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Fungal chitosan production on food processing by-products

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Abstract

Four fungal strains, *Aspergillus niger* TISTR3245, *Rhizopus oryzae* TISTR3189, *Zygosaccharomyces rouxii* TISTR5058 and *Candida albicans* TISTR5239, grown on soybean and mungbean residues were investigated for their chitosan production. Their chitosan yields were in a range of 0.4–4.3 g/kg of soybean residue and 0.5–1.6 g/kg mungbean residue. The highest amount of chitosan by *R. oryzae* on soybean residue was 4.3 g/kg. © 2002 Elsevier Science Ltd. All rights reserved.

Keywords: Chitosan; Mungbean residue; Solid state fermentation; Soybean residue

1. Introduction

Chitosan is a natural and biodegradable biopolymer. It can be used as a permeability control agent, as an adhesive, as a paper-sizing agent, as flocculating and chelating agents and as a chromatographic support [1,2]. In addition, it can be applied to immobilize enzymes or to deliver drugs to the target [3,4]. It is generally produced from chitin that is a waste product of the seafood processing industry [5]. However, it has heterogeneous and inconsistent physiochemical properties since supplies of the seafood wastes are seasonable and variable [1,2].

Several yeasts and filamentous fungi, for example, *Schizosaccharomyces pombe*, *Candida albicans*, *Saccharomyces cerevisiae*, *Mucor rouxii*, *Phycomyces blakesleeanus*, *Coprinus cinereus*, *Neurospora crassa*, *Trichoderma reesei*, *Rhizopus* spp., *Absidia* spp., *Mucor* spp., *Mortierella isabelina* and *Lentinus edodes*, have been reported containing chitin and chitosan in their cell wall and septa [2,6–8]. They can be readily cultured in simple nutrients and used as an alternative source of chitosan.

Soybean and mungbean residues are by-products from the manufacture of soybean milk and mungbean noodles which are popular foods in Thailand. They are annually generated as a waste in considerable volumes. The wastes could, however, be applied as an animal feed. Since legumes have been utilized by fungi as fermented foods such as soy sauce and tempeh in oriental countries, their residues could be used for fungal fermentation. Therefore, this investigation was aimed at utilizing these food-processing wastes for fungal growth and to examine chitosan produced by fungal cells.

2. Materials and methods

2.1. Cultures

Microorganisms used in this work were *Aspergillus niger* TISTR3245, *Rhizopus oryzae* TISTR3189, *Zygosaccharomyces rouxii* TISTR5058 and *C. albicans* TISTR5239. They were obtained from the Center of Culture Collection at Thailand Institute of Scientific and Technological Research, Thailand.

2.2. Substrates

Soybean and mungbean residues were supplied by a local factory and kept at 4 °C until used.

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2.3. Cultivation conditions

One ml of spore suspension (10^7 spores/ml) or 1 ml of yeast cell suspension (10^7 cells/ml) prepared from a 7-day-old slant was inoculated into 30 g of sterilized substrate without moisture adjustment in 500-ml Erlenmeyer flasks. The flasks were incubated at 30 °C for 16 days.

2.4. Analyses

Substrates were analyzed for moisture content, pH, fiber, fat, nitrogen content, carbohydrate and ash [9,10]. Growth was determined by glucosamine content on cell wall of fungal mycelia [11] and by viable cell counts on yeast malt extract agar for yeast.

Chitosan extraction was carried out by a modified method of Crestini et al. [2] and Rane and Hoover [12]. After cultivation, the residues were finely ground with a blender for 3 min, suspended with 1 N NaOH solution (1:30; w/v) and autoclaved at 121 °C for 15 min. Alkali-insoluble fractions were collected after centrifugation at 12,000 × *g* for 15 min, washed with distilled water and recentrifuged until neutral pH was obtained (pH 7). The residues were further extracted in 2% acetic acid (1:40; w/v) at 95 °C for 8 h. The extracted slurry was centrifuged at 12,000 × *g* for 15 min and the acid insoluble fraction discarded. The pH of supernatants was adjusted to pH 10 with 2 N NaOH. The solution was centrifuged at 12,000 × *g* for 15 min. Precipitated chitosan was then washed with distilled water, 95% ethanol (1:20; w/v) and acetone (1:20; w/v), respectively, and dried at 60 °C.

3. Results and discussion

As shown in Table 1, soybean and mungbean residues had similar composition except for fiber, fat and nitrogen content. They contain nutrients that can be utilized for microbial growth. However, it was observed that protein content expressed as nitrogen content in both residues, especially, mungbean was very limited since protein was mostly extracted from the beans during food processing.

Table 1
Proximate composition of soybean and mungbean residues

Compositions	Soybean residue[a]	Mungbean residue[a]
Moisture (%)	69.9 ± 0.2	77.5 ± 0.1
Fiber (%)	8.1 ± 0.4	15.4 ± 1.3
Fat (%)	10.3 ± 0.4	0.6 ± 0.1
Nitrogen content (%)	0.6 ± 0.1	0.002 ± 0.0
Carbohydrate (%)	18.4 ± 0.3	21.4 ± 0.2
Ash(%)	0.9 ± 0.2	0.6 ± 0.1
pH	4.6	5.5

[a] Mean ± S.D.

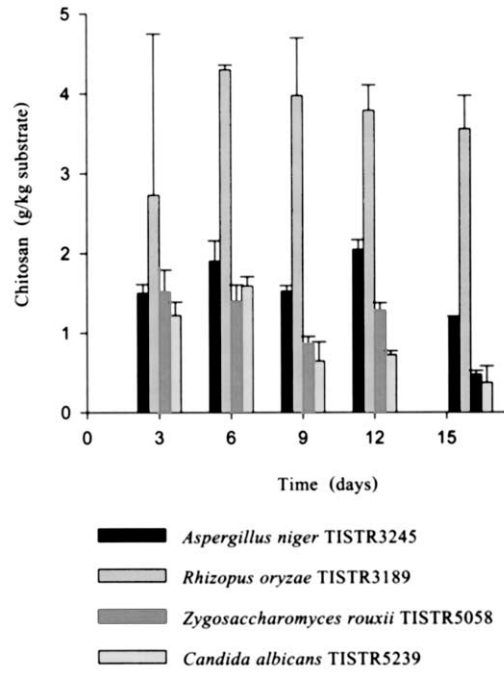

Fig. 1. Fungal chitosan production on soy bean residue

All strains grew well on both substrates especially *A. niger* TISTR3245 and *R. oryzae* TISTR3189. Growth reached a maximum after 3 days of cultivation and then remained nearly constant (data not shown). However, for yeasts, after 3 days of cultivation, growth declined (data not shown).

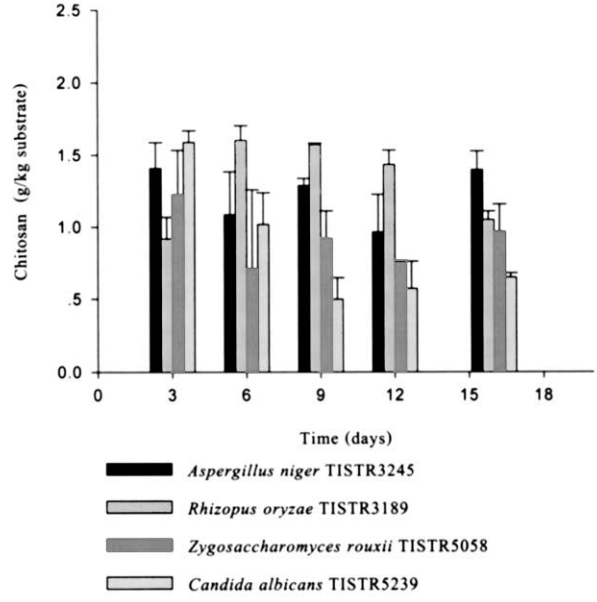

Fig. 2. Fungal chitosan production on mungbean residue

Fungal chitosan yields extracted from fermented soybean residue were higher than chitosan yields from mungbean residue (Figs. 1 and 2). Soybean residue supported better fungal growth than mungbean residue because of the higher protein content in soybean residue. However, the patterns of chitosan production from all strains were similar. *R. oryzae* TISTR3189 produced the highest yields of chitosan on soybean residue (4.3 g chitosan/kg substrate) and on mungbean residue (1.6 g chitosan/kg substrate) (Figs. 1 and 2). Fungal chitosan production had been studied on different media. Chitosan yields from *R. oryzae* ranged from 270 to 406 and 511–700 mg/l in corn and rice media, respectively [13]. Shimahara et al. [6] demontrated that chitosan yields from 32 strains of *Rhizopus* species ranged from 330 to 645 mg/l in a complex medium. Crestini et al. [2] reported that the chitosan yield from *L. edodes* grown on wheat straw reached a maximal value of 6.2 g chitosan/kg at 12 days after inoculation.

Microbial utilization of legume-processing wastes has been developed to produce value-added products. Soy meal waste could be used as a medium for cultivation of *Mortierella elongata* NRRL 5513 and *Pythium irregulare* ATCC 10951 for production of eicosapentaenoic and arachidonic acids [14]. On soybean curd residue, Okara, *Bacillus subtilis* NB22 produced iturin A, a lipopeptide antibiotic that can be used in the control of plant diseases [15]. In addition, soybean and mungbean could be applied as a support of inulinase cross-linking immobilization [16]. Organic wastewater could become a medium for fungal growth for chitosan production. Yokoi et al. [17] reported Shochu distillery wastewater was a good source for chitosan production from *Absidia atrospora* and *Gongronella butleri*. In this work, these food-processing wastes could be used as an inexpensive medium for fungal chitosan production.

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