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REVIEW ARTICLE

Review of fungal chitosan: past, present and perspectives in Brazil

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Abstract

Fungal chitosan is a polymer that has been discussed and studied since 1859 in the world with great advances occurring over the years. Due to its global importance, this review aims to expose the history of the production and application of fungal chitosan in Brazil. Data collection was done at the Scielo, Sciencedirect and Pubmed databases, considering the period of the last 50 years. The inclusion criteria were articles on pure or associated chitosan and, in particular, fungal chitosan produced or applied by Brazilian research groups. At the end of the review, it was noticed a fungal chitosan very studied in different continents, and in Brazil is still used in specific and small groups. With the present work, it is expected that the diffusion of the studies will be accelerated and that potential research groups for fungal chitosan may grow through interaction with the existing ones.

Keywords: *fungal chitosan, biopolymer, biomedical application, biotechnology industries.*

1. Introduction

The production of chitosan by the process of chemical deacetylation from the shell of crustaceans is financially advantageous for some industries. In Brazil, this process produces, in general, medium molecular weight chitosan and ~ 80% deacetylation degree. Chitosan with this standard is marketed nationally by the company Polymar Science and Nutrition S.A, located in Ceará and classified as food by the Ministry of Health. It is marketed only with weight reducer and cholesterol. This marketing line released by the Brazilian Ministry of Health takes into account literature reports on the purity of preparations containing chitosan obtained from α-chitin, which does not always meet the standards required in the areas related to pharmacy, medicine and food[1-4].

In order for the chitosan obtained from α-chitin to be used by the pharmaceutical, medical and food industries, it is necessary that several specific purification processes need to be done in order to correctly remove traces of proteins and pigments[5,6]. These processes raise production costs and decrease yield. These processes often depolymerize the resulting chitosan in a random manner[7,8], in addition to producing many chemical pollutants that are difficult to reuse or discard. This fact does not occur with chitosan originating from fungi, leaving its production potentially profitable financially.

Another argument is the difficulty of adapting a standard in the production of chitosan from the crustacean shell inside an industrial plant, in order to maintain the physicochemical properties of the biopolymer[9].

With this in mind, the microbiological production of chitosan from submerged fungi cultures has the advantage of being easier to set a standard for desirable physicochemical characteristics and to implement in an industrial plant because it has the advantage of yielding greater production in smaller areas[10-14]. As well as contributing to the reduction of environmental waste generated by the production of chitosan from the deacetylation of α-chitin.

Based on its biotechnological potential and global importance, this review intends to organize and catalog the researchers and research groups that work directly or indirectly with fungal chitosan in Brazil with a view to facilitating the exchange of knowledge between the groups. In addition to promoting the dissemination of what has been done about the production and application in the last 33 years, since it was published pioneering work in Brazil on the analysis of the cell wall of fungi belonging to the class of Zygomycetes, Mucorales order by Campos-Takaki et al.[15].

2. Fungal Chitosan

The fungal cell wall is mainly composed of a network of interconnected molecules consisting of proteins, glucans, chitin, chitosan, lipid and polyphosphates which may have the quantity and/or quality changed due to environmental conditions and intrinsic characteristics of their own species[15,16]. Among the compounds found in the cell wall of fungi, chitosan stands out for being associated to increased integrity, favoring protection against high temperatures and cell inhibitors to which the fungal strain may be subjected[17-19].

The production of chitosan in the cell wall of fungi occurs due to the activity of the chitin deacetylase enzyme (EC 3.5.1.41) on the chitin residues present in the cell wall itself. This synthesis was first described in 1974 by Araki and Ito in Mucor rouxii (Zygomycetes) showing that about 14% of chitin deacetylase directly involved in the natural synthesis of chitosan in fungi is present and associated with particles of cell fractions, 49% is associated with a soluble fraction of the supernatant, and about 37% is extracellular[20]. Complementing the route of synthesis of chitosan, Davis and Bartnicki-Garcia[21] found strong indications that the activity of the chitin deacetylase is highly efficient on residues from a growing chain of chitin, and has low efficiency in the preformed chitin molecule suggesting that changes in the culture medium in which the fungi grow can directly influence the production of chitosan. In the following years, new researchers studied the line of analysis of the wall and the confirmation of the presence of chitosan in different fungal species[22-24].

3. History of Fungal Chitosan in the World

The first chitin isolation reports are from 1811, when the French professor Henri Braconnot conducted tests with isolates from *Agaricus volvaceus*, *A. acris*, *A. Cantarellus*, *A. piperatus*, *Hydnum repandum*, *H. hybridum* and *Boletus viscidus* and obtained a crude extract formed by chitin-glucan complex, lacking proteins and pigments and presenting acetyl groups, which he called “fungine”. Odier, in 1823, named “chitin” the insoluble substance that he isolated from the exoskeleton of insects. Odier also realized this same substance could be found in the shell of crabs, and speculated that this could be a basic material present in the exoskeleton of insects[25].

In the nineteenth century, the researchers Odier described the isolation of chitin from the cuticle of insects as well as from mushrooms. This process was done by performing multiple treatments with a concentrated sodium hydroxide solution. With this report in mind, it is reasonable to believe that the material which resulted from such process had been chitosan, because when chitin is exposed to concentrated alkaline media, the deacetylation of this biopolymer is stimulated, and chitosan is obtained[26].

The description of the presence of an amine grouping at carbon 2 of the residues, determining characteristic of the formation of chitosan, was initially done by Rouget in 1859, however the name “chitosan” was proposed by Hoppe-Seyler in 1984[27].

Since its description and designation, chitosan was for many years the subject of basic research only. However, this scenario has been changing since the mid-1970s, when its vast potential in different applications was noted (Table 1).

4. Fungal Chitosan in the Industry

Companies that realized its marketable potential have used the fungal chitosan in industries worldwide. These companies have diverse uses for this biopolymer, more specifically in the medical field. Among the companies that promote bioproducts produced with fungal chitosan, we have:

Table 1. Overview of studies on the potential of chitosan in different applications regardless of the method used to obtain it and its physicochemical characteristics, from 1970 until now.

Period	Application potential	References
1970-79	Enzyme immobilization; Healing activity	Muzzarelli[28,29], Balassa[30], Leuba and Widmer[31,32], Kasumi et al.[33], Bissett and Sternberg[34]
1980-89	Treatment of industrial residues; Heavy metals biosorption	Muzzarelli et al.[35,36], Muzzarelli[37]
1990-99	Effluent treatment; Enzyme immobilization; Film production	Muzzarelli et al.[38], Muzzarelli[39], Dobetti and Delben[40], Hoagland and Parris[41]
2000-09	Degradation of dyestuff; Enzyme immobilization; Biosorption and adsorption of Heavy metals; Food conservation; Medical application	Jin and Bai[42], Amorim et al.[43], Chiou et al.[44], Franco et al.[45], Jeon and Ha Park[46], Rungsardthong[47], Zhao et al.[48], Crini and Badot[49], Baroni et al.[50], Perioli et al.[51], Cheung et al.[52]
2010-now	Cosmetics; Medical application; Food conservation; Biocapsules production; Effluent treatment; Adsorption of heavy metals Antiparasitic	Abdull Rasad et al.[53], Tajdini et al.[54], Gomathi et al.[55], Harris et al.[56], Li et al.[57], Batista et al.[58], Yu et al.[59], Moussa et al.[60], Paiva et al.[61], Oliveira et al.[62], Tayel et al.[63], Taillandier et al.[64], Souza et al.[65]

- Kitozyme: Belgian company pioneer in the production of highly pure non-animal chitosan. It holds some unique patents on the production processes of polymers, such as chitosan. Its products are KiOnutrime-CsG, which absorbs of fat in the human organism; KiObind, which is a combination of the previous with vitamin C; and KioCardio, which decreases cholesterol levels[66];
- InvivoGen: American company that uses chitosan as a vaccine adjuvant to promote protein adsorption on mucosal surfaces. Their commercial product is called Chitosan VaccGrade[67];
- nbiose: Belgian biotechnology company focused on providing carbohydrates with added value to their clients, aiming applications in various fields, such as nutrition, biomedical, pre-biotics, etc. They make use of a technology that yields good chitosan production using fermentation techniques. This allows reasonable costs and high productive yield[68].

5. History of Fungal Chitosan in Brazil

5.1 Past

The pioneering studies to identify and quantify fungal chitosan in Brazil were the works published by Galba Maria Campos-Takaki et al.[15]. In 1983, this group analyzed the cell wall of fungi, from the Zygomycetes class and Mucorales order, using cytochemical, ultrastructural and X-ray microanalysis techniques. In the said work, they confirmed the presence of chitosan in the cell wall of those fungi and that noted that the polysaccharide values vary from 26-28% of dry weight of the species analyzed.

From 2001, Amorim and colleagues, obtained for the first time chitosan from fungi, using strains of Mucorales. In addition, one of their results was the finding of a degree of N-acetylation of 49% for *Mucor recemosus* strain and 20% for *Cunnigamella elegans*, and quantities of D-glucosamine of 48% and 90%, respectively[10].

Continuing the research on chitosan extracted from strains of the Mucorales order, Amorim et al., (2003) obtained chitosan from *Syncephalastrum racemosum* with degree of deacetylation (DD) of 88.99%, and performed lipase immobilization using support film made from the chitosan they obtained[43]. The immobilization of the lipase with this biofilm reduced the catalytic activity of the enzyme in about 47%. These are similar results to those collected from studies that used the immobilization film made from crustacean chitosan, which demonstrates the potential of fungal chitosan in biotechnological production against similar products that are already commercialized for different applications, such as crustacean chitosan[40,42,44].

In 2004, Franco et al.[45] continued Brazilian research using fungal chitosan in bonds with metals, such as iron and copper. The work intended to use this polysaccharide as a bioremedy agent, assisting in the elimination of heavy metals from the environment. The experiment demonstrated good biotechnological potential for fungal chitosan obtained from *Cunninghamella elegans* in copper adsorption.

Having potential producers in mind and always comparing their samples to chitosan produced from chitin obtained from the shell of crustaceans. The same research group, in 2005, published a study that aimed to assess the production yield from the extraction of chitin and chitosan from a strain of *Cunninghamella elegans* (IFM 46109) when compared to other strains of the Zygomycetes class, and to the chitosan extracted from the shell of crustaceans. As a result, they demonstrate that fungal chitosan compares to chitosan derived from chitin obtained from crustaceans as to their physicochemical characteristics[69].

Stamford et al, 2007 conducted tests on various culture media and different incubation periods to optimize the production of chitosan from the fungus *Cunninghamella elegans*, they obtained chitosan with a degree of deacetylation of 85%[70].

In 2008, Fai et al.[71] expanded studies regarding chitosan by applying it as an additive to better food conservation over time, proving its efficiency and turning it into a promising molecule for large-scale use in the food industry.

In 2009, Bento et al.[72] investigated the production of chitosan from the fungus *Mucor rouxxi* UCP 064, obtaining deacetylation degree of 85% and the highest yield of the polymer with only 48 hours of cultivation. The same study applied the chitosan they obtained in an antimicrobial test against Listeria monocytogenes, finding good results on the inhibition of this microorganism. In the same year, Bento et al.[73] applied chitosan as an inhibitor of *Listeria monocytogenes* in meat products, and had satisfactory results on the inhibition of this microorganism.

5.2 Present

At the present moment, the Mucorales order Zygomycetes class, is the most representative and studied in the biotechnology field for containing most species that produce biocompounds of great commercial interest (Table 2).

Among many compounds of interest, chitosan should be highlighted, since these species have large quantities of this biopolymer in their cell wall when they are in their vegetative form[21,83].

Currently the work on fungal chitosan is divided into production, where studies concern more profitable and sustainable ways of obtaining this biopolymer; and application, in which one observes patents and marketable potential for this biotechnological product of international acceptance.

5.3 Production

In the nineteenth century, the scientist White et al.[84] developed a methodology that allowed the isolation of chitosan from fungal mycelium. From this study, several other scientists have begun to develop adaptations to seek improving the process efficiency[24,85,86]. Based on these studies, the scientist HU and coleages (1999), in the late twentieth century, developed an extraction protocol that is currently one of the most used in experiments with fungal chitosan. This protocol describes each step to be performed from cultivation period to specific pH values, which are essential for the success of the extraction[87].

Using the extraction process described by Hu et al.[87], many Brazilian researchers aim to acquire higher yield of fungal chitosan as well as decrease production costs in order

Table 2. Biopolymer production by species from the Mucorales order as depend of the cultivation conditions.The cultivation conditions interfere directly of the better biopolymer production.

Species	Biopolymer	Reference
Syncephalastrum sp.	α-amylase; chitosan;	Yu et al.[59], Batista et al.[74], Freitas et al.[75]
Cunninghamella sp.	Chitosan	Oliveira et al.[62], Tayel et al.[63]
Rhizopus sp.	chitin; chitosan	Cardoso et al.[76], Berger et al.[77]
Rhizopus stolonifer	Chitosan	Paiva et al.[78]
Mucor spp.	enzyme production; chitosan	Bento et al.[79], Fai et al.[80], Souza et al.[81]
Fusarium sp.	Chitosan	Berger et al.[82]

to compete commercially with chitosan obtained from chitin from crustacean's shell.

Cardoso et al.[76] performed the extraction of fungal chitosan by media comprised of by-products of the food agribusiness and were able to obtain 29.30 mg of chitosan per gram of dry mass of *Rhizopus arrhizus*; and Berger et al.[77] obtained 57.82 mg of chitosan per gram of dry mass of *Cunnigamella ellegans* UCP / WFCC 0542. Batista et al.[88], managed to increase yield working with a medium composed of the by-product of shrimp industry, reaching up to 89.95 mg of chitosan per 1g of biomass of *Rhyzopus* spp.

The values aforementioned, when compared to animal chitosan, obtained from the chitin extracted from the shell of crustaceans, still reflect lower yield. However, when taking into account the physicochemical characteristics, the ease of obtaining, the low cost purification and the little chemical waste generated by the production of fungal chitosan when compared to animal chitosan production, this difference becomes minimal[89,90].

Based on the synthesis path of chitosan in fungi, many researchers have shown that several factors can affect the production of this biopolymer by submerged fungi culture. These factors have also been described as important for the production and manipulation of physicochemical characteristics of chitosan according to the literature[91-95].

5.4 Some characterizations and applications

In Brazil, fungal chitosan has already been tested in different applications depending on its physicochemical characteristics. The most used characterizations in chitosan studies were infrared spectroscopy and molecular weight. The infrared technique is based on the Fourier Transform Infrared Spectroscopy, FT-IR, to obtain the degree of deacetylation as a function of Equation 1:

$$\boldsymbol{DD}(\%)=100-\left[\left(\frac{\boldsymbol{A}_{1655}}{\boldsymbol{A}_{3450}}\right)\boldsymbol{x}\frac{100}{1.33}\right] \quad (1)$$

where: A_{1655} is the absorbance at 1655 cm^{-1} of the amide I band, A_{3450} is the absorbance at 3450 cm^{-1} of the hydroxyl band and 1.33 is the value of the A_{1655}/A_{3450} proportion for a completely acetylated chitosan. Adapted from Martínez-Camacho et al.[96] and Khan et al.[97].

The molecular weight characterization is closely related to the intrinsic viscosity, where chitosan solutions are passed through Ubbelohde capillary in a water bath and the determination of the molecular weight occurs as a function of the Mark-Houwink-Sakurada Equation 2:

$$[\eta]=kMV^{a} \quad (2)$$

where [η] is the intrinsic viscosity, MV is the average viscometric molecular weight and both "κ" and "a" are empirical constants that depend of the polymer nature, the solvent and the temperature. "κ" and "a" values were 3.04×10^{-5} and 1.26, respectively adapted from Martínez-Camacho et al.[96] and Rinaudo et al.[98].

Other techniques that are used to characterize fungal chitosan are thermogravimetry, where it is possible to analyze if there are contaminants in the sample; Scanning electron microscopy, which demonstrates the structure of the polymer; and X-ray diffraction, which indicates the crystallinity of the material. These characteristics are very important for choosing a good polymer for an efficient application[43,60,62].

5.4.1 Antimicrobial activity application

Currently, a major concern in the use of antibiotics is the accumulation of synthetic substances in the body, in addition to the high rate in which the selection of microorganisms resistant to commonly known drugs increases. Therefore, some groups of Brazilian researchers conducted studies in which they used fungal chitosan as a bactericidal agent[62,65].

For the antimicrobial action three mechanisms are proposed: (1) related to the formation of polyelectrolytic complexes, because chitosan has positive charges present in its chain, due to its amine group, that bind selectively to the cell surface of the microorganisms, changing its activity, Permeability of the membrane and reducing the cellular components, resulting, then, in the inhibition or cell death; (2) chitosan acts as a chelating agent, binding to the ions that are necessary for the functioning of certain enzymes; (3) when chitosan is also low in molecular weight, being able to insert into the nucleus of the microbial cell, interacting with the DNA, and interfering with the activities of messenger RNA, consequently affecting protein synthesis[54,60,63,96].

In order for the antimicrobial action to occur, some studies show a connection with the degree of deacetylation above 75%; And molecular weight, that the lower, the greater the action of chitosan against microorganisms[43,45,54,60,62].

Paiva and colleagues, in 2014, tested the chitosan extracted from *Cunninghamella elegans*, as an antibacterial agent against *Escherichia coli* and *Staphylococcus aureus*, the study was successful in inhibiting bacteria in all times tested, proving the effectiveness of chitosan as an antibacterial agent[61].

In addition to the bactericidal activity, fungal chitosan may also have fungicidal activity. Oliveira et al.[62] extracted chitosan from *Cunninghamella elegans* to used as coating for fruit (grape - *Vitis labrusca* L.) and prevented the growth of pathogenic fungi. In this work, a chitosan was used in film form with 81% degree of deacetylation and average molecular weight. For this application in the form of film, in particular, it was pointed out that if the polymer under study were of low molecular weight, there would be a decrease in the tensile strength and permeability of the film. Within this experiment, they also found that such alternative coating did not influence the sensory conditions of this fruit.

5.4.2 Application of chitosan as biofertilizers

Stamford et al (2015) published a patent in Brazil describing the biofertilizer and bioprotector abilities of fungal chitosan. This chitosan was produced from mixed biofertilizer (phosphate and potassium rocks, along with sulfur and an inoculum of acidithiobacillus bacteria; organic matter inoculated with diazotrophs free life bacteria; and fungi that produce chitin and chitosan). This production was carried out in a purely biological way, and resulted in a product with a high fertilizer and protection effect against phytopathogenic microorganisms present in the soil, without the need to use external energy sources[99].

5.4.3 Enzyme immobilization

The enzyme immobilization technique is much valued in cosmetics, food and pharmaceutical industries. Having this in mind, Amorim et al.[43] used fungal chitosan as a film for perform enzyme immobilization. In their experiment, the degree of deacetylation of fungal chitosan was 88.9%. This characteristic was thought due to chitosan to provide as reactive points a smaller amount of acetamide clusters. In this way, there is a decrease in the density of the chitosan molecule and favoring its function as a support for the immobilization of enzymes. They aimed to immobilize an enzyme called lipase and watch its time of activity. As a result, they found 47% of initial catalytic activity after four reaction cycles. Therefore, these results are as efficient as those found in studies using animal chitosan (obtained from crustaceans shells).

6. Prospects

After its discovery, chitosan has become a source of numerous studies around the world due to its versatility of use. Initially, the most commonly used type of chitosan was that obtained from the shell of shrimp, however, with the discovery of the presence of chitosan in the cell wall of fungi, which is extracted with less production of waste during purification and no presence of allergenic substances, the fungal or biotechnological chitosan gained ground in worldwide research groups[100].

In Brazil, the first reports date back to the early 80's, with the research group of Professor Dr. Galba Maria de Campos-Takaki. After this first study, the others that followed tried to understand the structure of the fungus, how this chitosan behaves under different conditions, alternative culture media for growth of fungus and measuring the chitosan production, as well as physicochemical characterizations of this chitosan using techniques such as X-ray, infrared, thermogravimetry, zeta pontencial and others[15].

Currently, studies with fungal chitosan in Brazil seek a future biomedical application for this polymer. This can be in helping the body accept implants, such as dental, muscle or bone; using chitosan membranes to help with burn victims, establishing a relationship between the polymer and its reparative action on biological tissues; and in the antimicrobial activity of this polymer, helping to maintain aseptic conditions in hospital equipment.

The versatility of this polymer has also been found effective in the food engineering area, where research is focused on the production of biofilms and membranes that protect food from microorganisms, increasing its shelf-life and reducing financial losses due to contamination[59,63,77,101,102].

Another way that fungal chitosan is being used in Brazil is in the material engineering field, where the polymer is being used as an insulating material to composites, resulting in decrease of plastic material, thus avoiding the serious environmental damage that the plastic accumulation causes in the environment.

Concerning the companies in this sector, Brazil is still taking regular steps when compared to the reality of Europe and the United States. Even presenting characteristics and qualified personnel for the development of new technologies. What we can observe from the groups involved in this area are robust visions that contribute to making Brazil a leading producer of fungal chitosan for any feasible purpose.

For this, further studies concerning fungal chitosan are relevant in order to be sure that its use is feasible and safe for humans. Even though there is some resistance from the industry to this polymer obtained from fungi due to its lower yield when compared to chitosan of animal origin, it is important to note that the cost of purification and the amount of waste generated are much lower. These facts gives the fungal chitosan a prominent place in scientific research in Brazil, as well as in the world.

7. Conclusion

Given the knowledge acquired during this work, we see that the use of fungal chitosan in Brazil needs to be further explored. To date, Brazilian scientists have cataloged different biological activities of fungal chitosan. These studies, even if they are still on bench scale and with few deposited patents, are important because they demonstrate the biotechnological potential of some groups operating in the Brazilian Northeast, an area still with little financial investment for the research when compared to the Southeast region.

In the world fungal chitosan has already been reported for different purposes and has already been used in different patented formulations. In Brazil, there are still studies of production and small applications that normally do not transgress the academic area. With the dissemination of the papers and exposition of the research groups it is expected that new agreements will be set up and that agreements with industrial sectors be signed for the purpose of patents and commercial projection of fungal chitosan, as it happens in Europe.

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