

BPE-P04: THE EFFECT OF PLANT OILS FOR SUBMERGED FERMENTATION OF *Schizophyllum commune* PRODUCING MYCELIUM BIOMASS AND EXOPOLYSACCHARIDES

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Abstract

The effect of plant oils (olive, palm, corn, sunflower and soya bean) addition at different concentrations (0.5%, 1.0%, 2.0% and 4.0%) producing mycelium biomass (MBS) and exopolysaccharides (EPS) in submerged fermentation using a local strain *Schizophyllum commune* was determined. Additional of all type of plant oils exhibit higher yield of MBS and EPS than without plant oils. The highest production of MBS was obtained at 26.08 g/L in 4.0% palm oil, while in control (without plant oils) gave only 12.16 g/L of MBS. The maximum concentration of EPS was produced is 2.2 g/L from medium containing 1.0% and 4.0% of olive oil; and 2.0% of sunflower oil. The best treatment containing 4.0% of corn oil was selected since it showed high production of MBS (23.44 g/L) and EPS (2.1 g/L) compared to other treatments. However, the addition of 4.0% of palm oil in submerged culture of local strain *Schizophyllum commune* was preferred since it also showed the good result on production of MBS (26.08 g/L) and EPS (2.0 g/L). Palm oil is locally source and cheap, therefore, the MBS can be produced in local industry with cost-effective. The MBS will be used as liquid spawn (seed) or extraction of bioactive compounds in pharmaceutical industry.

Key word: *Exopolysaccharides, Liquid spawn, Mycelium biomass, Plant oils, Schizophyllum commune, Submerged fermentation.*

INTRODUCTION

Many mushroom species become attractive as a source for the development of drugs as they are able to produce exopolysaccharides (EPS), contain high variety of secondary metabolites with diverse biological activities as they are nutritionally functional food and a source of physiologically beneficial and non-toxic medicines [1].

Schizophyllum commune is an edible mushroom, which belongs to the phylum Basidiomycetes, Order Agaricales and Family Schizophyllaceae [2]. This species is being found on every continent and scattered grow saprophytically on dead wood or occasionally parasitically on living wood in coniferous and deciduous forest. Its sporocarp is leathery, fan-shaped bracket, 1-3.5 cm broad, frequently lobed or fused at the base with other brackets, upper surface densely hairy, light greyish-brown when moist, ashy grey to white when dry, lower surface light grey consisting of well spaced, longitudinally split gills, stipe usually absent, flesh thin, light grey to brown, tough. It can be found year round and fruiting after the fall rains [3].

Schizophyllan is a non-ionic, water soluble homopolysaccharide consisting of a linear chain of β -D-(1-3)-glucopyranosyl groups and β -D-(1-6)-glucopyranosyl groups [4]. Schizophyllan have high potential in pharmaceutical industry because it has an immunomodulating, antineoplastic and antiviral activities compared to other glucans [5]. It also been used in food and cosmetics industry and other industry applications like in enhanced mineral oil recovery as emulsifiers, stabilizers, binders, gelling agents, lubricants, and thickening agents [3]. Schizophyllan also been commercially produced as an antitumor agent in Japan [6]. Although many investigators have reported the positive results employing several plant oils, surfactants and fatty acids, there is still lack of knowledge on the stimulatory effect of those ingredients in fermentation processes.

In this study, we investigated the effects of several plants oil in attempt to enhance both MBS and EPS production of *S. commune* in shake flask culture.

MATERIALS AND METHODS

Microorganism

Schizophyllum commune strain was obtained in Perak, Malaysia and stored in Agro-Biotechnology Intitute, (ABI) Malaysia. The mycelium was maintained on media agar reported by Rau *et al.* [4]. The mycelium was activated on an agar plate at room temperature for 7 days. To maintain the strain activity, a mycelium disc (5mm in diameter) was transferred to a fresh agar every 7 days. All the experiments were carried out using the 7-day-old mycelium as the inoculums to the flask medium.

Inoculum preparation

The agar plate consisting 30 g/l glucose, 1 g/l yeast extract, 0.5 g/l $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$, 1 g/l KH_2PO_4 , 16 g/l bacto agar for mycelium activation culture was prepared; the pH was initially adjusted to 7, followed by autoclaving at 121°C for 15 minutes [4]. Activated mycelia agar discs (5mm in diameter) were obtained as inoculums in a shake-flask culture comprising optimized media of 34 g/l glucose, 1.2 g/l yeast extract, 0.5 g/l $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$, and 1 g/l KH_2PO_4 .

Shake Flask Culture Experiment

The shake flask culture experiments were performed in 250 ml baffled flasks comprising 50 ml of optimised media. After inoculating with 5 activated mycelia agar discs, the culture was incubated at room temperature on an orbital shaker at 150 rpm for 11 days and samples were collected from the shake flasks for analyzing MBS and EPS concentrations. Effects of plant oil additions on *S. commune* culture were studied by substituting various plant oils such as olive, palm, corn, sunflower and soybean oil for fermentation medium in one at a time fashion. The concentrations of plants oils used were ranging from 0.5% to 4.0% (v/v).

Plant oil separation

Fermentation broth was placed into a separatory funnel and *n*-hexane was added at 1:1 ratio. The mixture was allowed to shake and mix for 5 minutes, then it was left to stand to separate into two phases. The lower phase was extracted with equal volume of fermentation broth twice [7].

Analytical methods

1. Mycelium biomass (MBS).

The MBS was termed as the dry weight per unit volume. Separated fermentation broth was obtained and subjected to centrifuge at 10,000 rpm for 5 minutes at 20°C. Then, the sediment produced was dried to a constant weight.

2. Phenol sulphuric acid assay.

In order to determine the EPS production, 10 ml of fermentation broth filtrate was mixed with 20 ml of 96% isopropanol, shaken and then left overnight at 4°C. Next, the precipitated polysaccharides were collected by centrifugation at 10,000 rpm for 5 minutes at 20°C, and they were dried to remove residual isopropanol. The total polysaccharide concentration was determined by a phenol sulfuric assay [8].

RESULTS AND DISCUSSION

Effects of 0.5% (v/v) plant oils addition on MBS and the production of EPS

In this research, the effects of plant oils (olive, palm, corn, sunflower and soya bean) addition at different concentrations (0.5%, 1.0%, 2.0% and 4.0%) on *S. commune* were investigated. Based on the result obtained, plant oils were shown to have stimulatory effect on MBS and EPS production in mushroom cultures. The MBS of *S. commune* was increased with the addition of plant oils compared to control (12.165 g/l) except soybean oil (11.655 g/l) as shown in Figure 1. The MBS increased to 12.284 g/l, 15.676 g/l and 13.848 g/l with palm, corn and sunflower oil addition, respectively. The highest MBS (16.547 g/l) was obtained in the medium containing olive oil. On the EPS production by *S. commune*, the addition of plant oils led to a higher EPS production. The lowest EPS production (1.9 g/l), same with the control (1.9 g/l) was obtained when palm oil was added. The EPS production increased to 2.0 g/l with corn, sunflower and soybean oil addition, respectively. The highest EPS production (2.1 g/l) was obtained in the medium containing olive oil. This stimulatory effect of olive oil might be attributed to the main composition of the oil is oleic acid (84%), rather than linoleic acid (4%) [9].

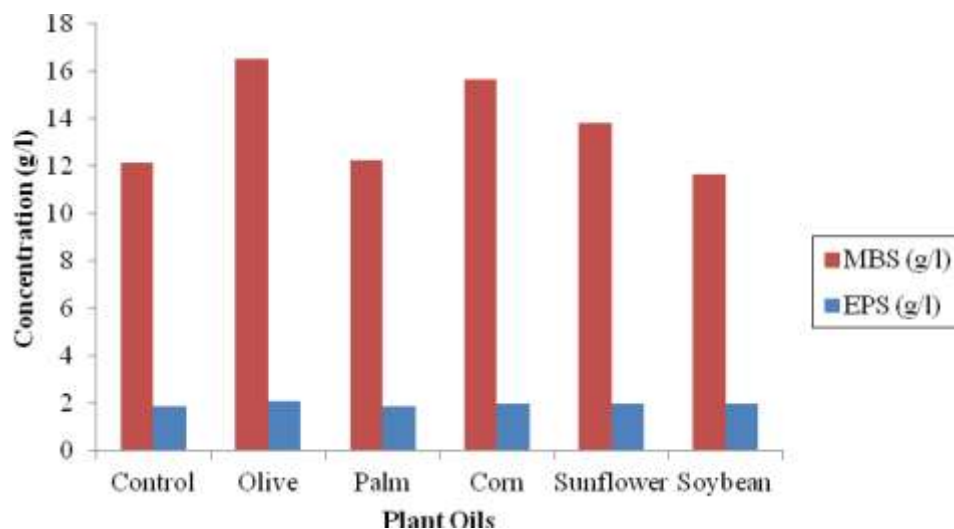


Figure 1: Effects of 0.5% (v/v) plant oils addition on MBS and the production of EPS

Effects of 1.0% (v/v) plant oils addition on MBS and the production of EPS

The MBS of *S. commune* was found to increase with the addition of plant oils compared to control (12.165 g/l) as shown in Figure 2. The MBS increased to 13.348 g/l, 13.338 g/l, 17.999 g/l and 13.655 g/l with olive, palm, corn and sunflower oil addition, respectively. The highest MBS (19.454 g/l) was obtained in the medium containing soybean oil. On the EPS production by *S. commune*, the addition of plant oils led to a higher EPS production compared to the control (1.9 g/l). The lowest EPS production (2.0 g/l) was obtained when palm, corn, sunflower and soybean oil was added, respectively. The highest EPS production (2.2 g/l) was obtained in the medium containing olive oil, consistent with the study by Bolla *et al.* [3].

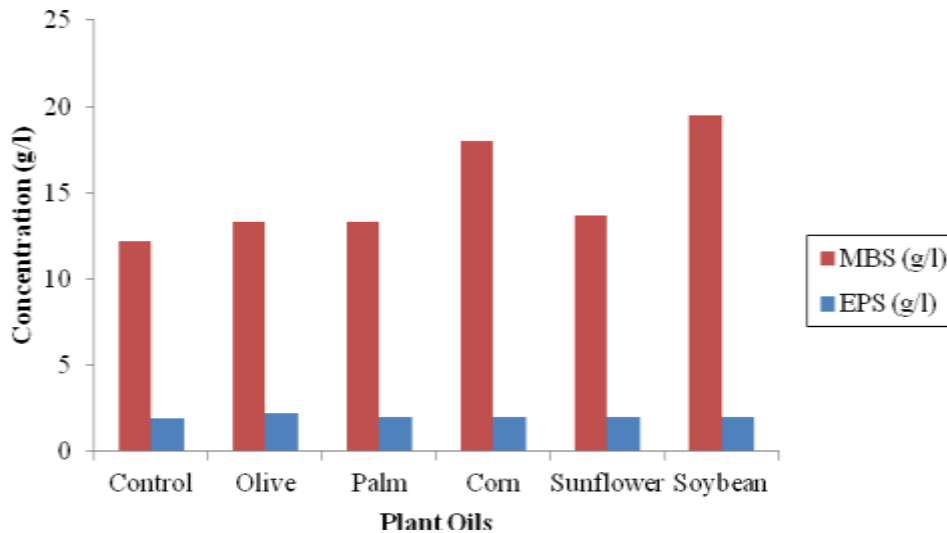


Figure 2 Effects of 1.0% (v/v) plant oils addition on MBS and the production of EPS

Effects of 2.0% (v/v) plant oils addition on MBS and the production of EPS

The MBS of *S. commune* was found to increase with the addition of plant oils compared to control (12.165 g/l) as shown in Figure 3. The MBS increased to 12.491 g/l, 20.630 g/l, 16.465 g/l and 14.598 g/l with olive, palm, sunflower and soybean oil addition, respectively. The highest MBS (21.256 g/l) was obtained in the medium containing corn oil. On the production of EPS by *S. commune*, the addition of plant oils led to a higher EPS production compared to the control (1.9 g/l). The lowest EPS production (2.0 g/l) was obtained when palm and soybean oil was added, respectively. The EPS production increased to 2.1 g/l with olive and corn oil addition, respectively. The highest EPS production (2.2 g/l) was obtained in the medium containing sunflower oil. The linoleic acid present in sunflower oil (66.2%), rather than oleic acid (21.3%) was the factor on MBS and EPS production. However, the result obtained was not consistent with Stasinopoulos and Seviour that demonstrated that linoleic acid had a strong inhibitory effect on the EPS production from *Acremonium persicinum* [10].

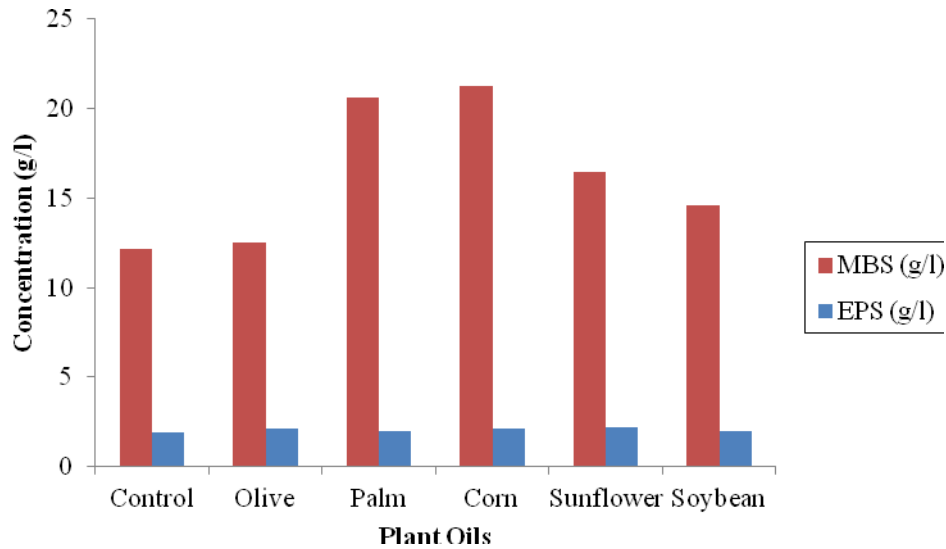


Figure 3: Effects of 2.0% (v/v) plant oils addition on MBS and the production of EPS

Effects of 4.0% (v/v) plant oils addition on MBS and the production of EPS

The MBS of *S. commune* was found to increase with the addition of plant oils compared to control (12.165 g/l) as shown in Figure 4. The MBS has increased to 16.771 g/l, 23.445 g/l, 19.679 g/l and 17.019 g/l with olive, corn, and sunflower and soybean oil addition, respectively. The highest MBS (26.077 g/l) was obtained in the medium containing palm oil. On the EPS production by *S. commune*, the addition of plant oils led to a higher EPS production compared to the control (1.9 g/l). The lowest EPS production (2.0 g/l) was obtained when palm oil was added. The EPS production increased to 2.1 g/l with sunflower and soybean oil addition, respectively. The highest EPS production (2.2 g/l) was obtained in the medium containing olive oil. This stimulatory effect may be caused by modification of membrane composition, increasing of membrane permeability or level of synthesis of enzyme involved in the production of polysaccharide by plant oils or fatty acids [10].

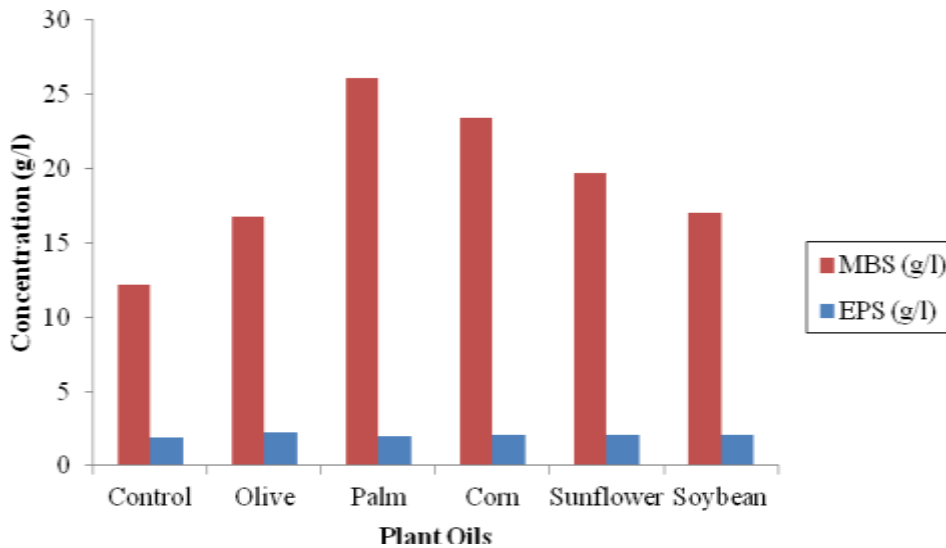


Figure 4: Effects of 4.0% (v/v) plant oils addition on MBS and the production of EPS

Effects of palm oil addition on MBS and the production of EPS

Palm oil was selected in this study because its local sources and cheaper price compared to other plant oil. This study would see the effect on the different concentration on the MBs and EPS production. Based on the result obtained, the MBS of *S. commune* was found to increase when palm oil concentration increased compared to control (12.165 g/l) as shown in Figure 5. The MBS increased to 12.284 g/l, 13.338 g/l and 20.630 g/l with 0.5%, 1.0% and 2.0% palm oil addition, respectively. The highest MBS (26.077 g/l) was obtained in the medium containing 4.0% palm oil. On the production of EPS by *S. commune*, the addition of plant oils led to a higher EPS production. The lowest EPS production (1.9 g/l), same with the control (1.9 g/l), was obtained when 0.5% palm oil was added. The highest EPS production (2.0 g/l) was obtained in the medium containing 1.0%, 2.0% and 4.0% palm oil.

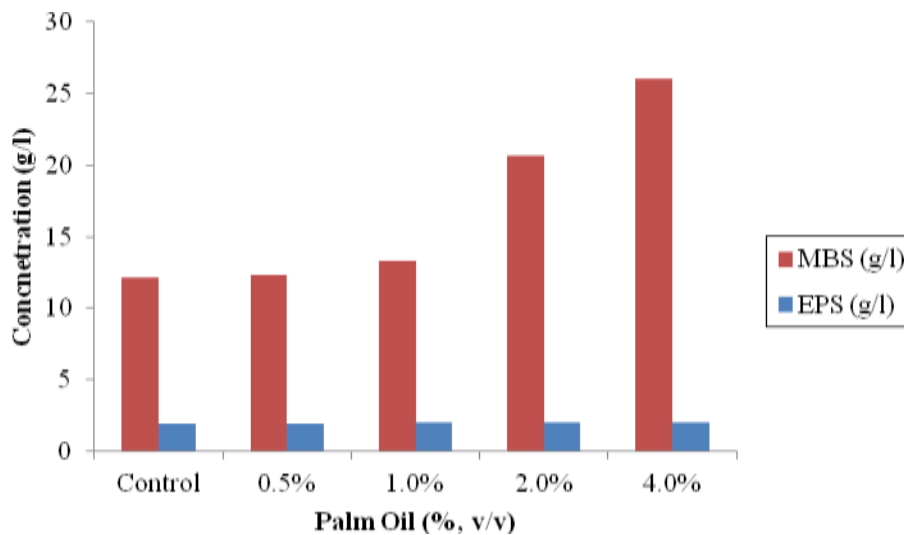


Figure 5: Effects of palm oil addition on MBS and the production of EPS

CONCLUSION

The addition of plant oils has shown a significant increase of MBS and the production of EPS of *S. commune*. The best type of plant oils to be added to the shake flask system is the palm oil because of the capability of producing high MBS and EPS production compared to other types of plant oils. Palm oil is main commodity in Malaysia, therefore it make palm oil cost effective and easily accessible for large scale production of *S. commune*.

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