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Influence of Plant Oils and Metals on Exopolysaccharide Production by *Fomitopsis feei*

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Abstract

Effect of ten types of plant oils and 11 types of minerals in the form of chlorides were tested on the production of exopolysaccharides from *Fomitopsis feei* in common production broth medium. Groundnut oil among the plant oils and sodium chloride among the minerals showed a positive effect on the production of exopolysaccharides from *Fomitopsis feei*. Thus, it creates a scientific basis to explore the exopolysaccharide effectively from *F. feei*, hence this medicinal mushroom will be effectively exploited in the future.

Keywords: Exopolysaccharide, *Fomitopsis feei*, shake culture, plant oils, and minerals.

INTRODUCTION

In general, factors influencing the exopolysaccharide production of fungi mainly include the composition of culture medium and environmental conditions. Plant oils, which were usually used as antifoam agents in fermentation, have been reported to be beneficial for the mycelial growth of several medicinal mushrooms and to increase the production of bioactive compounds (Yang *et al.*, 2000; Park *et al.*, 2002). The mineral components of the medium like phosphorus, sulfur, magnesium, and potassium influence not only yield of the fungal biomass but also polysaccharide production (EPS). Effects of some other additives including vegetable oils, surfactants, and vitamins were also studied and reported (Yang and He, 2008, Xiao *et al.*, 2010, Zhang and Cheung, 2011). EPS has been widely studied at home and abroad because of its strongly heavy metal adsorption capacity. The extracellular polymeric substances of white rot fungi play an important role in the adsorption of heavy metals (Ningjie *et al.*, 2018).

Fomitopsis feei is a brown rot fungus that belongs to the family Fomitopsidaceae. When screened for exopolysaccharide (EPS) production, it showed good results (data not shown here) hence the present study is carried out to identify the effect of plant oils and minerals under shaking condition. Optimization of the EPS production (Hima bindu and Singara Charya, 2017) and production, purification, and characterization of exopolysaccharides from this fungus were already published (Hima Bindu and Singara Charya, 2018).

MATERIALS AND METHODS

Isolation and identification of *Fomitopsis feei*

Fomitopsis feei fruit bodies were collected from Pakhal forest, Warangal during the rainy season and pure mycelial culture was developed and was identified at the molecular level by 28S rDNA analysis (Himabindu and Singara Charya, 2017).

Isolation and determination of exopolysaccharides

Exopolysaccharides were separated (Kim *et al.*, 2002) using 7 and 14 days old filtrates and precipitated with isopropyl alcohol in 1:4 ratios and incubated overnight at 4°C. After incubation, the pellet obtained by centrifugation was subjected to phenol-sulphuric acid (Dubois *et al.*, 1956) method with some modifications.

Plant oils

To investigate the effect of plant oils on the mycelial growth and exopolysaccharide production of *F. feei*, different plant oils i.e. castor, coconut, mustard, olive, palm, peppermint, groundnut, sesame, soybean and sunflower oils were supplemented at 0.5% v/v in common production

medium g/L (Peptone 1, Yeast extract 2, Dipotassium hydrogen phosphate 1, Magnesium sulphate heptahydrate 0.2, Ammonium sulphate 5, Glucose 20, pH 6) and control (without oil) was also maintained.

Metal ions

The effect of aluminum, barium, calcium, cobalt, ferric, mercuric, potassium, sodium, antimony, stannous, strontium was tested in the form of chlorides in the common production medium at 0.05% concentration on exopolysaccharide production.

RESULTS AND DISCUSSION

Effect of oils on EPS production

Pellet form growth of *F. feei* is presented in Fig.1 during shaking condition. Fig. 2 showed the experimental results concerning the effects of various plant oils on mycelial growth and exopolysaccharide production of *F. feei*. Since soybean oil contains linoleic acid, which had a strong inhibitory effect on the exopolysaccharide production showed less amount of exopolysaccharide production (1.5 g/L). In comparison with other plant oils, the stimulatory effect from groundnut oil was that there was no inhibitory effect on the production of both exopolysaccharide and biomass. This result seems to be linked to the fatty acid composition in groundnut oil i.e. the main fatty acids present in groundnut oil are 56% of oleic acid and 26% of linoleic acid.

On the other hand, a possible mechanism of stimulating effect of exopolysaccharide production has been proposed that oils modify cell membrane and thus increase permeability. Addition of groundnut oil resulted in the highest production (5 g/L) of exopolysaccharide and mycelial biomass (20.08 g/L). The growth of *F. feei* increased obviously from 7 to 14 days. Mustard oil supported good mycelial growth (17.48 g/L) after 7 days of incubation. Peppermint oil was not suitable for the stimulation of mycelial growth and exopolysaccharide production. The stimulation of cell growth by plant oils was attributed to a partial incorporation of lipids in the cell membrane, thereby facilitating the uptake of nutrients from the medium (Yang *et al.*, 2000). The oil addition can protect the cell from lysis for a longer time. The longer the cell stands under low pH, the more biomass and EPS can be produced.

Hsieh *et al.* (2008) evaluated the effect of plant oils on EPS production and reported that addition of 0.5% olive oil at the stationary phase of fungal growth induced maximum EPS (2.248 gm/L) production while same oil addition in early growth phage-induced mycelia growth and reduced EPS production. From the numerical analysis of Huang *et al.*, (2009), oil is more effective to biomass production than

to EPS production. Bolla et al. (2011) demonstrated that coconut oil stimulated the biomass production of the fungus. The EPS yields for olive oil (5%), sunflower oil (3%) and coconut oil (4%) were 5.94 g/L, 2.56 g/L, and 2.43 g/L, respectively. The olive oil and sunflower oil effectively affected the growth of the fungus and EPS biosynthesis. The results showed that the mycelial biomass and EPS production of *O. sinensis* fungus increased considerably compared to non-oil medium (Hang et al., 2017).



Fig.1. Growth of *F. feei* under shaking condition.

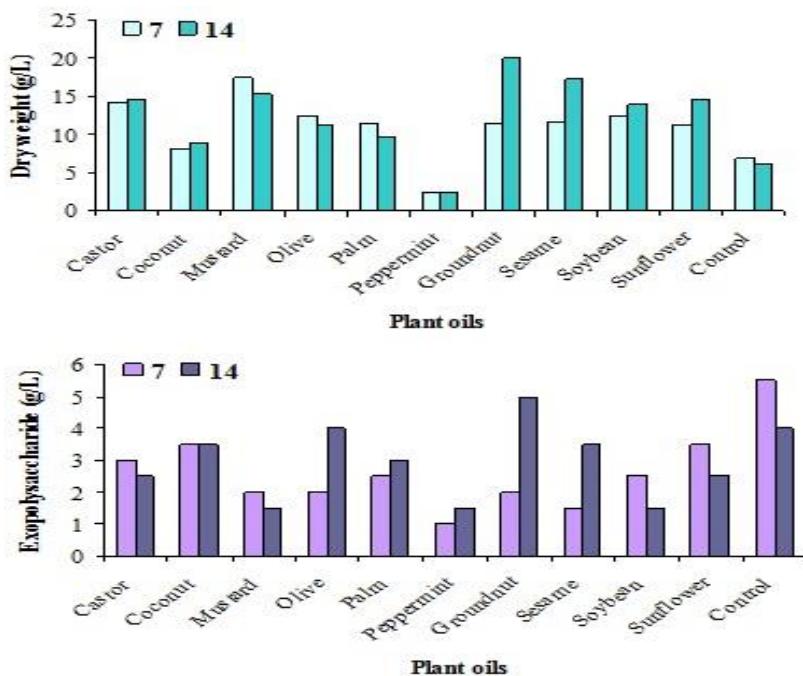


Fig. 2. Effect of plant oils on mycelial dry weight and exopolysaccharide production.

Effect of metals on exopolysaccharide production

The effect of metal ions in the media is an important factor that affects polysaccharide production due to they may act as inducers. Metal ions examined in this study had a negligible effect except for sodium chloride. Among the trace elements, the highest mycelial yield was obtained on strontium containing medium (8.8 g/L) (Fig. 3). This result implies that *Fomitopsis feei* can grow effectively in the presence of these metals. The inability of this fungus to utilize the two trace elements i.e. Cobalt and Mercury for growth may be related to their toxicity to fungal cells. A similar toxic effect was reported by Humfeld and Sugihara (1952) for *Agaricus campestris* and Chandra and Purkayastha (1977) for *Volvariella volvacea*.

Tang et al. (2008) practiced the one-variable-at-a-time approach and response surface methodology to evaluate

the effect of metal ions on EPS (*Tuber* polysaccharide) production by *Tuber sinense*. From their research, they concluded that 30 mM Mg²⁺ and 5 mM K⁺ maximized the EPS production (5.86 gm/L) in submerged culture, which was 130.7% higher compared to EPS produced in basal medium without metal ions. In our study potassium supported the growth of *F. feei* than the exopolysaccharide production. Metal ions indeed stimulate the synthesis of the polysaccharide may be because of the polysaccharide has many hydroxyl groups; these can bond metal ions, which may make it improve the pharmacological activity (Yue and Ke, 2011).

Optimal sodium chloride supplementation to culture media could be used to maximize exopolysaccharide production by manipulating the pellet form of fungi. Both copper and zinc are needed for fungal enzyme activities

but, in addition, zinc is needed for intermediary metabolism (Griffin, 1994). Poor mycelial yield, obtained on basal medium, suggests that *Psathyrella atroumbrinata* requires some trace elements for its growth (Griffin, 1994; Garraway and Evans, 1984). The maximum exopolysaccharide production was achieved in the media containing sodium chloride (6 g/L). These results are not in accordance with

those obtained by Jonathan and Fasidi (2001), Kim *et al.* (2002). The least growth recorded, on sodium, implies that it was needed for growth. The higher production of exopolysaccharide by sodium chloride supplementation was evident. Sodium chloride addition to culture media has been found to promote the pellet form of growth in fungi (Wang *et al.*, 2005).

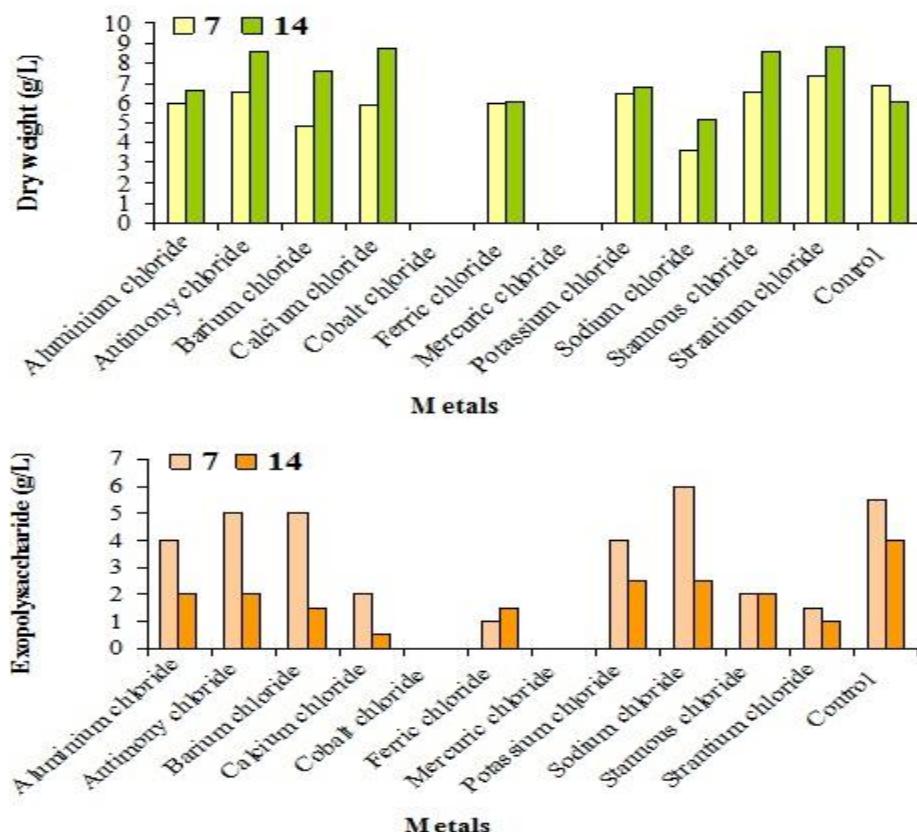


Fig.3. Effect of metals on mycelial dry weight and exopolysaccharide production.

CONCLUSION

This is the first report that a process parameter such as sodium chloride was also effective in manipulating polysaccharide formation from *F. feei*. The result is considered helpful for further investigation of the diversity of polysaccharide formation from this medicinal fungus. The findings are impressive from both the scientific angle and applicability. The hope is that this trend will continue to increase and will enrich both the scientific knowledge base and provide better assistance for life in the future.

CONFLICT OF INTEREST

The authors declare that no competing interests exist.

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