Submerged fermentation of the hybrid of *pleurotus tuberregium* and *pleurotus pulmonarius* in zobo and synthetic media

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ABSTRACT: This research work used the hybrid of ***Pleurotus tuberrigium*** and ***Pleurotus pulmonarius*** in Zobo and synthetic media, to investigate their ability to grow and produce mycelia mat during submerged fermentation. It was discovered that the ability of the hybrid of the two mushrooms in Zobo medium to form mycelia mat was lower compared to that of the synthetic medium; the mycelia mat weight in the synthetic medium and Zobo medium were 0.82g and 0.47g respectively. Moreover, the lag phase of the hybrid of the two mushrooms in the synthetic medium was shorter than that in the Zobo medium, this was shown by the turbidity of the medium on the second day while the Zobo medium became turbid on the third day. Changes in the colour of the Zobo medium showed that the hybrid of the two mushrooms has the ability of utilizing the medium for growth. There was a significant difference in the turbidity values of the Zobo medium compared to that of the synthetic medium, considering their means and t-test value of 5.58 and 7.80 for Zobo and synthetic medium respectively.
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INTRODUCTION

Mushrooms are highly nutritive, because they contain good quality protein, vitamins and minerals. They are low calorie food with very little fat and are highly suitable for obese persons. With no starch and very low sugars, they are the 'delight of diabetics' (Fasidi *et al.*, 1993).

There are many varieties of mushrooms, ***Pleurotus* sp.** are characterized by a white spore print, attached to gills, often with an eccentric strip or no strip at all. They are commonly known as Oyster mushrooms. ***Pleurotus tuberregium*** is a tropical and sub tropical sclerotial mushroom which has been gaining some interest in U.S. and it is common in southern Nigeria, ***P.tuberregium*** is used as both food and medicine. The sclerotium, which is hard is peeled and ground for use in vegetable soup. The sclerotium is expensive and considered a delicacy. ***P. tuberregium*** is used in some combination that are intended to cure headache, stomach ailment, cold and fever, as well as asthma, smallpox and high blood pressure (Zhang *et al*., 2001)

P. tuberregium seems to be adapted to a wide range of materials. This was proved by the work of Okhuoya and Okogbo (1991), which used slerotia to inoculate oil palm fruits fiber as spawning material; while Fasidi *et al*., (1994) used banana leaves, corn cobs, cotton wastes and rice straw for sclerotia production.

Pleurotus pulmonarius is a virtually indistinguishable from ***P. ostreatus***, and differs largely in it habitat preference for conifer woods. ***P. pulmonarius*** and ***P. ostreatus*** grow on variety of hardwoods with ***P. pulmonarius*** primarily a spring mushroom and ***P. ostreatus*** grow most prevalently in the summer fall. ***P. pulmonrius*** (Phoenix Oyster) is an aggressive mushroom that fruits easily on a wide range of substrates (Isikhuemhen *et al.,* 2000).

Much research work has been carried out on ***Pleurotus* sp** cultivation (Royse, 2002), biomass production using solid and liquid-state fermentation, medicinal properties; but so far, none about ability of the hybrid of ***Pleurotus tuberrigium*** and ***Pleurotus* pulmonarius** to grow in Zobo is known. Studies on mushroom cultivation have been focused on optimization of alternative substrate. It has been shown that a wide range of agricultural products, weeds and wastes can be used to produce food, feed, enzyme and medicinal compounds and in the degradation and detoxification of wastes (Gregori *et al.,* 2007).

Submerged fermentation with this genus of fungi can be more uniform and easy to reproduce, this is interesting for obtaining products, though this potential is yet to be recognized by Western biotechnology companies (Smith *et al.,* 2002). Sánchez *et al*. (2006) performed solid state fermentation with ***Pleurotus* sp.** on grapevine sawdust supplemented with synthetic medium and peroxidase activity was detected in all strains evaluated with the highest being ***P. ostreatus*** and ***P. pulmonarius.*** Márquez-Rocha *et al.* (1999); reported

on ***P. ostreatus***' growth in submerge liquid fermentation in a stirred tank bioreactor and showed that by varying impeller geometry and speed, aeration intensity, the growth rate and pellet size change. Also the lag phase of ***P. tubberegium*** growth in submerged liquid fermentation was shorter with glucose and fructose than with maize starch (Wu *et al.*, 2003).

Sanchez *et al.* (2002) showed the recycling of viticulture waste in solid state fermentation with ***P. ostreatus and P.pulmonarius*** yielding a high-fiber feed for limited use in ruminants. It has been shown that wheat straw supplemented with ***Lolium perenne*** grass chaff stimulated fruitification and mushroom yield of ***Pleurotus pulmonarius*** (Domondon *et al.,* 2004).

Rupak *et al.* (2005), showed that there was an increase in the biomass production of ***Pleurotus sajor-caju*** grown on whey when enhanced with plant growth hormones, 3.2g of biomass was produced, this showed an increase over the control which produced 2.5g.

Zobo drink, a non alcoholic drink produced from the petals of ***Hibiscus sabdarifa*** (Linn Roselle) by boiling and filtration (Ogiehor *et al.,* 2007). Zobo beverage has been shown to be a good source of natural carbohydrate, protein and vitamin C, which constitute the major reason(s) for consuming soft drinks and fruit juices (Okoro, 2003; Ogiehor and Nwafor, 2004).

The name 'Zobo' is derived from the local Hausa (Northern Nigeria) name for the Roselle plant that is 'Zoborodo'. The calyx contain about 8.3% moisture, 4% citric acid, 1.5% pigment (mainly anthocyanin), 6.9% protein and about 9% soluble solid with pH of 2.7 (Adenipekun, 1998).

The aim of this project work is to study the ability of hybrid of and ***P. tuberregium and Pleurotus pulmonarus*** to grow in Zobo and Synthetic media and produce mycelia mat; during submerged fermentation.

MATERIALS AND METHODS

The Organism

The hybrid of ***Pleurotus pulmonarius*** and ***Pleurotus tuberregium*** was produced by crossbreeding the two mushrooms on the slant. Slants of Potato dextrose agar and yeast extract were produced in MacCartney bottles, inoculated with the spawn of the hybrid of ***Pleurotus pulmonarius*** and ***Pleurotus tuberregium*** and allowed to grow at room temperature in the dark cupboard for four days.

Media

The Zobo medium was produced by boiling the dry calyx of ***Hibiscus sabdarifa*** (Roselle plant), the calyx is then filtered out and the pH regulated to 5.8. The synthetic medium was prepared by dissolving 15g of glucose, 5g of peptone, 3g of yeast extract, 1g of $MgSO_4.7H_2O$, 2g of KH_2PO_4 and 1g of Urea; all in 1l of distilled water and the pH was regulated to 5.8.

The media were sterilized in the autoclave at 121^0C for 15 minutes, and were pour into two different fermentors aseptically.

Fermentation

Two fermentors containing sterilized synthetic medium and zobo medium each were inoculated with the hybrid of the two mushrooms by washing organisms from three grown slants into each bioreactor, an aeration hose was introduced and the mouth sealed.

Growth and Harvesting of the Organisms

The fermentation process was allowed to run for 10 days at room temperature and then terminated. Organisms were harvested by filtration using Number 1 Whatman filter papers, then centrifuged and precipitated; cell debris was air dried and weighed.

RESULTS

Table 1 shows the result of the turbidity scores for the two media. The synthetic medium became turbid on the second day and the turbidity increased until the fourth day after which there was no detectable increase again in the turbidity of the medium. Zobo medium on the other hand became turbid on the third day and the growth was at the climax on the fourth day.

Table 1: Turbidity score for the two media as fermentation days increased

Day	Turbidity Score	
	Synthetic Medium	Zobo Medium
1	-	-
2	++	-
3	+++	+
4	++++	++
5	++++	++
6	++++	++
7	++++	++
8	++++	++
9	++++	++
10	++++	++

KEY:
+ : turbidity grade
-: no visible growth or turbidity

Result of the weighed mycelia mat and precipitate from the filtration and precipitation processes respectively are shown in Table 2. Weight

of the mycelia mat from synthetic and Zobo media are 0.83g and 0.47g respectively. While the weight of the precipitate from synthetic and Zobo media are 0.11g and 0.06g respectively.

Table 2: Mean weight of mycelial mat and precipitate of the hybrid of ***Pleurotus pulmonarius*** and ***Pleurotus tuberregium*** after ten days submerged fermentation

DRY MASS	SYNTHETIC	ZOBO MEDIUM
MYCELIAL MAT WEIGHT (G)	0.82 ± 0.01^{a}	0.47 ± 0.02^{b}
PRECIPITATE WEIGHT (G)	0.11 ± 0.02^{a}	0.06 ± 0.01^{b}

Values are means scores ± Standard Deviation
Mean values followed by the same superscript are not significantly different by Duncan's Multiple Range test ($P\leq0.05$)

Table 3: T-test result on the turbidity for the two media as fermentation progress

Medium	Mean Turbidity	T-Value	P value
Zobo Medium	1.50±0.27	5.58	$p\leq 0.05$
Synthetic Medium	3.30±0.42	7.80	$p\leq 0.05$

DISCUSION

Turbidity score shows the growth of the hybrid of ***Pleurotus pulmonarius*** and ***Pleurotus tuberregium*** based on the utilization of the media by the organism, and thus the turbidity. The faster growth rate of the organism in the synthetic medium compared to the Zobo medium may be in compliance with the work of Sánchez *et al.,* (2006), which reported that 'supplementing of solid media with synthetic medium produced high peroxidase activity in ***Pleurotus* sp.**.

The delayed turbidity in the Zobo medium until the third day may be in contrast to the report of Wu *et al.* (2003) that the lag phase of ***P. tuberregium*** in submerged liquid fermentation was shorter; this may be due to reduced ability of the organism to utilize the sugars available in Zobo.

The synthetic medium showed a higher yield of mycelia mat and precipitate than that of Zobo medium, but the two showed a lower yield compared to what Rupak *et al.* (2005) reported.

There was a significant difference in the t-test result of the turbidity of the two media, with a mean of 1.50 for zobo medium and 3.30 for the synthetic medium this agrees with the work of Wu *et al.* (2003).

CONCLUSION

The synthetic medium supported the growth of the hybrid of ***Pleurotus pulmonarius*** and ***Pleurotus tuberregium*** better than the Zobo medium as seen in the result. Addition of some nutrients to the Zobo medium may aid a better growth and yield of the hybrid of ***Pleurotus pulmonarius*** and ***Pleurotus tuberregium*** in this medium.

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