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Chitosan and its derivatives, natural polymers with potential for control of *Pyricularia oryzae* (Cav.)

Quitosano y sus derivados, polímeros naturales con potencial para controlar a *Pyricularia oryzae* (Cav.)

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ABSTRACT: Chitosan and its derivatives are natural compounds that have potential in agriculture for the control of one of the rice diseases, pyriculariosis (*Pyricularia oryzae*), of great importance worldwide. In general, this disease is controlled with synthetic fungicides belonging to the benzimidazole group; however, their use has generated adverse results to the environment, together with the low sensitivity of the fungus to them. This article provides a review of published research on chitosan, its physicochemical characteristics, general information on the fungus *P. oryzae*, the fungicidal action of chitosan and its derivatives in *in vitro* and *in situ* research on this fungus, and in general, the possible mechanisms of action of this compound.

Key words: antimicrobials, biocompounds, mechanisms of action, Magnaporthe, *Oryza sativa* L.

RESUMEN: El quitosano y sus derivados son compuestos naturales que tienen potencial en la agricultura, para el control de una de las enfermedades del arroz; la piriculariosis (*Pyricularia oryzae*), de gran importancia a nivel mundial. En general, esta enfermedad se controla con fungicidas sintéticos pertenecientes al grupo de los benzimidazoles; sin embargo, su uso ha generado resultados adversos al medio ambiente, aunado a la poca sensibilidad del hongo hacia ellos. En este artículo, se proporciona una revisión de investigaciones publicadas acerca del quitosano, sus características físicoquímicas, generalidades del hongo *P. oryzae*, la acción fungicida del quitosano y sus derivados en investigaciones llevadas a cabo *in vitro* e *in situ* sobre este hongo, y en general, los posibles mecanismos de acción de este compuesto.

Palabras clave: antimicrobianos, biocompuestos, mecanismos de acción, Magnaporthe, *Oryza sativa* L.

INTRODUCTION

Chitosan polysaccharide is a class of natural macromolecule that has an extremely bioactive tendency and is derived from the exoskeleton of crustaceans such as lobsters, crabs and shrimps (1). Chitosan, a partially deacetylated polymer of chitin, has the ability to be biodegradable, biocompatible and non-toxic, which is why it is considered a very attractive compound. In agriculture, it is used to stimulate germination, modify soils, as a fungicidal agent and as an elicitor of defensive responses in plants, among others. Also, in the area of food technology, it is

used in the elaboration of biodegradable films and antimicrobial packaging films (2,3).

Among the pathogens that this compound has been shown to have antifungal activity is *Pyricularia oryzae* (Cav.). This fungus produces the disease pyriculariosis, which is of great importance in rice cultivation. It causes great damage and it is widely distributed throughout the world (4). Chitosan and its derivatives have been shown to act directly on the fungus, inhibiting its mycelial growth and stimulating the defense mechanisms in the rice crop, protecting the plant from the attack of this pathogen (5-8).

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Chitosan. Chemical and physical characteristics

Chitosan is a polysaccharide obtained from chitin, the second most abundant polysaccharide in nature; it is a linear copolymer formed by residues of D-glucosamine units, to a greater extent, and N-acetyl D-glucosamine, to a lesser extent; randomly distributed and linked by β 1,4 bonds. According to the International Union of Pure and Applied Chemistry (IUPAC), it is 2-amino 2-deoxy-D-glucopyranose (D-glucosamine GlcN) and 2-acetamide-2-deoxy-D-glucopyranose N-acetyl glucosamine (1). Both the content and the sequence of these units will determine the physicochemical and biological properties of this polymer. Chitosan has a high nitrogen (N) content and has a regular distribution of free amino groups, which can be protonated by certain acids, becoming positively charged and this gives it a polycationic character. This fact allows explaining some of its properties, such as the ability to bind with negatively charged substances such as lipids, proteins, colorants, among others; as well as flocculant, adherent and adsorbent, in addition to the typical reactions of amines (9). This biopolymer has antimicrobial activity against a wide variety of microorganisms, including fungi, algae and some bacteria. Its functionality and activity depends on its characteristics, such as molecular mass, degree of acetylation, host cell, presence of natural nutrients, chemical or nutritional composition of the substrates and environmental conditions.

Pyricularia oryzae, causal agent of rice blast or rice scorch

Pyricularia and *Magnaporthe* are currently widely used generic names for the fungus that causes rice scorch. The *Pyricularial Magnaporthe* Working Group has considered the possibility of retaining the name *Magnaporthe* over *Pyricularia*. However, such retention would require a change in the type species of the genus *Magnaporthe* and would cause numerous name changes for those species currently placed in *Pyricularia*.

The asexually typed generic name, *Pyricularia*, is the correct name for the rice scorch fungus, which corresponds well with pathogenicity and ecological and evolutionary characteristics. Therefore, the name *Pyricularia oryzae* should be used for the fungus that produces this disease. However, the synonym *Magnaporthe oryzae* can continue to be referred to in publications as *Pyricularia oryzae* (syn. *Magnaporthe oryzae*). This practice will help to close a potential gap in the literature and knowledge of this important species (10). As for *grisea* and *oryzae*, they are very different species. *Grisea* is for strains of *Digitaria*, and *oryzae* is for strains of rice, wheat and other grasses; although in the literature, they are considered synonyms and all four names are used: *Pyricularia oryzae*, *Magnaporthe oryzae*, *Pyricularia grisea* and *Magnaporthe grisea*.

Pyricularia oryzae, known as pyriculariosis, blast, blight, blight, leaf blight or rice blight, is one of the most serious diseases of rice (*Oryza sativa* L.), which has caused

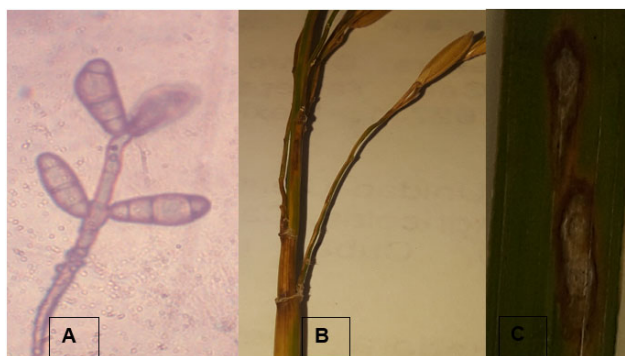


Figure 1. A) *Pyricularia oryzae* conidia, B) and C), rice stem and leaf infected by this fungus

significant losses in yields worldwide (11). It is a very effective plant pathogen, since it can reproduce sexually (teleomorph: *Magnaporthe grisea* Barr (It. Hebert) syn. *Magnaporthe oryzae*) and asexually (anamorph: *Pyricularia oryzae*). The distribution of the blast is worldwide, as it is found in all agroecosystems of the tropics and temperate zones where commercial rice is grown. Pyriculariosis generates large losses in grain production, both in rainfed and irrigated systems. This disease has been reported in at least 85 countries worldwide. It was discovered in Italy in 1560 and was later found in China (1637), Japan (1760), the United States (1960) and India (1913) (12). Pyriculariosis has caused significant losses, for example: in India 90 % (13), in China 70 % (14), in Thailand only 1900 ha were affected in 1987 and in 1988 it increased to 490 000 ha, in Spain and in the Mediterranean area it has also caused damages (15). Mexico has reported a drop in production of up to 30 % in rainfed crops. Cuba has been another country affected by this pathogen, and when conditions are favorable, losses have increased up to 70 %.

Taxonomic classification, morphology and symptomatology of *Pyricularia oryzae*

The causal agent of pyriculariosis is classified taxonomically in the class: Deuteromycetes, order: Moniliales, family: Dematiaceae, genus: *Pyricularia*, species: *Pyricularia oryzae*. *Pyricularia oryzae* has simple, tabulate, brownish conidiophores (Figure 1 A). The conidiophores are borne singly or in groups of three and carry conidia at their ends. These are hyaline, fusiform and are divided equidistantly by two septa. Their approximate size is 22 to 24 μm x 10 to 12 μm . More than one conidium can form on the conidiophore, the number of conidia ranges from 1 to 20.

It is a complex disease, due to the pathogenic variability and the speed with which this fungus overcomes the resistance of the rice plant. The mycelium of the fungus produces a toxic substance known as pyricularin, which inhibits the growth of tissues and disorganizes them. It attacks the aerial parts of the plant such as leaves, stems, nodes and spikes (16) (Figure 1, B, and C). The fungus produces spots or lesions on the leaves of elongated or elliptical to rhomboid shape, of uniform brown color, which

later will change to a grayish color in the central part, a fact that indicates the sporulation of the fungus; although its size and color vary according to environmental conditions and susceptibility of varieties (17). Its optimum growth temperature is between 22-29 °C and a high relative humidity, around 90 %, which fully coincide with the climatic conditions of tropical countries. The presence of high concentrations of nitrogen in the water also favors the development of the fungus and produces large quantities of spores. The spores arrive from crop residues from the previous season or from weeds, where the fungus has been harbored during the winter (18).

General strategies for control of *Pyricularia oryzae*

Disease control is mainly based on the application of systemic chemical fungicides, including benzimidazoles such as prochloraz, tebuconazole and propiconazole, among others. However, their use has had several disadvantages, since they cause contamination of the water table and adjacent bodies of water, generating harmful effects on various organisms. In addition, it is important to point out that these chemical fungicides are being damaged by the fungus, due to the emergence of populations with loss of sensitivity to their action mode (19). Due to this situation, there is an increasing number of fungicide cycles per year to protect crops against this disease, which implies an increase in production costs and in the negative effects of conventional fungicides on the environment, questioning their commercial use, being a priority the search for alternatives that allow complementing the integrated management of diseases.

In vitro and *in vivo* activity of chitosan and its derivatives on *Pyricularia oryzae*

The antimicrobial property of chitosan and its derivatives has received considerable attention in recent years due to the impending problem associated with synthetic chemical agents. These compounds have been shown to be fungicidal and fungistatic for the control of *Botrytis cinerea*, *Aspergillus flavus*, *Aspergillus parasiticus*, *Drechstera sorokiana*, *Fusarium acuminatum*, *Fusarium graminearum*, *Micronectriella nivalis*, *Rhizoctonia solani*, *Alternaria alternata*, *Colletotrichum gloesporioides*, *Penicillium* spp, *Fusarium oxysporum* and *Bipolaris oryzae*, among other fungi (20-24).

In this regard, the antifungal activity of chitosan and its derivatives has been observed in different stages of fungal development, such as affecting the growth and development of the pathogen, sporulation, viability and germination of spores and the production of fungal virulence factors (21,25,26). Some authors have demonstrated the fungicidal effect of these compounds at different concentrations and isolates of *P. grisea* (5,7,27). Also, chitosan oligomers have shown to have a better inhibitory effect on this pathogen, achieving the minimum inhibitory

concentration at a concentration higher than 2000 mg L⁻¹ (28).

Studies carried out in the laboratory of Oligosaccharins of the National Institute of Agricultural Sciences (INCA), with *P. grisea* indicate that chitosan and its oligomers in the culture medium, at a concentration of 1,000 mg L⁻¹ and pH 5.6, totally inhibited the mycelial growth of this fungus (5). However, it is important to consider the pH of the resulting solution, which affects the positive charge of the amino groups; since in another test, at pH 6, there was only a slight affectation of the fungus growth, although a total inhibition of sporulation was maintained (7).

Some research groups have begun to modify the chitosan molecule with the addition of hydrophobic groups to increase its biological activity against this pathogen. For example, N-sulfonated N-sulfobenzoyl chitosan (29), N,N,N-trimethyl chitosan (30), N,O-acyl chitosan (31), O-acyl chitosan (32,33), hydroxyethyl aryl chitosan (34), dimethylpiperazine chitosan (35), carboxymethyl chitosan (36), acyl urea thiourea chitosan (37), N-succinoyl chitosan (38) and N-heterocyclic chitosan (39). Researchers (40) have noted that N-alkylation or N-arylation of chitosan with aromatic or aliphatic aldehydes effectively increased its antifungal activity on *P. grisea*.

With the same methods and obtaining techniques, but using different types of aldehydes, scientists (29) observed antifungal activity of 24 new chitosan derivatives (N-benzyl chitosan derivatives), which had a greater inhibitory effect than native chitosans on the growth and spore formation of *P. grisea*; N-(m-nitrobenzyl) chitosan had the greatest effect at a concentration of 5 g L⁻¹. Also, these authors observed that the most active derivative was N-(2,2 diphenylethyl) chitosan, with a minimum inhibitory concentration of 0.3 g L⁻¹, against this pathogen. Moreover, great advances of chitosan and its oligomers on the direct control of rice diseases have been observed. Both chitin and chitosan have been shown to induce the accumulation of phytoalexin production, in this case, of momilactones A (41) and momilactones B upon infection with *P. grisea* in rice leaves at a concentration of 10 µg mL⁻¹.

A group of researchers published the effect of chitosan on the stimulation of defense responses in rice leaves (42). After treatment with 0.1 % chitosan, necrosis was clearly observed in the upper part of the rice leaf. However, treating rice seedlings with 5 mg L⁻¹ and inoculating them with *Magnaporthe grisea* 97-23-2D1 showed a better effect and control of the disease by more than 50 % (43). However, in 2007 it was evaluated, in semi-controlled conditions, where rice seeds were treated at different concentrations with two chitosans of different molecular weight (6). Eighteen days after seed germination, plants obtained were inoculated with *P. grisea* spores, and the activity of enzymes related to defense, such as PAL, glucanase, chitinase and chitosanase, was evaluated, observing an increase in the activity of the plants treated with the elicitors, with respect to the control. In addition, no disease symptoms were observed in the highest concentration of both compounds used.

Currently, research is being conducted on the application of chitosan-based nanoparticles with antifungal activity and for the control of diseases such as pyriculariosis (8,44,45). On the other hand, researchers (44) found that chitosan and silver (Ag) nanoparticles had a high antifungal activity on *Pyricularia oryzae*, at a concentration of Ag (2 ppm) and chitosan (4000 ppm). However, other scientists (8) applied 500 µl of a 0.1 % chitosan nanoparticle solution on rice leaves, and 24 h later, a spore suspension (1×10^5 spore mL⁻¹) of *P. grisea*, and after 10 days, no disease symptoms were observed.

Mechanisms of chitosan action

Mechanisms of chitosan action have not been fully established, although there are some hypotheses in this regard. In general, the various proposals to explain the antimicrobial activity of chitosan consider, as a fundamental characteristic, the polycationic nature of the molecule, which is given by the NH_3^+ groups of glucosamine, which confers important biological and physiological properties (46,47). In pH conditions, chitosan behaves as a linear polyelectrolyte, with a pH around 6.5, therefore, at low pH the glucosamine residues are positively charged due to the protonation of its amino residues, containing a high density of positive charges, which allows it to bind strongly to negatively charged surfaces (48). It is proposed that when the positive charge on the C-2 of the glucosamine monomer is below pH 6, chitosan is more soluble and has better antimicrobial activity than chitin (29,49).

Another proposed mechanism is the interaction between the positive charge of the chitosan molecule and the negative charge of the microbial membrane cells, or leading to the outgassing of proteins and other intracellular constituents (29). The most abundant of the sphingolipids is mannosyldiinositophosphate-ceramide ($\text{M}(\text{IP}_2)\text{C}$), which has two negative charges. Sites where chitosan could bind using its amino groups of glucosamine residues, which are able to interact with the negative components of $\text{M}(\text{IP}_2)\text{C}$ of the plasma membrane. In other studies, it has been observed that chitosan forms transport channels for molecules in artificial lipid bilayers, which provides evidence that this compound can disorganize the cell membrane (48). In turn, a working group (50,51) analyzed the action mode of chitosan on fungal cells and observed two aspects: that chitosan permeabilizes the fungal plasma membrane and penetrates fungal cells, a process that is ATP-dependent, and also showed that different cell types (conidium, germ tube and hyphae) exhibit different sensitivity to chitosan. In 2010, the same group of researchers demonstrated, through biological, biochemical, genetic and biophysical techniques, that the antifungal activity of chitosan depends on the fluidity of the fungal plasma membrane, which is determined by the composition of its polyunsaturated fatty acids, and this suggests a new strategy for antifungal therapy, involving treatments that increase the fluidity of the plasma membrane to make the fungus more sensitive to fungicides, such as chitosan.

Chitosan also acts as a chelating agent that selectively binds trace metals and thereby inhibits toxin production and mycelial growth (52). In addition, it activates some defense processes in host tissues (53), acts by inhibiting several enzymes; the binding of chitosan to DNA and the inhibition of mRNA synthesis and protein synthesis (54).

In the case of silver nanoparticles (Ag), it is based on the possibility that these adhere and penetrate the cell membrane causing osmotic imbalance in the spores, being very effective against *Magnaporthe grisea* (55).

CONCLUSIONS

- The reported literature shows that chitosan and its derivatives are capable of acting on *P. oryzae*, either directly by inhibiting mycelial growth and spore production, or by inducing defense mechanisms in the rice plant, so that these compounds could be used in agriculture, making it more sustainable.
- It is convenient to deepen in other lines of research, such as the evaluation of these compounds in field studies, the feasibility of developing commercial products based on this compound, focusing not only on control, but also on the possible mechanisms of action by which these compounds act on the fungus and on the plant.

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