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Chitinase Inhibitors and Chitin Mimetics for Crop Protection

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ABSTRACT

Pathogen and pest management is a key element in agriculture. The Integrated Pest Management concept relies on the availability of an array of protective measures having different but complementary mechanisms. As a consequence, alternative strategies have to be developed. Because chitin occurs in the cell wall of fungi and in various structural components of arthropods, its metabolism has been considered as a suitable target to manage pathogens and pests. Disruption of chitinase metabolism is one of the strategies that have been considered. Studies led to the discovery of new molecules which interact with chitinases and induce antibiotic, antifungal or insecticidal effects. These compounds present different ranges of activity, depending on their biochemical classification, their structure and the nature of the targeted chitinase. Some are oligo- and polysaccharides (e.g. allosamidin, FPS-1) and others are peptides (e.g. argadin, argifin, cyclic dipeptides or psammaplin A). These natural molecules directly interact with the catalytic site of chitinases, mimicking chitin structure. In this review we will present a synthesis of the recent works that aimed to disrupt chitin metabolism by chitinase inhibition with the ultimate objective of developing new applications for crop protection.

Keywords: allosamidin, cyclic peptides, fungicides, insecticides, oligosaccharides, psammaplin A

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INTRODUCTION

In the everlasting competition between humans, pests and pathogens, chemical pesticides play a major role worldwide in plant protection. However, as in any technologies, chemicals have their drawbacks, including potential toxicity for environment, negative impacts on human health, and development of resistant populations of pests and pathogens. As a consequence, new technologies or strategies must be considered to develop safe methods, notably some based on the use of natural compounds. One of these strategies is to control pest and pathogen populations by inhibiting their growth and development. A common characteristic of bacteria, fungi, arthropods and some microorganisms is the production of chitinases that are involved in the degradation and turnover of chitin.

Chitinases belong to glycosidases, a well known enzymatic family. Because they are involved in several biological processes, they are considered to be suitable targets to manage a number of pests. Studies have been conducted to enhance or reduce their activity. An increase or an inhibition of glycosidases activity can induce beneficial effects that are amenable in various fields, such as medicine and crop protection. Among the molecules inducing these effects, some oligo- and polysaccharides and peptides have been reported to interfere with chitinases activities, mimicking chitin structure or its derivatives.

In this paper, the effects of these compounds in medicinal and veterinary fields will not be discussed, as they have been reviewed extensively by Jollès and Muzzarelli (1999). We will focus on the disruption of chitin degradation by enhancement or inhibition of chitinase activities in the context of crop protection.

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Invited Mini-Review

CHITIN AND ITS TURNOVER

Chitin is a homopolymer of *N*-acetylglucosamine residues with β-1,4 linkages. With 10 gigatons naturally produced and degraded each year, it is the second most important polysaccharide worldwide, after cellulose. This structural component, that occurs in the cell wall of fungi and algae (between 22 to 44% of their mass), also represents a major part of the exoskeleton of various invertebrates, such as nematodes, molluscs and arthropods, and the peritrophic membrane, that lines the midgut of numerous insect species. Associated with various types of proteins and pigments, fibrils of chitin may confer rigidity and flexibility in arthropods. Rigidity and flexibility differ according to the arthropod body's parts. For instance, chitin may confer rigidity to support the exoskeleton and is thicker in some parts (e.g. pronotum) and thinner at appendance joints to allow flexibility and movements. Chitin turnover is under the control of few enzymes, among which chitin synthases participate to the synthesis of the chitinous structures; chitinolytic enzymes degrade them.

Chitin synthesis has been extensively studied. Some inhibitors have been commercialized as management tools against phytopathogens (Palli and Retnakaran 1999; Merzendorfer and Zimoch 2003). For example, polyoxines and nikkomycins, two analogues of chitin synthase substrates, competitively inhibit fungal and insect chitin synthases. Other compounds inhibit the synthesis of chitin fibrils or directly act on hormonal regulation of the moulting process (Cohen 1987; Merzendorfer 2006). Chitin degradation is chiefly realized by chitinases and, to a lesser extent, by other chitinolytic enzymes such as β-D-acetylglucosaminidases, chitin deacetylases, chitotriases or chitobiases.

CHITINASES AND THEIR FUNCTIONS

Enzyme classification

Chitinases belong to glycosidases, an enzymatic family which includes numerous hydrolases and catalyses the hydrolysis of oligo- and polysaccharide chains. Glycosidases are key enzymes in living systems and are involved in many functions such as digestion, biosynthesis, glycoprotein degradation or lysosomal metabolisms (von Figura 1982). Chitinases are essentially divided in the 18 and 19 families of glycoside hydrolases. Family 18 chitinases are found in bacteria, fungi, yeast, viruses, plants and animals whereas family 19 occurs almost exclusively in plants. Families 18 and 19 chitinases do not share protein sequence and have different structures and biochemical properties (Hamel *et al.* 1997; Henrissat *et al.* 1999).

Chitinase functions

Chitinases may play numerous functions. In bacteria, they are involved in digestive metabolism to provide carbon and nitrogen compounds. In fungi, chitinases allow numerous physiological processes such as hyphal growth or cell separation in yeasts by a reorganization of the cell wall structure. Chitin degradation is an essential step in the moulting process that allows growth of arthropods, notably in insects. Chitinases also play an important role in digestive enzymes compartmentalization because they are involved in the turnover of the peritrophic matrix (Cohen 2001), a non-cellular structure that lines and protects epithelial cells in the gut of most insect species, excluding Homoptera (Lehane and Billingsley 1996; Lehane *et al.* 1997). In plants, chitinases are involved in embryogenesis, morphogenesis, fruit maturation and also in defence responses against herbivorous and pathogens (Collinge *et al.* 1993; Kasprzewska 2003).

In that context, chitinases can be considered as suitable targets to develop new strategies to increase resistance and protection against pathogens and pests. In this paper, two approaches will be envisaged: the enhancement and the inhibition of chitinase activities.

Enhancement of chitinase activities

During the last decades, chitinolytic enzyme-encoding genes from various origins have been introduced into plant genomes to enhance crop resistance against phytophagous insects and other pests. Some antifungal activities were reported in transgenic plants expressing recombinant chitinases (Herrera-Estrella and Chet 1999; Giordanengo *et al.* 2008). Bacterial and plant chitinases did not show efficient insecticidal effects. The only paper reporting insecticidal effects of a plant chitinase expressed in transgenic plants exerted a slight increase of larval mortality of the chrysomelid *Leptinotarsa decemlineata* (Lawrence and Novak 2006). In contrast, significant insecticidal properties were reported for lepidopterans in transgenic tobacco plants transformed with an insect chitinase (Ding *et al.* 1998). In these two cases, the authors suggested that chitinases could directly interact with peritrophic membrane turnover. Our work on the effects of an insect chitinase expressed in transformed potato plants on the aphid *Myzus persicae* – deprived of peritrophic membrane – showed probiotic effects (i.e. reduction of pre-reproductive period, increase of nymphal survival and daily fecundity) on this pest (Saguez *et al.* 2005). Published evidences on insecticidal effects do not warrant commercial development at this time and relevance of a chitinase-based strategy in the context of crop protection is questionable, notably for the management of aphids.

Inhibition of chitinase activities

Glycosidase activities can be inhibited to address crop protection problems. Asano (2003) reviewed insecticidal and fungicidal effects of glycosidase inhibitors, like validoxylamine A, a pseudodisaccharide isolated from *Streptomyces* sp., which exhibits trehalase inhibitory activity and presents insecticidal properties against lepidopteran pests. Several glycosidase inhibitors discovered from cultures of *Streptomyces* species were also isolated from plants and microorganisms. Among these inhibitors, polysaccharides have been described as potent inhibitors of endoglycosidases. For example, amylostatins (Fukuhara *et al.* 1982) and bacterian liposaccharides (Ohno and Morisson 1989) respectively inhibit α-glucosidases and lysozymes.

During the two last decades, efforts have been made to find chitinase inhibitors, to develop new crop protection strategies. A patent for a method for screening new chitinase inhibitors (Silverman and Roosevelt 1996) shows interest to control the metabolism of chitinases. Because chitin, the natural substrate of chitinases, is a polysaccharide, some oligosaccharides or polysaccharides mimicking chitin have been tested for their potential to control growth of crop pests and pathogens (**Table 1**). Some peptides have also been isolated from different organisms and present variable antifungal and insecticidal effects (**Table 1**). A few present inhibitory activities and could be considered as chitin mimetics due to their structure homologies or their mode of interaction with the catalytic site of chitinases.

CHITIN MIMETICS, CHITINASE INHIBITORS AND THEIR APPLICATIONS

Polysaccharides

Chitosan

Chitosan is obtained by *N*-deacetylation of chitin and is composed of subunits of *N*-acetyl-D-glucosamine β-1,4 linked with D-glucosamine. So it could be considered as a structural mimetic of chitin. This polysaccharide is non toxic for mammals and largely used for biotechnological applications in agriculture and has some beneficial effects amenable for crop production (Ben-Shalom *et al.* 2000).

Chitosan is an elicitor of plant defence responses (Hadwiger 1999) and has been used as an antimicrobial agent. Acting on the cell wall of fungi, algae and bacteria, it indu-

Table 1 Selected chitinase inhibitors and mimetics and their inhibitory activities on various taxa.

Chitinase inhibitors or mimetics	Targeted organisms	Taxa	Effects	References
Chitosan	*Fusarium* sp.	Fungi	Growth inhibition, plant resistance	Cuero 1999
	Rhizopus stolonifer	Fungi	Postharvest protection of fruits	Kendra *et al*. 1987
	Botrytis cinerea	Fungi	Growth inhibition	Benhamou and Thériault 1992
	Pyricularia grisea	Fungi		El Ghaouth *et al*. 1992
				Rabea *et al*. 2006
				Liu *et al*. 2007
				Meng *et al*. 2008
	Spodoptera littoralis	Insect (L)	Food intake interruption	Rabea *et al*. 2006
	Helicoverpa armigera	Insect (L)	Developmental growth inhibition	
	Plutella xylostella	Insect (L)	Increase of mortality	
	Aphis gossypii	Insect (H)		
	Metopolophium dirhodum	Insect (H)		
	Hyalopterus pruni	Insect (H)		
	Rhopalosiphum padi	Insect (H)		
	Sitobion avenae	Insect (H)		
	Myzus persicae	Insect (H)		
Allosamidin and/or derivatives	*Streptomyces* sp.	Bacteria	Chitin synthesis induction	Suzuki *et al*. 2006
	Saccharomyces cereviciae	Yeast	Chitinase activity inhibition	Nishimoto *et al*. 1991
	Candida albicans	Fungi		
	Trichoderma sp.	Fungi		
	Onchocerca gibsoni	Nematode	Chitinase activity inhibition	Gooday *et al*. 1988
	Entamoeba invadens	Nematode	Inhibition of cyst formation	Villagomez-Castro *et al*. 1992
	Artemia salina	Crustacean	Chitin synthesis induction	
	Bombyx mori	Insect (L)	Ecdysis disturbance	Sakuda *et al*. 1987
	Leucanobia separata	Insect (L)	Increase of mortality	
	Tineola bisselliella	Insect (L)	Increase of larval mortality	Blattner *et al*. 1997
			Ecdysis disturbance / growth inhibition	
	Lucilla cuprina	Insect (D)	Increase of larval mortality	
	Aedes aegyptii	Insect (D)	Reduction of peritrophic membrane penetration	Shahabuddin *et al*. 1993
	Myzus persicae	Insect (H)	Increase of larval mortality	Saguez *et al*. 2006
			Reduction of fecundity	
FPS-1	*Spodoptera littoralis*	Insect (L)	Growth inhibition	Nitoda *et al*. 2003a, 2003b
DP2S	*Myzus persicae*	Insect (H)	Increase of larval mortality	Bultel *et al*. 2007
	Macrosiphum euphorbiae	Insect (H)	Growth inhibition	
			Reduction of fecundity	
Cyclopentapeptides (Argifin / Argadin)	*Serratia marcescens*	Bacteria	Chitinase activity inhibition	Arai *et al*. 2000b
				Omura *et al*. 2000
				Houston *et al*. 2002a, 2002b
	Aspergilus fumigatus	Fungi	Chitinase activity inhibition	Rao *et al*. 2005
	Periplaneta americana	Insect (B)	Increase of larval mortality	Arai *et al*. 2000b
			Growth inhibition	Omura *et al*. 2000
	Lucilia cuprina	Insect (L)	Chitinase activity inhibition	Arai *et al*. 2000b
Cyclic dipeptides	*Serratia marcescens*	Bacteria	Chitinase activity inhibition	Houston *et al*. 2004
	Saccharomyces cereviciae	Yeast	Cell separation inhibition	Izumida *et al*. 1996b
	Candida albicans	Fungi	Hyphal growth inhibition	
	Myzus persicae	Insect (H)	Increase of larval mortality	Saguez *et al*. 2006
			Growth inhibition	
Psammaplin A	*Staphyloccocus aureus*	Bacteria	Antibiotic activities	Kim *et al*. 1999a, 1999b
	Bacillus sp.	Bacteria	Chitinase activity inhibition	Tabudravu *et al*. 2002
	Streptomyces sp.	Bacteria		
	Actinomyces sp.	Bacteria		
	Serratia marcescens	Bacteria		
	Plasmopora viticola	Fungi	Growth inhibition	Tabudravu *et al*. 2002
	Drosophila melanogaster	Insect (D)	No or poor effects	Tabudravu *et al*. 2002
	Heliotis virescens	Insect (L)		
	Plutella xylostella	Insect (L)	Increase of mortality	
	Myzus persicae	Insect (H)	Increase of larval mortality	Saguez *et al*. 2006
	Macrosiphum euphorbiae	Insect (H)	Growth inhibition	
			Reduction of fecundity	

For insects: (L): Lepidoptera, (D): Diptera, (H): Homoptera, (B): Blattodea

ces plant resistance against pathogens and plays a protective role on harvested grains and fruits (Cuero 1999). Eliciting the accumulation of secondary plant metabolites such as phytoalexins or monoterpenes (Miller *et al.* 1986; Cuero 1999), this compound induces the accumulation of pathogenesis-related proteins among which protease inhibitors, chitinases and chitosanases and favours the bioconversion of linolenic acid into jasmonic acid. For example, in *Fusarium solani*, chitosan fragments inactivate the growth of the pathogenic fungus (Kendra and Hadwiger 1987). Chitosan possesses high antifungal activities, increasing polyphenoloxidase, peroxidase, and phenolic compounds in fruits and vegetables (Liu *et al.* 2007; Meng *et al.* 2008). Applications of chitosan prevent lesions caused by *F. oxysporum* on tomato leaves and roots (Benhamou and Thériault 1992). Chitosan also protects strawberries against the postharvest fungal pathogen *Rhizopus stolonifer* (El Ghaouth *et al.* 1992). Likewise, chitosan induces resistance of wheat seeds against *F. graminearum*. Antifungal effects of chitosan were also reported against *Botrytis cinerea* and *Pyricularia grisea*. In

these cases, yields were improved due to the inhibition of hyphal growth and spore formation but also the reduction of roots symptoms and plant mortality (Rabea *et al.* 2006).

These authors also reported insecticidal properties of chitosan on the lepidopteran pests *Spodoptera littoralis*, *Helicoverpa armigera* and *Plutella xylostella*, and on the aphids *Aphis gossypii*, *Metopolophium dirhodum*, *Hyalopterus pruni*, *Rhopalosiphum padi*, *Sitobion avenae* and *Myzus persicae*. Insect larvae intoxicated with chitosan presented impaired larval development associated with a dysfunctional food intake and increased mortality. In some cases, moulting processes were interrupted (Rabea *et al.* 2006). The insecticidal effects of chitosan are likely associated with the increase of plant defence responses such as callose and lignin synthesis. These effects could result in antixenosis and antibiosis effects.

The above facts and the common origin of chitosan and chitin and their structural homologies strongly suggest that chitosan and its derivatives can directly interact with glycosidases and chitinases through competitive inhibition.

Allosamidin

Allosamidin was the first chitinase inhibitor isolated from a culture of *Streptomyces* sp. (Sakuda *et al.* 1987). Chrystallographic studies have shown that allosamidin possesses a structure similar to that of chitin and mimics a catalytic intermediate of its catalysis (van Aalten *et al.* 2001). It is composed by two *N*-acetylallosamine subunits linked to an allosamizoline subunit (Bortone *et al.* 2002; Fusetti *et al.* 2002; Rao *et al.* 2003). This non- hydrolysable pseudotrisaccharide is a potent chitinase inhibitor reported to be only specific against family 18 chitinases, including fungal and insect chitinases (Blattner *et al.* 1997; Sakuda *et al.* 1987; Sakuda and Sakurada 1998). Allosamidin exerts a competitive inhibitory activity against chitinases at very low concentrations. Injection of allosamidin in larvae of the silkworm *Bombyx mori* and the armyworm *Leucanobia separata* strongly disturbed larval ecdysis and increased mortality rate of these lepidopteran pests (Sakuda *et al.* 1987). Allosamidin and its derivatives highly increased mortality of the blowfly larvae (*Lucilia cuprina*) after contact applications or feeding tests (Blattner *et al.* 1997). In the webbing clothes moth *Tineola bisselliella*, consumption of allosamidin resulted in larval mortality associated with severe morphological alterations (delayed growth and interrupted moulting) that occur during larval development (Blattner *et al.* 1997). Allosamidin also induces aphicidal effects, increases larval mortality and reduces fecundity of the aphid *Myzus persicae* (Saguez *et al.* 2006).

Inhibition of chitinases by allosamidin has been extensively studied due to its high inhibitory activity. Allosamidin and its derivatives, among which demethylallosamidin, methylallosamidin, glucoallosamidin A et B, and methyl-*N*-demethylallosamidin (Spindler *et al.* 1997), were also tested against different human pests and pathogens such as plasmodium (Shahabuddin *et al.* 1993; Tsai *et al.* 2001; Filho *et al.* 2002) and nematodes (Gooday *et al.* 1988; Villagomez-Castro *et al.* 1992). Allosamidin also possesses antibacterial (Sampson and Gooday 1998) and antifungal activities against the pathogenic fungi (Dickinson *et al.* 1991; McNab and Glover 1991; Nishimoto *et al.* 1991; Sandor *et al.* 1998; Sami *et al.* 2001; Bortone *et al.* 2002).

Allosamidin has been reported to elicit chitin synthase in arthropods (Peter and Schweikart 1990). In *Streptomyces* species that produce allosamidin, this inhibitor favours production of an allosamidin-insensitive chitinase, necessary for fungi growth (Suzuki *et al.* 2006). Although allosamidin and its derivatives are potent chitinase inhibitors, none are presently used in agricultural or medicinal fields because of difficulties in their synthesis and high production costs. As a consequence, screening programs are aiming at the discovery of new compounds having inhibitory activities against chitinases.

Other oligo- and polysaccharides

Recently, a fungal polysaccharide named FPS-1 showed inhibitory activities in the same range than allosamidin, with insecticidal effects against the lepidoptera *Spodoptera litura* (Nitoda *et al.* 2003a, 2003b). We synthesized a new disaccharide and reported toxic effects on aphids, resulting in larval mortality, reduced body length and fecundity (Bultel *et al.* 2007). It has been shown that some molecules cross the digestive epithelium to reach the hemolymph where they could interact with different metabolisms. In aphids, chitinase inhibitors may affect cuticular chitinase activities of the mother and the offspring. While the target of this new disaccharide is unknown, the effects on aphid postembryonic development and morphology alterations strongly suggest its interaction with chitinases. Oligosaccharides could also directly interact with other glycosidases of the digestive tract (e.g. sucrases) which are involved in osmoregulation processes.

Mimetic peptides

Because of the difficulties of synthesis and the cost of production of oligosaccharides, alternatives have been explored to find new chitinase inhibitors. Among the diversity of biological molecules that can be used as substitutes for oligo- and polysaccharides, peptides are single molecules that can be easily characterized and chemically or biologically synthesized in different systems (transformed plants, bacteria, insects cells, viruses), reducing the cost of production. Some peptides have been discovered which mimic the chemical conformation of carbohydrates and inhibit glycosylhydrolases, binding their catalytic site. Among these peptides of low molecular weight (dipeptides and cyclopentapeptides), some of them exhibit antifungal and antibacterial activities (Beauvais and Latge 2005).

Cyclopentapeptides

Efforts to find new chitinase inhibitors led to the discovery of two new cyclopentapeptides, argifin (Arai *et al.* 2000a; Omura *et al.* 2000) and argadin (Arai *et al.* 2000b), respectively isolated from the fungal cultures of *Gliocladium* sp. and *Clonostachys* sp. Both argifin and argadin interact with specific amino acids of the chitinase active site mimicking a carbohydrate substrate. Houston *et al.* (2002b) studied the interactions between *S. marcescens* chitinase and cyclopentapeptides and showed that they mimic the interactions of the enzyme with the chitooligosaccharides substrate. Rao *et al*. (2005) described the tight interactions between argifin and argafin, and a "bacterial-like" chitinase secreted by the fungus *Aspergillus fumigatus.*

Argifin and argadin respectively exert weaker and stronger inhibition than allosamidin (Houston *et al.* 2002b): the higher the affinity between chitinase and their inhibitor, the higher the inhibitory activity. Chrystallographic studies showed the dimethylguanylurea fragment of argifin establish significant interactions with chitinase catalytic site binding it with only one intramolecular hydrogen bond, whereas there are four intramolecular hydrogen bonds in argadin-chitinase complex (Houston *et al.* 2002b). This may explain the 1000-fold greater inhibition by argadin over argifin. Argadin strongly inhibits blowfly *Lucilia cuprina* chitinases at very low concentrations (1 nM). Its inhibitory activity is about 20-fold more potent than that of allosamidin (Arai *et al.* 2000b). Although its low inhibitory activities on chitinases 18 family, argifin inhibits the growth of the cockroach larvae *Periplaneta americana* (Arai *et al.* 2000a; Omura *et al.* 2000). Recently chemically synthesized (Dixon *et al.* 2005), this cyclopentapeptide was shown to affect chitinase B1 of *Aspergillus fumigatus* and human chitinases (Dixon *et al.* 2005; Rao *et al.* 2005). Argadin poorly affects microorganisms chitinases but its inhibitory activities were reported on the dipteran chitinases of *Lucilia cuprina*. Furthermore, injection of this inhibitor in cockroach larvae induced an

increase of 60% mortality compared with the non-injected larvae (Arai *et al.* 2000b).

Cyclic dipeptides

The first cyclic dipeptide discovered, cyclo(L-Arg-D-Pro) also named CI-4, has been isolated from *Pseudomonas sp.*, a marine bacterium (Izumida *et al.* 1996a). Presenting a moderate activity against chitinases (Izumida *et al.* 1996a; Houston *et al.* 2002a), CI-4 induces *in vivo* inhibition of cell separation of the yeast *Saccharomyces cerevisiae* (Izumida *et al.* 1996b). Hyphal growth and elongation of the filamentous fungus *C. albicans* are disrupted by CI-4 (Izumida *et al.* 1996b). Crystallographic studies associated with *in vivo* bioassays have shown that inhibitory activity is correlated with the affinity of the inhibitor for the active site of chitinase and particularly with specific amino acids from the catalytic site (Houston *et al.* 2004). CI-4 inhibits chitinases at micromolar concentrations and shows mimetic structures and a similar mode of interaction than allosamidin with the chitinase active site. Other cyclic dipeptides were studied: cyclo(L-Arg-L-Pro), cyclo(L-Gly-L-Pro), cyclo(L-Tyr-L-Pro) and cyclo(L-His-D-Pro). Proline residues appeared to be essential for the interaction of these compounds with chitinases. However, the numerous interactions established between the Histidine residue and the enzymatic active site of ChiB from *S. marcescens*, confers to cyclo(L-His-D-Pro) a higher affinity than other cyclic dipeptides. Its inhibitory activity on cell separation of *S. cerevisiae*, is lower than that of CI-4 (Houston *et al.* 2004). Moreover, these interactions are weaker than allosamidin which tightly binds chitinases. Saguez *et al.* (2006) showed that cyclo-(His-Pro) and cyclo-(Tyr-Pro) had poor effects on aphids.

Psammaplin A

Psammaplin A, made of two brominated tyrosine linked by a disulfur bond, has been extracted from the sponge *Aplysinella rhax* (Tabudravu *et al.* 2002). This phenolic compound presents interesting medicinal properties (Kim *et al.* 1999a, 1999b; Shim *et al.* 2004) and was studied for its potential inhibition of chitinases. Psammaplin A binds near the active site of chitinases and its inhibitory activity is lower than that of allosamidin. Moderate and variable inhibitory effects were described in *Bacillus* sp., *Streptomyces* sp., *Actinomyces* sp. and *S. marcescens* bacterial chitinases. Psammaplin A inhibits the growth of vine mildew *Plasmopora viticola*. Variable effects have been reported on insects. While no insecticidal effects were induced in the dipteran *Drosophila melanogaster* and the lepidopteran *Heliothis virescens*, psammaplin A induces 55% mortality in the lepidopteran *Plutella xylostella* (Tabudravu *et al.* 2002) and causes 100% mortality in the aphids *Myzus persicae* and *Macrosiphum euphorbiae* (Saguez *et al.* 2006). Moreover, intoxicated with low doses of this compound, *M. euphorbiae* showed a significant reduction of fecundity and abnormal development, including uncompleted moult.

CONCLUSIONS AND PERSPECTIVES

The variability of chemical nature and structure of chitinase inhibitors and their interactions with the catalytic site of chitinases are key factors of their inhibitory activities. Mimicking chitin or chitin degradation intermediates, all these compounds differently interact with chitinases catalytic site, changing different rates of inhibitory activities which also depend on chitinase origin. These substrate-specific inhibitory activities constitute an interesting property to obtain species-specific inhibitors that would selectively inhibit chitinases and target pests or pathogens. Because they induce antibiotic, antifungal and insecticidal effects, oligosaccharides, polysaccharides chitin mimetics and mimetic peptides could offer new tools to be developed as agrochemical agents**.** Some of these inhibitors, such as chitosan, also elicit plant defences. They can be considered as potential alternatives to synthetic and chemical pesticides in crop protection programs. However, up to now, no compound is commercially available to manage pathogens and pests because of their cost of production and their difficulties of synthesis. A better knowledge of the interaction between chitinase inhibitors and their targeted enzymes is a prerequisite that would allow to select compounds having specific actions on pests and pathogens. Furthermore, in addition to the demonstration of their field efficacy, the properties of these inhibitors such as their environmental fate (i.e. stability, biodegradation, remanence) must also be taken into account. Effects on non-target beneficials, such as predators and parasitoids, have to be studied to better frame their usefulness in the context of Integrated Pest Management programs. In a near future, concerted efforts should lead to the identification of new natural or chemically synthesized chitinase inhibitors for crop protection, reducing environmental risks due to chemical pesticides.

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