*Review Paper:*

# Chitinases as bio-pesticides: An eco-friendly approach for sustainable agriculture

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## *Abstract*

*The negative effect of the enormous utilization of synthetic pesticides on the earth and on human health has invigorated the quest for inviting practices to control plant ailments and pests. In the agriculture green revolution, the escalation of pesticide uses has conveyed into center the enduring perilous effect of such practices to general public health and the earth. Between them, bio-control, which depends on utilizing advantageous life forms or their products, grips the best potential and is viewed as an agriculture pest management. Chitinases are especially appealing to this field, subsequently they have fungicidal, insecticidal and nematicidal activities.*

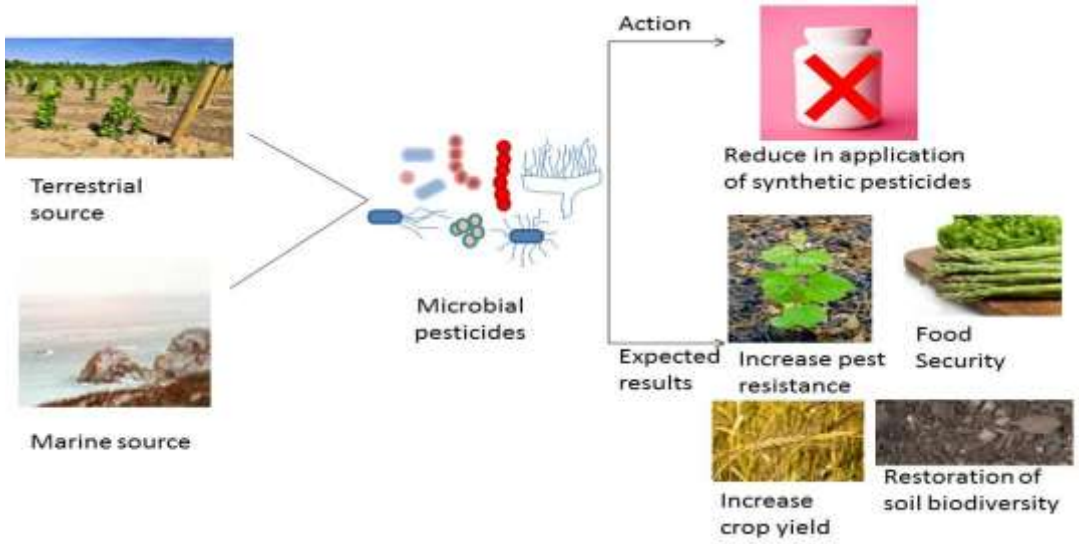
*On the other hand, utilization of chitinolytic microorganisms of agriculture significance for sustainable harvesting and ailment management is a successful methodology for supplanting conservative agrochemicals. The review uncovers extraordinary potential for further utilization of microbial derived chitinase in plant defense, bio-pesticides of microbes and their potential use as fungicidal, insecticidal and nematicidal agents.*

**Keywords**: Chitinase, Bio-pesticides, Microorganisms.

## Introduction

Agriculture has been fronting the negative actions of various pests such as fungi, weeds and insects since ancient times resulting in decreases of crop yield and production. So there is an incessant practice of use of fertilizers and chemical pesticides which has established a severe risk to mankind, ecosystem and soil microbiota. The pests may be exotic organisms, which intrude into new areas, either accidentally or through deliberate actions. As a consequence of global trade, the amount of hostile non-native species of pests in the new areas has increased. These antagonistic species pose a serious threat worldwide as they are becoming difficult to control[76].

Furthermore, degradation of xenobiotic is very slow leading to their bioaccumulation and bio-magnification through the food-chain and triggers the damage of biodiversityalong with ground-water pollution[50]. Hence, to produce agricultural products with high quality and enhanced yield, a green alternative is necessary. Therefore, a rapid demand for the development of bio-pesticides has begun. Bio-pesticides can efficiently curb agricultural pests without any ecological contamination or serious damage to the living organisms. They can be categorized into three different types: (a) micro-organisms, (b) bio-chemicals and (c) semiochemicals[16]. The bio-pesticides obtained from environmental sources have been categorized according to their sources such as microbial pesticides, zooid pesticides, botanical pesticides and genetically modified plants[16].




**Graphical abstract**

They are becoming ideal preference for control of pests because there is no damage in application area. Bio-pesticides have been more species specific than chemical pesticides. The advantage of the bio-pesticides is biological safety of non-target organisms. High performance with low residue, minimal side effects and compatibility with the ecosystem mark the characteristics of bio-pesticides. The targeted organisms do not easily develop resistance towards bio-pesticides, unlike in various cases of their chemical counterparts. Bio-pesticides are rapidly becoming a latest trend[76].

In the past few years, biological control of mycotoxin has established promising results in pre- and post- yield harvests on account of its eco-friendliness, specificity, ability to eliminate toxins, conserving the food character and feed with generation of less toxic residues. Furthermore, most of the microbial bio-control agents and growth promoters of plant have been sourced from soil plant tissue, soil surface and rhizosphere. A very few reports have been found with microorganisms sourced from marine environments which could have enhanced control of post-harvest diseases.[37,93]

Marine waters hold distinctive morpho-physiological characteristics of assorted microbial taxonomic groups which have not yet been widely assessed. The marine micro-organisms have the advantage to grow even in intense habitats due to their distinct metabolic and physiological characteristics and generate novel metabolites that are usually not found in terrestrial microbes. Numerous marine bacteria are chitinivorous i.e. they can consume chitin as the unique source of carbon and nitrogen.

Besides these attributes, marine microbes synthesize metabolites that could be powerful bio-control agents. The marine ecosystem, in current scenario, has been widely acknowledged as a resource loaded with novel bio-control agents and approaches have been initiated to investigate the feasibility of the marine strains in bio-controlling plant diseases[89]. The application of chitinases from marine bacteria and chitin oligomersin various fields such as medicine, biotechnology, agriculture, industries and waste management having insecticidal, antifungal, antimalarial, hypocholesterolemic, antihypertensive properties help to improve the food quality. Thus, chitinases have been gaining more attention for being used as the bio-control of fungal phyto-pathogens and for their capability to hydrolyze components of chitin in the fungal cell wall[108]. This review focuses on bio-pesticides, microbial chitinases, their mechanism of action and application in environmental friendly agricultural sectors. Moreover, it also suggests mycotoxin contamination levels using bio-pesticides.

## Bio-pesticides

Bio-pesticides refer to the pesticides derived from living organisms or their extracts. Their sales have speeded around the world because of unavailability and driven along environmental and health issues of synthetic pesticides. But certain reports specify the probable inflow of bio-pesticides into a new era of mainstream use instead of the niche market products[89]. The bacteriacidal, fungicidal, insecticidal and nematicidal activity of bio-pesticides are along with other secondary action like mammal and bird repellent.

The synergistic mechanisms revolving around the bio-control action of bio-pesticides include: (1) generation of antimicrobials and other secondary metabolites (e.g. *Pseudomonas spp.* produces phenazines, *Bacillus spp*. produces lipopeptides and *Rhizobia* produces hydrocyanic acid) and (2) secretion of lytic and protection catalysts (e.g. peroxidases, chitinases, glucanases, phenylalanine ammonia lyases and polyphenol oxidases formed by *Fusarium*, *Rhizoctonia*, *Serratia*, *Trichoderma*, *Bacillus strains* and *Streptomyces*).[57,85]

As a matter of fact, the second class microbial items constitute biochemical pesticides like environmentally happening compounds released via microorganisms or by their synthetic analogues. This class incorporates microbial secondary metabolites such as the bug spray Spinosad comprising macrolides from *Saccharopolyspora spinosa* and glucanases, proteases, lipases and chitinases as hydrolytic enzymes.

The above mentioned metabolites can either be used individually or in a combination; they even could be added to chemical pesticides which would favor their actions, hence limiting the use of synthetic pesticides on natural ecologies. Furthermore, some alive organisms with adhoc biochemical mixtures provide the advantage of enhancing each component when necessary (benefitting from the contemporary methods of metabolites and protein engineering), adjusting the total composition of various pests under different situations.

Bio-pesticides have four major classes:

1. Microbial Bio-pesticides
2. Plant Incorporated Protectant
3. Biochemical Bio-pesticides
4. Botanical Bio-pesticides

**Microbial Bio-pesticides:** Microbial bio-pesticides are those derived from naturally occurring bacteria, fungi (involving some protozoa and yeasts) and viruses. These compounds consist of a crucial part of the bio-pesticide industry[5]. Conventionally, microbial bio-pesticides have been manufactured through few small industries commercializing products to manage pests in specialty markets[55]. But, the medium and large companies have also initiated to adopt the trend and market to large number ofmicrobial bio-pesticides[5]. They target specificity and security to non-targeted organisms due to their lowered environmental toxicity, so the microbial bio-pesticides have gained much popularity.

In recent years, the commercial level production of microbial bio-pesticides has gained thrust in India. Overall 188 mycoinsecticides, 39 myconematicides, 51 bacterial insecticides and 27 nucleopolyhedrovirus products were registered by the CIBRC in the year 2017[78].

**Plant-incorporated Protectants (PIPs):** The second type of bio-pesticides contains plant-incorporated protectants synthesized via transgenes presented in genetically modified crops[94]. For the safety of cotton, corn, potato, plum soybean and papaya crops, almost 31 plant-incorporated protectant lively components have been accepted currently in US[80]. They generally contain Cry toxins derived from *B. thuringiensis* and a little amount of viral proteins[10]. However, PIPs need to be ingested and surpass both physical and digestive barriers and subsequently reaching the target site where they operate. There are existent systems that can overcome analogous challenges to achieve activity in target hosts by means of oral delivery and could be considered as models to develop PIP technologies[80].

The expression of viral, bacterial and fungal genes encoding for chitinases has been expressed into transgenic plants. Although it is possible to introduce plant-incorporated protectants in the EU, it is really restricted because of strict guidelines and low open acknowledgment over the utilization of hereditarily modified living organisms[43]. Plants produce pesticidal substances like plant-Incorporated protectants (PIPs) as a result of genetic manipulation.[5,57]

**Biochemical Pesticides:** These types of pesticides are composed of bio-chemicals obtained from naturally present substances produced by microbes or synthetically derivedequivalents that have minimal risk having non-toxic action mode on pests[5]. The secondary metabolites (insecticide Spinosad containing macrolides from *Saccharopolyspora spinosa*) and hydrolytic enzymes as glucanases, proteases, lipases and chitinases of microbes are included in this class. These substances can be brought into action individually or combinedly. They can be utilized in combination with synthetic pesticides with the possibility to improve their actions, hence lessening the involvement and effect of chemical pesticides on environment[10].

**Botanical Pesticides:** The use of substances derived from aromatic plants has shown promising results towards agricultural pests control. These plant derived compounds can be extremely efficacious with numerous action mechanisms as well as exhibit low rates of toxicity towards non-targeted organisms. But due to their poor stability and additional technological issues, the huge-scale function of these compounds for controlling pests is restricted.

## Chitinases

These are substances that hydrolyse chitin which is an insoluble linear homo-polymer of N-acetylglucosamine (GlcNAc) residues with β-(1,4)-glycosidic linkages. After lignicellulose, chitin is second abundant natural biopolymer on earth. It is broadly distributed in both terrestrial and aquatic ecosystem. Chitin plays a basic structural role for fungal cell wall, comprising upto 45% of its dry weight and situated at the inner cell wall in alliance with proteins and carbohydrates[35]. It makes the exoskeleton of insects and other arthropods have an important role in the structure and function of peritrophic matrix, a thin cellular sheath made up of chitin fibrils linked with proteoglycans and glycoproteins and lines the midgut epithelium of insects[36].

**Bacterial chitinase:** Several bacteria produce the chitinolytic enzyme which convert chitin to carbon and nitrogen that act as energy sources. Most bacterial chitinases are though belonging to the GH18 family, certain chitinases synthesized from actinomycetes and purple bacteria are found in GH19 family. These two families include enzymes that are different in position of their three-dimensional structural arrangement, sequence and catalytic action[3]. GH18 chitinases are characterized by a distinctive (β/α)8 triose phosphate isomerase barrel fold as well as a catalytic domain marked through the existence of highly conserved regions (D/N)G(L/I/V/M/F)(D/N)(L/IV/M/F)(D/N)xE, Y(D/N) and SxGG(10) . On the basis of the structure similarity of their catalytic domain, GH18 family is further branched into three sub-families namely A, B and C[61].

Chitinases are mainly found in the bacteria such as *Arthrobacter, Aeromonas, Serratia, Chromobacterium, Bacillus, Pseudomonas, Clostridium, Klebsiella, Vibrio, Beneckea* etc.[47] But the recent research aims on microbial-derivedchitinase that includes both gram-negative bacteria like *Aeromonas, Serratia and Pseudomonas;* and gram-positive bacteria like *Bacillus, Nocardia, Clostridium* and *Streptomyces*[84]. Many of the bacterial chitinases that have been characterized and derived from isolated chitin-degrading microbes (both terrestrial and aquatic habitats) cultured on solid media comprising chitinous substrates (colloidal or swollen chitin as fluorigenic or chromogenic engineered chitin analogs)[40].

**Fungal chitinase:** The chitiases found in eukaryotic and prokaryotes have essential role for pathogen inhibition in both terrestrial and marine environment. A surplus amount of genes presumably encoding chitinolytic enzymes are found in fungal genomes.[31,45]

These genes have numerous utilization for mycology with remodeling of cell wall during autolysis, growth, exogenous chitin hydrolysis to take nutrient and cell wall attack of competing fungi for ecosystem niches.[8,12,22,100] Mycoparasitic fungi, utilizing chitinases for attacking their fungal prey show the most intense type of competition.[31,71]

On the basis of their amino acid sequences, chitinases can be categorized into two glycoside hydrolase groups: 19 (GH19) and18 (GH18)[67]. The fungal chitinases absolutely belong to the GH18 whereas the latter is found in both plant and bacteria[46].

Moreover, according to their preferred types of cleavage, chitinolytic enzymes can be categorized into exochitinases and endochitinases. The chitin polymer is cleaved from the exposed ends by exochitinases, whereas the polymers are cut at arbitrary positions by endochitinase[1,39]. According to phylogenetics, fungal chitinases GH18 can be classified into 3 different groups viz. A, B and C which can be again divided into subgroups, A-II, A-IV, A-V, B-I to B-VI, C-I and C-II.[45,97] The enzymes of fungus belong to subgroup B-V code for encode endo-b-N-acetylglucosaminidases.[112,104,111]

**Viral chitinase:** At present, only few types of viral chitinases placed in GH18 family are acknowledged. These chitinases assist viral infection and/or release nascent viruses from the infected cells by deteriorating the structural barrier of host. ChiA from Auto graphical ifornicamulticapsidnucleopolyhedro virus (AcMNPV) is the most investigated enzyme which is closely related to *S. marcescens* ChiA.

This suggests that the chitinase genes might have been acquired by viruses from bacteria and conferred with both insecticidal and fungal properties. The other identified chitinases from viruses have been found to be encoded in genomes of *Cydiapomonella* , *Epinotiaaporema*[96] and *Pierisrapae*[82] granulo viruses, in Chlorella virus PBCV-1 and in the nucleopolyhedrovirus of *Dendrolimuskikuchii matsumara*[116]. Amusingly, this type of enzyme shows powerful insecticidal activity[116] (Table 1).

**Table 1**
**Microorganisms with pesticidal and fungicidal activity**

| Virus | Target insect species | Chitinase name | Expression system |
|---|---|---|---|
| *Clonostachysrosea* | *Sclerotiniasclerotiorum*<br><br>*Rhizoctonia solani* | Chi67- 1<br><br>CrChi1 | Homologous Host[29,106] |
| *Aspergillus terreus* | *Candida albicans*,*Rhizoctonia solani, Penicillium oxysporium, Fusarium solan*and *Aspergillus spp.* | na | Homologous host[27] |
| *Talaromyces flavus*CGMCC 3.4301 | *Verticillium dahliae, Magnaporthe grisea, Fusarium moniliforme, Alternaria alternata, Rhizoctonia solani and Sclerotinia sclerotiorum* | Chit41 | Homologous host[23] |
| *Fusarium Chlamydosporum* | *Puccinia arachidis* | na | Homologous host[73] |
| *Monascus purpureusCCRC31499* | *Fusarium spp.* | na | Homologous host[117] |
| *Thermomyces lanuginosus* | *Penicillium verrucosum* and *Aspergillus niger* | Chit2 | Homologous host[119] |
| *Trichoderma Harzianum* | *Botrytis cinerea, Rhizoctonia solani* and *Alternariaspp* | Chi42 | Heterologous expression (*Solanumtuberosum* and*Nicotianatabacum*)[70] |
| *TrichodermaAtroviride PTCC5220* | *Sclerotinia sclerotiorum, Alternaria alternate* and *Rhizoctonia solani* | Chimeric Chi42 | Heterologous host (*Escherichia coli*) – in mix with a CBM from *Serratiamarcescens*[74] |

| Bacteria | Target fungal species | Chitinase name | Expression system |
|---|---|---|---|
| *Aeromonashydrophila*SBK 1 | *Fusarium oxysporum* and*Aspergillus flavus* | na | Homologous host[33] |
| *Bacillus cereus 28-9* | *Botrytis elliptica* | Chimeric ChiCW | Heterologoushost (*Escherichia coli*) - in mix with a CBM from *Bacillus circulans*[10] |
| *Achromobacter xylosoxidans (Alcaligenes xylosoxidans)* | *Aspergillus niger* | na | Homologous host[10] |

| | | | |
|---|---|---|---|
| *Bacillus cereus IO8* | *Fusarium spp., Verticillium dahlia, Botrytis cinerea, Penicillium occitanis, Alternaria spp., and Aspergillus nidulans* | ChiO8 | Homologous host[34] |
| *Bacillus licheniformis NM120-17* | *Aspergillus spp.* | na | Homologous host[30] |
| *Bacillus thuringiensissub sp. colmeri* | *Physalospora piricola, Botrytis cinerea, Penicillium spp., Sclerotinia fuckelian* and *Rhizoctonia solani* | ChiA | Heterologous host (*Escherichia coli*)[63] |
| *Bacillus subtilis* E1R-J | *Botrytis cinerea, Bipolaris sorokiniana, Gibberellazeae* and *Valsa spp.* | Ep-2 | Homologous host[115] |
| *Bacillus Pumilus* | *Trichoderma harzianum* and *Rhizoctonia solani* | Chimeric ChiS | Heterologous host *(Bacillus subtilis)* - in mix with aspore coat protein from *Bacillus pumilu*[95] |
| *Paenibacillus elgii* HOA73 | *Botrytis cinerea and Cladosporium spp.* | PeChi68 | Heterologous host *(Escherichia coli)*[51] |
| *Serratia marcescens*B4A | *Alternaria spp. and Bipolaris* | Chit62 | Heterologous host *(Escherichia coli)*[6] |
| *Streptomyces coelicolor* | *Mucor javanicus, Trichoderma spp.and Fusarium solani* | Chi18b A and Chi19F | Heterologous host *(Escherichia coli)*[49] |

| **Virus** | **Target fungal species** | **Chitinase name** | **Expression system** |
|---|---|---|---|
| *Autographa californica multicapsid nucleopolyhedro virus* | *Alternaria alternate ChiA* and *Botrytis cinerea* | ChiA | Heterologous host *(Escherichia coli* or *Nicotiana tabacumcv. Samsun NN)*[20] |

**Microorganisms with insecticidal activity**

| **Fungus** | **Target insect species** | **Chitinase name** | **Expression system** |
|---|---|---|---|
| *Beauveria bassiana* | *Myzuspersicae* and *Galleria mellonell* | Chimeric BbChit1 | Homologous host - in mix with a protease *enzyme* from *B. bassiana*[26] |
| *Isaria fumosorosea* | *Plutellaxylostella* | IfChit1 | Homologous host[41] |
| *Trichoderma Viride* | *Bombyxmori* | Fusion ofminimum4 chitinases | Homologous host (commercial establishment)[9] |
| *Pochonia chlamydosporia* | *Bombyx mori* | PcChi44 | Heterologous host (*Escherichia coli*)[75] |
| *Metarhizium anisopliae* | *Dysdercus peruvianus* | Chi2 | Homologous host[12] |

| **Bacteria** | **Target insect species** | **Chitinase name** | **Expression system** |
|---|---|---|---|
| *Bacillus thuringiensis subsp. colmeri* | *Helicoverpa armigera* and *Spodoptera exigua* | ChiA | Heterologous host *(Escherichia coli)*[63] |
| *Pseudomonas fluorescens*MP-13 | *Helopeltis theivora* | na | Homologous host[105] |
| *Serratia marcescens*SEN | *Spodoptera litura* | Mixture oftwo chitinases | Homologous host[10] |
| *Yersinia Entomophaga* MH96 | *Adoryphorus couloni,Costelytrazealandica,Plutellaxylostella*and*Acrossidiustasmaniae* | Chi1 and Chi2 | Heterologous host *(Escherichia coli)*[13,42] |

| | | | |
|---|---|---|---|
| *Serratia marcescens* WW4 | *Helicoverpa armigera* and *Malacosoma Neustria* | ChiA, ChiBand ChiC | Heterologous host *(Escherichia coli)*[21] |

| Virus | Target insect species | Chitinase name | Expression system |
|---|---|---|---|
| *Dendrolimuski kuchii* Matsumara Nucleopolyhedro Virus | *Lymantria dispar,Helicoverpa armigera, Spodoptera exigua* | DkChi | *Heterologous host* (*Escherichia coli*)[116] |

**Microorganisms with nematocidal activity**

| Fungus | Target nematode species | Chitinase name | Expression system |
|---|---|---|---|
| *Pochoniachlamydosporia (Verticillium chlamydosporium)* | *Meloidogynein cognita (egg)* | PcChi44 | Heterologous host *(Escherichia coli)*[75] |
| *Isariajavanica (Paecilomycesjavanicus)* | *Meloidogynein cognita (egg, juveniles)* | PjChi-1 | Heterologous host (*Solanum lycopersicum*)[15] |
| *Monacrosporiumthaumasium* NF34 | *juveniles* of *Panagrellus redivivus (egg)* | Mixture of two chitinases | Homologous host[102] |
| *LecanicilliumPsalliotae (Verticilliumpsalliotae)* | *Meloidogyne incognita* | LpChi | Heterologous host (*Pichiapastoris*)[29] |

| Bacteria | Target nematode species | Chitinase name | Expression system |
|---|---|---|---|
| *Pseudomonasaeruginosa* | *Caenorhabditiselegans (adult, egg)* | $PaChi_{N35D}$ | Heterologous host *(Escherichia coli)*[18] |
| *Bacillus thuringiensissubsp. tenebrionis*YBT-9602 | *Caenorhabditiselegans (juvenile)* | Chi9602 | Homologous host[88] |
| *Pseudomonas aeruginosa* | *Caenorhabditiselegans (egg, adult)* | Chimeric PaChi | Heterologous host *(Escherichia coli)*-single or mixwith a δ- endotoxin crystal protein synthesizedfrom *Bacillus thuringiensis*[19] |
| *Bacillus thuringiensissubsp. tenebrionis*YBT-9602 | *Caenorhabditiselegans (juvenile)* | Chi960235 -459 (truncated and mutagenized form) | Hetrologous host *(Escherichia coli)*[81] |
| *Lysobactercapsici* YS1215 | *Meloidogyne incognita (juvenile, egg)* | na | Homologous host[59] |

na-Not available

## Protective mechanisms of chitinases

Chitinases have been stated to display various functions i.e. parasitism, defense, nutrition, immunity, morphogenesis, pathogenesis and growth regulation. Chitinases have unique affinity towards polymer chitin for degradation and result in low-molecular-weight COS (chitooligosaccharides) and GlcNAc (N-acetylglucosamine).

Chitinase can act as endo or exo on the basis of mode of action. Exochitinases (chitobiodidases and 1,4 β-glucosaminidases) display catalytic action from the non-reducing end of chitin whereas endochitinases arbitrarily act at an internal site of chitin chain[54]. The chitinase-induced degraded products; COS and GlcNAc are of chitin having more attention because they have massive utilization for enhancement of growth and plant protection.

Chitinase expression is promoted by hormones of plant such as jasmonic acid, salicylic acid and ethylene, these are resistant to pathogens. A lysine motif (LysM) carrying receptor protein (OsCEBiP) triggers immune responses on encounter with fungal chitin in rice.[44,64]

Chitinase, however can allow symbiosis interaction with mycorrhizal fungi or nitrogen-fixing bacteria[48]. *Vibrio cholera* releases chitinases and dwells in fish gut. *V.cholera* secrets chitinase and other relevant enzymes, thereby assisting the fish to assimilate chitin-containing prey[98] (Table 2).

**Table 2**
**Action mechanism of microbes**

| Microbes | Target pathogen species | Mechanism of action |
|---|---|---|
| *Bacillus megaterium* | *Aspergillusflavus* | Degradation of membrane chitin[52] |
| *Bacillus pumillus* | *Fusariumsolani* | Degradation of RST25 membrane chitin[32] |
| *Streptomyces* sp. | *Aspergillus* sp., *Fusarium* sp., *Penicilliumsp.* and *Pythiumsp.* | Degradation of chitin of cell wall[32] |
| *Trichodermasp.* | *Aspergillus* sp., *Rhizopus* sp. and *Mucorsp.* | Degradation of chitin of membrane[32] |
| *Pseudomonas fluorescens* | *Helopeltistheivora* | degrade the chitin present in the gut lining of insects[105] |
| *Bacillus velezensis* NKG-2 | *Fusariumoxysporum*, *Fusariumgraminearum*, *Botrytis cinerea*, *Alternariaalternata*, *Fulviafulva* and *Ustilaginoideavirens* | Degradation of fungal cell wall membrane[79] |
| *B. thuringiensi subsp. colmeri* | *Spodopteraexigua andHelicoverpaarmigera* | degrade the chitin present in the gutlining of insects[76] |
| *B. thuringiensissubsp. aizawaiandits* | *Rhizoctoniasolani, Penicilliumchrysogenum, Physalosporapiricola* and *Botrytis cinerea* | Inhibits sporegermination of fungi[77] |
| *Bacillus subtilis*BCC 6327 | *Aspergillusflavus* | Hydrolyze dried myceliumof alfatoxigenic fungi[109] |
| *B. megaterium*MB3, *B. subtilis*MB14,*B. subtilis*MB99 and *B. amyloliquefaciens*MB101 | *Rhizoctoniasolani* | Inhibit the growth of fungus[109] |
| *Brevibacilluslaterosporus* | *Plutellaxylostella* | Inhibit the growth of insect larvae[87] |
| *Pseudomonas sp.* | *Collectotrichumgleosporioides, Alternariabrassicola, A. brassiceae and A. alternate* | Lysis of fungal cellwall[76] |

*Pichiaanomala* produces exo-chitinase and ß-1, 3- glucanase which displayed the antifungal activity against aflatoxigenic *Aspergillus flavus* growth inhibition by hyphallysis. Moreover, *W. anomalus* species are available commercially as biological control products to manage an extensive series of post-harvest fruit diseases[38].

Chitinases from prokaryotes differ extensively in their size ranging from 20 to ~90 kDa. They are active above the pH 4.0 and wide range of temperature, depending on the source from where bacteria have been isolated[101].

**Mechanism of Bacterial chitinases:** Recent research mostly aims on bacteria for microbial-derived chitinases for example both gram-negative bacteria such as *Pseudomonas*, *Serratia* and *Aeromonas* and gram-positive bacteria such as *Clostridium*, *Nocardia*, *Bacillus* and *Streptomyces*[110]. Certain researchers are also working on efficient production of chitinase or improving the chitinase expression levels by utilizing heterologous expression systems.[68,113]

Till date, the chitinase expression have been observed successfully in *Escherichia coli*[56,83], *Pichiapastoris*[58,66] and *Manducasexta* from various sources[4]. Heterologous expression of proteins in *Escherichia coli* is however vulnerable to inclusion bodies formation.

The necessity to mechanically disrupt cell for the expression of intracellular enzymes might lead to inactivation of enzyme or lowered enzyme functions and hamper chitinase production and application in huge-scale.

Swiontek et al[107] reviewed that dual culture assays of chitinolytic bacteria can inhibit *in vitro* fungal growth and hence it enhances inhibition of phytopathogenic fungi by a greenhouse experiment results disease free plant growth.

Although a lot of mechanisms are involved concurrently in the procedure but sometimes difficult to understand the relation between antifungal activity and chitinase secretion. Due to the presence of chitinases, the antifungal activity of live bacteria has been confirmed. In one case, for the treatment of cotton infected with the plant pathogen *R. solani*, three *Enterobacter agglomerans* were tested to secrete atleast four different chitinases[10].

Chitinases from bacteria have also been suggested as prospective biocontrol agent against insects[7]. The mortality rate went higher when broth culture of *S. marcescens* strain SEN was added to the diet as the broth contains minimum 2 chitinases active at alkaline pH. *S. marcescens* WW4 secretes 3 type of chitinases (ChiA, ChiB and ChiC) which demonstrate insecticidal action on *Malacosomaneustria* and larvae *of H. armigera*[22]. One study showed that after the ingestion of purified chitinase from *Bacillus subtilis* orally to *S. litura* larvae results a poor growth[17].

Chandrasekaran et al[17], proved that chitinases of *B. laterosporus* Lak1210 have insecticidal action on diamond back moth *P. xylostella*[87]. Several studies have established the fact that chitinases are involved in the bacterial nematicidal effect when the nematodes are exposed to purified chitinase[2,14,91] or partially purified enzymes like the chitinases synthesized from *P. fluorescens*[60]. The bacterial chitinases have almost similar effects on nematodes to that of chitinases from fungi (e.g. disruption of growth and misregulation of hatching).

**Mechanism of Fungal chitinase:** GH18 fungal chitinases are phylogenetically classified into 3 different groups viz. A,B and C, which are again subclassified into subgroups, A-II,A-IV, A-V, B-I to B-VI, C-I and C-II (111) . It has been well-established that the chitinases from entomopathogenic fungi like *Isaria, Beauveria, Trichoderma* and *Metarhizium* have a role in attacking the insects by penetrating directly into the cuticle. When nematophagous fungi attack living nematodes, they release a group of hydrolytic enzymes such as chitinases, collagenases and proteases[118].

The fungicidal action of fungal chitinase has been explored less in comparison to bacterial ones. These are released by filamentous fungi with *Trichoderma* strains producing majority of them. Amongst them, the *T. harzianum* Chi33, Chi36, Chi42 and their homologues in several *Trichoderma*strains showed efficacious inhibition of many plant pathogens like *Fusarium spp., R. solani, Marchophominiaphaseolina and* others.[53,62,114] The substantial and versatile anti-fungal property was shown by the endochitinase Chi42 .

The chitinases have a crucial role in biological measures of controlling insects (92) and pathogenic fungi of plant[24]. They have involvement in ingestion and molting procedures of insects. The chitinolytic enzymes targets the cuticle, results in death or affects the survival of insects. The changes of peritrophic membrane cause disturbance to its selective permeability properties leading to imbalance in nutrition due to improper digestion and retention or excretion of water (Fig. 2).

**Fig. 1: Structure of chitinase**

Several degradative extracellular enzymes, like proteolytic and chitinolytic enzymes are produced by certain entomopathogenic fungi such as *Metarhiziumanisopliae, Beauveriabassiana, Nomuraearileyi* facilitating the pathogens to invade barriers and accelerate infections. Because of this degrading property of chitin, it might be postulated that on application onto insect or on injection into insect larval gut, significant damage may occur to the peritrophic membrane structural arrangement which would lead to improper feeding of larvae and consequent death[11].

The formulation of spraying types chitinase as a potent biocontrol agent is possible. On basis of this methodology, the current investigation aims at possible progression to formulate enzyme-based potential biocontrol agent in the form of spraying type against *Helicoverpa* larvae[86].

The ability to attack motile or non-motile phases of nematodes in one mode or the other is known to be unique feature of some fungi (*Basidiomycota, Ascomycota, Chytridiomycota, Oomycota, Zygomycota*)[69]. The nematicidal function involves first nematodes getting immobilized on exposure to toxins and then later addition of hyphae and digesting the nematode by enzymes. This line of attack is mainly typical for nematophagous fungi. A characteristic feature of nematoparasitic fungi is their ability to produce an enzyme complex that can kill eggs and larvae.

These fungi produce various compounds (toxins, attractants, enzymes: chitinases, proteases, lipases) and mechanisms to trap nematodes in their motile stages (sexually developed adult specimens and larvae)[65]. On the basis of nematophagous fungi as *Arthrobotrys* and *Paecilomyces*, the most recognized biopreparations are carried out (patents: US 5,989,543; CN 101422168; CN 101081982; DE 102005024783; CAN 2059642; RU 2636550; RU 2634390). Those bioformulations can selectively affect non-motile and motile phases of certain phytohelminths species[99] (Fig. 3).

**Mechanism of Viral chitinase:** The only viral chitinase i.e. ChiA synthesized from AcMNPV has been established to show fungicidal action on *A. alternata* and *B. cinerea* till date. *In vitro* assays revealed that the fungal growth and elongation of germ tube were inhibited by the purified protein. Moreover, inoculation of transgenic tobacco leaves

(protein expression in plants) with spores of *B. cinerea*, resulted in minor lesions in comparison to control mechanism[20].

The viruses belonging to family of *Baculovirida* are capable to damage insect cells and specifically AcMNPV have been investigated a lot, though their realistic application has limitations like slow effect of killing mechanism and also commercial manufacture has high cost in contrast to synthetic chemical insecticides. The final liquefaction of infected host cells can be promoted by the protein AcMNPVChiA. Endo- and exo-chitinolytic functions are characteristics of the enzyme and active in the pH ranging from 4 to 10. Primarily, it was synthesized in *E. coli* as a recombinant protein and tested on peritrophic matrix isolated from *in vitro B. mori* larvae[90]. The permeability of peritrophic matrix got amplified by the protein in different doses by changing the structural arrangement.

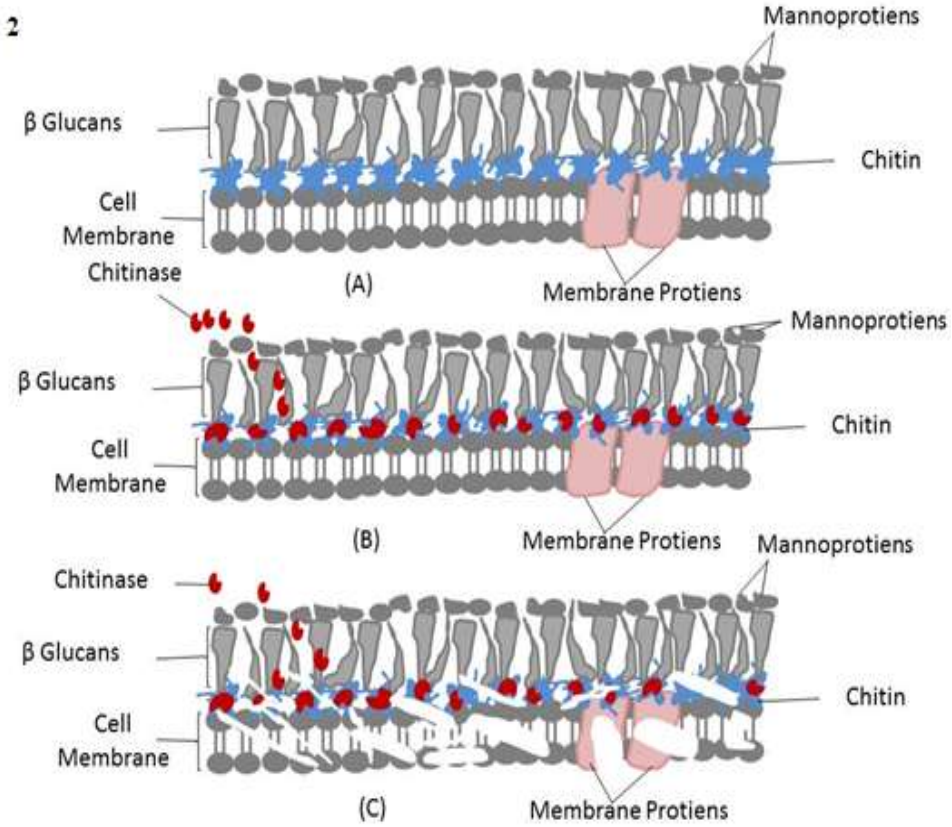



**Fig. 2: (a) Fungal cell wall (b) Binding of chitinase to chitin of cell wall and (c) Degradation of cell wall**

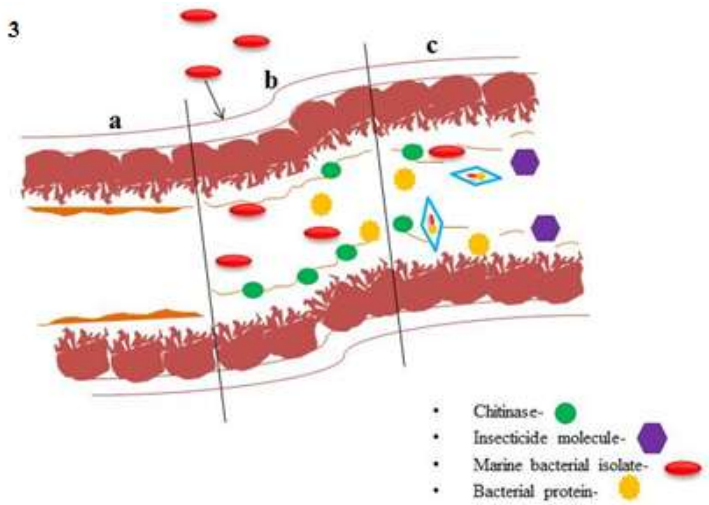



**Fig. 3: Schematic representation of mode of action and possible synergism associate with chitinases in insect midgut. (a) Native midgut lumen with insect peritrophic membrane (b) Disruption of peritrophic membrane by chitinases and (c) Inability of disrupted peitrophic membrane to protect epithelial cells that leads to increased attachment as rapid action of insecticidal compounds**

Thus, a fast and dose-dependent increase in mortality of larvae was observed on administration of a diet supplemented with the recombinant enzyme to *B. mori* larvae[90]. A transgenic plant of tobacco expressing AcMNPVChiA was also produced by the same research group which showed that a single chitinase gene expression in plants raised resistance against herbivorous pests and fungal pathogens with no harm to non-target pest[20]. The lines of transgenic tobacco restricted development of harmfullarvae pest *Heliothisvirescens*. A consistent increase in peritrophic matrix permeability of larvaeof *H. virescens* and *B. mori* was observed with the chitinase purified from tobacco leaves[72].

Moreover, a combination of AcMNPVChiA and *Aedesaegypti* Trypsin-Modulating Oostatic Factor (Aea-TMOF) was also used[28]. A peptide Aea-TMOF that binds receptors situated in midgut cells of basolateral membrane, hindering trypsin production that impairs the insect digestive activity. The transgenic tobacco plants co-expressing both Aea-TMOF and AcMNPVChiA were fed to the *H. virescens* larvae.

It was observed that there was a significant scaling down in the developmental time, growth rate and larval mortality fed on those plants that expressed only 1 of the 2 molecules (28). Hence, it could be concluded that the toxicity of bioinsecticides targeting hemocelic receptors can be enhanced by using ChiA as an effective oral ingestion bio-control agent. According to the information, another viral chitinase, DkChi was reported from the nucleopolyhedrovirus *D. Kikuchii Matsumara* with only insecticidal activity. On addition of such a chitinase to the artificial diet, high mortality rates were observed in the larvae of *Hyphantriacunea*, *S. exigua*, *L. dispar* and *H. armigera*[116].

## Immobilization of chitinase

Chitinase immobilization is essential for the re-application of the enzyme and hydrolysis of oligosaccharides. The immobilization of enzyme with high effectiveness is searching suitable carriers which could be simply applied in industries and relatively low cost is always the aim of many recent studies. The immobilization technique would enable the reusability of enzymes for tens times.

There are numerous procedures to immobilize enzymes, for example, encapsulation, entrapment, adsorption, cross linking and covalent. Every procedure has its pros and cons; however, covalent technique has the advantage of keeping the enzymes well bound to the carrier, avoiding enzyme diffusion and for this reason it is extensively used in industries (25). So far there is less report concerning immobilization of exo-chitinase.

## Conclusion

The present circumstances have greater effort and concentrated harvesting frameworks other than expanding efficiency levels and brought about harvest explicit irritation of certain pest complex. The over dependence and undiscriminating utilization of synthetic chemical pesticides to deal with those pest complexes results in negative effects on environment as well as human health. The principle of chitin dependence via a larger part of pestilent organisms, extensive event of chitinases and their built up destructiveness proposes an extraordinary chance of chitinolytic microorganisms as perfect applicants in pest management methods.

Their use in dissimilar harvesting systems against various pest was causing difficulties specifically against soil borne pathogens either unaccompanied or in mix with supplementary pesticidal mixture which may result in unique pest management in board strategies.

Most of reports share chitinases as a unique mechanism in agriculture management by chitinolytic microorganisms to control pathogenic pests. According to biological activity, chitinase or chitinolytic microorganisms can be tried on non-target organisms to create an eco-environmental approach.

## Future perspectives

In future, analysts should focus and put more endeavors on understanding that the usage of chitinases as bio-pesticides is at present narrowed by a couple of basic focuses. Hence specialists should focus and keep more endeavors on comprehension of various expression and regulatory mechanisms related with utilization of engineered protein of chi genes and pesticidal genes for developed adequacy prompting commercial use of chitinases and chitinolytic microorganisms with complex advantages to the biological system and that must be approved for formulation of chitinase commercialization. The initial one is essential to deliver them in huge scale creating consistent and vigorous synthesis procedure at sensible expenses.

Mostly fermentations in liquid batch, fed-batch, continuous and solid- state, processes are applied for chitinase production, however the gap among the academic community and industry still should be filled. Suggesting an opinion, the utilization of chitinases in industries and health measures, more endeavors are ought to be made in the regions of identification and characterization of putative strains and related chitinases in perspective on various circumstances. The tremendous microbial diversity certainly maintains unique chitinases and chitinolytic microorganisms as bio-pesticide, therefore discoverer certainly proposes an ecofriendly practical security of agricultural yielding.

The second basic point is ongoing periods, a noteworthy number of examinations were done on chitinases of marine bacteria. A large portion of these identified were characterized, extracted and purified. The fate of chitinases depends on the discovery and advancement of methods for industrial activity which will diminish the cost. Numerous headings could be laid out as planned for functioning of

marine microbial chitinases in industry, for instance, significant predictions for upcoming investigation into uses of marine microbial chitinases and chitinolytic microorganisms in the fields of agriculture management, medical and other industries.

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