve the enzyme production. The variation sources investigated were p H of culture, age of inoculum, type of fermentation- submerged (SF) or solid (SSF) including support and moisture content.

Time and pH of chitinase production:

Chitinolytic activity of filamentous fungi generally increases with culture time incubation, also it depends on other factors such p H, temperature and water availability. The enzyme production starts from 20 to 40 hrs during the exponential phase of the growths. It has been reported in *B. brassiana, A. fumigatus, Lecanicillium lecanii, L. ungicola, Metarhizium spp.* and *Fusarium spp. L. lecanii* produces chitinase in media with added colloidal chitin as sole carbon source starting from 24 hours although the maximum activity is reached at 72 hr. The addition of 0.2% glucose produced a delay in the time of production of about 24 hr. but it improved the yeilds. Matsumoto *et al.* increased the enzyme productivity when replaced colloidal chitin with raw chitin that was obtained from biological chitin purification with 14% of residual protein. *B. brassiana* was grown with insect cuticle as carbon source produced proteases during 48 hr. of culture and later on it begins to produce the chitinases (96 hr. of fermentation). St. Leger *et al.* inoculated mycelia of *M. anisopliae, B. brassiana* and *A. flavus* instead of spores, reducing the time of chitinase production from 30 to 40 hr. These authors detected first endo-chitinases followed by exo-chitinases. The chitinases production depends on the p H of the media. Usually this p H is in the same range as the one in which the enzyme displays its maximum activity. *Stachybotrys elegans* produced the maximum enzyme activity at p H 5, whereas with p H 8 and 9, the production diminished significantly. *L. lecanii* produced chitinases among p H 4 to 7 with the highest activity at p H 5. The p H affects the expression of the genes of chitinases in *M. anisopliae* that showed the maximum transcription at p H 5, although it was also detected at p H 8 but not at p H 3. The enzymatic activity of *L. fungicola* remarkably increased in liquid culture when the p H varied from acid to alkali. A variation of the enzymatic pattern was observed and therefore there was an increased production of endochitinase at low p H values whereas the proteolytic activity increased in alkaline media.

Chitinases production in solid state fermentation (SSF)

Solid state fermentation has gained importance recently in the production of microbial enzymes obtaining economic advantages over conventional submerged culture (SF). Several groups of microorganisms have been used in SSF, especially the filamentous fungi that have been exploited for their abilities to produce a wide range of extracellular enzymes and to grow on solid complex substrates. Several enzymes including amylases, cellulases, pectinases, proteases and glucoamylases have been produced in solid state fermentation.

However reports of chitinase production in SSF are scarce. Suresh and Chandrasekaran produced chitinases from *B. brassiana* using SSF in koji cultures and wheat bagasse as support and colloidal chitin as carbon source. The initial moisture content affected significantly the production of enzymes in SSF, being a critical factor in fungal growth and yield of chitinases. The temperature also has a strong influence on the yield of the chitinases and the time of the enzyme production. Another factor that presented a strong influence on the production of chitinases was the p H of the media. Maximum production was obtained at p H 9.6 and the second peak was observed at p H 6.

The chitinases production in SSF is constitutive and inducible as it is reported for SF. Colloidal chitin was added to the media in SSF and the chitinases production was significantly increased comparing with the media without inducers. The use of mycelia as inoculum, instead of spores that had been grown with raw chitin produced more chitinases in shorter time than with spores as inoculum of *L. lecanii* with sugarcane bagasse as support. Similar results were reported by Suresh and Chandrasekaran using mycelia instead of spores, the chitinases production increased from 117.2 U/g IDS to 109.2 U/g IDS. Also the time of production was reduced 20 hr. with mycelia.

Applications of chitinases

Physiological role of chitinases

Chitinases have been detected in a great variety of organisms, including those that contain chitin, such as insects, crustaceans, yeasts and fungi, and also organisms that do not contain chitin, such as bacteria, higher plants and vertebrates. In arthropods, chitinases are involved in molting and digestion. Insects periodically shed their old cuticles and resynthesize new ones. This process is mediated by the elaboration of chitinases in the molting fluid that accumulates between the old cuticle and the epidermis. The products of hydrolysis are recycled for the synthesis of the new cuticle. Often larvae will ingest the old cuticle. Apparently, chitinases found in the gut have a digestive function in addition to their role in breaking down chitin present in the gut lining.

Chitinases in biocontrol of plant pathogenic fungi and insects

Chitin is a structural component of the cell wall of phytopathogenic and entomopathogenic microbes. Chitin has been considered as a target molecule to be attacked by biological agents that might contain chitinases, chitin synthesis inhibitors or microorganisms as antagonists. For example, the chitinases from *Myrothecium verrucaria* that degrades the cuticle of mosquito *Aedes aegypti* and those from *B. brassiana* that attacks Galleria mellonella. Another application of chitinases from *Nomuraeariley* increasing virulence to larvas of *Trichoplusiani*. In biological control area, it is known that the plants have a complex defensive system to be protected by the attack of phytopathogenic fungi which include important elements like chitinases and β- 1, 3 glucanases. According to this premise, transgenic plants of tobacco have been produced containing both their own chitinases and also inserted chitinase gene from yam which shows higher resistance to phytopathogens. The genes of *Trichoderma* spp. were cloned and engineered to tobacco and potato plants and they became more resistant when combined with bioinsecticides. Chitinases have also been suggested to act against important fungal infections, although there are immunological constraints to take into account before using these enzymes like therapeutic agents. Biological control of some soil-borne fungal diseases has been correlated with chitinase production. Fungi- and

bacteria-producing chitinases exhibit antagonism against fungi, and inhibition of fungal growth by plant chitinases has been demonstrated. Insect pathogenic fungi have considerable potential for the biological control of insect pests. Entomopathogenic fungi apparently overcome physical barriers of the host by producing multiple extracellular enzymes including chitinolytic enzymes, which help to penetrate the cuticle and facilitate infection.

Chitinase as a target for biopesticides

Chitin is present in the exoskeleton and gut linings of insects. The insect molting enzyme, chitinase has been described from *Bombyx mori*, *Manduca sexta,* and several other species. Similarly, chitinases have been implicated indifferent morphogenetic events in fungi. The pseudotrisaccharide, allosamidin, is a potent inhibitor of chitinases from most of the sources. The allosaminidin was foundto be inhibitory after ingestion to the growth of mite, *Tetranychus urticae* and a larva of a housefly, *M. domestica*. However, there is no report for the inhibition of lepidopteran insects by oral or topical application. Nevertheless, chitinase inhibitors can be explored as potential biopesticides.

Production of chitooligosaccharides

There is a growing appreciation of the potential of biologically active chito-oligosaccharides. They act as elicitors of plant defense, involved in the signalling for root nodule formation, and are potentially useful in human medicine too. For example, chitohexaose and chitoheptaose showed anti-tumor activity. A chitinase from *Vibrio alginolyticus* was used to prepare chitopentaose and chitotriose from colloidal chitin. *N*,*N*9-Diacetylchitobiose has been widely used as a starting material for synthesis of biologically active compounds. A chitinase preparation from *S. griseus*was used for the enzymatic hydrolysis of colloidal chitin. The chitobiose produced was subjected to chemical modifications to give novel disaccharide derivatives of 2-acetamido-2-deoxy-D-allopyranose moieties that are potential intermediates for the synthesis of an enzyme inhibitor.

Single cell protein production

For the effective utilization of chitinous waste, a parallel concept used for cellulose bioconversion to single cell protein (SCP) was suggested by Revah–Moiseev and Carrod. They used chitinase from *S. marcescens* to hydrolyze chitinous material and yeast, *Pichiakudriavzevii* for SCP that was acceptable as aquaculture. Fungi, in general used as the source for SCP are *Hansenula polymorpha, M. verrucaria, Candida tropicalis, P. kudriavzevii, S. cerevisiae,* etc. The criteria used to evaluate SCP production are growth yield, total protein and nucleic acid contents. The protein contents in the organisms used were between 39 to 73% whereas the nucleic acid contents were 1–11%. The best reported was *S. Cerevisiae* that exhibited .60% protein and 1 to 3% nucleic acid contents. The *P. kudriavzevii* had 45% protein and 8 to 11% nucleic acid content. Vyas and Deshpand used *M. verrucaria* chitinase preparation for chitin hydrolysis and *S. cerevisiae* for SCP. The high *N*-acetylglucosaminidase activity in the culture filtrate of *M. verrucaria* yielded high levels of GlcNAc. Thetotal protein contents were reported to be 61% with very low contents of nucleic acids (3.1%).

Miscellaneous

Fungal protoplasts have gained substantial importance in the mycological research as well as in strain improvement program for biotechnological applications. One of the major components of the fungal cell wall lysing enzyme complex is chitinase/chitosanase. It has been seen using various mycolytic enzyme preparations singly or in combination for protoplast isolation that high chitinase levels permit effective fungal mycelia degradation. The lectins due to their specific monosaccharide-binding properties can be used to locate sugar residues in thin sections of plants and fungi. Similarly hydrolytic enzymes like chitinases can also be employed to locate fungal pathogens that have chitinous cell wall. The chitinase- gold complex can be used for this purpose. A wide spread ability to produce *N*-acetylglucosaminidase has been observed in fungi in presence and absence of added chitin in the growth medium. Although *N*-acetylglucosaminidase activity was found to be expressed in a limited number of bacteria. Therefore, *N*-acetylglucosaminidase activity measured on fluorogenic substrates of the soil samples was significantly correlated with the estimate of fungal biomass, based on the levels of phospholipid fatty acid and ergosterol. Similarly, using chitinase and chitin-binding protein a method has been suggested for the detection of fungal infection in humans.The enzyme like tannase is used in the food industry to remove unwanted tannins and for producing gallic acid that is used as preservative. The enzyme produced by *Aspergillus niger*, is strongly bound to the mycelium and its release by chemical and physical means is not efficient. Enzymatic hydrolysis of the cell walls using chitinase preparation was found to be effective in the recovery of tannase enzyme.

Futuristic considerations

The success in using chitinases for different applications depends on the supply of highly active preparation at a reasonable cost. Most of the suppliers use either natural microbial biodiversity or genetically engineered chitinase overproducing microbial strains to obtain efficient preparations. The use of chitinases for the biocontrol of plant pathogens, and for developing transgenic plants is one of the major applications. Chitinases can also be employed in human health care, such as making opthalmic preparations with chitinases and microbicides. The understanding of biochemistry of chitinolytic enzymes will make them more useful in a variety of processes in near future.

Concluding remarks

All together, allow us to envisage chitinases as an important factor in the development of improved agents and novel strategies for biological control. Further work on cloning and characterization of chitinases will provide us the tools and understanding needed to make better use of these genes. The potential of chitinases is likely to be enhanced by combining them with other bioactive peptides and lytic enzymes, such as glucanases, as is found in natural systems. Thus, special emphasis should be made of the use of combinatorial strategies. The enormous potential of genetic engineering will allow us to combine the natural responses of plants with transgenes of microbial and insect chitinases, other bioactive peptides and improved microbial bio control agents.

On the basis of the results of the previous studies reported in the production of enzymes, particularly chitinases, the research is being focused on the exploration of other extrinsic factors related to the regulation mechanism of induction-repression, morphology of fungi, on different size and shape of the supports, and their physiology of solid state and submerged fermentations. The role of chitinases in biological control and their potential use in the improvement of bio control agents and crop plants by genetic engineering is analysed in view of recent findings.

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