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Review

Current status of action mode and effect of chitosan against phytopathogens fungi

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Chitosan is a deacetylated derivative of chitin, consisting mainly of glucosamine units, commercially obtained from crustacean waste. This natural compound is biodegradable and nontoxic and has diverse applications in agriculture, among which highlights the control of fungal diseases in crops of agricultural interest. This review focuses on some basic studies about the mode of action and the effect of chitosan on different phytopathogens fungi. In general, it is known that molecules of this polymer can act on extracellular (plasma membrane) and intracellular level (penetration of chitosan into the fungal cell). The study of the effect of chitosan on different phytopathogens fungi evidence that the response is variable; in some investigations, it was found that the spores are more sensitive than hyphae to the application of chitosan. Even though the progress in understanding the mode of action of this polymer and the various effects that can cause damage are known, it is necessary to carry out more studies about the biological activity of these molecules to propose better control strategies of the phytopathogens fungi.

Key words: Antifungal activity, polymers, *in vitro* studies, plasma membrane, fungal cell, chitosan.

INTRODUCTION

Chitin is present in the exoskeleton of crustaceans and insects and the cell walls of some fungi and is considered the second most abundant polymer in nature (Rinaudo, 2006). Chitosan is a deacetylated derivative of chitin mainly composed of glucosamine units, 2- amino-2 deoxy-β-D-glucose (Freepons, 1991). The commercial chitosan is obtained in different countries from waste crustaceans fisheries and food industry through food processing process, the major sources of production include shrimp, crab and lobster (Du et al., 2009; Al Sagheer et al., 2009; Falcón et al., 2008). This natural compound is biodegradable and nontoxic, and has a positive charge that confers to this polymer numerous physiological and biological properties with great potential in diverse industries (Rinaudo, 2006; Aranaz et al., 2009), among them in the food industry and in the agriculture (Chien et al., 2007a; No et al., 2007). Recently, it was demonstrated that the application of the chitosan treatment (0.02 gmL^{-1}) could be used to reduce deteriorative processes, maintain quality and increase the shelf life of fresh-cut papaya stored at 5°C (González-Aguilar et al., 2009). Other studies suggest that it is feasible to elaborate antifungal chitosan films, with good thermal stability and acceptable mechanical properties for food packaging (Martínez-Camacho et al., 2010). Additionally, the biological activities of chitosan depend on its physicochemical properties (Kim and Rajapakse, 2005).

In Agriculture, it has several applications, which highlights the control of fungal diseases in crops of

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agricultural interest. However, its antimicrobial activity and mode of action must be studied in greater depth, particularly if one takes into account the large potential for production and application of chitosan in Agriculture in Latin America (Lárez-Velásquez, 2008). In recent years, chitosan coatings have been used for preservation and to extent the shelf life on fruits (Chien et al., 2007b; Fisk et al., 2008; Ponce et al., 2008; Sangsuwan et al., 2008). Additionally, it is reported that chitosan applications on fruits helps to maintain quality, induces resistance to decay and stimulates defense reactions in plants (Meng et al., 2008; Trotel-Aziz et al., 2006; Zhu et al., 2008).

The factors that influence the mode of action of chitosan have been classified into four types like intrinsic, environmental, microorganism and physical state (Kong et al., 2010). There are several scientific papers focused on chitosan antimicrobial effect. In this review, we mentioned some of them and analyzed the results obtained in previous investigations that could contribute to propose better strategies of control of phytopathogen fungi taking account a sustainable technology development.

MODE OF ACTION OF CHITOSAN

In general, it is known that the mode of chitosan action on phytopathogens fungi could development in an extra level (plasma membrane) and intracellular level (penetration of chitosan on fungal cell) (Guo et al., 2008; Palma-Guerrero et al., 2008).

Action of chitosan on the plasma membrane

Several studies suggest that chitosan neutralizes the electronegative charges on cell surfaces and the cell permeability is changed; therefore, this interaction causes the leakage of intracellular electrolytes and proteinaceous material of the cell (Guo et al., 2008). In previous reports was demonstrated that chitosan provoked the leakage of amino acids and proteins of the *Rhizopus stolonifer* cell (El Ghaouth et al., 1992a). Similar results was obtained on three isolates on *R. stolonifer* grew in minimum medium, in that study there were an increased release of compounds at 260 and 280 nm with chitosan of different molecular weight (Guerra-Sánchez et al., 2009). In other studies, potassium ion leakage was demonstrated by effect of chitosan on fungal cell, being more pronounced for the first 5 min (Singh et al., 2008; García-Rincón et al., 2010).

In general, it is known that chitosan treatment causes changes in the membrane integrity of spores, modifications in pH media and the proteins release. This effect was different depending on the isolate, kind of chitosan and used concentration (Hernández-Lauzardo et al., 2010a). On the other hand, the membrane integrity of *P. expansum* and *B. cinerea* spores was affected by chitosan. *P. expansum* was more sensible than *B. cinerea*; and the effect was related with the fungal species (Liu et al., 2007). In other studies, chitosan affected the membrane integrity on *S. sapinea* allowing the outflow of cell components (Singh et al., 2008). Besides, chitosan could be affecting the plasma membrane properties. It was demonstrated that this polymer caused a decrease in the H^+-ATPase activity on plasma membrane of *R. stolonifer*; this effect could provoke the accumulation of protons inside the cell, which would result in the inhibition of the chemiosotic driven transport that allows the H^+/K^+ exchange (García-Rincón et al., 2010).

Current research suggests that the plasma membrane forms a barrier to chitosan in chitosan-resistant but not chitosan-sensitive fungi. Additionally, it was reported that the plasma membranes of chitosan-sensitive fungi had more polyunsaturated fatty acids than chitosan-resistant fungi, suggesting that the permeabilization by chitosan may be dependent on membrane fluidity (Palma-Guerrero et al., 2010).

PENETRATION OF CHITOSAN ON FUNGAL CELL

Few reports demonstrated that chitosan could penetrate the fungal cell. Recent studies of chitosan-fungal cell interactions showed that the polymer penetrates the cell and cause intracellular affectations. It was found that chitosan by an energy-dependent process quickly penetrated the conidia of *F. oxysporum* (less than 15 min) and caused ultrastructural alterations (disorganized cytoplasm, retraction of the plasma membrane and loss of intracellular content) in the treated spores (Palma-Guerrero et al., 2008). However, is evident that a chitosan tracer is needed to evaluate the capture and dissemination within the cell.

Previous report showed that oligochitosan penetrated the fungal cell and caused disruption on endomembrane system of *Phytophthora capsici*, such as, distortion and disruption of most vacuoles, thickening of plasmalemma and appearance of unique tubular materials (Xu et al., 2007a). Additionally, other studies in this plant pathogenic fungus with oligochitosan marked confirmed that, the polymer penetrated the membrane and binds to nucleic acids (Xu et al., 2007b).

EFFECT OF CHITOSAN ON PHYTOPATHOGENS FUNGI

The effect of chitosan on phytopathogens fungi has been evaluated on different researches. In general, the obtained response is variable and dependent of the fungal cell, previous studies revealed that the spores are

more sensitive than the hyphae to chitosan application. The effect of the polymer has been attributed to several factors such as, the degree of deacetylation, molecular weight and concentration. Most physiological activities and functional properties of chitosan depend on their molecular weight (Rabea et al., 2003). *In vitro* studies on *Botrytis cinerea* showed that the antifungal activity increased when the chitosan molecular weight decreased (Badawy and Rabea, 2009). Similar results were found in *Aspergillus niger*, the highest antifungal activity was observed with low molecular weight chitosan (Xiao-Fang et al., 2008). Also, low molecular weight chitosan showed a marked inhibition of mycelial growth of *B. cinerea* and *Penicillum expansum* (Liu et al., 2007). However, other studies demonstrated that high molecular weight chitosan caused a noticeable inhibition of mycelial growth of *Fusarium oxysporum*, *Alternaria solani* and *Valsa mali* (Guo et al., 2006). It is known that the molecular weight of chitosan also influences in the physiological-biochemical response. For example, on three isolates of *R. stolonifer*, it was observed that the medium molecular weight chitosan showed a major effect on the release of protein while the highest glucose consumption was induced with chitosan of low molecular weight (Guerra-Sánchez et al., 2009). Moreover, it has been reported that chitosan and oligochitosan inhibited the mycelial growth and germination of spores of the fungal patho-gens, *Alternaria kikuchiana* and *Physalospora*, the effect was most evident on the fungal mycelium (Meng et al., 2010).

It was demonstrated that the low molecular weight chitosan was more effective for inhibition of mycelial growth of *R. stolonifer* while the high molecular weight chitosan affected spore shape, sporulation and germination (Hernández-Lauzardo et al., 2008). Other studies showed that chitosan completely inhibited spore germination of *Fusarium oxysporum* and *Verticillium dahliae*; the spores were clearly more sensitive to chitosan than hyphae (Palma-Guerrero et al., 2008). Similar results were found on *Botrytis cinerea* and *P. expansum* (Ben-Shalom et al., 2003; Liu et al., 2007). The effectiveness of chitosan has been demonstrated on spores of *Fusarium oxysporum* treated with a low concentration (0.01%) of this polymer (Tikhonov et al., 2006). Also, it was reported that chitosan derivatives affected more than 90% germination of *F. oxysporum* (Rabea et al., 2009). On the other hand, oligochitosan was more effective than chitosan in inhibiting mycelial growth of *P. capsici* and its inhibition on different stages in life cycle of this fungus was observed. Furthermore, rupture of release of zoospores was showed (Xu et al., 2007a).

Chitosan severely affected the morphology on *Sphaeropsis sapinea*. It was observed that an increase concentration of chitosan provoked excessive branching, vacuolation and reduced the hyphal diameter (Singh et al., 2008). Other morphological change on phytopathogens by chitosan effect has been observed. *R. stolonifer* showed morphological change in their hyphae (excessive branching) by effect of 3 mg mL^{-1} of chitosan. Moreover, their mycelial growth was inhibited (Ghaouth et al., 1992b). However, in other studies chitosan inhibited the mycelial growth of *R. stolonifer* with lowest concentration of this polymer, therefore the application of chitosan to control the plant pathogen may be more effective (1.0, 1.5 and 2.0 mg mL^{-1}) (Hernández-Lauzardo et al., 2008). Other studies demonstrated that the mycelial growth and sporulation of the three isolates of *R. stolonifer* (from peach, papaya and tomato) were markedly inhibited at all tested chitosan concentrations (1.0, 1.5 and 2.0 mg mL^{-1}). The highest antifungal indexes and sporulation reduction were observed with chitosan at 2 mg mL^{-1}. Additionally, the morphological characteristics of the spores of *R. stolonifer* showed different behavior depending on the evaluated isolates (Hernández-Lauzardo et al., 2010b).

Oligochitosan was more effective than chitosan in inhibiting mycelial growth of *Phytophthora capsici*, although both were effective in controlling different phytopathogens. Thus, the molecular weight of chitosan influences the antifungal activity of this polymer (Xu et al., 2007a). It is important to comment that in other research's the results showed that deacetylated degree influenced on antifungal activity of chitosan on *Phytophthora parasítica*; the lowest deacetylated degree caused the highest inhibition of mycelial growth (Falcón et al., 2008). Another important stage of development of fungi is the sporulation; the effect of chitosan on the same has been evaluated in some phytopathogens of interest (Cruz et al., 2004; Manjunatha et al., 2008). It is known that chitosan with different molecular weights can affect the sporulation on isolates of *R. stolonifer* (Hernández-Lauzardo et al., 2008). In previous studies, a decrease on the formation of sporangios when *R. stolonifer* grew in the presence of chitosan (0.75 – 6.0 mg mL^{-1}) was observed. However, there were no counts of spores of this fungus (El Ghaouth et al., 1992b). On the other hand, it have been demonstrated that the spores of *R. stolonifer* treated with chitosan of high molecular weight (2 mg mL^{-1}) showed the highest variation on their shape (elliptical form factor) and the highest relative frequency of globose spores was observed with high molecular weight chitosan for all concentrations tested. Additionally, studies of scanning and transmission electron microscopy revealed numerous and deeper ridge ornamentations of the spores treated with chitosan of different molecular weight (Hernández-Lauzardo et al., 2008).

Additionally to previous studies mentioned in this paper, in some of the studies evaluates the effect of chitosan combined with different natural alternativ es. For example, the combined effect of chitosan with yeast

(*Pichia guillermondii*) for control to *Penicillum digitatum* was studied. The results demonstrated that the biopolymer and yeasts presented an additive effect, since chitosans were effective to delay spore germination, whereas *P. guillermondii* decreased the fungal growth (Pacheco et al., 2008).

CONCLUSIONS

Several researches have been developed around the mode of action of chitosan to explain the effect of this polymer. All have produced results that support the different modes of action of the molecule; probably the size or the molecular weight of chitosan influences the mode of action that primarily is triggered. Given the above research, one might infer that the greater the number of chitosan molecules to penetrate the intracellular level, there will be more likely that the polycationic polymer to interact with intracellular structures and molecules provoking damage and causes affect their development. Despite the progress in understanding the mode of action of chitosan and the various effects that can result in different fungal pathogens still need to generate scientific information to understand more precisely the biological activity of chitosan molecules. Comprehensive knowledge of the action of chitosan in fungal cells will increase the chances of successful application of this natural alternative in the control of fungal pathogens.

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