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OPTIMIZATION OF CULTURE CONDITIONS TO PRODUCE SECONDARY METABOLITES BY PLEUROTUS OSTREATUS

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ABSTRACT

The specie of mushroom *Pleurotus ostreatus* grown on the Potato Dextrose Agar (PDA) medium for the activation and then inoculated in the fermentation medium to obtain maximum production of metabolite. Maximum production of metabolites produced by *Pleurotus ostreatus* at different incubation time periods. The effect of different carbon sources glucose, galactose, fructose, sucrose and starch was checked on the growth and production of metabolite by *Pleurotus ostreatus* and the higher production was achieved with glucose. The effect of different nitrogen sources like potassium nitrate, sodium nitrate, ammonium sulphate, casein and urea were checked on the growth and production of metabolites by *Pleurotus ostreatus. A*mong the different nitrogen sources maximum metabolite were obtained with casein. The effect of pH on the growth and production of metabolites by *Pleurotus ostreatus* was studied in the range of 4.5-8.5 and maximum production was noted with 4.5 pH. The effect of temperature was checked on the growth and production of metabolite by *Pleurotus ostreatus* in range of 25℃-35℃ and higher production of metabolites were achieved at 30℃.

Key words secondary metabolites and *Pleurotus ostreatus*

INTRODUCTION

All organism needs to convert and transform a large variety of organic compounds to enable their selves to reproduce and grow. They need to provide energy to themselves in the form of ATP and to construct their own, tissues also have a need of supply of building blocks. For this purpose, an integrated network of enzyme mediated and chemically regulated reactions are used which is referred as intermediary metabolism. The pathways involved are called metabolic pathways primary metabolic pathways which can synthesize, degrade and interconvert compounds are commonly found in all organisms whereas another area of metabolism whose compound have limited distribution in nature is called secondary metabolites (Berdy, 2005, Martin, 1978). Secondary metabolites specifically special metabolites, which are not consumed by the producing micro-organisms but they are harmful to other microorganisms. They are not necessarily required by the cell for its growth, however secondary metabolites are considered as important survival factor in nature (Juan, 1980). Secondary metabolites are also called as idiolites (Walkers, 1974). Because they are synthesized during the idiophase (Berdy, 1974). It has been seen that secondary metabolism is regulated by overall regulatory controls which operate as functions of growth rate and Specific regulatory effects on individual pathways (Martin, 1978).

Growth phase of secondary metabolite is termed as “tropophase” whereas, production phase is termed as “idiophase” (Bu'Lock, 1975). Phenolics and flavanols possess antioxidant, anticancer, antimutagenic, antimicrobial and antiradical properties (Sahu and Green, 1997, Amarowicz, et al., 2004). Growth, reproduction and protection to the plants against pathogens and predators is facilitated by Phenolics compounds (Bravo, 1998). Due to the presence of antioxidant properties of flavonoids and phenolics, lipid peroxidation and shelf life of the product is increased by adding them in food, which consists of lipids and its associated foods (Yasoubi, 2007). Several physio-chemical factors such as nutrient supply, oxygenation, temperature and pH have profound influence on the production process of secondary metabolites. To enhance the production of metabolites these factors have traditionally optimized and controlled in industrial fermentation. Additionally, different pharmaceutical industries used traditional mutagenesis programs to improve strain and yield production (Carlos, et al., 2008). Some of the secondary metabolites such as digitalis, morphine and quinine are considered as plant metabolites while penicillin, cephalosporin, ergot rate and the statins are known as fungal secondary metabolites. Although few common biosynthetic pathways are followed by the chemically diverse secondary metabolites for their production in collabo-ration with morphological development. Recently using modern techniques such as molecular biology, bioinformatics and comparative genomics, it has been revealed that genes which are involve in production of fungal

metabolites are clustered and found near telomeres (Nancy, et al., 2005).

In this paper, we have tried to investigate the optimum culture conditions to produce metabolites from *Pleurotus ostreatus*.

MATERIALS AND METHODS

Culture collection: *P. ostreatus* was gifted by Department of Biotechnology, Sindh Agriculture University Tando Jam.

Preparation of stock culture: Stock culture was prepared by activating the culture on PDA media (potato 200g dextrose 20g agar 1.5g in 1L). The pH of media at 6.5 was adjusted by using 1N NaOH and 1N HCl. The sterilized media was inoculated with *P. ostreatus* culture. After inoculation, the petri plates were covered and incubated at 25℃ for one week in growth room.

Preparation of spore suspension solution: Spore suspension solution was prepared by using 50 ml of sterilized distilled water. Sterilized distilled water was added into a culture test tube and gently rub the surface of test tube in such a way that it gets mixed with sterilized distilled water and prepare spore suspension solution.

Preparation of culture media: Starter culture was prepared for fermentation broth media containing 25g glucose, 1.5g corn steep liquor, 5g peptone, 0.5g NH_4NO_3 in 500ml distilled water and the pH of media was adjusted at 6.5 by using 1N NaOH and 1N HCl. The media was sterilized in autoclave at 121℃ for 20 minutes at 15 psi. 50 ml sterilized media was taken in conical flasks. The sterilized media was inoculated with 5 ml of spore suspension solution in each flask. After inoculation flasks were covered and incubated at 25℃ for 12 days in growth room.

Analysis of secondary metabolite: The fermentation media was filtered through Wattman filter paper and filtrate was used for analysis of flavanols, reducing sugar, tannin contents, reducing power, antioxidant activity, and ascorbic acid contents under optimized culture conditions.

Determination of total flavanol: Total flavanol concentration was determined by spectrophotometric method as reported by Kumaran and Karunakan (2007) with slight modification and results were calculated from standard graph using quercetin.

Determination of tannin content: The tannin content from the culture broth was determined by Tamilselvi et al., (2012) using gallic acid as standard.

Reducing Sugar Determination: Reducing sugar content was determined by the method reported by Miller (1959). According to this method, 2.0ml (0.2ml sample + 1.8d.H_2O) of test solution was mixed with 2.0ml of dinitrosalicylic acid in test tube. The mixture was heated in boiling water bath for 5 minutes. The tubes were cooled in tap water and color intensity was read against blank at 540nmon Spectro-UV-Vis Double PC spectrophotometer, LaboMed, USA 11DV-60Hz or 220V-50Hz Serion Number 001151. Instead of sample distilled water was used for the preparation of blank. The concentration of reducing sugar was calculated from the standard graph that was prepared in same manner as test sample by using different concentration of the glucose.

Reducing Power: The reducing power of ethanol extracts of plants was checked as by the scheme of Oyaizu [13]. Took 0.5 ml sample solution, 102.5 ml phosphate buffer (6.6pH) and 2.5 ml 1% potassium ferricyanide. The reaction mixture was kept in water bath at 50°C for 20 minutes. Then 2.5ml of 10% T.C.A. was mixed and centrifuged at 1000 rpm for 10 minutes. The supernatant (2.5 ml) was added in 2.5 ml distilled water and 0.5 ml of 0.1% of ferric chloride. Absorbance was read at 700 nm on Spectro-UV-Vis Double PC spectrophotometer, LaboMed, USA 11DV-60Hz or 220V-50Hz Serion Number 001151. Absorbance of the sample is directly proportional to the reducing power of sample. Higher will be the reducing power with the increase of absorbance.

Total Antioxidants Activity: Total antioxidant's capacity was measured by modifying phosphomolybdate method as reported by Prieto et al., (1999) using α- tocopherol as a standard. An aliquot of 0.4 ml of plant extracts was mixed with 4 ml of reagent (0.6M sulphuric acid, 28 mM sodium phosphate and 4mM of ammonium molybdate). All tubes were capped and kept in boiling water bath at 95˚C for 90 minutes. Then the samples were cooled to room temperature and the absorbance was read at 695 nm on Spectro-UV-Vis Double PC spectrophotometer, LaboMed, USA 11DV-60Hz or 220V-50Hz Serion Number 001151 against a blank solution prepared in the same conditions by replacing the sample with 0.1 ml of methanol. The total antioxidants activity was expressed as mg/ml equivalents of α- tocopherol. The standard error was calculated by using Microsoft excel.

Absorbance = 0.4544 (alpha tocopherol) + 0.0131

Determination of Ascorbic acid: Ascorbic acid was determined by spectrophotometric method as reported by Bajaj and Kaur (1981). The concentration of ascorbic acid was calculated from the standard graph that was prepared in same manner as test sample by using different concentration of ascorbic acid.

Optimization of culture conditions: Optimization of culture conditions includes time of incubition, carbon sources, nitrogen sources, pH and temperatures for effective production of secondary metabolite.

Effect of time of incubation: The effect of time of incubation was analyzed to find best time of incubation for production of secondary metabolite after interval of 3days, 6 days and 12 days.

Effect of carbon source: The carbon source was optimized separately by using same concentrations of galactose, starch, sucrose and fructose along with glucose to find effective production of secondary metabolite for optimized time of incubation.

Effect of nitrogen source: The effect of different nitrogen sources such as ammonium sulphate, sodium nitrate, potassium nitrate, casein and urea instead of sodium sulphate were incorporated in fermentation media separately which contain best time of incubation and best carbon source to find maximum production of secondary metabolite.

Effect of pH: The effect of different pH was analyzed between the range of 4.5 to 8.5 in fermentation media having best time of incubation, best carbon and nitrogen source for effective production of secondary metabolite.

Effect of temperature: The effect of different temperatures (℃) was analyzed such as 25, 30, and 35in fermentation media which containing best time of incubation, best carbon and nitrogen source along with best pH to obtain best production of secondary metabolite.

RESULTS AND DISCUSSION

The effect of incubation time: The effect of incubation time (6 days, 9 days, 12 days) on growth and production of metabolite is shown in figure 1. It shows that higher amount of tannin production obtained at 12 days whereas flavanol and antioxidant production was maximum after 9 days. The present results are consistent the reported results of Gökhan, et al., (2015) using edible mushrooms including *P. ostreatus* consist of higher contents of flavanol and phenols after incubation of 8 days to 60 days.

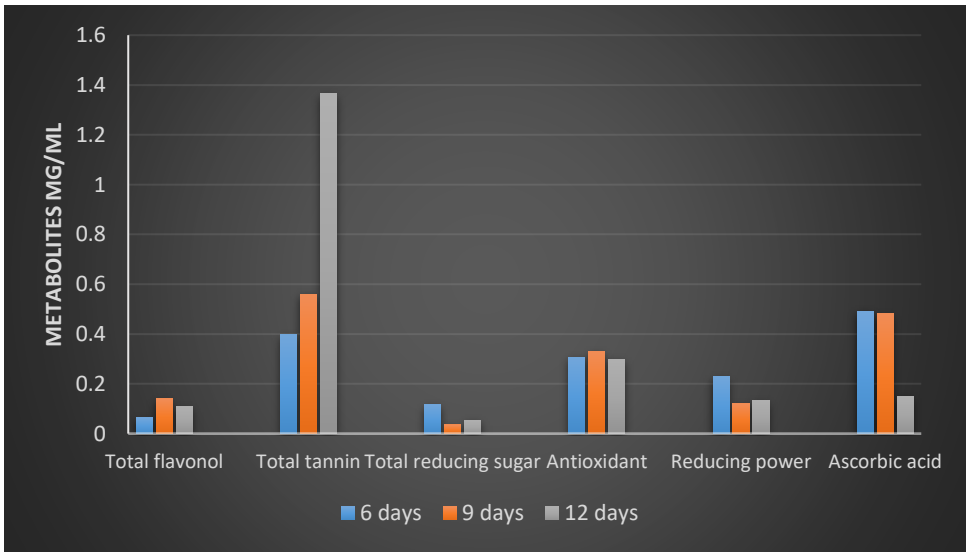

Figure 1: Metabolites production by *P. ostreatus* grown at different time using glucose as carbon source and incubated at 25℃ with pH 6.5.

The effect of carbon sources: The effect of carbon sources (glucose, galactose, fructose, sucrose and starch) on the growth and production of metabolites were checked as shown in fig 2, and higher amount of tannin flavanol 0.0501 mg/ml were achieved with glucose when *P. ostreatus* grown in a fermentation media at 25˚C, 6.5 pH. Similarly, different carbon sources were used by Chang (1989), Wang (1993) and Yang (1996) for maximum production of mycelium and glucose referred as suitable source for growth and mycelium growth of various mushrooms.

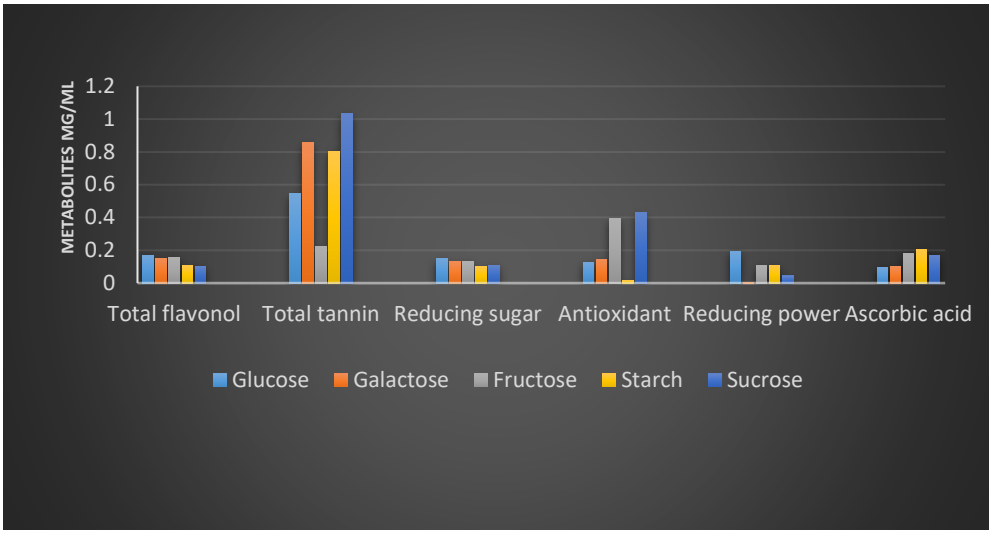

Figure 2: Metabolites production by *P. ostreatus* grown on different carbon source at 25˚C with pH 6.5

The effect of nitrogen sources: The effect of nitrogen sources (potassium nitrate. ammonium sulphate, sodium nitrate, casein and urea) was checked to investigate the growth and production of flavanol as shown in figure 3, among all these nitrogen sources casein gave higher amount of tannin 0.9 mg/ml when *P. ostreatus* grown in fermentation media containing glucose as carbon source at 25˚C and at 6.5 pH. The result is in contrast with the experiment performed by Hoa and Wang (2015) using different nitrogen sources and among them ammonium chloride and ammonium sulphate appeared as best nitrogen source for growth and mycelium production by *P. ostreatus* and *P. cystidiosus.*

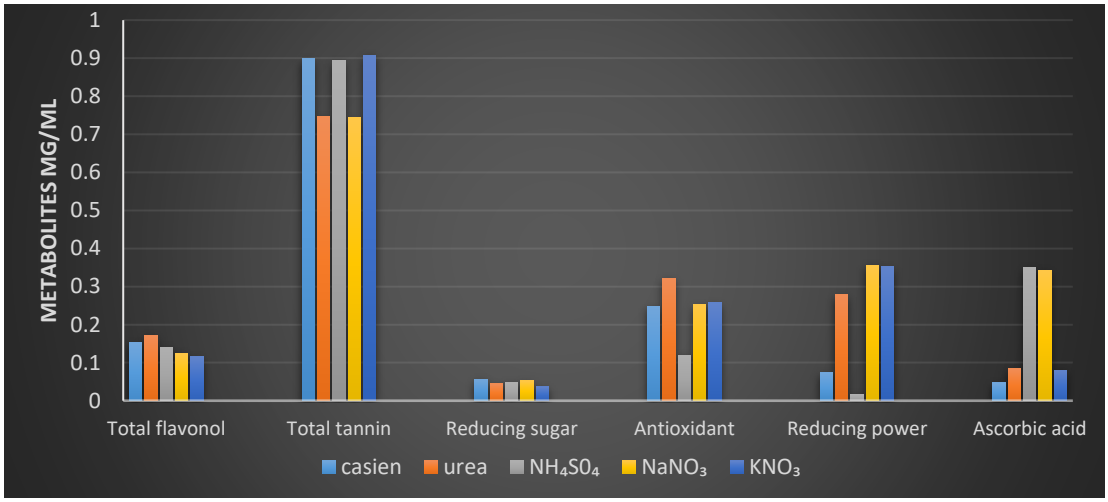

Figure 3: Metabolites production by *P. ostreatus* grown with different nitrogen sources with glucose as carbon source and 6.5 pH

The effect of pH: The effect of pH from 4.5 to 8.5 was checked to determine the growth and production of metabolites as shown in figure 4, among different ranges 5.5 pH appeared as best for growth and maximum production of tannin 0.225 mg/ml in a fermentation medium containing glucose as carbon source and casein as nitrogen source at 25℃ and most of the organisms produce various compounds at different pH ranges and their production rate is also affected by pH.

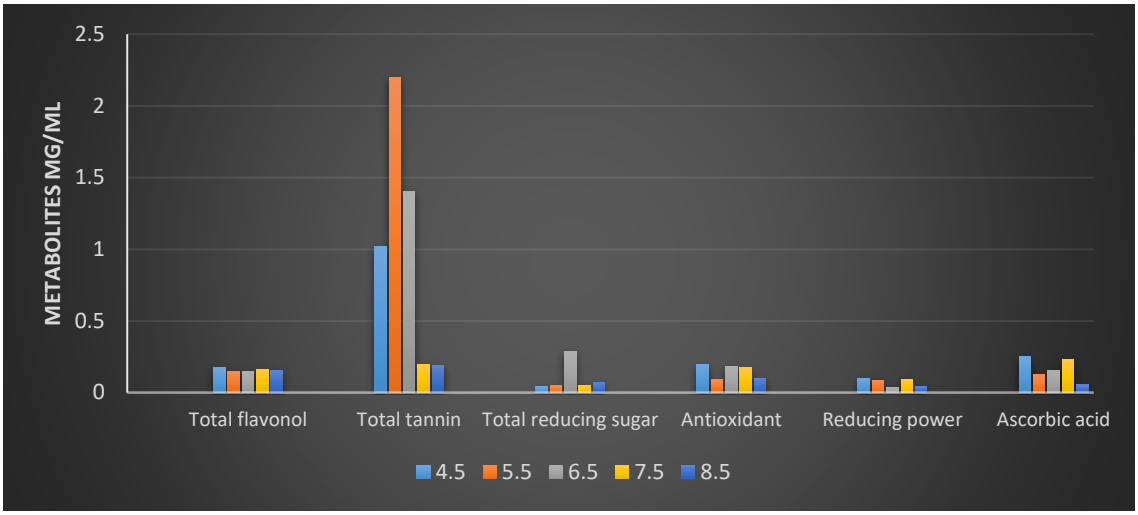

Figure 4: Metabolites production by *P. ostreatus* grown with different ranges of pH.

The effect of temperature: The effect of temperature like 25℃, 30℃ and 35℃ on the growth and maximum production of metabolites was checked as shown in figure 5 and the maximum production of tannin 1.1mg/ml and reducing power 0.5mg/ml achieved at 25℃, whereas flavanol 0.584 mg/ml, antioxidant 0.42mg/ml and ascorbic acid 0.6mg/ml at 30℃ were achieved when P. ostreatus grown in fermentation media with glucose as carbon source and casein as nitrogen source at 8.5 pH. These results are nearly similar to the results reported by Hoa and Wang (2015) where highest mycelium growth of *P. ostreatus* was obtained at 28℃.

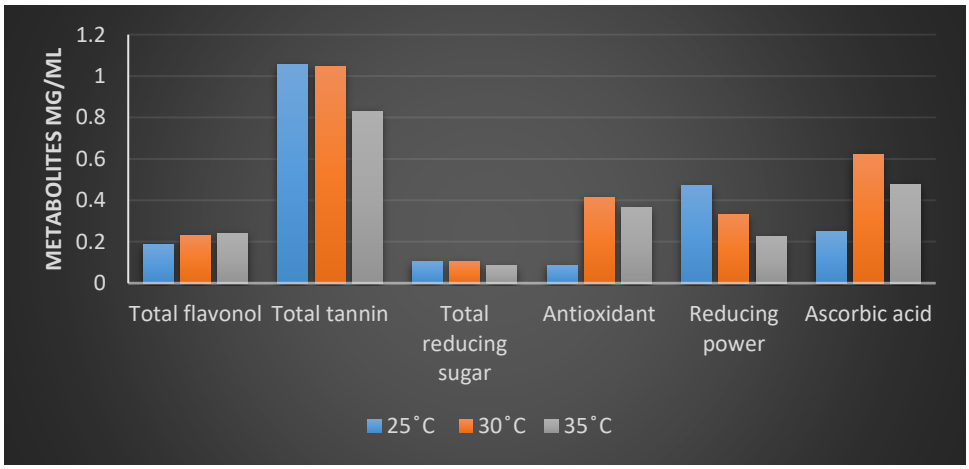

Figure 5: Effect of temperature on the growth of *P. ostreatus* and production of metabolites

CONCLUSION

On the light of the results obtained from this study, it could be concluded that *Pleurotus ostreatus* is capable to produce different metabolite but higher amount was found of tannin in comparison to other constituents.

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