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Short Communication

Production of fungal chitosan by solid state and submerged fermentation

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Abstract

The growth of the fungus *Gongronella butleri* USDB 0201 was compared in solid state fermentation (SSF) and submerged fermentation (SMF) using various nitrogen sources. The optimal production of biomass and chitosan by SMF was around 1.5–2.5 times higher than SSF. Urea is the best nitrogen source tested. SSF is to be preferred for the production of lower MW chitosan. © 2001 Elsevier Science Ltd. All rights reserved.

Keywords: Chitosan; Solid state fermentation; Submerged fermentation

1. Introduction

Chitosan is an important component of the cell wall of certain fungi, particularly the class of Zygomycetes (Bartniki-Garcia, 1968). Therefore, chitosan-producing fungi might offer an attractive alternative source for commercial production (Stevens, Win, Ng, Pichyangkura, & Chandrkrachang, 1997; Nitar, Chandrkrachang, & Stevens, 2000). Tan, Tan, Wong, and Khor (1996) evaluated the yield of chitosan from several Zygomycetes fungi and concluded that *Gongronella butleri* USDB 0201 gave the highest value.

In this report, chitosan production by *G. butleri* USDB 0201 using solid state fermentation (SSF) and submerged fermentation (SMF) is described. In both cases, different nitrogen sources were added as supplement. The amount of chitosan produced, its degree of deacetylation and molecular weight, were measured.

2. Materials and methods

2.1. Preparation of culture media

G. butleri USDB 0201 was obtained from the Department of Biological Sciences, National University of Singapore. For SSF, mineral solution was prepared containing per 1 distilled water 5 g $(NH_4)_2SO_4$; 1 g K_2HPO_4; 1 g NaCl; 0.5 g $MgSO_4 \cdot 7H_2O$ and 0.1 g $CaCl_2 \cdot 2H_2O$ and 1 l solution was utilized for 1 kg of sweet potato pieces. For SMF, a 10% extract of sweet potato was prepared. The resulting slurry was filtered and the filtrate collected. Filtrate (9 l) was mixed with 1 l of mineral solution. The effect of supplementary nitrogen source was studied by supplying peptone, ammonium sulfate, sodium nitrate and urea to the culture medium. The supplementary nitrogen supply is equivalent to 2 g per kg sweet potato. Sweet potato medium without additional nitrogen source was used as a control. Finally, all culture media were adjusted to pH 4.5 with 0.5 M H_2SO_4.

2.2. Solid state fermentation

The medium was inoculated with spore suspension (1.8×10^9 spores/kg) and incubated in a tray fermentor under filtered and humidified air at a flow rate 0.8 l/min, 28 °C for 7 days, which was earlier established as the late exponential growth phase.

2.3. Submerged fermentation

The SMF medium was inoculated with the same spore concentration as SSF and incubated in a shaking incubator set at 28 °C and 200 rpm, for 3.5 days, which was established as the late exponential growth phase.

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Table 1
Biomass (dry weight) and chitosan yield obtained by SSF and SMF with different nitrogen sources (SSF basic medium: sweet potato pieces impregnated with mineral solution; SMF basic medium: sweet potato 10% with minerals)

Growth condition	Biomass (g/kg sweet potato, w.b.)		Chitosan yield (% biomass)	
	SSF	SMF	SSF	SMF
Basic medium	24.6	49.0	8.2	9.3
Basic medium and peptone	28.5	52.7	10.5	7.9
Basic medium and $(NH_4)_2SO_4$	19.8	52.0	11.7	11.6
Basic medium and $NaNO_3$	25.6	54.7	10.2	9.1
Basic medium and urea	29.3	56.3	12.7	9.2

2.4. Extraction of chitosan

Dried mycelia were treated with 40 ml of 46% NaOH with 0.05 g of sodium borohydride per g mycelia at 46 °C for 13 h. The alkali insoluble material (AIM) was collected, and dried. Dried AIM was treated with 200 ml of 2% acetic acid/g at 95 °C for 5 h. The suspension was centrifuged and the supernatant was subjected to vacuum filtration through GF/C filter paper. Chitosan was precipitated at pH 9 with 1 M NaOH solution. The precipitate was centrifuged and dried. The chitosan was redissolved and treated with β-glucanase (1 ml/100 ml) to remove bound glucan at pH 7.5, 30 °C for 5 h. Chitosan was recovered as a precipitate by adjusting the pH to 9. The precipitate was centrifuged and dried.

2.5. Characterization of chitosan

The degree of acetylation was determined by the first derivative ultraviolet spectrophotometry method (Muzzarelli and Rocchetti, 1985) with the modification of Tan, Khor, Tan, and Wong (1998). Relative molecular weight was determined by gel permeation chromatography (GPC) in a Waters HPLC equipped with Ultrahydrogel 2000, 1000 and 500 columns and a Waters™ 486 Tunable Absorbance Detector.

3. Results and discussion

3.1. Solid state fermentation

The highest dry mycelia yield, 29 g/kg sweet potato pieces was obtained when urea was added. Ammonium sulfate, as a nitrogen source, gave the lowest mycelia yield (Table 1). The yields of chitosan from mycelia grown on substrate enriched with urea or ammonium sulfate were significantly higher ($p < 0.05$) compared with the basic medium.

In fungal cell walls, chitosan is associated with β-glucan. Muzzarelli, Ilari, Tarsi, Dubini, and Xia (1994) reported that the glucan could be removed by 25% NaOH. Under the conditions used in this study, glucan is usually removed efficiently. In most samples, the acetic acid extracts are clear solutions. When additional ammonium sulfate was included however, turbid extracts were obtained. This was attributed to suspended glucan particles of less than 1.2 μm that can pass through the GF/C filter paper. Treatment with β-glucanase removes these particles. The relative number average molecular weight and degree of deacetylation of chitosans obtained ranged from 0.25×10^5 to 0.38×10^5Da and 92–96%, respectively.

3.2. Submerged fermentation

Urea was also the best nitrogen source and mycelia production was approximately 56 g/kg sweet potato (Table 1). A similar result, 55 g, was obtained when using $NaNO_3$. All the culture media with the additional nitrogen supply result in higher biomass formation compared to medium without an additional nitrogen source. It is apparent that biomass obtained from the medium with peptone comprises lowest chitosan (≈ 8%) compared to the rest of the culture media (10%). The absolute amount of chitosan is about 5 g/kg of sweet potato material regardless of the type of nitrogen supply ($P < 0.05$). There is no significant effect on characteristics of the resultant chitosan in SMF by using different nitrogen sources. The range of degree of deacetylation and relative molecular weight were found to be 94–96% and 0.7×10^5–1.2×10^5 Da, respectively.

3.3. Comparison of solid state and submerged fermentation

The results of SSF and SMF can only be compared to a limited extent. At the end of either type of fermentation, the amounts of mycelia and chitosan obtained from same amount of sweet potato used can be quantified, as well as molecular properties of the chitosan like the degree of deacetylation and the molecular weight, however, the decision to compare the data on basis of the amount of sweet potato used is arbitrary. Sweet potato is a cheap carbohydrate source and will only make a small contribution to the cost of the process. However, in the absence of a better criterion, data will be presented per kilogram of untreated sweet potato.

Although amounts of sweet potato materials used for SSF and SMF media preparations were balanced, other contents of fermentation media cannot be identical, as different

Table 2
Nitrogen contents and pH of nutrients used for SSF and SMF

Growth condition	Sweet potato pieces (medium sweet potato extract impregnated)			
	N content (g N/kg, w.b.)	pH	N content (g N/kg)	pH
Basic medium	2.72	5.34	2.59	4.43
Basic medium and peptone	3.28	5.23	4.59	4.61
Basic medium and $(NH_4)_2SO_4$	3.86	5.28	4.59	4.42
Basic medium and $NaNO_3$	2.48	5.30	4.59	4.45
Basic medium and urea	3.32	6.28	4.59	7.44

procedures have been employed. With regard to additional nitrogen supply, calculations on nitrogen content for SSF are not simple as additional nutrients are supplied in liquid form that are allowed to penetrate into the sweet potato pieces during the impregnation and sterilization steps. At the end of the entire preparation, i.e. after sterilization, the nitrogen contents of SSF culture media were determined (Table 2). Surprisingly, addition of $NaNO_3$ results in a decrease of nitrogen content in sweet potato. Unlike SMF, this variation occurs in SSF because of decantation of unabsorbed nutrient solutions from the solid medium. N contents of culture media in SMF were about 1.5 times higher compared to SSF.

In addition to nutrient compositions, there is also a discrepancy in environmental factors such as pH and water surroundings. In both fermentations, regardless of increasing the pH to 6/7 (Table 2), urea offers the preferred growth condition. Under the conditions specified, the yield of mycelia mass in SMF was 2 times higher than that of SSF.

In SMF, Muzzarelli et al. (1994) obtained about 1.8 g/l of chitosan with *Absidia coerulea* using YPG medium while Davoust and Persson (1992) reported a 2.8 g/l yield using glucose, yeast and minerals medium. A yield of 0.47 g/l was obtained by Tan et al. (1996) from *G. butleri* USDB 0201. Crestini, Kovac, and Giovannozzi-Sermanni (1996) reported a production yield of chitosan 0.12 g/l and 6.18 g/kg in SMF and SSF on a unit basis of cultivation medium. In this study, yields of 0.6 g/l (equivalent to 6 g/kg) and 3.7 g/kg were obtained in SMF and SSF, respectively.

A DD of chitosan above 92% was obtained by both processes. The DD values found are similar to the results of Crestini et al. (1996) who reported a range of 87–95%. As shown in Table 3, the relative molecular weight of chitosan produced from SSF (3×10^4 Da) is lower than that from SMF (1×10^5 Da). This indicates that chitosan polymers with a lower molecular weight can be produced by SSF. Chitosan of low molecular weight and high DD has a large charge density and a high solubility. It is useful in pharmaceuticals, biomedicals and food. A series of chitosan products of low molecular weight ($4–10 \times 10^4$ Da) have been successfully used for the preparation of microcapsules-containing cells (Duoxian and Anjie, 1994).

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