Influence of Different Nutrient Sources on Exopolysaccharide Production and Biomass Yield by Submerged Culture of *Trametes versicolor* and *Coprinus* sp.

Bukola Christianah Adebayo-Tayo and Edwin Emeka Ugwu

Department of Microbiology, University of Ibadan, Ibadan, Oyo State, Nigeria
E-mail: <bukola_tayo@yahoo.com; emekaedwin@yahoo.com>

Abstract

Influence of different nutrient sources on Exopolysaccharide (EPS) production and Biomass yield by submerged culture of Trametes versicolor and Coprinus sp. was investigated. The best EPS production by Trametes versicolor and Coprinus sp. was stimulated by sucrose. EPS production ranged within 1,011-17945 mg/l and 200-18,765 mg/l, respectively. The least EPS production was recorded in xylose and glucose, respectively. Xylose and sorbitol supported the highest biomass yield (1.9 gdryw/l and 2.0 gdryw/l) by Trametes versicolor and Coprinus sp., respectively. Yeast extract induced the highest EPS production (7,835 mg/l) by T. versicolor. The EPS ranged within 701-7,835 mg/l. Casein stimulated the highest production of EPS by Coprinus sp. and it ranged within 563-7,474 mg/l. Yeast extract and $NaNO_3$ induced the highest biomass yield by Trametes versicolor and Coprinus sp., respectively. Biomass yield ranged within 1.0-1.6 gdryw/l and 2.0-1.9 gdryw/l. The highest EPS production by the strains was stimulated by glutamic acid and it ranged within 272-3,684 mg/l and 209-8,899 mg/l, respectively. The least stimulatory amino acids were alanine and glutamate, respectively. The best amino acids for biomass production by the isolates were aspartic acid and asparagines, respectively, and it ranged within 1.0-15.0 gdryw/l and 1.0-16.0 gdryw/l.

***Keywords**: Trametes versicolor, Coprinus sp., EPS production, biomass, nutrient sources.*

Introduction

The *Trametes versicolor* (formerly known as *Coriolus versicolor*) mushroom, commonly called the “turkey tail” mushroom in the United States and used in Chinese medicine under the name “Yun Zhi”, has a special place among medicinal mushrooms. Polysaccharide-K (Krestin, PSK, PSP) produced by *Trametes versicolor* has been approved as an anticancer agent in Japan in 1980s by the Health and Welfare ministry (ACS 2011). Polysaccharide-K, used with chemotherapy, has increased the survival time of cancer patients in randomized control studies with the following types of cancer: stomach cancer, colorectal cancer, and non-small cell lung carcinoma (Kobayashi *et al.* 1995; Oba *et al*. 2007; Mitomi *et al*. 1992; and Hayakawa *et al*. 1997). The protein-bound polysacccharides (PSK) and (PSP) from *Trametes versicolor* were found to have antiviral effect on HIV and cytomegalovirus in in-vitro tests (Tochikura *et al*. 1989), and they also contribute to the antiviral activity, such as the inhibition of HIV-gp120 to immobilized CD4 receptor and the reverse transcriptase activity of viruses (Collins and Ng 1997).

Coprinus comatus is an edible mushroom; it is cultivated in China as food and is used as a hypoglycemic food or medicine for hyperglycemic people. The hypoglycemic activity of *Coprinus comatus* has been tested on Alloxan and adrenalin-induced hyperglycemic mice (Han and Liu 2009). Inhibitory effect of *Copronus comatus* extract on the proliferation and viability of the androgen-sensitive human prostate adenocarcinoma cell line LNCaP has been reported by Dotan *et al*. (2010). This work aimed at investigating the influence of different nutrient sources on exopolysaccharide production and biomass yield by submerged culture of *Trametes versicolor* and *Coprinus* sp.

Materials and Methods

Sample Collection and Preparation of Inoculums

Trametes versicolor and *Coprinus* sp. cultures were obtained from the culture collection of our previous work in the Department of Microbiology University of Ibadan, Ibadan, Oyo State, Nigeria. The stock cultures were maintained on potato dextrose agar (PDA) supplemented with 0.5% yeast extract incubated at 25^oC for 7 days and stored at 4^oC. The seed culture was grown in a 250 ml flask containing 50 ml of basal medium prepared by adding 5% glucose, 12-15% peptone, 1.5% malt extract and 1-5% yeast extract. The pH of the medium was adjusted to 6.0 and the medium was autoclaved for 15 min. The medium was then inoculated with mycelia from the stock culture and incubated for 5 days.

Effect of Carbon Sources on Biomass and EPS Production by the Strains

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. Also, 97.5 g carbon of each carbon source (lactose, glucose, sucrose, maltose, mannose, galactose, fructose, xylose, sorbitol and starch) was the supplement in the basal medium. The pH of the basal medium was adjusted to pH 6. The basal medium without any carbon source served as the control. Further, 100 ml of each basal medium was dispensed into a corresponding 500 ml flask and autoclaved at 121^oC for 15 min. In order to suppress bacterial growth, 1 ml of streptomycin was added. Then the flasks were purposely inoculated with 10% of 5-day-old actively growing cultures of *Trametes versicolor* and *Coprinus* sp. Finally, the flasks were incubated at 25^oC for 2, 4, 7 and 14 days, respectively. The fermentation medium was consequently analyzed for biomass and EPS production.

Effect of Nitrogen and Amino Acid Sources on Biomass and EPS Production by the Strains

The basal medium used was made up of glucose (10.0 g), NaCl (0.1 g), $CaCl_2$ (0.1 g), KH_2PO_4 (0.5 g), $MgSO_4$, $7H_2O$ (0.5 g), thiamine hydrochloride (0.5 mg) and distilled water (1,000 cm^3). The medium was supplemented separately with amino acids and inorganic nitrogen sources at the rate of 1.0 g per liter. Complex nitrogen (casein, urea, yeast extract and peptone) sources were supplemented at a concentration of 2.0 g/liter. The liquid without any nitrogen source served as the control. Further, 100 ml of each liquid medium was dispensed into a conical flask and treated as described in the carbon medium experiment.

Estimation of Mycelia Yield and EPS Production by the Strains

The biomass concentration was determined by the dry mass method involving filtration of broth samples through pre-weighed filter discs (Whatman No. 1). The fungal biomass was washed twice with distilled water and quantified as dry weight (treated at 105^oC 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^oC the precipitated biopolymer was separated by centrifugation (8,000 rpm for 10 minutes) and the EPS quantity was determined by using the phenol sulphuric acid method of Dubois (1956). The pH was determined by using the method of AOAC (1990) or by using a digital pH meter equipped with a glass electrode.

Results and Discussion

All the carbon sources had a profound influence on EPS production and biomass yield by the isolates. The influence of different carbon sources on EPS production and biomass yield by *Trametes versicolor* is shown in Fig. 1a. The EPS production by *Trametes versicolor* ranged within 1,011-17,945 mg/l. Sucrose was found to stimulate the highest EPS production after 7 days of fermentation, followed in order by xylose and galactose after 2 days of fermentation. The least EPS production was recorded with xylose after 4 days of fermentation (Fig. 1a). Among the carbon sources used for biomass yield, xylose supported the highest biomass yield (19.0 g/l) after 7 days of fermentation. Biomass yield by *Trametes versicolor* ranged within 1.0-1.9 gdryw/l as shown in Fig. 1b.

Coprinus sp. produced different quantities of EPS in all the carbon sources used. Sucrose was the best utilizable carbon for EPS production. It ranged within 200-18,765 mg/l. The highest quantity was produced after 4 days of fermentation (Fig. 2a). Glucose was the least utilizable sugar. Sorbitol supported the highest biomass yield (2.0 gdryw/l) by *Coprinus* sp. after 7 days of fermentation as shown in Fig. 2b.

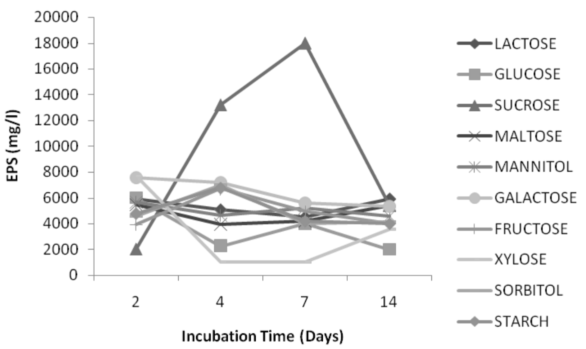

Fig. 1a. Influence of carbon sources on EPS production by *Trametes versicolor*.

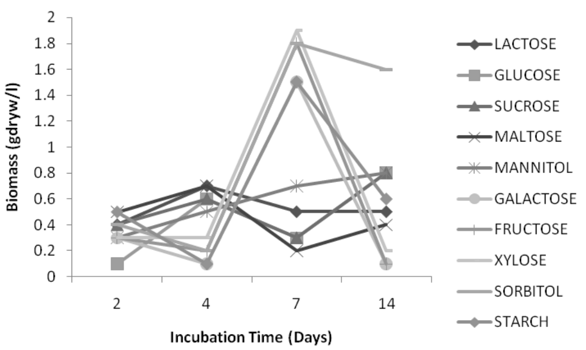

Fig. 1b. Influence of different carbon sources on biomass yield by *Trametes versicolor*.

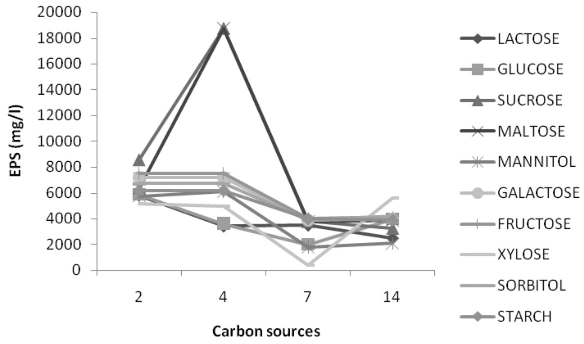

Fig. 2a. Influence of different carbon sources on EPS production by *Coprinus* sp.

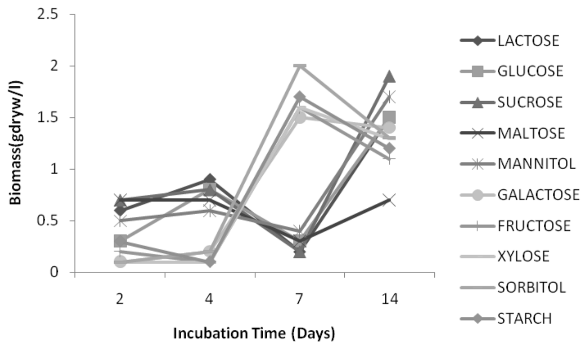

Fig. 2b. Influence of different carbon sources on biomass yield by *Coprinus* sp.

Trametes versicolor and *Coprinus* sp. have the ability to use different carbon sources, all of which have varied degree of stimulatory effect on EPS and biomass production. The stimulatory ability of sucrose on EPS production is in contrast with the reports of Michel *et al*. (1987), Burns *et al*. (1994) and Adebayo and Sola (2010) on the cultures of *Epicoceum purpurascens, Pleurotus florida* and *Pluroteus sajor-caju*, respectively. This report is in agreement with the report of Mahmoud *et al*. (2004). Different carbon sources had different effects of catabolic repression on the cellular secondary metabolism. Such phenomenon was also demonstrated in submerged cultivation of different kinds of mushrooms (Hwang *et al*. 2003; Kim *et al*. 2003). Sucrose has been reported to stimulate EPS production in many studies (Kim *et al*. 2002). The ability of xylose and sorbitol to stimulate the highest biomass yield by *Trametes versicolor* and *Coprinus* sp. is in contrast with Alofe (1985) and Kadiri (1990) who reported that glucose and fructose were the most readily utilized carbohydrate sources for the growth of *P. tuber-regium* and *P. squarnosulus*, respectively. The result from this study was quite different from the said report. This result indicates that the carbon source can be utilized to improve mycelia growth and EPS production, and that high mycelia growth seems not to be a determinant factor for high production of EPS by *Trametes versicolor* and *Coprinus* sp.

The nitrogen sources had a profound influence on EPS production by the strains. *Trametes versicolor* and *Coprinus* sp. produced different quantities of EPS in the nitrogen sources used. Yeast extract stimulated the best EPS production (7,835 mg/l) by *Coriolus verscolor* after 2 days of fermentation. The EPS production ranged within 701-7,835 mg/l as shown in Fig. 3a. Yeast extract stimulated the highest biomass yield by Trametes versicolor after 7 days of fermentation. The biomass yield ranged within 1.0-1.6 gdryw/l as shown in Fig. 3b.

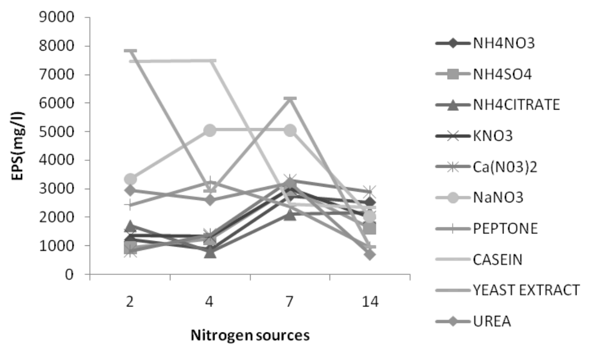

Fig. 3a. Influence of different nitrogen sources on EPS production by *Trametes versicolor*.

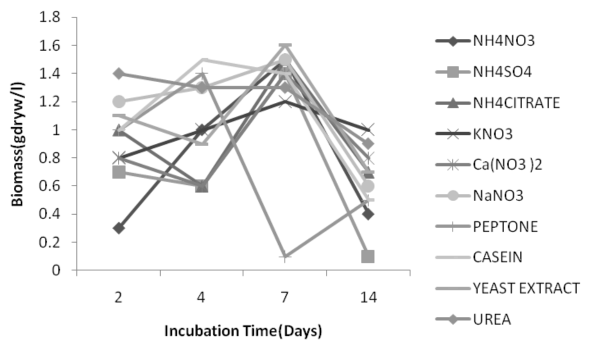

Fig. 3b. Influence of different nitrogen sources on biomass yield by *Trametes versicolor*.

The casein stimulated the best EPS production by *Coprinus* sp. after 2 days of fermentation. The EPS production ranged within 563-7,474 mg/l as shown in Fig 4a. Among the nitrogen sources used for the cultivation of *Coprinus* sp., yeast extract supported the highest biomass yield (1.9 gdryw/l) after 7 days of fermentation. The biomass yield ranged within 2.0-19.0 g/l as shown in Fig. 4b. The least biomass yield was recorded with KNO_3 after 14 days of fermentation.

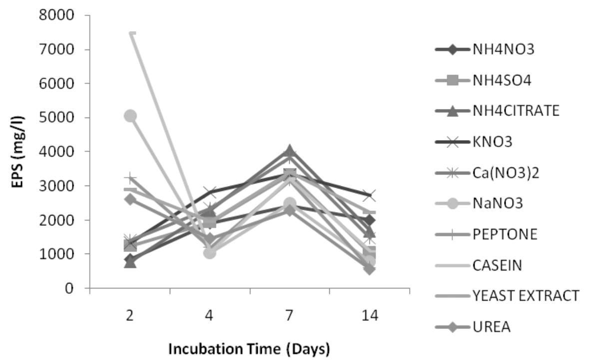

Fig. 4a. Influence of different nitrogen sources on EPS production by *Coprinus sp.*

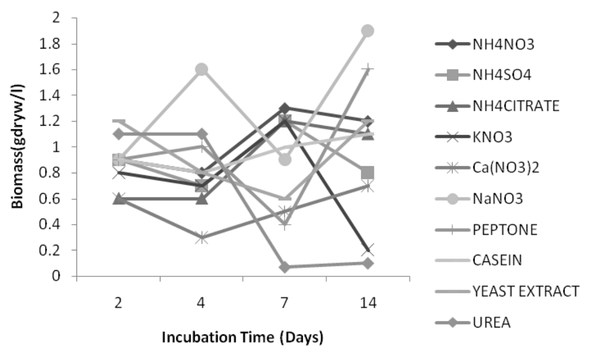

Fig. 4b. Influence of different nitrogen sources on biomass yield by *Coprinus* sp.

The highest stimulatory effect of organic nitrogen sources, compared to inorganic nitrogen sources, which gave rise to relatively lower EPS production and biomass yield was in accordance with that reported by Park *et al.* (2002), who observed a high level of mycelia growth in *C. miltaris* in media containing corn steep powder in submerged cultures. A similar result was also obtained by Kim and Nho (2004) who observed that protease peptone was the most useful nitrogen source in submerged cultivation for the production of angiotensin-converting-enzyme (ACE) inhibitor. However, for industrial processes, cost considerations mean that inorganic nitrogen sources are often preferred over organic sources. It was observed that EPS production and biomass yield were very dependent upon the type of nitrogen sources provided for the organisms.

Among the amino acids used, glutamic acid stimulated the highest EPS production by the isolates. The EPS production by *Trametes versicolor* ranged within 272-3,684 mg/l. The highest quantity was produced after 14 days of fermentation as shown in Fig. 5a. The least quantity was recorded with alanine after 4 days of fermentation. *Trametes versicolor* had the highest biomass yield in aspartic acid after 4 days of fermentation. The biomass yield ranged within 1.0-1.5 gdryw/l. The least quantity was recorded with arginine as shown in Fig. 5b.

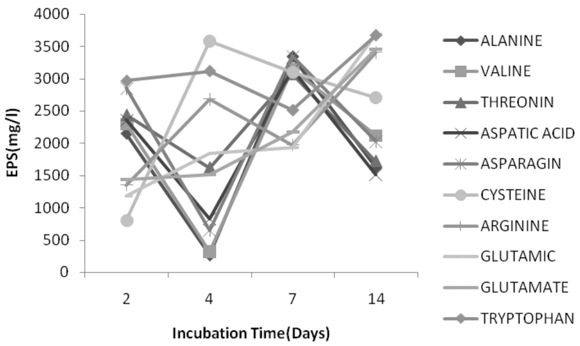

Fig. 5a. Influence of different amino acid sources on EPS production by *Trametes versicolor*.

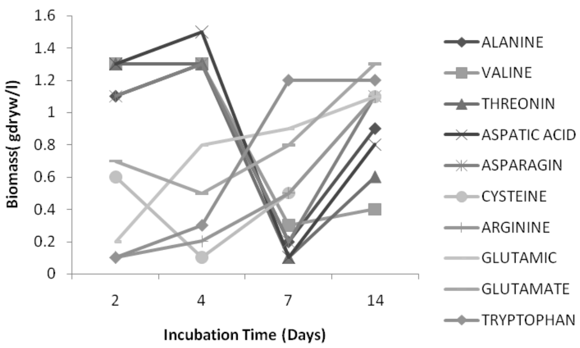

Fig. 5b. Influence of different amino acid sources on biomass yield by *Trametes versicolor*.

The EPS production by *Coprinus* sp. ranged within 209-8,899 mg/l, in which the highest quantity was produced with glutamic acid after 14 days of fermentation. The least quantity was recorded with glutamate after 4 days of fermentation as shown in Fig. 6a. Asparagine was the best amino acid for biomass yield by *Coprinus* sp. after 2 days of fermentation. The biomass yield ranged within 1.0-1.6 gdryw/l as shown in Fig. 6b.

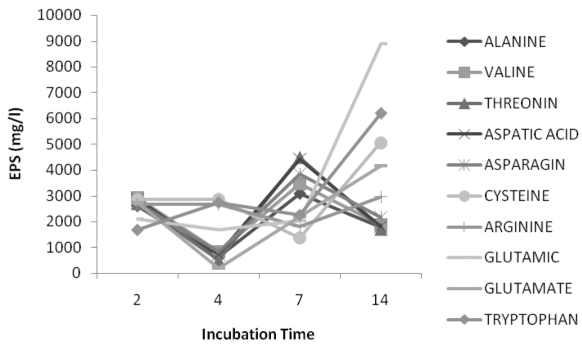

Fig. 6a. Influence of different amino acid sources on EPS production by *Coprinus* sp.

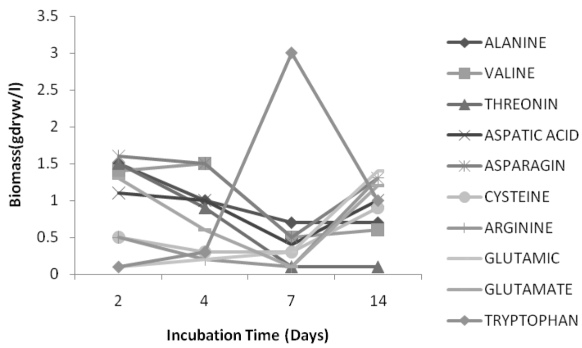

Fig. 6b. Influence of different amino acid sources on biomass yield by *Coprinus* sp.

The preference of glutamic acid to other amino acids may be due to its role in the glycolytic pathway (Garraway and Evans 1984). Gbolagade (2006) reported similar result for *Lepiota procera*. This result is in contrast to that obtained by Adebayo-Tayo and Sola (2010) on *P. sajor-caju*.

Conclusion

This work has shown that nutrient sources have a profound influence on Exopolysaccharide production and biomass yield by submerged culture of *Trametes versicolor* and *Coprinus* sp. Maximum EPS production is attainable by cultivating the isolates in sucrose, xylose and sorbitol as carbon source for EPS production and mycelia yield, respectively. Yeast extract, casein, glutamic acid and aspartic acid were the best nutrients for EPS production by the isolates. This result may provide sustainable means of adding value to submerged cultivation for the production of this promising medicinal bio-molecule which can exhibit hematological, antiviral, antitumor, antibiotics, antibacterial and immune-modulating activities.

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