

Full Length Research Paper

Effect of oils on the production of exopolysaccharides and mycelial biomass in submerged culture of *Schizophyllum commune*

Krishna Bolla*, Syed Zeenat Shaheen, Kandukuri Vasu and M. A. Singara Charya

Department of Microbiology, Kakatiya University, Warangal – 506 009, Andhra Pradesh, India.

Accepted 11 December, 2012

The effect of oils (olive, castor and peppermint) addition at different concentrations on the cell growth and production of exopolysaccharides (EPS) in a submerged culture of *Schizophyllum commune* was determined. The highest cell growth (8.2 g.dr.w/l) was observed on the 14th day of incubation in medium containing 1% of the olive oil. EPS production was slightly enhanced by olive oil and castor oil, but significantly inhibited by peppermint oil. Mycelial growth was increased with the oils used at different concentrations, except 1% at 7 and 14 days of incubation. Amongst the three oil sources examined, the highest EPS production was observed in medium with 0.5% castor oil after 7 days of incubation.

Key words: *Schizophyllum commune*, exopolysaccharides, submerged culture, oil.

INTRODUCTION

Many fungi are able to produce extracellular polysaccharides. They fulfill different tasks during growth on natural substrates, for example, adhesion to surfaces, immobilization of secreted enzymes and prevention of hyphae from dehydration and increased residence time of nutrients inside the mucilage (Rau, 1999). Many of them contain - (pullulan) or -linked (for example, sclera-glucan and schizophyllan) glucose units. The alignment and disposition of linkage and branching affect the three-dimensional structure and determine the physicochemical characteristics of the gum. The branched -glucans are biologically active and consequently, are used in medicine and biotechnology, as well as additives in food and cosmetics (Manzoni and Rollini, 2001).

Schizophyllum commune is an edible mushroom, which belongs to the phylum Basidiomycetes, order Agaricales and family Schizophyllaceae (Alexopoulos et al., 1996). The fungus usually grows abundantly during the rainy

season and frequently appears on dead wood (Zoberi et al., 1978). This is a small, whitish fungus with no stalk, which grows on dead trees throughout the year. It is a very common fungus and has a world-wide distribution (Hobbs, 1995). Pharmacologically it is extremely important because it produces the polysaccharide schizophyllan, which shows considerable anti-cancer activity in xenograph and clinical practice. Key active constituents of *S. commune* are -glucans that act as antitumour and immuno-modulating agents. It is, nevertheless, a very good source of protein, vitamins, lipids and mineral elements for those who value the mushroom.

Schizophyllan, a non-ionic water-soluble homopolysaccharide, consisting of a linear chain of -D-(1-3)-glucopyranosyl groups and -D-(1-6)-glucopyranosyl groups, produced by fermentation of the filamentous fungus *S. commune*. Since schizophyllan exhibits antitumor and immuno-modulating activity (Tabata et al., 1981), the possible relationship between the biological activity of EPS and chemical structure has attracted a wide range of attention. The above-mentioned situation led to this study on the effect of oils on EPS and biomass production of *S. commune*.

*Corresponding author: E-mail: bollakrishna@gmail.com.
Mobile: +91 9949857949

MATERIALS AND METHODS

Organism

S. commune used in this study was obtained from the Microbial Collection Culture Lab, Kakatiya University, Warangal, India. The cultures were maintained on malt extract agar slants. The slants were inoculated and incubated at 25°C for 7 days, and then stored at 4°C (Hsieh et al., 2006).

Inoculum preparation

The medium selected for studies on exopolysaccharide production by basidiomycetes comprised of the following (g/l): Peptone 1.0; yeast extract 2.0; K₂ HPO₄ 1.0; MgSO₄·7H₂O 0.2; (NH₄)₂SO₄ 5.0; glucose 20.0; pH 6.0 (Cavazzoni et al., 1992). The pH was initially adjusted to 6, followed by autoclaving at 121°C, 15 lbs for 15 min. Erlenmeyer flasks containing 100 ml of sterilized culture medium were inoculated with the suspension in sterile water of fungal mycelium grown on malt extract agar slants. Incubation was done at 25°C in a rotary shaker incubator at 150 rpm for 7 and 14 days.

Flask culture conditions

The flask culture experiments were performed in 250 ml flasks, containing 100 ml of the above medium. After inoculating with 10% (v/v) of the seed culture, the culture was incubated at 25°C in a rotary shaker incubator at 150 rpm. Samples were collected at 7 and 14 days after incubation for determination of biomass dry weight and exopolysaccharides (EPS). The effects of oils additions on the cell growth and the production of EPS by the *S. commune* culture were studied by adding olive, castor and peppermint oil to fermentation medium. The concentrations of oils used ranged from 0.1 to 1% (Hsieh et al., 2006).

Analytical methods

Biomass dry weight: The culture was filtered to separate fungal biomass, which was washed twice with distilled water and quantified as dry weight (105°C to constant weight).

Determination of polysaccharides: In order to determine the extracellular polysaccharides, isopropanol was added 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 15 min). The precipitated EPS was dried to remove the residual isopropanol and quantified as dry weight (Maziero et al., 1999).

RESULTS AND DISCUSSION

Effects of oil on cell growth and the production of exopolysaccharides were observed. Oil, which has the function of an antifoam agent in fermentation, has been reported to be favorable to mycelial growth in several medicinal mushrooms and to increase the production of

bioactive metabolites (Yang et al., 2000; Peacock et al., 2004; Park et al., 2002). In this research, effects of the additives of olive, castor and peppermint oil on *S. commune* in the submerged fermentation were studied at volume fractions of 0.1 – 1% with 2% glucose. The mycelial growth of *S. commune* was found to increase when the concentration of castor oil and olive oil was increased, but decreased with peppermint oil addition. The highest cell growth (8.2 g.dr.w/l) was obtained on the 14th day in the medium with 1% olive oil (Figure 1).

The pH of the broth was found to decline with increasing oil addition, except with peppermint oil. The lowest pH (0.5) was found in 0.1% of olive oil after 14 days of incubation. The stimulation of cell growth by oil in this study might be caused by the partial incorporation of lipids in the cell membrane, thereby facilitating the uptake of nutrients from the medium (Yang et al., 2000). The lower pH in oil addition media might be due to the uptake of oils as the carbon source, which supply for the cell growth continuously. This result is also consistent with a previous study that various carbon sources were suitable for the cell growth of *Cordyceps sinensis* and the cell could grow better with lower final pH of the fermentation broth (Hsieh et al., 2005).

Regarding the production of EPS by *S. commune*, an increased concentration of all the oils used led to a higher EPS production (Figure 1). The results also showed that the EPS production was significantly inhibited by all the oils at 1% concentration. The lowest EPS production (1.6 g.dr.w/l) was obtained when 0.1% olive oil was supplemented.

On the other hand, the EPS production increased to 5 g.dr.w/l when the concentration increased up to 0.5% of castor oil. The fatty acids present in oils seemed to play a critical role in inhibiting the production of polysaccharides. Yang et al. (2000) reported that linoleic acid drastically suppressed polysaccharide formation during submerged culture of *Ganoderma lucidum*. Stasinopoulos and Seviour (1983) also demonstrated that linoleic acid had a strong inhibitory effect on the polysaccharide production from *Acremonium persicinum*.

It was concluded that the supplementation of the oils in the media substantially increased the EPS production and 0.5% concentration proved to be ideal. The present study signifies that during the process of EPS production in large scale the amendment of oil certainly enhances the production rate of EPS.

ACKNOWLEDGEMENT

The authors are thankful to Prof. S. Ram Reddy, Head, Department of Microbiology, Kakatiya University, Warangal for providing facilities during the tenure of the work.

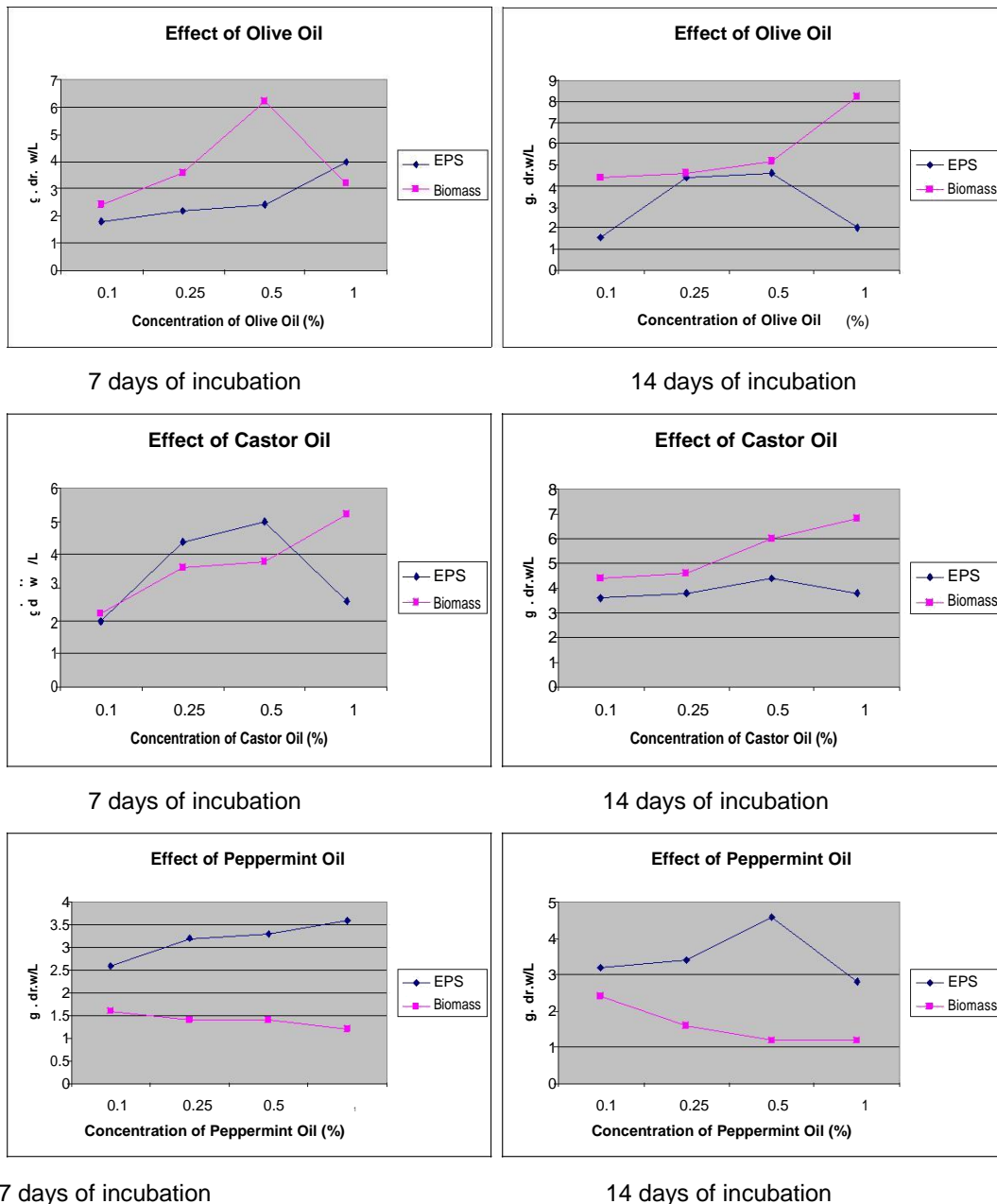


Figure 1. Effects of plant oil on cell growth and the production of polysaccharides.

REFERENCES

- Alexopoulos CJ, Mim CW, Blackwell M (1996). *Introductory Mycology* (4th ed.). New York: John Wiley.
- Hsieh C, Tsai MJ, Hsu TH, Chang DM, Lo CT (2005). Medium Optimization for polysaccharide production of *Cordyceps sinensis*. *Appl. Biochem. Biotechnol.* 120: 145–157.
- Hsieh C, Liu CJ, Tseng MH, Lo CT, Yang YC (2006). Effect of olive oil on the production of mycelial biomass and polysaccharides of *Grifola frondosa* under high oxygen concentration aeration. *Enzyme Microbiol. Technol.* 39: 434-439.

- Cavazzoni V, Adami A (1992). Exopolysaccharides produced by mycelial edible mushrooms. *Ital. J. of Food Sci.* 1: 9-15.
- Yang FC, Ke YF, Kuo SS (2000). Effect of fatty acids on the mycelial growth and polysaccharide formation by *Ganoderma lucidum* in shake flask cultures. *Enzyme Microbiol. Technol.* 27: 295–301.
- Hobbs C (1995). *Medicinal Mushrooms: An Exploration of Tradition, Healing and Culture*. Botanica Press, Santa Cruz, A p.251
- Park JP, Kim SW, Hwang HJ, Cho YJ, Yun JW (2002). Stimulatory effect of plant oils and fatty acids on the exo-biopolymer production in *Cordyceps militaris*. *Enzyme Microbiol. Technol.* 31: 250–255.
- Tabata K, Ito W, Kojima T, Kawabata S, Misaki A (1981). Ultrasonic

- degradation of schizophyllan, an antitumor polysaccharide produced by *Schizophyllum commune* Fries. *Carbohydr. Res.* 89:121–135.
- Peacock L, Ward J, Ratledge C, Dickinson FM, Ison A (2004). How *Streptomyces lividans* uses oils and sugars as mixed substrates, *Enzyme Microbiol. Technol.* 32:157-166
- Manzoni M, Rollini M (2001). Isolation and characterization of the exopolysaccharide produced by *Daedalea quercina*. *Biotechnol Lett.* 23: 1491-1497
- Rau U (1999). Production of Schizophyllan. In: Bucke C (ed) *Methods in biotechnology, carbohydrate biotechnology protocols*. Humana, Totowa, 10: 43-57
- Maziero R, Cavazzoni V, Bononi VLR (1999). Screening of basidiomycetes for the production of exopolysaccharide and biomass in submerged culture. *Revista de Microbiologia.* 30:77-84
- Seviour RJ, Hensegen K (1983). Extra cellular glucan production by *Acremonium diospyri*. *FEMS Microbiol. Lett.* 16: 343–347.
- Zoberi MH (1978). *Tropical macrofungi: some common species*: Macmillan Press. London, p. 158.