

## VEGETATIVE GROWTH OF DIFFERENT STRAINS OF *PLEUROTUS* AND *LENTINULA* SPECIES ON CASSAVA (*MANIHOT ESCULENTA*) AND YAM (*DIOSCOREA ROTUNDATA*) WASTES IN GHANA

DZOMEKU M<sup>1\*</sup>, NARHMENSAH DL<sup>1</sup>, OBODAI M<sup>1</sup>, PREMPEH J<sup>1</sup>, TAKLI RK<sup>1</sup>, KOMLAGA G<sup>1</sup>,  
DZIEDZOAVE NA<sup>1</sup> AND SONNENBERG A<sup>2</sup>

<sup>1</sup> CSIR-Food Research Institute, P.O. Box M20, Accra, Ghana

<sup>2</sup> Plant Research International, Wageningen, Netherlands

matildadela@yahoo.com

### ABSTRACT

Mushrooms are fast becoming important components of diets worldwide and it is necessary to find out appropriate substrates on which they can be grown. Eight strains of two mushrooms: *Pleurotus ostreatus* strain MES11797, 03416, 03772, 03364, 03216 and *Lentinula edodes* strain MES 02008, 02052, 12060 were cultivated on substrate formulated from cassava and yam wastes such as Cassava peels, Cassava sticks and Yam peels. Sawdust of *Triplochitons cleroxylon*, which has been the traditional substrate for the cultivation of *Pleurotus* spp. in Ghana was used as the control substrate. Cassava peels agar supported the best growth for four *Pleurotus* spp. (MES 11797, MES 03772, MES 03216 and MES 03416) and one *Lentinula* strain (MES 12060). Yam peels however, support the best growth for *Pleurotus* strain MES 03364. Cassava sticks supported the best growth for *Lentinula* strain MES 02008. Sawdust supported the best growth for *Lentinula* strain MES 02052.

**Keywords:** substrates, mushroom, yield, *Pleurotus*, *Lentinula*

### INTRODUCTION

Ancient Egyptian empire considered mushrooms as a delicacy reserved for Pharaohs and the Romans and they ate mushrooms at feasts and believed that mushrooms provide strength for warriors during battle [1]. The Chinese on the other hand use mushrooms more because of the medicinal values they have [2]. Mushrooms are a delicacy in Ghana and are used in the preparation of many traditional and exotic dishes [3]. They are known to have anti cancerous, anti cholesterol and anti tumorous properties and are useful against ailments such as diabetes, high blood pressure, ulcer and lung diseases [4]. Edible Mushrooms are a good source of protein, vitamins and minerals [5]. Fresh mushrooms contain about 85-95% water, 3% Protein, 4% Carbohydrates, 0.1% fats, 1% minerals and vitamins [6]. Mushrooms contain appreciable amounts of potassium, phosphorus, copper and iron but have low levels of calcium. Mushroom protein is intermediate between that of animals and vegetables [7, 8]. Mushrooms such as the genus *Pleurotus* are known to be among the largest of fungi composed of filaments and survive very well in a damp or moist condition [9].

Cassava (*Manihot esculenta* Crantz) is the third most important source of calories in the tropics, after rice and maize. Millions of people depend on cassava in Africa, Asia and Latin America [10]. Cassava processing, especially in areas where the industry is highly concentrated, is regarded as polluting and a burden on natural resources [11]. In many places where cassava is cultivated and processed, heaps of cassava peels are discarded along the roads and causes unpleasant odours and unhygienic conditions [12].

Cassava is cultivated in Ghana on a large scale and some of the waste is given to livestock as feed. The rest is left to the weather and eventually this creates a high pollution problem. Over 90 per cent of the peels are either burnt or left unattended to at dumping sites. A total of 3.6 million tonnes of cassava waste peels are discharged parts during the peeling process which are generated annually. Peels represent around two thirds of the waste, and about 200,000 tonnes of cassava can be saved through more efficient peeling which translates into potential savings of almost \$37 million [13]. In the case of yams, waste is mostly generated at the consumption level through households, chop bars and food vendors. Since yam processing is very limited, it is done by a few small and medium enterprises. Yam (*Dioscorea rotundata*) peels constitute

about 14 per cent of the volume of yam consumed and approximately five per cent of volumes of the crop tend to go waste [13]. The cassava and yam peels can therefore be used as efficient substrate for mushroom cultivation.

This study seeks to evaluate the use of cassava and yam wastes as suitable substrates for the growth of five strains of *Pleurotus* spp. and three strains of *Lentinula* mushrooms within Ghanaian environmental conditions.

## **MATERIALS AND METHODS**

### **Mushroom cultures used**

The cultures used for this work were obtained from the Plant Research International of the University of Wageningen, Netherlands. These were: *P. ostreatus* strain MES 11797, MES 03416, MES 03772, MES 03364, MES 03216 and *L. edodes* strain MES 02008, MES 02052, MES 12060.

### **Agar media preparations**

Initial studies were carried out on agar media to ascertain the effectiveness of these media. The media used were: cassava peels agar -CPA, cassava sticks agar-CSA, yam peels agar-YPA and sawdust agar-SDA. Cassava and yam peels, cassava sticks were each solar dried for five days and milled mechanically using a hammer mill to a fine powder and used to prepare the agar media. This was prepared by soaking 20 g of each substrate in 200 ml of water and allowed to stand for four hours. Then strained and the supernatant topped with distilled water up to 1 liter. Fifty grams of select agar and 20 g of glucose were added and mixed. This mixture was put on a hot plate and stirred continuously till granules of the agar dissolved. Then it was sterilized for 1 hour at 121 °C and poured into Petri dishes to set. Each substrate had two replicates. Sawdust of *Triplochiton scleroxylon* agar was prepared as above.

### **Measurement of mycelia growth rates**

The vegetative growth of mycelium of the mushroom on the different media were assessed by measuring growth of the fungus along two diameters drawn at right angles at the bottom of the Petri plates prior to inoculation. Measurements were made daily for five days.

## **RESULTS AND DISCUSSION**

### **Mycelia growth on solid media**

Mycelia extension of various strains varied on different solid media. *L. edodes* strain MES 02008 inoculated on YPA showed the lowest mycelia extension by the third (0.7 cm) and fifth days (1.0 cm) of incubation in comparison to all the other strains of mushrooms studied. Although the recorded low mycelia extension was not significantly different from values recorded for the same strain on the CPA, CSA, and SDA, the value was significantly lower than that recorded for *L. edodes* strain MES 12060 on all the solid media. Among the *P. ostreatus* strains, the lowest mycelia extension on the third day of incubation was 0.8 cm and was recorded for *P. ostreatus* strain MES 11797 inoculated on CSA (Table 1).

On the fourth day of incubation, the lowest mycelia extension (0.9 cm) was recorded for *L. edodes* strain MES 02052 inoculated on YPA. *P. ostreatus* strain MES 03364 recorded the highest mycelia extension of 1.9, 2.4 and 3.1 cm on days 3, 4, and 5 of incubation, respectively on YPA. With the exception of the value recorded for strain MES 12060 on CPA and YPA, the high mycelia extension recorded for strain MES 03364 on YPA on the third day of incubation (2.4 cm) was significantly higher ( $P < 0.05$ ) than values recorded for the same strain inoculated on all the other solid media (Table 1). Significantly lower mycelia extension of strain MES 03364 was also recorded when inoculated on CSA and SDA. Among the other MES strains (11797, 03416, 03772, 03216) on the various media, significantly lower mycelia extensions were recorded for strain MES 03216 on SDA and YPA and strains MES 03416 and MES 11797 on all the solid media.

The lowest, mean and the highest mycelia growth rates recorded were 0.1 cm/day for strain MES 02008 on YPA, 0.4 cm/day for various strains and solid media, and 0.7 cm/day for strain MES 12060 as well as strain MES 03364 both incubated

on CPA (Table 1). Among the *L. edodes* strains studied, strain MES 12060 recorded the fastest mycelia growth on the solid media, whereas the other two strains showed comparable mycelia growth rates for the various solid media. On the other hand, among the *P. ostreatus* strains studied, strain 03364 showed the fastest mycelia growth ranging from 0.5-0.7 cm/day (Table 1), closely followed by strain MES 03216 with 0.5 and 0.6 cm/day on CSA and SDA, and CPA and YPA, respectively (Table 1).

In a study by Furlan *et al.* [14], the authors reported growth rates of 0.38 cm/day on potato dextrose agar (PDA) and 0.4 cm/day on both wheat dextrose agar (WDA) and malt extract agar (MEA) incubated in the dark at 30 °C for *L. edodes* and growth rates of 0.75, 0.6 and 0.4 cm/day under the same condition on PDA, WDA and MEA, respectively for *P. ostreatus*. Although the reported mycelia growth rates by Furlan *et al.* [14] are comparable to those reported herein, some of the growth rates reported for *L. edodes* herein are lower. However, both studies indicate that mycelia growth rate of both mushroom species varies on the growth media on which the mycelium is incubated. Furlan *et al.* [14] showed that the difference in mycelia growth rate on the solid media was independent of the pH of the media and the incubation temperature. Inglet *et al.* [15] have recorded *L. edodes* mycelia growth rate of 0.6 cm/day using whey permeate at 40 g of lactose/L, temperature 23.6 °C, and pH 5.0. Strain- and growth media –dependent variations of *Lentinus squarrosulus* mycelia growth rate have also been reported [16]. As such, it appears reasonable to infer that variations in *L. edodes* and *P. ostreatus* mycelia growth rates are strain and growth media dependent and that this may be a general characteristic of mushroom mycelia growth regardless of the species or strain.

Knowledge about media requirements by strains of mushrooms is essential in tissue culturing for the further steps in mushroom studies or cultivation. Moreover, the information is required to assess the viability of the strain in question. For instance, knowledge of behavior of a particular mushroom species or strain at specific conditions (media and incubation parameters) would ensure that viable, vibrant cultures are not discarded with the assumption that they are not vibrant enough, especially when classical biotechnology techniques are being applied.

Across the strains, the slowest and fastest growth rates recorded on CPA were 0.4 and 0.7 cm/day, respectively, with a mean value of 0.45 cm/day (Fig. 1). Growth rates recorded for the strains on YPA showed wide variations, with values falling within the extreme ranges of 0.1-0.7 cm/day with a mean value of 0.38 cm/day (Fig. 1). Mean mycelia growth rates of 0.46 and 0.44 cm/day were recorded on CSA and SDA, respectively (Fig. 1). Mycelia densities recorded across the mushroom species and strains on CPA had various degrees of mycelia density (ranged from 2-4), those for CSA were quite dense (2 and 3), and YPA were generally highly dense (ranged from 3-5) (Fig. 2). The lowest mycelia densities (1 and 2) were observed on SDA (Fig. 2).

The mycelia densities of the strains studied across the solid media are shown in Fig. 3. The strains showed comparable mycelia densities on the various media, although strain MES 03216 showed relatively low mycelia densities across the

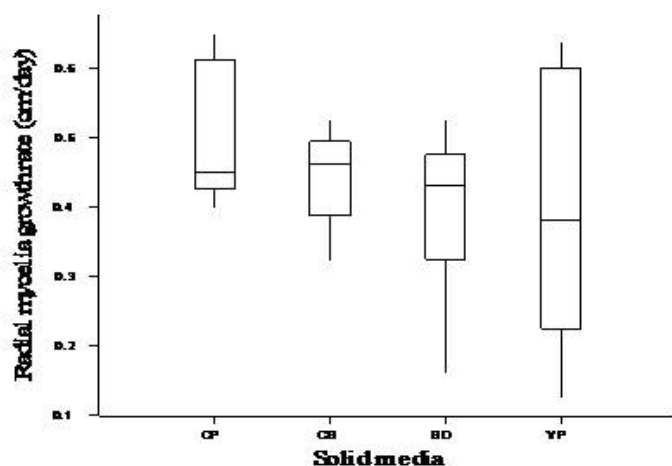


Figure 1. Box-and-whisker plots showing mycelia growth rate on the solid media

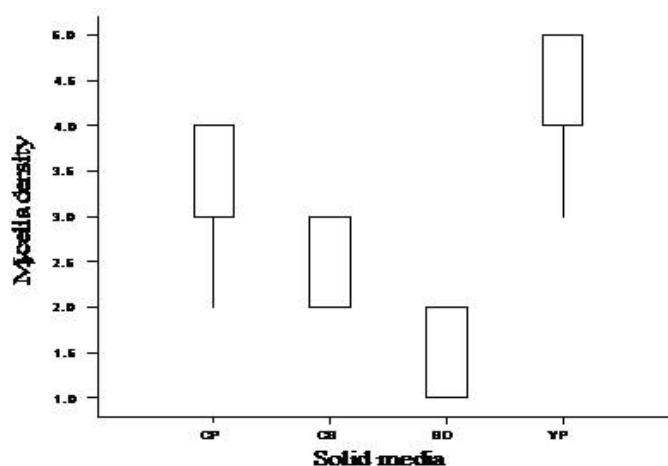


Figure 2. Box-and-whisker plots of MES strain mycelia density on the solid media

media. Mycelia densities of MES 03772 differed when incubated on the different solid media. These results indicate that mycelia growth rate does not directly correspond to the mycelia density. In fact, a weak negative correlation of -0.0635 was obtained between values recorded for mycelia growth rate and mycelia density for all the mushroom strains studied on the various solid media. Thus, there is a very low chance (less than 1%) that cultures with high mycelia growth rates would have low mycelia densities. As such, it is reasonable to infer that there is no correlation between the two parameters. This relationship between mycelia growth rate and mycelia density have been reported on *Lentinus squarrosulus* mycelia growth [16].

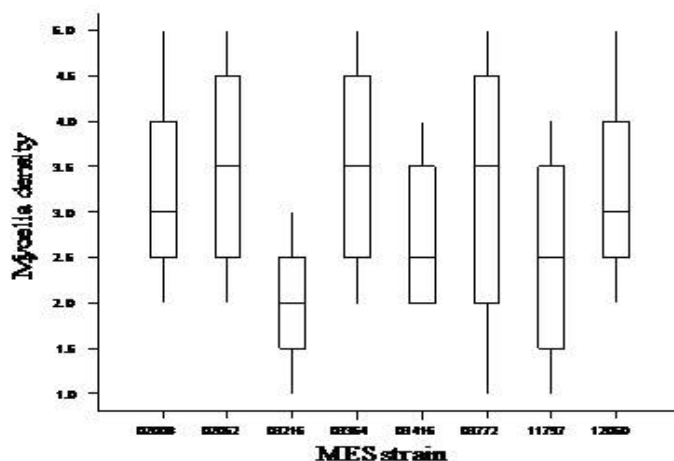


Figure 3: MES strain mycelia density on solid media

However, in selecting mushroom cultures for further work on mushroom cultivation, it is more reasonable to select mycelia with higher growth rates rather than higher mycelia densities as faster mycelia growth would enable the mushroom to rapidly fully colonize the substrate in order to outgrow contaminants in the substrate if there are any contaminants. Nevertheless, mycelia density is also an important factor as it has been observed that pinhead or primordial formation occurs only after the mycelia thickens or becomes dense on the substrate, indicating that basidiocarp formation and yield would be affected by the mycelia density on the substrate. Culture behavior of mushrooms have been indicated [17] to be directly linked to cultivation and pharmaceutical aspects of mushrooms.

### Morphological description of mycelial growth on different substrate media

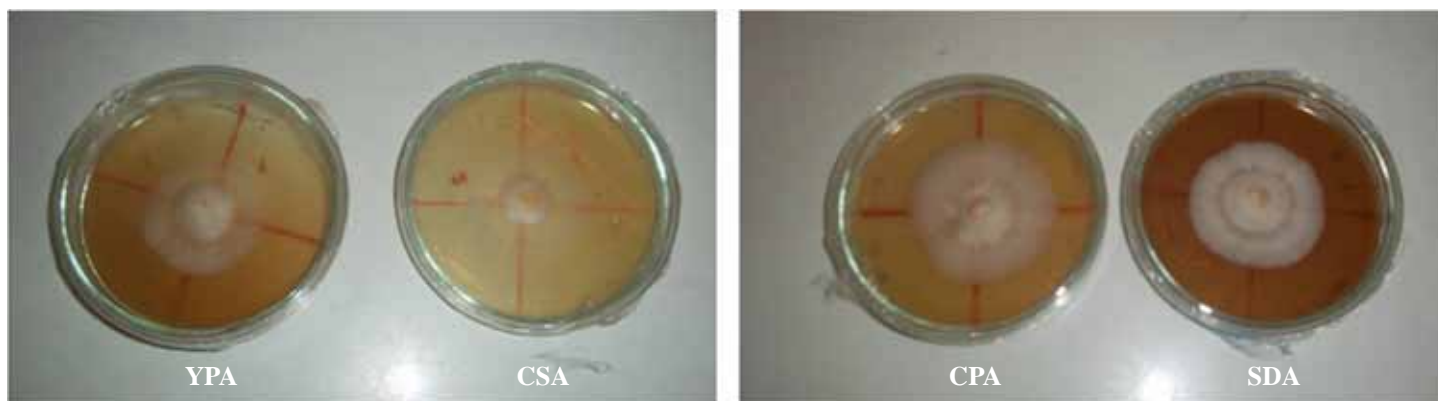
Various strains had different morphological characteristics during growth on the different media. MES 11797 strain showed clear zonation at the edge of the colony (Fig. 4a). In addition, longitudinal radial mycelia were observed on both sawdust and cassava sticks agar. Concentric longitudinally radial growth on YPA was quite prominent but not as prominent as on CPA. In the strain MES 03416 (Fig. 4b), the mycelial morphology on all media showed similar concentric longitudinally radial growth. Green mould contaminant partially restricted mycelia growth. Strain MES 03772 (Fig. 4c), growing on CPA and YPA had a concentric longitudinally radial mycelia growth while CSA and SDA showed a longitudinally radial mycelia growth. Growth of mycelia was partially inhibited by bacterial contaminant as well. Mycelia morphology of strain MES 03364 on all the substrates was either concentric, longitudinally or radial. The concentric morphology was prominent. Mycelial morphology of MES 02008 strain were similar on SDA, CSA and CPA (Longitudinally radial). The mycelial morphology of strain MES 02052 had similar morphology to that of MES 02008 on SDA, CSA and CPA (longitudinally radial). However, there was an uneven growth on YPA. For strain MES 03216 (Fig. 4d), the morphology was different on each different media: SDA, CSA and CPA had longitudinally radial mycelia growth, YPA had a concentric longitudinally radial growth and a dense mycelia but the strain was denser on CPA. The strain MES 12060 (Fig. 4e) had clear area (zone) formed at the periphery of the colony, this is possibly caused by an exudate from the mycelia. This was especially pronounced on YPA and is likely to be due to the presence of extracellular enzymes. The morphology of the strain MES 12060 on the medium SDA and CSA both showed a longitudinally radial growth.

### CONCLUSION

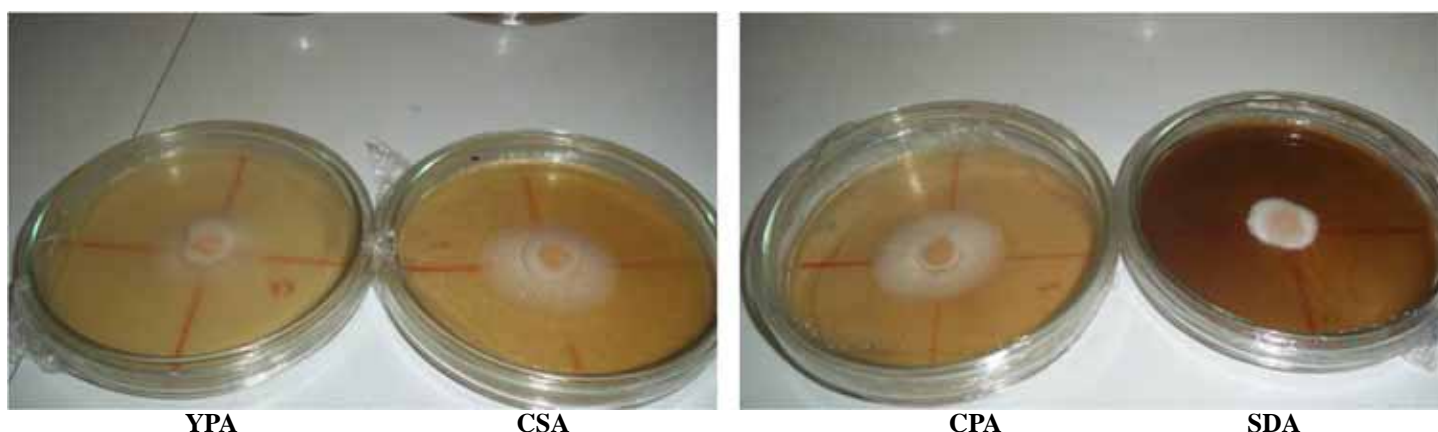
Different agar media supported the growth of the different strains of mushrooms with cassava peels agar supporting majority of the strains (*Pleurotus ostreatus* strain MES 11797, 03416, 03772, 03216 and *L. edodes* MES 12060).

### ACKNOWLEDGEMENTS

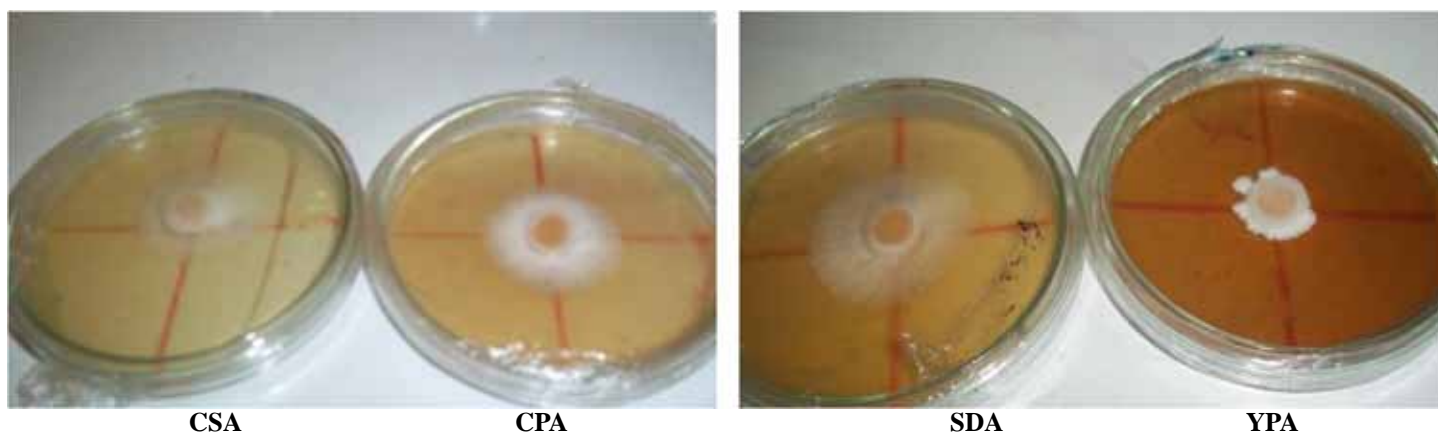
This work was funded by the Gains from losses of Root and Tuber Crops (GRATITUDE), an EU funded project.



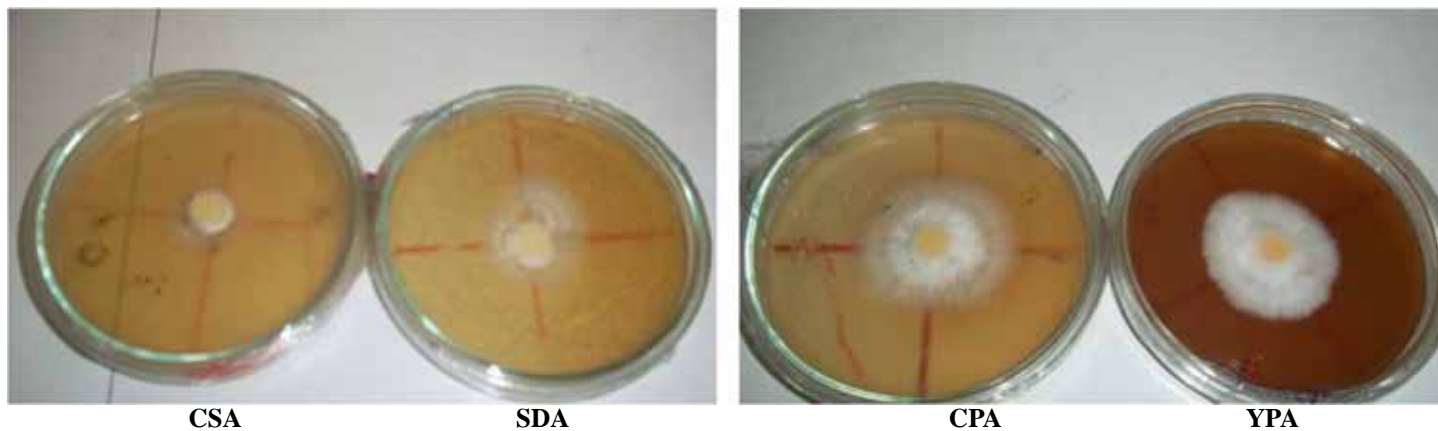
**Figure 4a.** Morphology of mycelial growth of *P. ostreatus* MES 11797 on various solid media on the second day of incubation



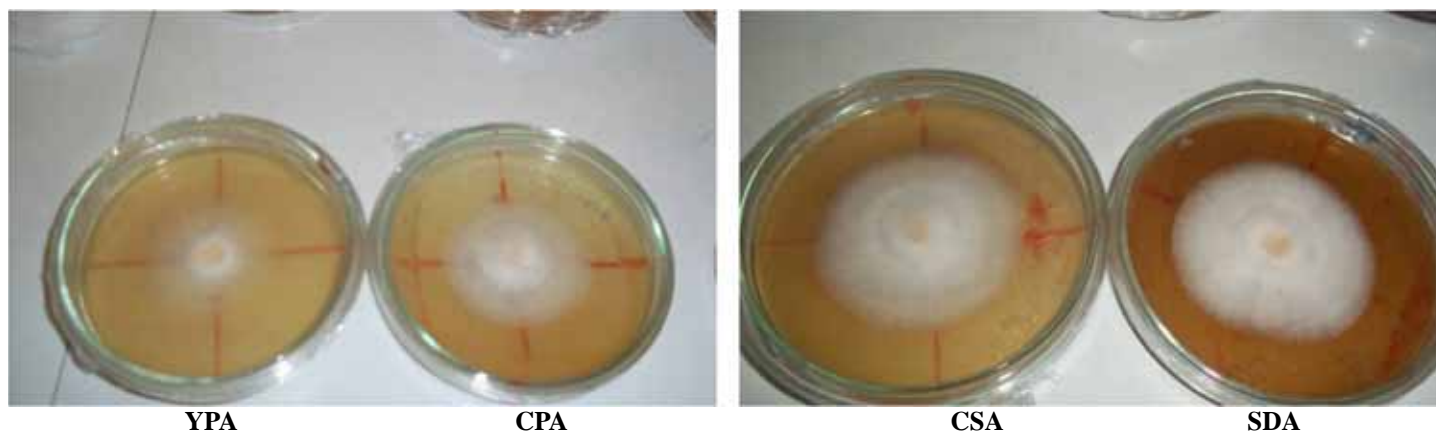
**Figure 4b.** Morphology of mycelial growth of *P. ostreatus* MES 03416 on various solid media on the second day of incubation



**Figure 4c.** Morphology of mycelial growth of *P. ostreatus* MES 03772 on various solid media on the second day of incubation



**Figure 4d.** Morphology of mycelial growth of *P. ostreatus* MES 03216 on various solid media on the second day of incubation



**Figure 4e.** Morphology of mycelial growth of *L. edodes* MES 12060 on the various solid media on the fifth day of incubation

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