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Review

# Can fungi compete with marine sources for chitosan production?

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ABSTRACT

Chitosan, a β-1,4-linked glucosamine polymer is formed by deacetylation of chitin. It has a wide range of applications from agriculture to human health care products. Chitosan is commercially produced from shellfish, shrimp waste, crab and lobster processing using strong alkalis at high temperatures for long time periods. The production of chitin and chitosan from fungal sources has gained increased attention in recent years due to potential advantages in terms of homogenous polymer length, high degree of deacetylation and solubility over the current marine source. Zygomycetous fungi such as *Absidia coerulea, Benjaminiella poitrasii, Cunninghamella elegans, Gongrenella butleri, Mucor rouxii, Mucor racemosus* and *Rhizopus oryzae* have been studied extensively. Isolation of chitosan are reported from few edible basidiomycetous fungi like *Agaricus bisporus, Lentinula edodes* and *Pleurotus sajor-caju.* Other organisms from mycotech industries explored for chitosan production are *Aspergillus niger, Penicillium chrysogenum, Saccharomyces cerevisiae* and other wine yeasts. Number of aspects such as value addition to the existing applications of fungi, utilization of waste from agriculture sector, and issues and challenges for the production of fungal chitosan to compete with existing sources, metabolic engineering and novel applications have been discussed to adjudge the potential of fungal sources for commercial chitosan production.


**Contents**



## 1. Introduction

This review is intended to revisit the issues and challenges in application potential of fungi as a source of chitin and chitosan. Henri Braconnot (1780–1855) discovered chitin in fungi such as *Agaricus, Hydnum* and *Boletus* species, while cellulose, a β-1,4-linked glucose polymer, a major component of plant cell walls was discovered later almost after 30 years [1]. Nevertheless cellulose has become one of the major polymers in having lot of commercial applications. The possible reason could be ease of obtaining indus-

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| Advantages | Limitations |
|---|---|
| • No seasonal variation<br>• Demineralization is not required<br>• Homogenous preparation<br>• Free of heavy metals as nickel, copper<br>• Low molecular weight and low viscosity<br>• High degree of deacetylation<br>• High polydispersity<br>• Biocompatible<br>• Not heavily melanised<br>• Can be a by-product of fungi-based industries<br>• Useful in healthcare | • The availability and quantity of raw material is not comparable to marine sources<br>• Production cost for practical grade chitosan is high<br>• Cannot be feasible for high volume low cost applications, such as industrial effluent water treatment; land filling in agriculture |

**Chart 1.** Fungal sources for commercial production of chitosan.

trial quantities and lesser complexity as compared to chitin and its decetylated derivative, chitosan [2].

In view of the large scale isolation of chitin and chitosan for a variety of applications, it was concluded that marine waste of shrimp, prawn and crabs as a source would be difficult to replace with other organisms at least in near future [2,3]. However, it was suggested that fungal organisms cultivated on a large scale for different purposes could be used as an alternate source for these raw materials.

The following sections would appraise the fungal organisms as the commercial source of chitin and chitosan.

### 1.1. Structural complexities of protective covers

Chen et al. [4] studied the structure and mechanical properties of sheep crab (*Loxorhynchus grandis*) exoskeleton. According to them, it is a natural composite consisting of highly mineralized chitin-protein (protein, 20–40%; Ca carbonate, 20–50%; chitin, 15–40%) fibers arranged in a twisted pattern. Further it was reported that the exoskeleton was twice harder than the endoskeleton. This indicated that in general, for the isolation of chitin from different marine sources, chemical treatments like deproteinization with hot alkali (1N NaOH at 65–100 °C for 1–72 h), demineralization with acid to eliminate calcium carbonate (0.275–2M HCl at near 100 °C for 1–48 h) and decoloration to remove pigments are necessary.

The fungal cell wall is composed of a polysaccharide-based three-dimensional network [5]. In most of the fungi, a branched β-1,3- and β-1,6- glucan linked with chitin via β-1,4 linkage is a main structural component. Feofilova [6] reviewed extensively the contributions of the structural and matrix components of the cell walls, such as chitin/chitosan, α- and β-glucans, proteins, lipids, uronic acids, hydrophobins, sporopollenin and melanins. For isolation of chitin from the fungal cell walls the chemical treatments used are not as harsh as reported for marine sources [7].

The α, β and γ are the three manners for chitin assembly in nature. The strong intermolecular bondings are present in α-chitin, which is composed of antiparallel chains of β-1,4 linked *N*-acetyl-D-glucosamine (GlcNAc). Shells of crustaceans like shrimp and crabs and fungi have α-chitin. β-Chitin has parallel alignment of chains, which is commonly found in squid pens. While γ-chitin present in insects, has two chains parallel in one direction and a third chain goes antiparallel to them [8]. In nature, pure form of chitin is present only in diatoms. The extracellular β-chitin spines of the centric diatoms such as *Thalassiosira fluviatilis* are completely acetylated and not associated with other substances [9]. However, in most of the organisms it is a polymer of both GlcNAc and glucosamine with varying percentages. Usually, in chitin >70% acetylation is expected while by definition chitosan has degree of acetylation <30–40%.

The chitin synthesis using chitin synthase (EC 2.4.1.16) has been extensively studied in different organisms, particularly in fungi [10,11]. There is no separate pathway for the synthesis of chitosan. Usually chitosan formation is achieved in any organism by the deacetylation of chitin using chitin deacetylase (CDA, EC 3.5.1.41) [12,13].

## 2. Commercial production of chitin/chitosan

So far, the main commercial sources of chitin are crab and shrimp shells. However, under marine sources, squids, oyster, and cuttlefish are also used. Almost $10^{12}$ kg of chitin is synthesized and degraded per year. According to earlier estimates, more than 80,000 t of chitin is obtained per year from the marine by-products [14,15]. In India, the chitin and chitosan production is being mainly carried out in Kerala state from lobsters, crabs and insects. The variety of applications for chitosan have been reported [16]. In most of the countries, chitin from crab shells is processed annually to chitosan using alkali treatment for its commercial exploitation. As alkali treatment is not eco-friendly, use of CDA is one of the feasible alternatives to obtain chitosan.

However, due to dis-continuous supply, seasonal variations of the marine sources, use of fungi could be a viable alternative (Chart 1). Moreover, the fungi can be readily grown in the laboratory on cheap nutrients, wall material can be recovered by simple chemical procedures and constant quality and supply of the raw material is possible.

## 3. Fungal biodiversity and cell wall composition

Fungi are defined as the organisms which contain chitin as a main structural component in the cell walls. This is a second largest group of organisms on earth with estimated number 5,100 thousands while known species are more than 70,000 [17,18]. The cell wall of a particular fungus is composed mainly of chitin, chitosan, β-glucan and mannan. The main components of particular classes of fungi are: Zygomycetes (chitin/chitosan), Chytridiomycetes (chitin/β-glucan), ascomycetes (chitin/mannan/β-glucan), Basidiomycetes (chitin/β-glucan), Chitin comprises 22–44% of cell walls of fungi [7,19]. The zygomycetous fungi are the potential sources for chitosan production. The recent observations suggested that non-zygomycetous plant- and insect- pathogenic fungi also have high proportion of chitosan in the cell walls [12,13].

### 3.1. Different fungal sources for chitin/chitosan production

As compared to the marine sources, chitin production using fungi is negligible, however, the mucoraceous fungi, having high