

Chapter 194

Biotechnological advances in the production of fungal chitosan



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Fungal biology has made important advances in recent decades, enabling the extraction of molecules of biotechnological interest, such as chitin, one of the most versatile and abundant biopolymers in nature. Several studies suggest that fungal organisms cultivated on a large scale can be used as an alternative source for obtaining this polysaccharide. Thus, this review presents the most recent biotechnological advances in the production of chitin and its derived products, evaluating the potential use of fungal organisms as a commercial source of this raw material.

Keywords: Fungal chitin, Chitosan, Biotechnology, Mucorales.

ABSTRACT

1 INTRODUCTION

The biology of fungi has presented important advances in recent decades, enabling the extraction of molecules of biotechnological interest, such as chitin, one of the most versatile and abundant biopolymers in nature, after cellulose (SOUZA et al., 2011). In addition to being the main constituent of the exoskeleton of insects and crustaceans, chitin is also found in fungi, mollusks, and yeasts (CREMAR et al., 2018).

It is a linear copolymer formed by repetitive monomeric units of β -1,4 N-acetyl D-glucosamine. With the use of alkali solutions, chitin undergoes deacetylation reactions giving rise to its copolymer, chitosan, which can also be naturally found as a structural component in the cell wall of some fungi, such as those belonging to the order Mucorales (BERGER et al., 2018).

The applications of chitin and chitosan have been growing rapidly due to their diverse properties such as biocompatibility, hemostatic activity, non-toxicity, adsorption, and anti-infectious properties, enabling their use in agricultural, environmental, medical, and pharmaceutical areas (GHORMADE; PATHAN; DESHPANDE, 2017). In addition, its high biodegradability and production from renewable substrates further elevate its importance as a sustainable product, making it increasingly attractive to scientific and technological advances (BURANDE; DHAKITE; RAWAT, 2018).

Currently, the commercial production of chitin and its derived products occurs through the process of thermochemical deacetylation of chitin obtained from marine sources, such as shells of crustaceans (TOLESA; GUPTA; LEE, 2019). However, the supply of this source is seasonal and limited, resulting in

variability of the source material (ADNAN et al., 2017; BERGER et al., 2014). In addition, the physicochemical properties of chitin derivatives obtained from such processes are, for the most part, heterogeneous, and may present different molecular weights and degrees of deacetylation (KANNAN et al., 2010). Another concern of this process of obtaining is the use of strong alkaline solutions that constitute important sources of pollution for the environment (CAMPANA-FILHO et al., 2007) in addition to the presence of protein residues in the final product, which can cause allergic reactions in susceptible human beings (FRANCO et al., 2004).

In this sense, several studies suggest that fungal organisms grown on a large scale could be used as an alternative source for obtaining chitin. Thus, this review presents the latest biotechnological advances in the production of chitin and its derived products, evaluating the potential use of fungal organisms as a commercial source of these raw materials.

2 CHEMICAL AND MOLECULAR STRUCTURE OF CHITIN

Chitin is a natural polysaccharide, semicrystalline in a solid state and that performs a structural function in nature, constituting, for example, the exoskeleton of arthropods, conferring protection and mechanical resistance to these organisms (ZARGAR; ASGHARI; DASHTI, 2015). It is highly hydrophobic, being insoluble in water and most organic solvents (PILLAI; SWAMP; SHARMA, 2009), presents low chemical reactivity (LARANJEIRA; FÁVERE, 2009) and biodegradability (DUTTA; DUTTA; TRIPATHI, 2004).

As for its structure, chitin is presented as a linear polymer, whose repetitive unit is the disaccharide formed by 2-acetamide-2-deoxy-D-glucopyranose and 2-amino-2-deoxy-D-glucopyranose joined by glycosidic binding of type β (1 \rightarrow 4), thus defining the reducing and non-reducing terminals of the polymer chains, which correspond to the ends containing free hydroxyl group of the glucopyranose ring (CAMPANA-FILHO et al., 2007).

The elucidation of the molecular structure of chitin and its chitosan copolymer took decades to complete. The molecular weight of these polysaccharides can reach 10^6 Da. The degree of N-acetylation and the degree of polymerization, which in turn decides the molecular weight of the polymer, are two important parameters that dictate the use of chitosan for various applications. Its influence on the development of the viscosity of aqueous solutions has significant roles in the biochemical and biopharmacological importance of chitin and chitosan. Typically, in chitin acetylation, >70% is expected, while by definition chitosan has a degree of acetylation <30–40%. There is no separate pathway for chitosan synthesis. Chitosan formation is commonly achieved in any organism by deacetylation of chitin using chitin deacetylase (GHORMADE et al., 2010).

Chitin is also known for exhibiting polymorphism, where it can be found in different forms of arrangements (α , β , and γ -chitin) being in its solid state, according to the arrangement of its polymeric chains in the leaves or lamellae that constitute the crystalline domain (ARANAZ et al., 2009). In α -chitin,

which is the most abundant in nature, the polymer chains are arranged in an antiparallel way, which favors the existence of numerous inter- and intra-chain hydrogen bonds of the same lamella and neighboring lamellae, resulting in a dense packaging (BATTISTI; CAMPANA-FILHO, 2008). In β -chitin the chains are arranged in a parallel arrangement, making it difficult to establish intermolecular and interlamellar hydrogen bonds, which results in less dense packaging (JANG et al., 2004). In γ -chitin, there seems to be a combination of the arrangements α and β , where every two lamellae are in a parallel arrangement, and a third lamella are arranged antiparallel (ARANAZ et al., 2009).

3 OCCURRENCES OF CHITIN AND CHITOSAN

It is estimated that the annual production of chitin and chitosan is almost as much as cellulose, becoming of great interest as a functional biomaterial, presenting a high potential for applicability in several fields (ZARGAR; ASGHARI; DASHTI, 2015). One of the main factors guiding the demand for chitosan is the easy availability of its raw material, chitin, whose main source comes from the residual product of the fishing industry.

In addition to being present in the carapace of crustaceans, chitin is also found in the matrix of the skeletal structure of invertebrates of various phyla, such as annelids, other types of arthropods, mollusks, and coelenterates, in diatomaceous algae, and in the cell wall of some fungi, especially in the species belonging to the order Mucorales (Table 1) (BERGER et al., 2018).

Table 1. Sources of obtaining chitin and chitosan

Classification	Phylum	Family	Example species	Reference
Marine organisms	Annelida	<i>Siboglinidae</i>	<i>Oligobrachia ivanovi</i>	(BLACKWELL et al., 1965)
	Mollusca	<i>Facelinidae</i>	<i>Pilgrim Cratena</i>	(MARTIN et al., 2007)
	Cnidaria	<i>Schizopathidae</i>	<i>Parantipathes larix</i>	(BO et al., 2012)
	Arthropoda	<i>Penaeidae</i>	<i>Litopenaeus vannamei</i>	(SANTOS et al., 2019)
	Arthropoda	<i>Potamidae</i>	<i>Potamon potamios</i>	(BOLAT et al., 2010)
	Arthropoda	<i>Palinuridae</i>	<i>Polinurus vulgaris</i>	(ACOSTA et al., 1993)
	Arthropoda	<i>Euphausiidae</i>	<i>Euphausia superba</i>	(WANG et al., 2013)
	Brachiopoda	<i>Lingulidae</i>	<i>Anatina Lingula</i>	(WILLIAMS et al., 1994)
Arachnids	Arthropoda	<i>Buthidae</i>	<i>Mesobuthus gibbosus</i>	(KAYA et al., 2016)
	Arthropoda	<i>Lycosidae</i>	<i>Geolycosa vultuosa</i>	(KAYA et al., 2014)
Insects	Arthropoda	<i>Formicidae</i>	<i>Camponotus floridanus</i>	(WANG et al., 2012)
	Arthropoda	<i>Scarabaeidae</i>	<i>Scarab beetles</i>	(BADAWY; MOHAMED, 2015)

	Arthropoda	<i>Blattidae</i>	<i>American per planet</i>	(BADAWY; MOHAMED, 2015)
Microorganisms	Mucoromycota	<i>Cunninghamellaceae</i>	<i>Cunninghamella elegans</i>	(FRANCO et al., 2005)
	Ascomycota	<i>Saccharomycetaceae</i>	<i>Saccharomyces cerevisiae</i>	(BULIK et al., 2003)
	Microsporidia	<i>Unikaryonidae</i>	<i>Encephalitozoon cuniculi</i>	(PEUVEL-FANGET et al., 2006)
	Bacillariophyta	<i>Thalassiosiraceae</i>	<i>Thalassiosira fluviatilis</i>	(MCLACHLAN et al., 1965)
	Ascomycota	<i>Trichocomaceae</i>	<i>Penicillium chrysogenum</i>	(TIANWEI et al., 2002)
	Blastocladales	<i>Blastocladiaceae</i>	<i>Allomyces arbuscula</i>	(MITCHELL; DEACON, 1986)

Source: Prepared by the author (2023).

4 SYNTHESSES OF FUNGAL CHITIN

Fungal chitin has been discussed and studied in the world since 1859, having achieved important advances over the years. In Brazil, the first studies presenting techniques for the identification and quantification of biopolymers of microbiological origin were published by Campos-Takaki in mid-1978 (CAMPOS-TAKAKI, 1978) and early 80s (CAMPOS-TAKAKI, 1984). Currently, studies with chitin and fungal chitosan in Brazil and the world seek applications in the medical and pharmaceutical areas, given its antibacterial and antifungal properties against pathogenic microorganisms and ability to inhibit the proliferation of cancer cells (DENG et al., 2014).

Chitin can be found in the formation of mycelium of different fungal species, such as *Cunninghamella elegans* (SILVA et al., 2022a), *Mucor circinelloides* (FAI et al., 2011), *Mucor rouxii* (ABASIAN et al., 2020), *Absidia coerulea*, *Gongronella butleri* (NWE et al., 2011), *Aspergillus niger* and *Rhizopus arrhizus* (VIEIRA et al., 2020) have been considered as possible sources of chitin and chitosan due to their abundance in cell walls. Every chitin found in the inner wall of the fungal cell as a microfibril serves to compensate for the turgor pressure of the cells (DHILLON et al., 2013; HUQ et al., 2022).

The synthesis of chitin in fungi is highly compartmentalized. The key enzyme chitin synthetase occurs in the form of zymogen where it is distributed to specific regions on the cell surface in specialized vesicles called schistosomes. Macromolecular assembly begins outside the cytoplasm where the protease (activator) on the cell surface activates the zymogen. Once energy-rich uridine diphosphate-N-acetylglucosamine is produced (from glucose), chitin synthetase successfully catalyzes the transfer of GlcNAc to chitin-forming primers (THARANATHAN; KITTUR, 2003). In most fungi, chitin is synthesized from the polarized growth sites of the cell wall. A period of isotropic growth begins in large cells with buds, where the material is deposited on the entire surface of the yolk. After nuclear splitting, a repolarization phase begins where the material is directed to the parent button neck to prepare for

cytokinesis. In hyphal or filamentous forms, cell extension is a continuous and indefinite apical process (HUQ et al., 2022).

4.1 MUCORALES

The order Mucorales comprises ubiquitous organisms, mainly saprotrophic (HOFFMANN et al., 2013), also known as sugar fungi, given their enzymatic ability to catabolize glucose and/or sucrose by rapidly consuming the entire substrate of the medium. They are economically important as fermenting agents of soybean products and producers of enzymes, but also as parasites of plants and deteriorating organisms (HOFFMANN et al., 2013; WALTHER et al., 2013). As one of the largest orders in basal fungi, Mucorales is also one of the most studied groups in physiological and biochemical aspects, as well as its taxonomy and potential applications in different industries (SPATAFORA et al., 2016).

The fungal species belonging to the order Mucorales are the ones that present the greatest potential for chitosan extraction, when compared to other fungi (BERGER et al., 2018) since they naturally present chitin and chitosan as a structural component of their cell wall, which is an important physiological and taxonomic characteristic of the species (STAMFORD et al., 2007).

The deacetylation process of chitin occurs naturally in Mucorales with the participation of the enzymes chitin deacetylase and chitin synthetase (YUTANI et al., 2011). Chitin deacetylase is involved in the nutrition, morphogenesis, and development of fungi (GHORMADE et al., 2010), participating in the formation and integrity of the cell wall (BAKER et al., 2007), in germline adhesion (GEOGHEGAN; GURR, 2016), in the formation of spores, in fungal autolysis (WHITE et al., 2002) and the defense mechanisms during host infection (SÁNCHEZ-VALLET; MESTERS; THOMMA, 2015). Studies indicate that the activity of chitin deacetylase and its function in the biosynthesis of chitosan in the cell wall of the fungus indicates that enzymatic deacetylation has much milder conditions and that it overcomes most of the disadvantages found in the alkaline deacetylation process, aiming at increasing the microbiological production of this polymer (PAREEK; SINGH; GHOSH, 2011).

5 MICROBIOLOGICAL PRODUCTION OF CHITIN AND CHITOSAN

The production of chitin from the mycelial biomass of fungi has become the target of several studies that indicate that the content of polysaccharides present in the cell wall may suffer variations according to the fungal species, fermentation techniques, and nutritional conditions of the medium, especially concerning the carbon sources used in the production process (AKILA, 2014; CAMPOSTAKAKI, 2005), as shown in Table 2.

The growth of several species of fungi, especially those representing the order Mucorales, is evaluated in several studies that seek to achieve greater biomass production and, consequently, higher yields of chitin and microbiological chitosan. For this, different synthetic culture media and low-cost alternative media have been tested. Among the alternative means are the residues of industries, and products or agro-

industrial residues such as jacatupé (FAI et al., 2011), maize, and manipueira (SILVA et al., 2022b), aiming at the possibility of large-scale production.

Table 2. Production of biomass, chitin and chitosan by Mucorales cultivated in different media reported in the literature.

Microorganisms	Substrate	Biomass (g.L ⁻¹)	Chitin (mg. g ⁻¹)	Chitosan (mg. g ⁻¹)	Reference
<i>R. arrhizus</i>	Corn and manipueira	8,80	54,38	20,51	(BERGER, 2013)
<i>R. arrhizus</i>	Corn and juice of papaya peel	3,75	137,14	77,76	(BERGER, 2013)
<i>R. arrhizus</i>	Molasses and corn	24,6	83,2	49,31	(BERGER et al., 2014)
<i>R. arrhizus</i>	Corn and bee honey	20,6	-	29,3	(CARDOSO et al., 2012)
<i>R. arrhizus</i>	Corn 4%	13,00	30,40	12,85	(LINS et al., 2010)
<i>R. arrhizus</i>	Mamycin and glycerin	22,5	-	44,46	(SILVA, 2015)
<i>R. arrhizus</i>	Corn 8%	16,8	575	416	(SILVA, 2007)
<i>R. microsporus</i>	Molasses, malt pomace and tomato	12,6	485,71	150	(ARAÚJO, 2018)
<i>R. oryzae</i>	Corn and manipueira	1,24	-	44,67	(LIMA et al. , 2011)
<i>R. oryzae</i>	Corn straw	14,6	-	8,57	(OMOGBAI; IKENEBOMEH, 2013)
<i>R. oryzae</i>	Corn and manipueira	8,0	-	115,6	(LIMA et al. , 2011)
<i>A. coerulea</i>	Soybean bagasse	21,38	-	5,88	(JIANG et al. , 2011)
<i>A. corymbifera</i>	Mamycin and glycerin	21	-	88	(SILVA et al. , 2010)
<i>M. circinelloides</i>	Jicama	20,70	500	64	(FAI et al., 2011)
<i>M. nightingale</i>	Soybean meal and husk	-	-	34,4	(MONDALA et al., 2015)
<i>C. elegans</i>	Jicama	24,30	440	66	(STAMFORD et al., 2007)
<i>C. elegans</i>	Corn and manipueira	6,375	-	101,7	(SILVA et al., 2022a)
<i>C. elegans</i>	Corn and manipueira	5,67	89,39	57,82	(BERGER et al., 2014)
<i>C. elegans</i>	Corn and juice of papaya peel	4,11	126,0	77,78	(BERGER, 2013)
<i>C. elegans</i>	Maize and papaya husk	-	-	37,25	(BERGER et al., 2018)
<i>C. elegans</i>	Medium YPD	25,0	-	20,5	(AMORIM et al., 2001)
<i>C. elegans</i>	Andrade et al.	11,6	238	78,0	(SOUZA et al., 2011)
<i>C. echinulata</i>	Corn 1% and green banana flour 5%	1,1643	-	154,2	(PEREIRA- GOMES et al. , 2013)
<i>C. bertholletiae</i>	Cane juice	7,70	-	128	(AMORIM et al., 2006)
<i>G. bulteri</i>	Apple pomace	0,091	-	178,3	(STREIT et al., 2009)
<i>A. niger</i>	Broth Potato Dextrose	9,00	-	107	(POCHANAVANICH; SUNTORNSUK, 2002)
<i>Z. nightingale</i>	Yeast malt extract broth	4,4	-	36	(POCHANAVANICH; SUNTORNSUK, 2002)
<i>C. albicans</i>	Yeast malt extract broth	1,8	-	44	(POCHANAVANICH; SUNTORNSUK, 2002)
<i>M. racemosus</i>	Medium YPD	15,0	-	35,1	(AMORIM et al., 2001)
<i>S. racemosum</i>	Maize and tangerine husk	25	-	30	(MILK, 2014)

<i>S. racemosum</i>	Avocado peel	17	-	16,4	(MILK, 2014)
<i>S. racemosum</i>	Banana peel	8	-	25	(MILK, 2014)
<i>S. racemosum</i>	Sugarcane and maize bagasse	32	-	23,5	(MILK, 2014)

Source: Prepared by the author (2023).

Thus, the use of mycelial biomass has proven to be a good alternative since it is an easy and economically viable process, and simultaneous extraction of chitin and chitosan can be performed (BERGER et al., 2018). Another advantage is in the extraction methods since the cultivation of the fungus is independent of seasonality and also reduces the environmental impact generated by the residual products resulting from the deacetylation of the α -chitin, in which the varied purification processes of the biopolymer extracted from crustaceans produce large amounts of chemical pollutants (GHORMADE; PATHAN; DESHPANDE, 2017).

6 CONCLUSION

Chitin has attracted everyone's attention, given its versatility of application, being of great interest to the industry and scientific community. With the advances of fungal biotechnology, numerous advantages have been offered in the simultaneous production of fungal chitin over classical processes, since this biomaterial can be produced with simple and economical extraction processes, since many fungal species have great potential in the bioconversion of alternative substrates, such as agro-industrial waste. It is anticipated that its additional specific applications will be realized shortly.

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