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Full Length Research Paper

Optimization of growth conditions for mycelial yield and exopolysaccharride production by *Pleurotus ostreatus* cultivated in Nigeria

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Optimum culture condition for mycelia and exopolysaccharides production (EPS) by *Pleurotus ostreatus* in submerged liquid culture was investigated. The optimum temperature for mycelia yield and exopolysaccharide production was 25 and 15°C in day 14 with yields of 1.40 g/ml and 2700 mg/l respectively. Optimum pH was found to be 8 with respective yields of 2.5 g/ml and 2665 mg/l in day 12 for mycelia and EPS mannitol (9.75 g/litre) and raffinose stimulated the highest mycelia growth (3.0 g/ml) and EPS production (3000 mg/l) at day 14 respectively. Mycelia yield (2.10 g/ml) and EPS production (2700 mg/l) was optimally supported by urea. Glycine (1.0 g) and leucine (1.0 g) supported optimum production of mycelia yield (2.5 g/ml) and EPS concentration (2925 mg/l) respectively while folic acid and ascorbic acid induced the moderate mycelia yield and EPS concentration respectively. These results have shown that significant improvement in mycelia yield and EPS production by *P. ostreatus* could be enhanced through submerged cultivation under appropriate optimized conditions.

Key words: Culture, *Pleurotus ostreatus*, mycelia yield, exopolysaccharride-production.

INTRODUCTION

Mushrooms are group of organisms which possessed unlimited source of polysaccharides with antitumor and immune stimulating properties. Antitumour action of mushroom polysaccharides such as Lentinan from *L. edodes,* Krestin from *C. versicolor* and schizophyllan from *Schizophyllum commune* have been reported (Chilara et al., 1970; Tabata et al., 1981; Ng, 1993).

Edible *Pleurotus species* are very good dietary food which has been reported to contribute positive effect on body metabolism, where it helps in decreasing the free lipid triglycerides thus preventing arterioscleroses (Ginter and Bobeck, 1987; Trinci, 1992; Jong and Birgmingham, 1993). It was also reported that medicinal mushrooms exhibit hematological, antiviral, antitumor, and antibiotic and immuno-modulating activities (Cohen et al., 2002; Gbolagade and Fasidi, 2005; Jonathan et al., 2008; Olawuyi et al., 2010).

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The fungal biomass has various applications as far as fermentation is concerned (Maziero et al., 1995; Gbolagade et al., 2006a; 2006b). The biomass can be used as Single cell protein (SCP), sources of lipids, flavors (Jong and Birgunghan, 1993; Gbolagade et al., 2006b; Jonathan et al., 2009) and other metabolites such as enzymes and polysaccharides. Fungal biomass also finds its usefulness in wound healing. Healing capacity of chitin was reported by Hamyth and Schmidt (1994).

In this present study, Optimization of growth condition for mycelia yield and exopolysaccharride production by submerge culture of *P. ostreatus*, an edible mushroom was investigated.

MATERIALS AND METHODS

Source of the fungal sample

P. ostreatus was obtained from Plant Physiology and Biochemistry laboratory, Department of Botany and Microbiology University of Ibadan, Ibadan Nigeria. The stock culture was maintained on potato

dextrose agar (PDA) supplemented with 0.5% yeast extract incubated at 25°C for 7 days (Jonathan and Fasidi, 2003).

Culture preparation

The mycelial starter culture was inoculated onto a 250 ml flask containing 50 ml of basal medium prepared by adding 5% glucose, 12.5% 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 at 121°C for 15 min and then inoculated with mycelia from the stock culture and incubated for 5 days.

Effect of carbon sources on biomass and EPS production by *P. ostreatus*

The liquid medium used consisted of $MgSO_4$. $7H_2O$ (0.2 g), K_2HPO_4 (1.0 g), NH_4SO_4 (5.0 g), yeast extract (3.0 g), peptone (1.0 g) and 1000 cm^3 distilled water 9.75 g carbon of each carbon source (glucose, sucrose, fructose, lactose, maltose, and mannitol) was supplement in the basal medium according to the procedure of Jonathan et al. (2006b). The pH of the basal medium was adjusted to 6.0. The basal medium without any carbon source served as the control. 100 ml of each of the basal medium was dispensed into 500 ml flasks and autoclaves 121°C for 15 min. 0.5 mg/l of streptomycin sulphate was added after sterilization to suppress bacterial growth. The flask was then inoculated with 10% of a 5 day old actively growing culture of *P. ostreatus.* The flasks were then incubated at 25°C for 2, 4, 6, 8, 10, 12 and 14 days respectively. The fermentation medium was then analyzed for biomass and EPS production (Hwang et al., 2003).

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 (100°C to 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 biopolymer was separated by centrifugation (8,000 rpm for 10 min) and the EPS was quantified by using phenol sulphuric acid method of Dubois (1956). pH was determined by using the method of AOAC (1990).

Effect of inorganic/ organic nitrogen and amino acids sources

The liquid medium used consisted of D-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 (1000 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 concentration of 2.0 g/l. The liquid without any nitrogen source served as control. 100 ml of the liquid medium was dispensed into a conical flask and treated as described in the carbon media experiment (Jonathan et al., 2006b)

Effect of Vitamins

The vitamins used include riboflavin, ascorbic acid, pyridoxine and folic acid. 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 (9.0 g), yeast extracts 3.0 g, peptone (1.0 g) and 1000 cm^3 distilled water. The vitamins are supplemented at the rate of 0.1%. The pH of the media is adjusted to 6.0. 100 ml of the liquid media was dispensed into 500 ml conical flask and treated as described in the carbon experiment (Jonathan and Fasidi, 2003).

Effect of Temperature and pH

The influence of temperature and pH on biomass and EPS production was determined. For temperature, 100 ml each of the basal medium was dispensed into different conical flasks. The flasks were autoclaved at 121°C for 15 min and inoculated with 2 ml of 5 day, old culture of *P. ostreatus* incubated at 15, 25, 35 and 45°C for 2, 4, 6, 8, 10, 12 and 14 days respectively and analyzed as described in carbon experiment.

For the pH, same basal medium was employed but the medium was adjusted to pH 6, 8 and 10. 100 ml of each treatment was dispensed into 500 ml conical flask they were autoclaved, inoculated and incubated and analyzed as described in the carbon experiment.

RESULTS AND DISCUSSION

Temperature had a remarkable effect on mycelia yield and EPS–production by *P. ostreatus.* The minimum and maximum temperatures for growth were 15 and 35°C (Figures 1a and 1b). Optimum temperature for growth and EPS –production by *P. ostreatus* contrasts the report of Mahmond et al. (2004) who obtained very good EPS-production by *P. plumonarius* at 30°C. EPS-production varied considerably with the change in incubation temperature. The optimum temperature for the mycelia growth of *P. ostreatus* was 25°C. This observation agree favorably well with the findings of Chi et al. (1996) and that of Jonathan and Fasidi (2003) who reported optimum temperature for the growth of *Phellinus linteus and Psathyerella atroumbonata* as 25 to 30°C.

Initial pH had a profound effect on the studied parameter by *P. ostreatus*. pH 8 and 2 were optima for mycelia growth (2.5 g/l) and EPS production (2180 mg/l) by *P. ostreatus*. Significant difference was observed in mycelia yield and EPS-production ($P \leq 0.05$). Effect of pH on mycelia yield and EPS-production by *P. ostreatus was* shown in Figures 2a and 2b. Ability of *P. ostreatus* to grow and produced EPS optimally at pH 8 is in contrast to those reported for other EPS that several kinds of mushrooms have more acidic pH optima for mycelia yield during their submerge cultivation (Kim et al., 2003). The pH value has a profound effect on exopolysaccharide and biomass production by *P. ostreatus. P. ostreatus* appears to be able to grow over a wide range of pH value from 4.0-8.0 and pH optimal for both EPS and biomass production are quite different. The pH of medium is often a neglected environmental factor but it has a remarkable influence on morphology of fungi mycelia which further affect biomass accumulation and metabolite formation (Wang and McNeil, 1995; Shu and Lung, 2004; Gbolagade et al., 2006b; Jonathan et al., 2009).

Investigation was done to determine the best carbon and nitrogen sources on mycelia yield and EPS-

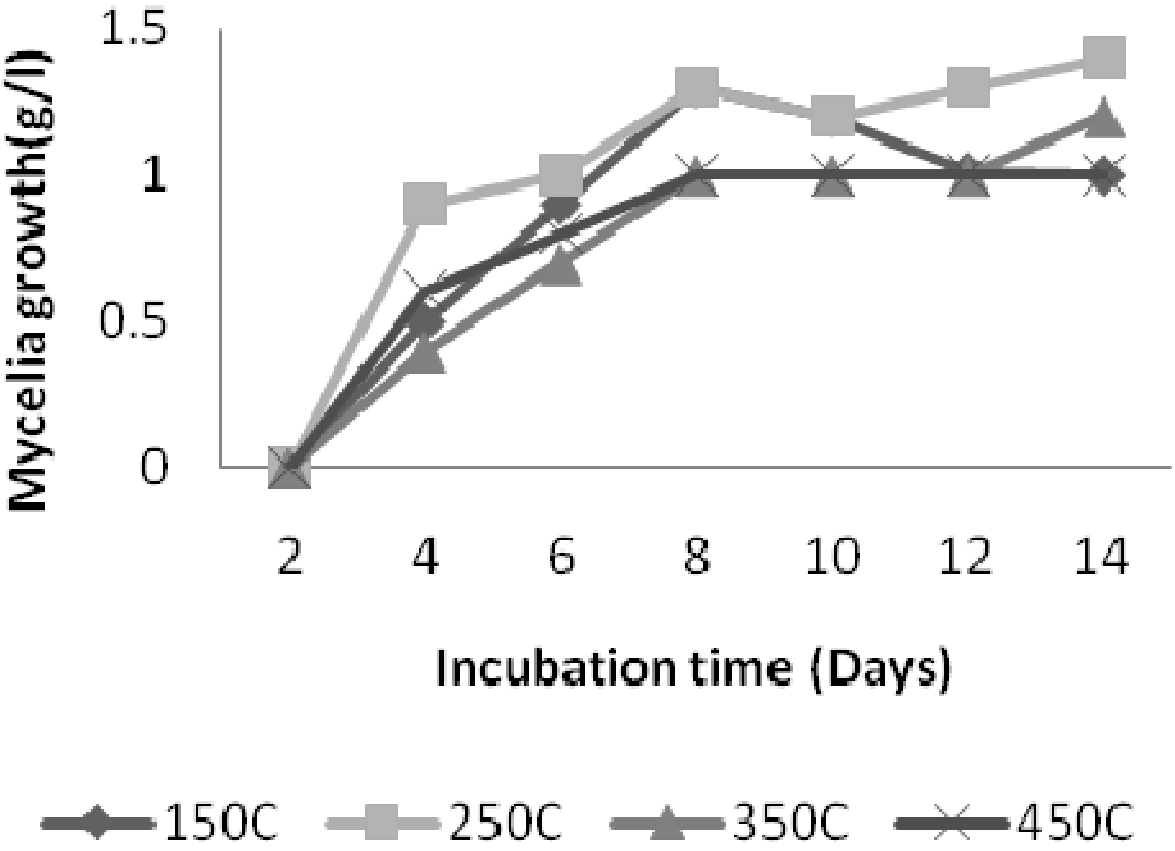

Figure 1a. Effect of temperature on mycelia growth by *P. ostreatus.*

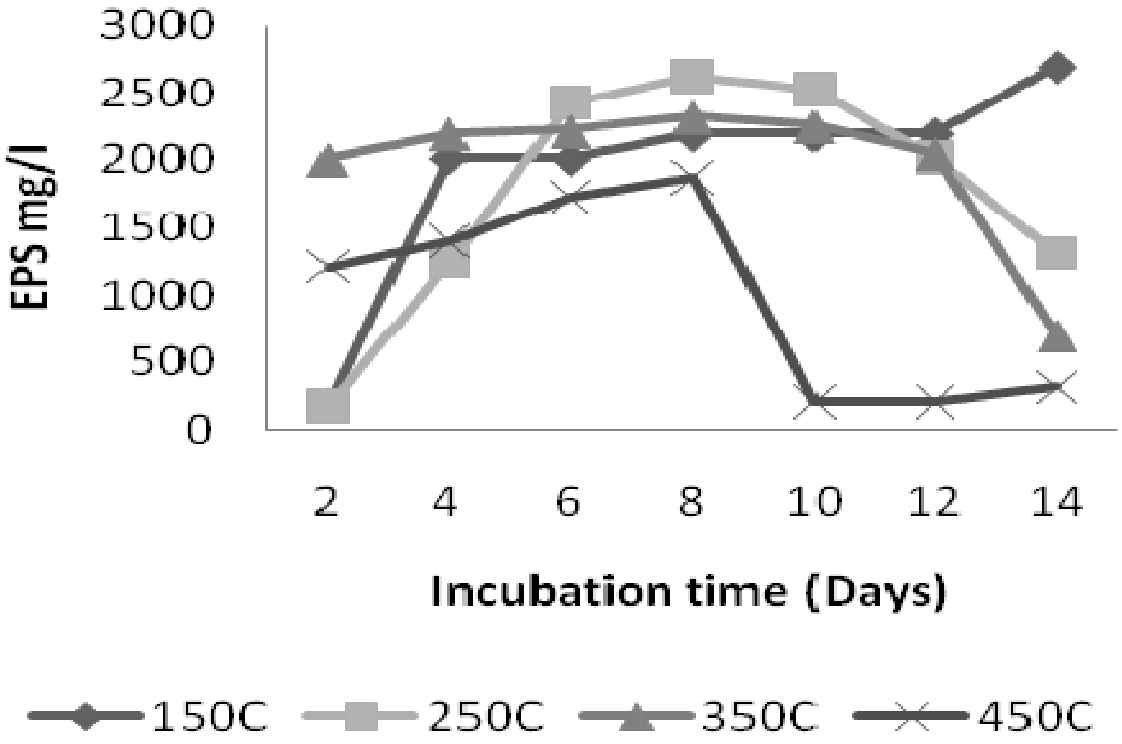

Figure 1b. Effect of temperature on EPS-production by *P. ostreatus.*

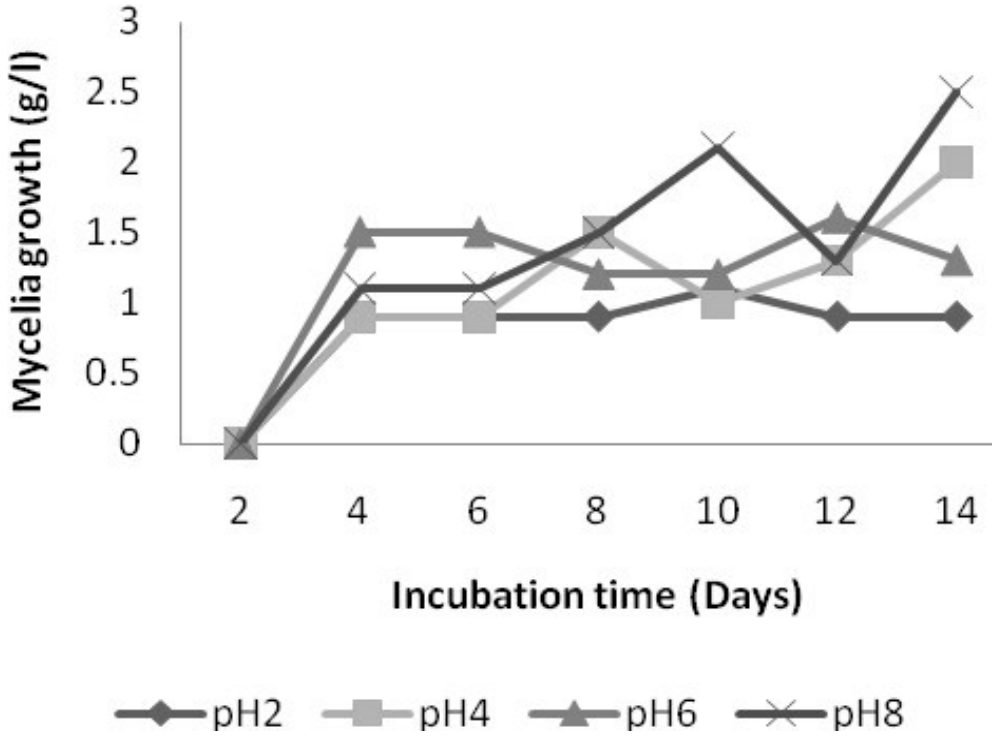

Figure 2a. Effect of pH on mycelia growth by *P. ostreatus.*

production by *P. ostreatus.* It was observed that among the 7 carbon sources used mannitol and raffinose supported the highest mycelia yield (3.0 g/ml) and EPS-production (300 mg/100 ml) by *P. ostreatus* respectively. The least biomass production was observed in raffinose (Figures 3a and 3b). Optimum mycelia growth and EPS-production was achieved when urea was used as nitrogen sources as shown in Figures 4a and 4b.

P. ostreatus has the ability to use a large number of sugars all of which have varied degree of stimulatory effect on EPs and biomass production. Stimulatory ability of raffinose on EPS production is in contrast with the report of Michel et al. (1987) and Burns et al. (1994) by the culture of *Epicoceum purpurascens and Pleurotus florida*. Different carbon sources had different effects of catabolic repression on the cellular secondary metabolism. Such phenomenon was also demonstrated in submerge cultivation of different kinds of mushrooms (Jonathan and Fasidi, 2001; Hwary et al., 2003; Kim et al., 2003). Alofe (1995) and Kadiri (1990) reported that glucose and fructose were the most readily utilized carbohydrate source for the growth of *P. tuber-regium and P. squarrosulus* respectively, the result from this study was quite different from this report. This result indicate that the carbon source can be utilized to improve good mycelia and EPS production and that high mycelia growth seem not to be a determinant factor for high production of EPS by *P. ostreatus.*

Comparatively organic nitrogen supported optimum production of mycelia and EPS concentration by *P. ostreatus*. Poor biomass growth and EPS-production was reported when inorganic nitrogen was used. It has been reported that nitrate ions have an inhibitory effect on growth of some basidiomycetes and sulphate ions (SO_4^{2-}) is large radicals which may be difficult to transport across the fungal membrane where it can promote growth (Griffin, 1994; Garraway and Evans, 1984). Urea has been recorded as the best nitrogen source for exo-biopolymer and biomass production by *P. ostreatus,* This observations is in contrast to the results obtained by Sevior and Kristiansen (1983) for *Acremomium pullulan,* Michel et al. (1987) for *E. purpurasceus* and Stasinopoulos and Seviour (1992) for *Acremnium persicinum* and Mahmoud et al. (2004) for *P. ostreatus.*

Among the vitamins tested, ascorbic acid and folic acid had the highest stimulatory effect on mycelia yield (2.4 g/ml) and EPS-production (2775 mg/l) respectively as shown in Figures 5a and 5b. Preference of the isolate for ascorbic and folic acid for the production of biomass and EPS is in contrast to the report of Adebayo-Tayo and Ekerete (2010) on *P. sajor-caju* in which pyridoxine had the highest stimulatory effect on biomass growth. Folic acid supported optimum EPS-production. This is in contrast to the report of Lilly and Barnett (1987) who observed that fungi synthesized the vitamins they required for growth. This result showed that *P. ostreatus* is not in this category.

Some of the amino acids used during this study have a stimulatory effect on mycelia yield and EPS-production by *P. ostreatus*. Optimum mycelia growth (2.5 g/l) and EPS

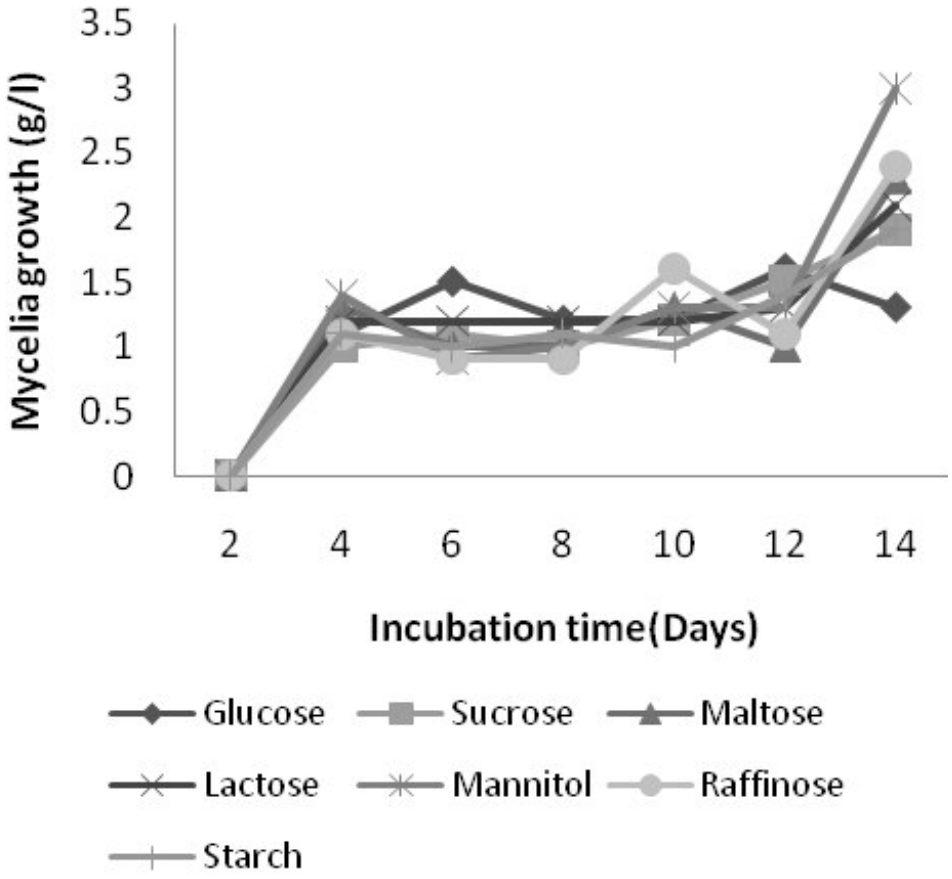

Figure 3a. Effect of carbon source on mycelia growth by *P. ostreatus.*

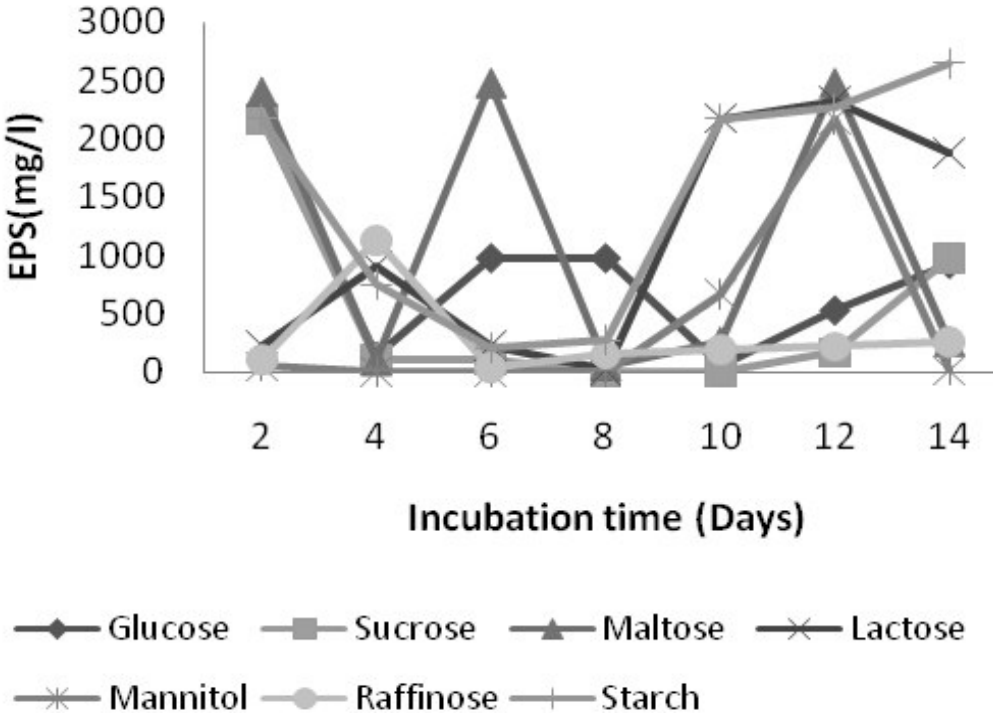

Figure 3b. Effect of carbon source on EPS-production by *P. ostreatus.*

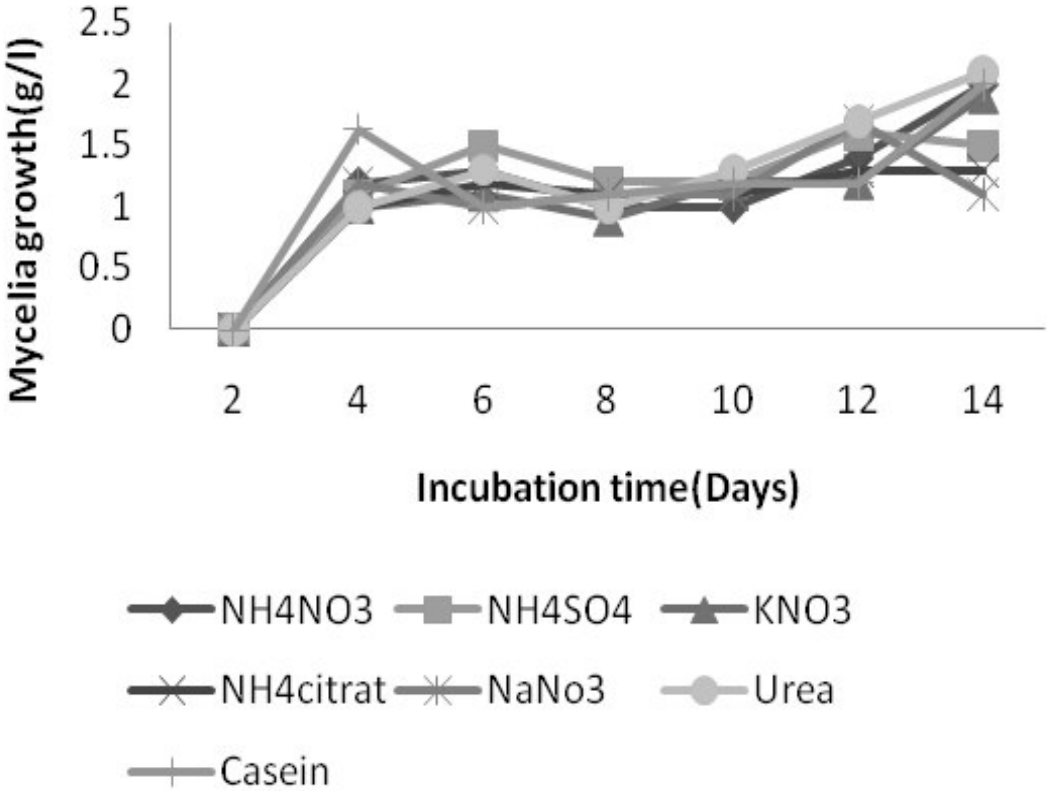

Figure 4a. Effect of Nitrogen source on mycelia growth by *P. ostreatus*

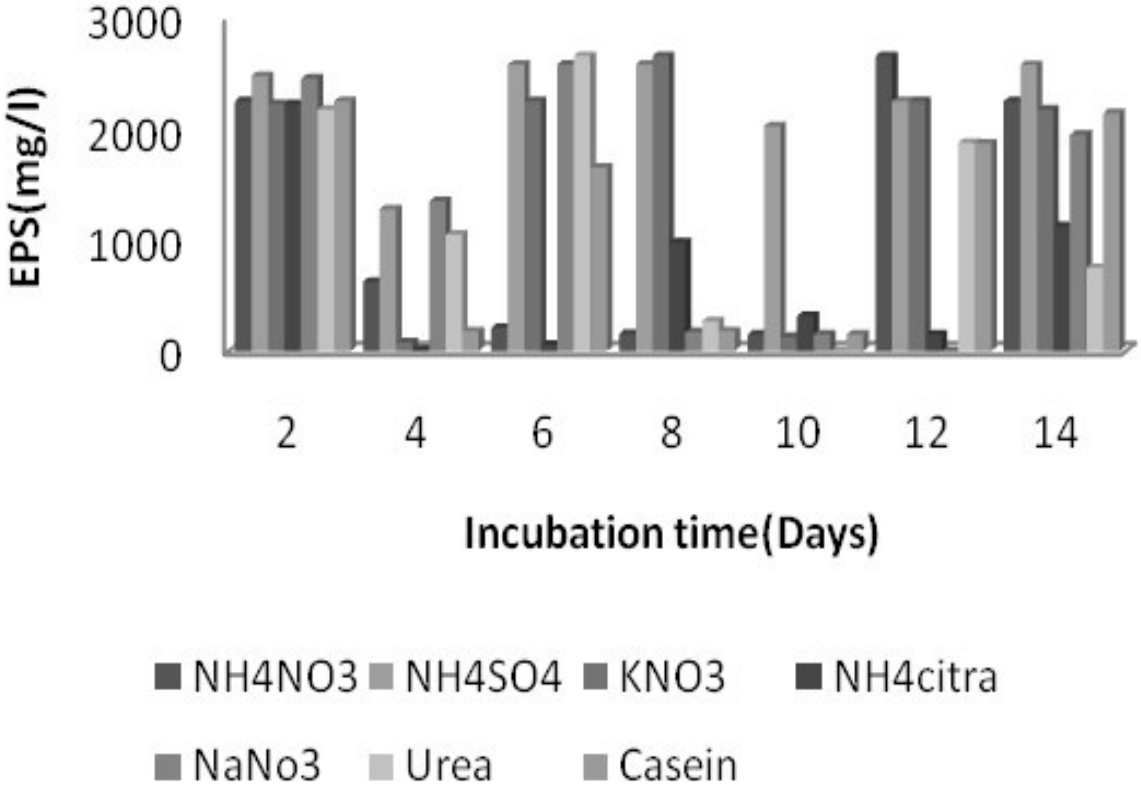

Figure 4b. Effect of Nitrogen source on EPS-production by *P. ostreatus.*

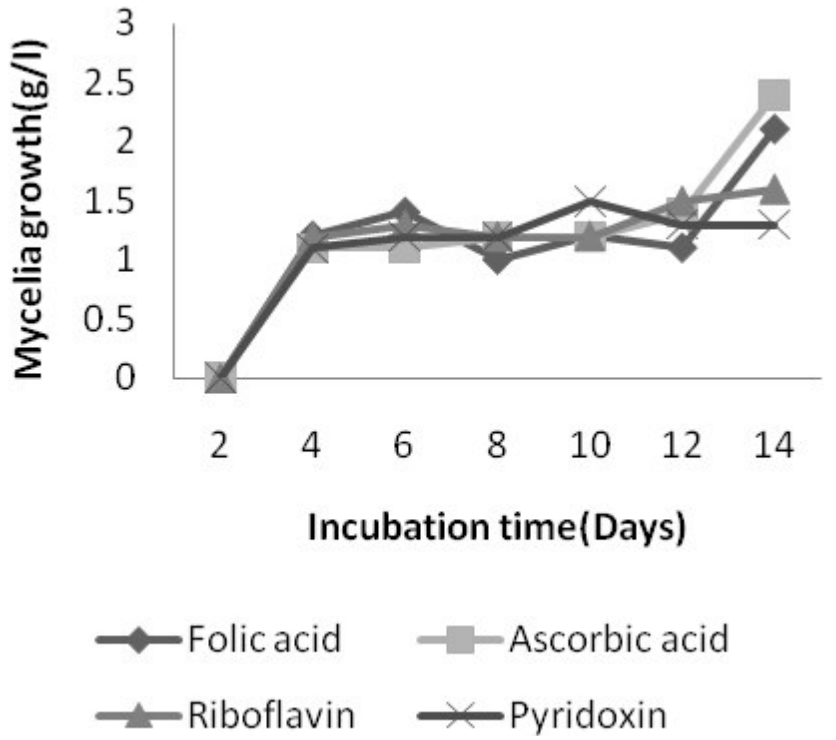

Figure 5a. Effect of Nitrogen source on EPS-production by *P. ostreatus.*

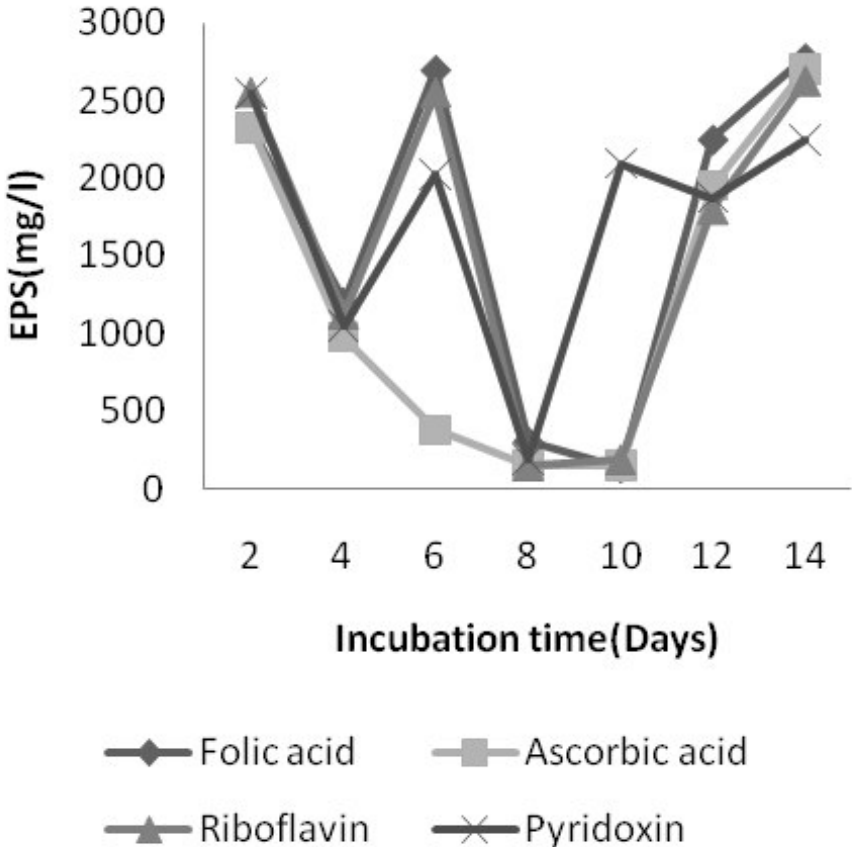

Figure 5b. Effect of vitamins on mycelia growth by *P. ostreatus.*

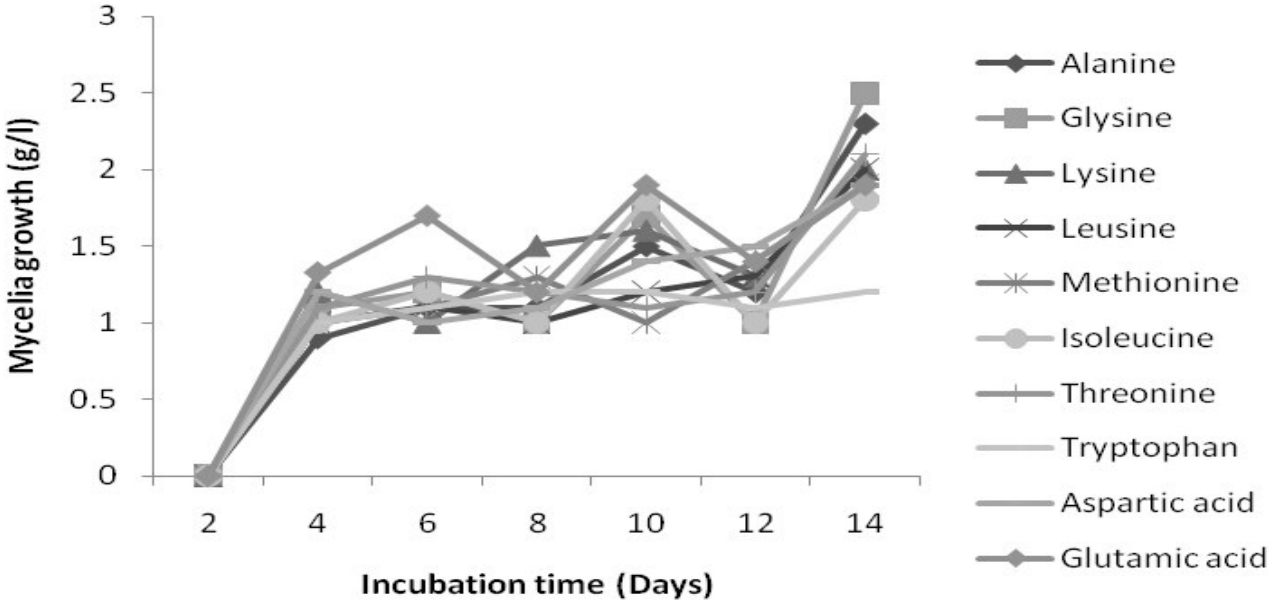

Figure 6a. Effect of Amino acids on mycelia growth by *P. ostreatus.*

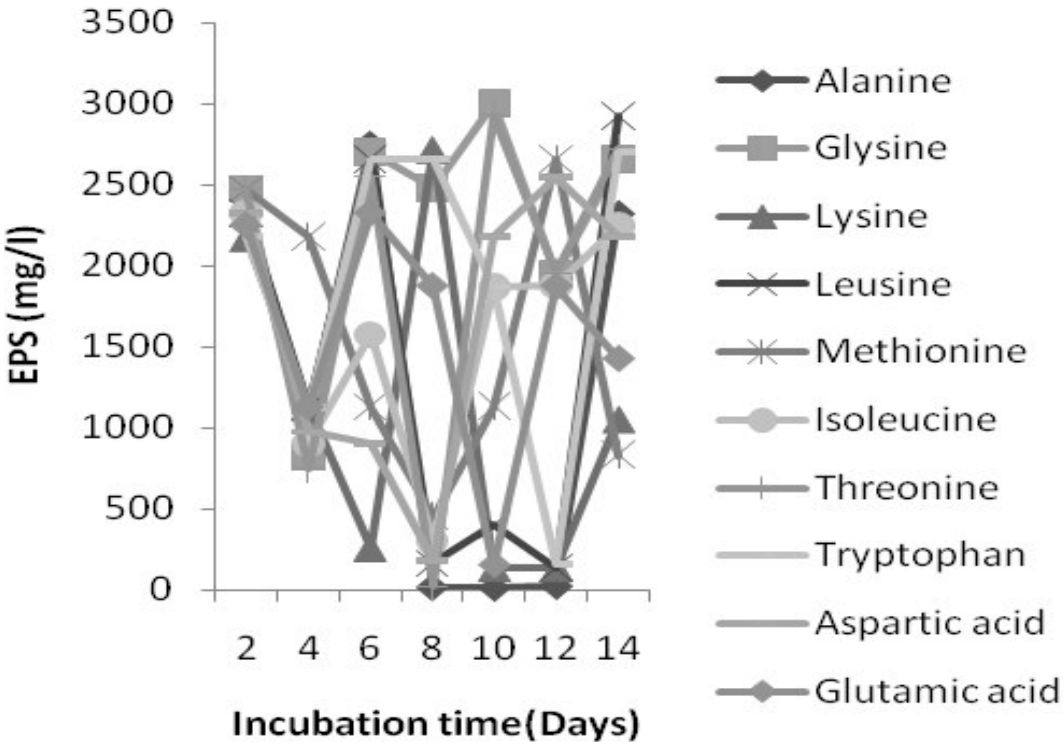

Figure 6b. Effect of Amino acids on EPS-production by *P. ostreatus.*

concentration (3000 mg/l) was recorded when glycine was used (Figures 6a and 6b). The preference of glycine to other amino acids may be due to the ease at which it is being transported across the fungal cell membrane. This result is in contrast to that obtained by Adebayo-Tayo and Ekerete (2010) on *P. sajor-caju.*

Conclusion

This work has shown that optimum mycelia and EPS-production by *P. ostreatus* could be attained through submerge cultivation of the fungus at 25 and 15℃, pH 8, mannitol and raffinose as carbon source respectively,

urea, glycine leucine, ascorbic acid and folic acid respectively. This result may provide a sustainable means of adding value to submerge cultivation of which will result in production of this promising medicinal fungus which can exhibit hematological, antiviral, antitumor, antibiotics, antibacterial and immune-modulating activities.

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