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Effect of additives on *Pleurotus ostreatus* Growth on Agar medium

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Abstract : *Oyster mushroom (Pleurotus ostreatus) is one of the most widely grown mushrooms worldwide. This type of fungus have been grown for centuries in green houses to produce mycelium and fruiting bodies and marketed as highly nutritious food. Like many other mushrooms, oyster mushroom characterized by lower growth rate which consume long time on agar medium for inoculum preparation for spawn preparation. Therefore, design of new cultivation medium to shorten the cultivation time on solid medium is necessary to reduce time and produce inoculum with less cost. This work was focused on optimization of agar medium (using the conventional potato dextrose agar medium PDA as base medium for growth and supplemented with other components such as malt extract, yeast extract, and sodium nitrates). The results clearly demonstrate that addition of malt extract and yeast extract support dense cell growth but not fast radial growth on agar medium. Addition of sodium nitrate in addition to malt and yeast extract, increase both of radial cell growth and dense growth as well. Therefore, for fast growth of Pleurotus ostreatus on agar medium its recommended to supplement the growth medium with malt extract, yeast extract, and sodium nitrate.*

Keywords: *Pleurotus ostreatus, Agar medium, Growth rate, medium composition, Mushroom growth*

I. Introduction

Mushrooms have been used since centuries in different cultures in the world as food and medicine. Nowadays, they are widely cultivated in solid state cultivation in green house and also in submerged cultivation system for the production of wide range of bioactive metabolites [1-3]. Of different types of mushrooms cultivated worldwide, strains belong to the genus *Pleurotus* such as *P. ostreatus, P. sajor-caju,* and *P. florida* are widely cultivated as source of food and many nutraceuticals and biotherapeutic molecules [4-7]. The main interesting features of this type of mushroom is the ability to grow under different environmental conditions, ability to grow on wide range of lignocellulosic biomasses, ease of cultivation in both solid state and submerged cultivation systems, rich of high nutritious compounds (carbohydrates, proteins, vitamins, trace elements), and ability to produce many medicinally important metabolites acting as anticancer, immunomodulator, antioxidant, anti hyperglycemic, antimicrobial, anti-inflammatory, and many other therapeutic functions [8-15] . Therefore, different studies have been carried out related to classification, cultivation, bioactive compounds isolation/characterization, and preclinical research in both *in vivo* and *in vitro* models to assess the functionality of the therapeutic molecules of *Pleurotus* sp [16-20]. However, like many other mushrooms, low growth rate is usually is one of the main issues which increase the production cost and thus become of high interest for many researchers to develop new medium to enhance the growth rate of mushrooms. In our previous research, we developed solid culture medium to enhance cell growth for short time inoculum preparation of *Cordyceps militaris* using potato dextrose agar (as based medium) supplemented with yeast extract and malt extract [21]. In this work, attempts were carried out to study the effect of different medium supplements to increase the growth rate of *Pleurotus ostreatus* on solid agar medium through addition of different types of growth enhancing nutrients to shorten the cultivation time of this mushroom during the stage of inoculum preparation in solid medium.

II. Materials and Methods

Pleurotus ostreatus (WICC-F18) obtained from Wellness Industry Culture Collection (WICC), Institute of Bioproduct Development (IBD), Unviersiti Teknologi Malaysia (UTM, Malaysia) was using in this study. This strain was preserved on potato dextrose agar medium (PDA) at the first stage for mycelium production. The inoculated agar plates were incubated at 26 ºC for 14 days and stored thereafter at 4 ºC. This was used as master bank for further strain activation. Sub-culturing were made from this first generation culture

on PDA agar medium and used further as working cell bank. The initial experiment for characterization of cell growth was carried out on PDA agar. After this step, medium modifications were carried out using PDA medium supplemented with malt extract (MA), yeast extract (YA), glucose (G), and Sodium nitrate (SN).

The initial preliminary experiment to investigate the growth of mycelium on agar medium was carried out using standard PDA medium without any supplementation. After 12 days cultivation, full growth on agar medium was observed. From this medium, agar disk of mushroom mycelium was transferred into the center of the petri dish using crock porer. The vegetative radial growth of mycelium was measured every 2 days. The radial growth was an average of 12 measures (4 measures from the one petri dish, and the experiment run in triplicate) Figure 1 shows the typical growth of *P. ostreatus* on agar medium during 12 days cultivation.

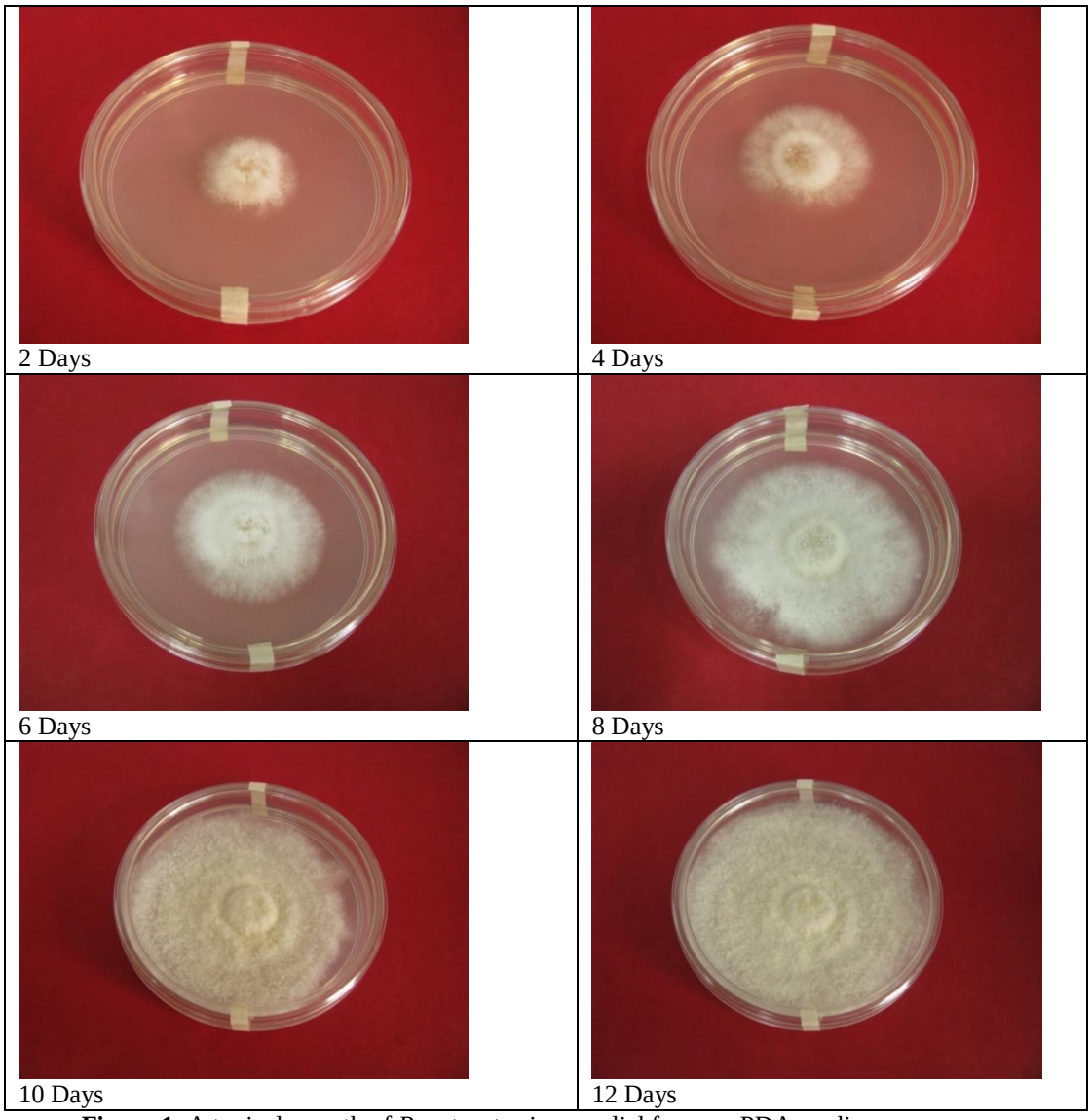

Figure 1. A typical growth of *P. ostreatus* in mycelial form on PDA medium.

III. Results and Discussion

At first cultivations were carried out using standard PDA agar medium to study the growth kinetics. As shown in figure 1. In addition, cultivations were also conducted using the same methods using PDA supplemented with 5 g/L malt extract and 2.5 g/L yeast extract as these combined supplements provided higher growth rate in solid culture in our previous study for cultivation of *Cordyceps militaris* [21]. As shown in figure 2, when following the diameter of growth on all media, no big difference was observed between PDA medium and other medium supplemented with either malt extract or yeast extract or a combination of these two nutrients. However, there was some differences in the density of mycelium growth in these modified PDA media. It have been shown that PDA plat supplemented with both malt extract and yeast extract exhibited more dense cell growth compared to other culture, whereas PDA medium without any supplementation showed the

less dense growth on the plat (fig. 3). This could be attributed to that mycelial growth was enhanced by exogenous addition of other sugars in malt extract and nitrogen source of yeast extract.

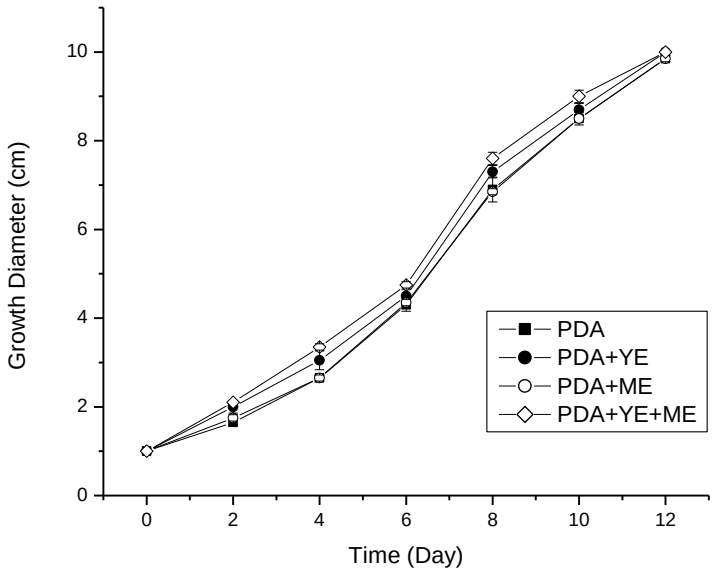

Figure 2. Change of *Pleurotus ostreatus* mycelial growth diameter during cultivation on agar media of different composition. PDA: Potato dextrose agar medium; PDA+YE: Potato dextrose agar medium supplemented with 2.5 g/L yeast extract; PDA+ME: Potato dextrose agar medium supplemented with 5 g/L malt extract; PDA+YE+ME, Potato dextrose agar medium supplemented with 2.5 g/L yeast extract and 5 g/L malt extract.

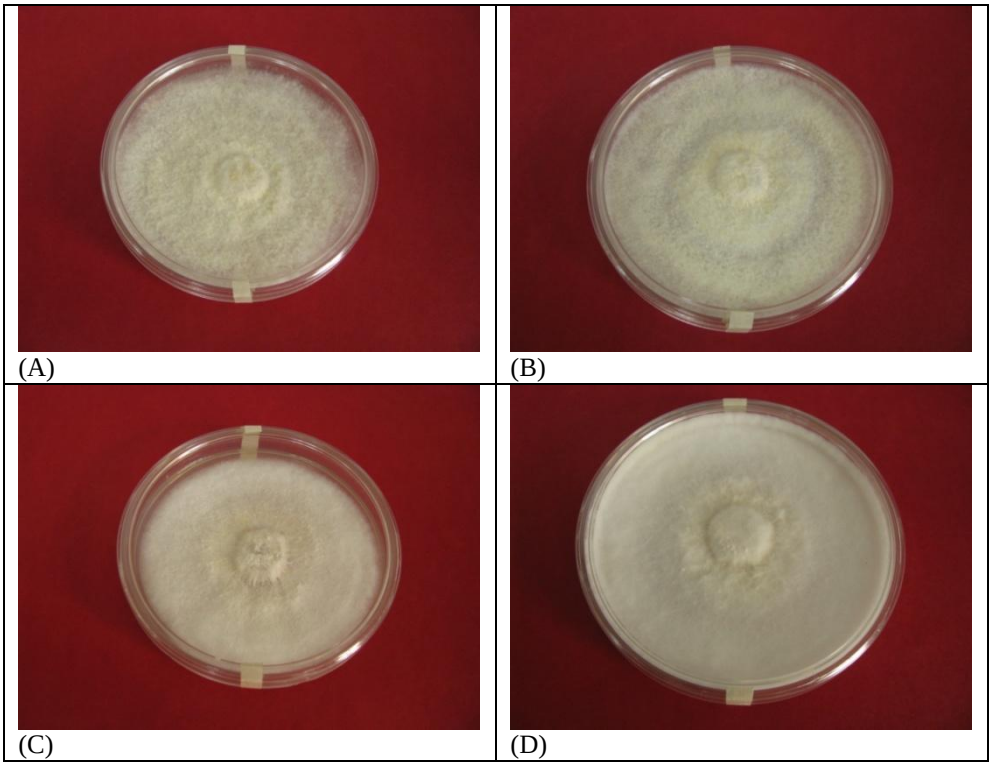

Figure 3. Macro-morphological cell growth of *P. ostreatus* on agar media: (A), PDA; (B), PDA+Malt extract; (C), PDA+Yeast extract; (D), PDA+Yeast extract+ Malt extract.

For further study on enhancement of radial cell growth of this mushroom, cultivations were carried out using base medium composed of (PDA+ yeast extract +malt extract) and supplemented with different

concentrations of sodium nitrate to enhance cell growth. The preliminary experiments showed that addition f sodium nitrate alone without malt extract and yeast extract, didn't show significant effect on radial cell growth compared to the standard PDA medium when applied in concentration range between 0 up to 6 g/L. However, when sodium nitrate added the modified PDA medium (medium supplemented with YE and ME), it showed positive influence on the increase of radial growth rate when applied in concentration of 3 g/L, further increase beyond this concentration didn't show positive result on cell growth rate. Figure 4 shows a comparison between cell growth of standard PDA medium, PDA medium supplemented with YE and ME, PDA medium supplemented with YE, ME, and sodium nitrate. For many years, PDA is considered as standard medium for fungal cell cultivation and usually considered as the first choice for mushroom cell cultivation to support high cell growth. However, this medium is not fully optimize to enhance mushroom growth and shorten the time needed for inoculum preparation and supplementation with YE and ME showed positive impact on cell growth rate in our previous work for cultivation of *C. militaris* [21]. These based on the fact that ME is very rich source carbohydrates mixtures of different structures (maltose, sucrose, and other sugars) which support cell growth. In addition, yeast extract is well known as one of the best sources not only for nitrogen but also for vitamins, growth factors, and trace elements which are all needed for growth and production of different types of primary and secondary metabolites [22,23] The results in figure 4 clearly demonstrate that addition of sodium nitrate to the cultivation medium increased the rate of radial cell growth significantly when used in concentration of 3 g/L. By addition of YE, ME, and sodium nitrate in combination, the growth diameter reached 10 cm after only 10 days instead of 12 days in other cultivation media. The growth was also characterized by dense mycelium as in YE and ME cultures.

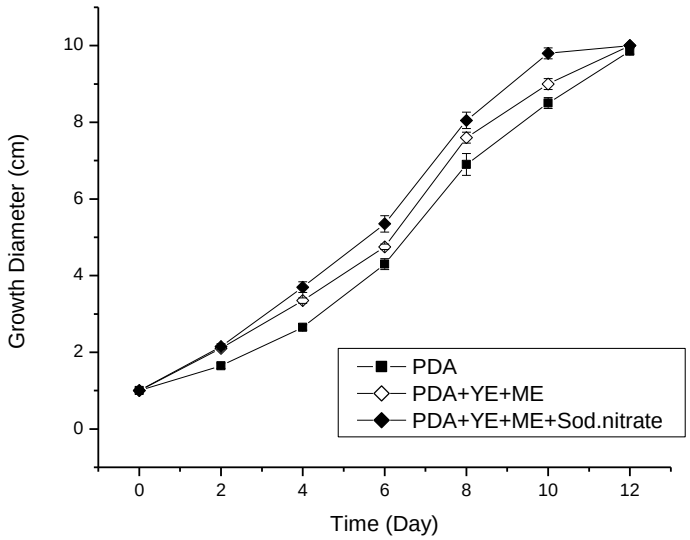

Figure 4. Radial growth of *P. ostreatus* when grown on standard agar medium (PDA) and PDA supplemented with yeast extract, malt extract, and sodium nitrate.

IV. Conclusion

The obtained results in this work confirmed the supportive effects of malt extract and yeast extract for mushroom cell growth when added the traditionally used PDA for surface growth. Both of yeast extract and malt extract enhance more dense growth, whereas, addition of sodium nitrate to solid medium increase radial growth. Therefore, its recommended that during cultivation of *P. ostreatus* on solid agar medium, supplementation with yeast extract, malt extract and sodium nitrate is necessary to shorten the cultivation time and to increase the cell growth rate.

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