Review Article

Microbial Chitinase and its potential application for biological control of plant parasites

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Received: 25/01/2018
Revised: 10/05/2018
Accepted: 17/12/2018

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Abstract:

Chitin is the second most abundant polysaccharide in nature after cellulose, which is mainly found in the exoskeleton of insects, fungi, yeast, algae, and in the internal structures of other vertebrates, which are mostly plant parasites. At present, chemical pesticides are the major means of controlling these disease-causing agents. However, as these chemicals cause potential harm to the environment, human and animal health, new strategies are being developed to replace or reduce the use of such compounds in agriculture. Chitinase are enzymes that degrade chitin, resulting in severe damage and even death in pathogens and pests whose external and internal surfaces contain this polymer. In this perspective, chitinolytic microorganisms are likely to play an important role as biological control agents in agriculture fields against plant pathogens. In this review, various types of microbial chitinase and their role as biological control agents are thoroughly discussed.

Keywords: Microbial chitinase, fungicidal, pesticidal, nematicidal

INTRODUCTION

Chitin, a linear polymer of β-1, 4-N-acetylglucosamine (GlcNAC), is the second most abundant biopolymer on the planet after cellulose (Shahidi and Abozaytoun 2005). It is found in the shells, exoskeletons, and gut linings of arthropods mainly crustaceans and insects. It also comprises the cell walls of many fungi, including some yeasts, and makes up the structural frameworks of certain Protista as well as of nematode eggs (Stoykov et al. 2015). According to previous studies, estimated amount of crop loss due to diseases in the field was up to 40% preharvest- and additionally 10% postharvest (Chandler et al. 2011). The main cause of these losses are insects, weeds, and plant parasites. Subsequently, crops worldwide are completely dependent on the use of chemical pesticides to reduce crop loss, but the major problem of using these chemicals is that the target organisms often develop resistance to them (Russell, P.E. 2006), and non-target organisms and the surrounding environment too face the hazardous effects of chemicals employed. Many human health problems have been linked to pesticide use. Hence, it is important to find out an alternative to these chemical pesticides that is not harmful to humans and to the surrounding environment, besides degrading the chitin present in various plant parasites.

STRUCTURE OF CHITIN

Chitin is a white, hard, inelastic polysaccharide having high percentage of nitrogen (6.89%). It exists in three crystalline forms i.e. α-chitin, β-chitin γ-chitin, which differ in the arrangement of polymer chains giving them different mechanical properties (Jang et al. 2004). These forms of chitin vary in packing and polarities of adjacent chains in the succeeding sheets (Chen et al. 2010). In the environment, chitin is found in fully acetylated state to its completely deacetylated form which is known as chitosan (Beier and Bertilsson 2013). Because of the ubiquity of chitin in the environment, its degradation has been extensively considered (Nagpure et al. 2014). There is a dire need of a biological substitute for harmful chemicals used as chitin degraders. Insect cuticle, fungal cell wall and nematodes eggshells comprises chitin biopolymer as a protective barrier against harmful chemicals and environmental factors. So based on these findings, microbial chitinase can be employed to degrade chitin to control these plant parasites.

DEGRADATION OF CHITIN BY CHITINASE

Chitinase enzyme degrades chitin. It is secreted by a wide range of organisms for carrying out various functions such as morphogenesis, nutrient cycling, as well as in defense against chitin containing pests and parasites (Dahiya et al. 2006). The catabolism of chitin takes place in 2 steps involving the initial cleavage of the chitin polymer by chitinase into chitin oligosaccharides, and further cleavage to N-acetylglucosamine. Whereas, monosaccharides are cleaved by chitobiases (Suginta et al. 2000). Chitinase (E.C 3.2.2.14) are glycosyl hydrolases with their size ranging from 20 kDa to about 90 kDa (Bhattacharya et al. 2007). Chitinase have been divided into 2 main groups *viz* endochitinase and exochitinase. The endochitinase randomly split chitin at internal sites, thereby forming the dimer di-acetylchitobiose and soluble low molecular mass multimers of GlcNAc such as chitotriose, and chitotetraose. The exochitinase have Chitobiosidases, which are involved in catalyzing the progressive release of di-acetylchitobiose cleaving the oligomeric products of endochitinase and chitobiosidases, thereby generating monomers of GlcNAc (Sahai et al. 1993).Based on amino acid sequence of the enzyme, chitinases are grouped into different categories of glycoside hydrolase (GH) families such as GH18, GH19, and GH20. Most bacterial chitinase belong to the GH18 family (Adrangi and Faramarzi 2013). Chitinase are produced by wide range of organisms such as bacteria, fungi, plants, arthropods, and humans. Chitinase have been receiving an increased attention due to their role in the biocontrol of plant parasites (Mathivanan et al. 1998). Microbial chitinase weaken and degrade the important structures of many pests and pathogens, thereby exhibiting fungicidal, insecticidal, or nematicidal activity (Edreva, A. 2005). Chitinolytic enzymes will become a more obvious and important solution towards overcoming the environmental hazards that result from the application of synthetic pesticides and fungicides. Chitinase producing bacteria have been isolated from soil, shellfish waste, garden and park waste compost, and hot springs (Yuli et al. 2004). In marine environments, they are involved in the nutrient cycling of the substantial amount of chitin derived from arthropod shells and other sources (Souza et al. 2011). In the soil and rhizosphere, bacteria use chitin from insects and fungi as a carbon and nitrogen source (Cohen-Kupiec and Chet 1998, Geisseler et al. 2010).

In this review, we intent to broaden the understanding regarding chitinase, how it degrades the chitin, microbial chitinase and its potent application as biological control agents against plant parasites.

MICROBIAL SOURCES OF CHITINASE

Bacterial Chitinase

Microorganisms, particularly bacteria, form one of the major sources of chitinase (Bhattacharya et al. 2007). Bacteria produce chitinase mainly to degrade chitin and utilize it as an energy source. Microbial chitinase can hydrolyze chitin and partially N-acetylated chitosan as well (Mitsutomi et al. 1995). Bacterial chitinases are receiving increased attention due to their wide range of biotechnological applications especially in the production of chito-oligosaccharides and N-acetyl D-glucosamine (Pichyangkura et al. 2002), biocontrol of pathogenic fungi, preparation of sphaeroplast and protoplasts from yeast and fungal species (Balasubramanian et al. 2003), bioconversion of chitin waste to single cell protein (Dahiya et al. 2005), as well as one of the potential enzymes for applications in agriculture, pharmaceutical, waste management, biotechnology and industry (Gupta et al. 1995). Their high demand and varied uses has led to the discovery of new strains of microorganisms that are capable to produce enzymes with novel properties and the development of low cost industrial media formulations

(Saito et al. 2009).

Fungal Chitinase

Fungal chitinase, like bacterial chitinase, have multiple functions as they play an important role in nutrition, morphogenesis, and fungal development processes. Chitin is a major cell wall component of fungi (Sahai et al. 1993). The chitinase production in filamentous fungi is seen throughout its life cycle. The main role of this enzyme is to help the fungi in releasing the spores and in hyphal elongation and branching (Muzzarelli et al. 2012). In yeast, chitinase helps in budding especially in cell separation. It also plays an important role in septa dissolution and gamete fusion (Gooday and Gow 1990). Chitinase is essential not only in the various developmental stages of fungi but also in fungal nutrition (Gunaratna and Balasubramanian 1994). It helps in utilization of chitin for its carbon and nitrogen requirements. In certain cases, it is also associated with pathogenicity of the organisms (Narayana and Vijayalakshmi 2009).

CHITINOLYTIC MICROORGANISMS AS POTENTIAL BIOLOGICAL CONTROL AGENTS

Among the bacteria used as biocontrol agents, the primary ones are species of *Streptomyces*, *Bacillus*, and *Pseudomonas* (Bélanger, R.R. 2001). Recent studies are focused on the search for alternatives to chemical pesticides and *B. thuringiensis* toxin, since plant parasites have developed resistance against both control agents. To this end, bacteria from different orders have been found to be effective biocontrol agents (Kalia and Gosal 2011).

Actinobacteria are important saprophytic soil bacteria which are known for antibiotic and secondary metabolite production, as well as for the synthesis of chitinolytic enzymes (Barka et al. 2016). They are among the most important taxa in the soil microbial chitinolytic community (Gonzalez-Franco et al. 2003). The purified chitinase from *Streptomyces rimosus* exhibited in vitro antifungal properties against *Fusarium solani* and *Alternaria alternate* (Brzezinska et al. 2013). Similarly, *Streptomyces viridificans* was found to lyse the fungal cell walls of *Rhizoctonia*, *Colletotrichum*, *Aspergillus*, *Fusarium*, *Sclerotinia*, *Curvularia*, and *Pythium* in vitro (Gupta et al. 1995).

Bacillus thuringiensis is a well-known biocontrol agent that has been in use for decades for pest control in agriculture. Many *B. thuringiensis* strains that constitutively express chitinase have been described (Liu D et al. 2010, Chen et al. 2007). Hollensteiner et al. (2017) isolated *B. thuringiensis* from tomato roots which exhibited in vitro antifungal activity against *Verticillium spp.* *Paenibacillus illinoisensis* isolated from coastal soil in Korea was reported to have strong in vitro chitinolytic activity when assayed on colloidal chitin. It also deformed and destroyed the eggshell of the root-knot nematode *Meloidogyne incognita* (Jung et al. 2002).

BIOCONTROL OF FUNGAL PHYTOPATHOGEN BY MICROBIAL CHITINASE

The fungal phytopathogens cause severe problems worldwide in the cultivation of economically important plants (Brimner and Boland 2003). Chitinase enzymes are able to lyse the cell wall of numerous fungi and the microbes which produce these enzymes are capable of eradicating these fungal pathogens. Normally chemical fungicides are used to reduce

Table: 1 Chitinase producing key agents for biocontrol of fungal plant pathogens **(published from 2007 onwards)**

Microbial antagonist	Fungal pathogen	References
Bacillus licheniformis	*Gibberella saubinetii, Aspergillus niger, Rhizoctonia solani*	Xiao et al 2009, Kamil et al 2007
Bacillus pumilus	*Rhizoctonia solani, Verticillium spp., Nigrospora sp., Stemphyllium botryosum, Bipolaris spp. Alternaria brassicicola*	Ghasemi et al 2010
Bacillus subtilis	*Aspergillus niger, Aspergillus flavus, Penicillium chrysogenum.*	Karunya S.K 2011
Pseudomonas spp	*Fusarium oxysporum*	Velusamy et al 2011
Serratia marcescens	*Rhizoctonia solani, Bipolaris spp., Alternaria raphanin, Fusarium solani, Aspergillus flavus*	Zarei et al 2011, Zeki and Muslim 2017
Aspergillus niger	*Fusarium culmorum, Fusarium solani, Rhizoctonia solani*	Brzezinska and Jankiewicz 2012
Trichoderma viride	*Rhizoctonia solani, Fusarium oxysporum, Pythium ultimum*	Fahmi et al 2012
Streptomyces griseus	*Fusarium oxysporum, Alternari alternate, Rhizoctonia solani, Fusarium solani, Aspergillus flavus*	Anitha and Rabeeth 2010

Table: 2 Microbial agents for pest management (published from 2007 onwards)

Microbial antagonist	Insect/Pest	Reference
Bacillus subtilis	*Spodoptera litura*	Chandrasekaran et al 2012
Bacillus thuringiensis subsp. *colmeri*	*Spodoptera exigua, Helicoverpa armigera*	Liu et al 2010
Pseudomonas fluorescens MP-13	*Helopeltis theivora*	Suganthi et al 2017
Serratia marcescens SEN	*Spodoptera litura*	Aggarwal et al 2015
Isaria fumosorosea	*Plutella xylostella*	Huang et al 2016
Pochonia chlamydosporia	*Bombyx mori*	Mi et al 2010
Trichoderma harzianum	*Helicoverpa armigera*	Binod et al 2007
Trichoderma viride	*Bombyx mori*	Berini et al 2016

fungal pathogens. But the extreme use of chemical fungicides had led to environmental problems along with induced pathogen resistance. These chemical compounds may be toxic to beneficial insects and microorganisms in the soil, and may also enter into the food chain (Budi et al. 2000). Biological control of plant pathogens by soil bacteria is a well-known fact and chitinase production by these bacteria has been shown to play an important role in suppressing them (Hong and Hwang 2006). Bolar et al. (2001) studied synergistic activity of endochitinase and exochitinase from *Trichoderma atroviride* against the pathogenic fungus *Venturia inaequalis* in transgenic apple plants. The principal chitinase producers for controlling fungal phytopathogens are enlisted in Table: 1.

MIRCOBIAL CHITINASE AS A BIOPESTICIDE

In an agricultural system, pesticides are used to protect plants/crops from damage by disease causing plant pathogens. Acknowledging the side effects of these chemicals, control of crop pests by use of biological agents is prerequisite (Table: 2). In insects, chitin functions as scafflold material that supports the cuticles of epidermis and trachea as well as the gut epithelium lining. Hence if chitin is targeted it would result in degradation of insect's

structures. Chitinase produced by *Bacillus spp*. is found to control *Aphis gossypii* (Nurdebyandaru et al. 2010). Daizo K (2005) sprayed chitinase directly to the strawberry plants and observed that plants were free from insects. Recently, Suganthi et al. (2017) isolated chitinase from *Pseudomonas fluorescens* that showed insecticidal activity against tea mosquito bug. These findings open up the possibility of using chitinase as a biopesticidal agent for integrated pest management strategies.

MICROBIAL CHITINASE AS A NEMATICIDAL AGENT

Global estimates of agricultural losses due to infections by plant-parasitic nematodes are of $ 130 billion annually (Becker, J.O. 2014). At present, chemical pesticides control nematodes but enzymes and the microbes which produce these enzymes (Table: 3) having nematicidal action are receiving an augmented attention. Cuticle in adult nematodes and eggshell play an important role in preventing nematophagous infection. The main component of cuticle is collagen which is degraded by microbial proteases during infection of adult nematodes. Eggshell is a more complex protective structure resulting in the increased survival rate of enveloped eggs, embryos or larvae against chemical and biological nematicides. In many studies, it was found that the chitinase has nematicidial effect on eggshell of nematodes (Wharton, D. 1980), and it was first investigated by (Mankau and Das 1969). They found that the chitin amendments controlled the citrus nematode *Tylenchulus semipenetrans* and the root-knot nematode *Meloidogyne incognita*. Later, chitin amendments were used to control *Tylenchorhynchus dubius* (Miller et al. 1973), *M. arenaria* (Mian et al. 1982), *M. javanica*, and the cereal cyst nematode *Heterodera avenae* (Spiegel et al. 1989). Purified chitinase inhibited egg hatch of *Globodera rostochiensis* up to 70% in vitro, and the chitinase-producing bacteria *Stenotrophomonas maltophilia* and *Chromobacterium sp*. reduced egg hatch of nematode both in vitro and in soil (Cronin et al. 1997). *Pseudomonas chitinolytica* reduced *M. javanica*

Table: 3 Microbial agents for biocontrol of plant parasitic nematodes (published from 2007 onwards)

Microbial antagonist	Target Nematode	Reference
Bacillus thuringiensis subsp. *tenebrionis*	*Caenorhabditis elegans* (juveniles)	Ni et al 2015
Pseudomonas aeruginosa	*Caenorhabditis elegans* (eggs, adult)	Chen et al 2017, Chen et al 2015
Pseudomonas fluorescens HN1205	*Meloidogyne incognita* (eggs)	Lee and kim 2015
Monacrosporium thaumasium NF34	*Panagrellus redivivus* (juveniles)	Soares et al 2015
Paecilomyces javanicus	*Meloidogyne incognita* (eggs, juveniles)	Chan et al 2010
Pochonia chlamydosporia	*Meloidogyne incognita* (eggs)	Mi et al 2010
Verticillium psalliotae	*Meloidogyne incognita* (eggs)	Gan et al 2007

infection and improved growth of tomato, *Lycopersicon esculentum* (Spiegel et al. 1991). The chitinolytic fungus, *Paecilomyces lilacinus*, destroyed nematode eggs and efficiently controlled *M. incognita* (Morgan-Jones et al. 1984).

CONCLUSION

Application of biological agents as a substitute to chemicals pesticides holds great possibilities to control a range of plant pathogens. More study on microbial chitinase by using genetic engineering tools will offer better understanding about chitinase genes, its overexpression resulting in better yield of enzymes and biotechnological applications in agriculture sector. By understanding biochemistry and protein engineering, one can produce microbial chitinase with specific functions that will make them more useful for various processes in near future.

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