Screening of Some Basidiomycetes for Bio-polymers and Biomass Production in Submerged Cultivation

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Abstract

Twenty-one species of edible and non-edible basidiomycetes were screened for bio-polymers and biomass production in submerged cultivation. All the basidiomycetes produced bio-polymers and there was variation in quantities of bio-polymers produced by the strains at different incubation times. The bio-polymers production ranged within 44 - 9,177 mg/l, 63 - 14,525 mg/l and 69 - 6,367 mg/l at days 2, 7 and 14, respectively. Coriolus versicolor produced the highest yield at day 7. The biomass production at days 2, 7 and 14 ranged within 0.12 - 0.23g dry w/l, 0.13 - 0.25g dry w/l and 0.13 - 0.30g dry w/l in which C. versicolor also had the highest yield. The highest biomass production was recorded on day 14.

***Keywords**: Basidiomycetes, screening, bio-polymers, biomass, Coriolus versicolor.*

Introduction

Mushroom is a macro fungus with a distinctive fruiting body, which can either be hypogenous or epigeous, large enough to be picked by hands. Their number is estimated at 140,000, yet only 10% (approximately 14,000 named species) are known. Less than 25 species of mushrooms are widely accepted as food and even fewer have attained the status of commerce. In particular, mushrooms comprise vast and yet largely untapped resources of new pharmaceutical products in modern medicine (Chang and Miles 1992; Hamlyn and Schmidt 1994; Hawksworth 2001; Wasser *et al*. 2000).

Mushrooms represent unlimited sources of bio-polymers and due to wide industrial applications of microbial bio-polymers much attention has been focused on their production (Ricciardi *et al*. 2002). Over 70,000 tons of bio-polymers per year are used in the food industry worldwide as thickening agents, stabilizers and texturizers (Liu 1990).

Several groups of bio-polymers from edible and medicinal mushrooms are attractive because they have potent biological and pharmaceutical activities including immuno-stimulating, anti-tumor, and hyoglycemic activities (Borchers *et al*. 1999; Ooi and Liu 2000). Some mushroom polysaccharides such as lentinan from *Lentinus edodes*, schizophyllan from *Schizophyllum commune* and krestin from *Coriolus versicolor* are commercially available (Chihara *et al*. 1970; Tabata *et al*. 1981).

This work aimed at the screening of edible and non-edible basidiomycetes for bio-polymers and biomass production in submerged cultivation.

Materials and Methods

Sample collection

Twenty-one different species of edible and non-edible basidiomycetes were collected from the botanical garden, University of Ibadan, Ibadan, Nigeria, and its environs. They were identified and the mycelia of these fungi were obtained using a modified tissue culture method of Quimio *et al*. (1990) and maintained on potato dextrose agar supplemented with 0.5% peptone.

Inoculums Preparation

The seed culture was grown in seed culture medium containing (g/l) peptone 1.0, yeast extract, 2.0, K_2HP0_4, 1.0, $MgS0_4$, 0.2, $(NH4)_2S04$, 5.0, glucose, 39.0 (Cavazzoni and Adami 1992). The pH was adjusted to 6.0 and the medium was autoclaved at 121°C for 15 min. The medium was then inoculated with mycelia from the stock culture and incubated for 5 days.

Fermentation medium

The basal medium used contained $MgSO_4$. $7H_2O$ (0.2 g), K_2HPO_4 (1.0 g), NH_4SO_4 (5.0 g), D-glucose (9.75 g), yeast extract (3.0 g), peptone (1.0 g) and 1,000 cm^3 distilled water. Erlenmeyer flasks containing 100 ml of sterilized basal medium were inoculated with 5 mm diameter of a sterilized cork borer Agar disk from the margin of 5-day young culture from Petri-dishes. The flasks were inoculated for 2, 7 and 14 days at 25°C on shaker at 150 rpm.

Mycelia yield and EPS quantification

Biomass dry weight was determined by filtering the culture to separate fungal biomass which was washed twice with distilled water and quantified as dry weight (at 105°C to reach constant weight). The EPS was determined by adding isopropanol to the culture filtrate ($1:1^v/v$) and after 24 h at 4°C the precipitated bio-polymer was separated by centrifugation (8,000 rpm for 10 minutes) and the EPS quantity was estimated by using the phenol sulphuric acid method of Dubois *et al*. (1956). The pH was determined by using the method of AOAC (1990).

Results and Discussion

The bio-polymer produced by the screened basidiomycetes is shown in Table 1. All the basidiomycetes produced bio-polymer and there was variation in quantities of bio-polymer produced by the strains. At day 2, the bio-polymer production ranged within 44 - 9,177 mg/l in which *Lentinus* sp. produced the highest quantity. About 20% of the strains produced bio-polymer bellow 1,000 mg/l while 80% produced bio-polymer above 1,000 mg/l. At day 7, the bio-polymer ranged within 63 - 14,525 mg/l in which the highest was produced by *Coriolus versicolor*. About 80% of the strains produced reasonable quantity of bio-polymer at day 7. At day 14, the bio-polymer production ranged within 69 - 6,367 mg/l in which *Formes* sp. was found to be the best producer. 50% of the strains produced larger amount of bio-polymer at day 14 whereas 50% of the strains produced the quantity below 1,000 mg/l.

Table 1. Total bio-polymers production by the screened Isolates.

Serial number	Isolates code	Incubation Time (Days)		
		2	7	14
1	*Lentinus subnudus*	1,519	7,177	6,171
2	*Termomyces mammiformis*	6,918	1,601	424
3	*Lepiota morgani*	1,627	8,424	2,184
4	*Psathyrella atroumbcuta*	6,082	2,124	1,975
5	*Agaricus* sp.	1,677	1,348	5,798
6	*Coriolus versicolor*	1,354	14,525	1,069
7	*Pleurotus florida*	2,298	4,386	2,703
8	*Ganoderma lucidium*	44	7,709	2,639
9	*Marasmius* sp.	1,006	7,608	506
10	*Fomes sp.*	6,589	1,139	6,367
11	*Grifola frondosa*	7,728	6,589	171
12	*Tricholoma lobayensis*	7,722	5,538	766
13	*Polyporus* sp.	2,620	2,595	69
14	*Coriolus ocidentalis*	1,139	1,639	1,703
15	*Ganoderma* sp.	930	1,101	563
16	*Coprinus* sp.	6,165	487	2,291
17	*Chlorophyllum molybditis*	3,196	1,791	1,241
18	*Pleurotus ostreatus*	1,025	3,842	2,538
19	*Lentinus* sp.	9,177	4,399	633
20	*Sparasis crispa*	8,076	63	829
21	*Coprinus* sp.	4,228	8,266	879

Incubation time had profound effect on bio-polymers production by the strains. There was variation in bio-polymers production among the same species, for example, *Lentinus subnudus* and *Lentinus* sp. in which 1.519 and 9,177 mg/l, 7,177 and 4,399 mg/l, and 6,771 and 637 mg/l bio-polymer was produced at days 2, 7 and 14, respectively. Similar trend was also observed for *Coriolus versicolor* and *Coriolus ocidentalis*, the bio-polymer produced at days 2, 7 and 14 were 1,354 and 1,139 mg/l, 14,522 and 1,639 mg/l, and 1,069 and 1,703 mg/l, respectively. However, for *Ganoderma lucidium* and *Ganoderma* sp. 44 - 930 mg/l, 7.709 - 1,101 mg/l and 2,639 - 563 mg/l bio-polymers were produced at days 2, 7 and 14, respectively. Polymer production in this study was higher than the one reported by Cavazzoni and Adami (1992) on *Schizophyllum commune*. This result confirms the diversity of bio-polymer producing ability of the individual strains as it has been reported by Maziero *et al*. (1999).

Biomass production ranged within 0.12 - 0.23g dry w/l in which *Sparasis crispa* and *Chlorophyllum molybditis* had the highest and *Coprinus* sp.(2) had the least. At day 7, the biomass growth ranged within 0.13 - 0.25 g dry w/l in which *Coriolus versicolor* had the highest and the least was produced by *Coprious* sp. (1). However, at day 14 it ranged within 0.13 - 0.30 g dry w/l. The highest was recorded for *Coriolus versicolor* while *Ganoderma lucidium* produced the lowest. Different strains of *Coprinus* sp., *Ganoderma* sp., show different results not only for bio-polymers but also for biomass production. This result is in agreement with the report of Maziero *et al*. (1999).

Though bio-polymers production had no correlation with biomass yield, the highest biomass was recorded on day 14. There was color consistency of the filtrates. Sometimes, the filtrate was very clear, while at other times it was turbid and very viscous. Agglomeration of mycelia without a defined form was observed which agreed with the reports of Maziero *et al*. (1995) and Maziero (1996). The submerged cultures showed different features according to the fungal species. After a long period of incubation, the mycelia was shrunk and some broken, this was attributed to the glucose depletion in the medium.

The result showed that most of the screened isolates were good potential bio-polymers and biomass producers. There is a need for systematic studies of mushroom applications in medical fields and industries. Also, there is a need for further investigations on the potentiality of Nigerian basidiomycetes.

Table 2. Total biomass production (g dry w/l) by the screened isolates.

Serial number	Isolates code	Incubation Time (Days)		
		2	7	14
1	*Lentinus subnudus*	0.15	0.15	0.26
2	*Termomyces mammiformis*	0.21	0.18	0.23
3	*Lepiota morgani*	0.20	0.14	0.23
4	*Psathyrella atroumbcuta*	0.19	0.19	0.25
5	*Agaricus* sp.	0.21	0.20	0.19
6	*Coriolus versicolor*	0.20	0.25	0.30
7	*Pleurotus florida*	0.21	0.14	0.26
8	*Ganoderma lucidium*	0.24	0.15	0.13
9	*Marasmius* sp.	0.15	0.18	0.24
10	*Fomes sp.*	0.17	0.14	0.26
11	*Grifola frondosa*	0.16	0.20	0.21
12	*Tricholoma lobayensis*	0.21	0.15	0.25
13	*Polyporus* sp.	0.15	0.14	0.19
14	*Coriolus ocidentalis*	0.15	0.20	0.17
15	*Ganoderma* sp.	0.16	0.17	0.29
16	*Coprinus* sp. (1)	0.22	0.13	0.26
17	*Chlorophyllum molybditis*	0.23	0.16	0.18
18	*Pleurotus ostreatus*	0.21	0.15	0.15
19	*Lentinus* sp.	0.18	0.24	0.15
20	*Sparasis crispa*	0.23	0.17	0.24
21	*Coprinus* sp.(2)	0.12	0.17	0.21

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