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Full Length Research Article

Mycelial Growth and Antibacterial Metabolite Production by Wild Mushrooms

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ABSTRACT
Russula sp. and Pycnoporus cinnabarinus (wild mushrooms) were subjected to laboratory cultivation by spore germination and tissue culturing on Sabouraud dextrose agar plates. Subsequently, the growth and production of metabolite(s) were monitored in submerged fermentation for 7days using agar diffusion method. The result obtained showed that metabolite production peaked on the fourth day in Russula sp. and on the fifth day in Pycnoporus cinnabarinus with subsequent decrease in activity of the fermentation extract. Dry weight increases with fermentation time in both mushrooms.

Keywords: *Wild mushroom, Spore germination, Tissue culturing, Antibacterial metabolite*

INTRODUCTION

Some mushrooms contain compounds, which can make a contribution to the general health of man (Elliot, 1997). As mushrooms are widely distributed all over the world, some of them have been used in traditional medicine as anti-inflammatory, analgesics, hemostatic, diuretic, nourishment, antibechic and antitumour agents (Koyama *et al*., 1997).

Most of the medicinal extracts from mushrooms are different forms of polysaccharides and all of them are strengtheners of the immune system with little or no side effects (Oei, 1991, 1996; Gao *et al*., 1997). For example, a sizofiran, antitumour polysaccharide extracted from the culture broth of *Schizophyllum commune* is an effective immuno-therapeutic agent for cervical carcinoma because it stimulates a rapid recovery of the immunological status impaired by radiotherapy (Miyazaki *et al*., 1995). *Lentinus edodes* is another immunological strengthener. Some mushrooms are used for the treatment of gastric ulcer, duodenal ulcer and chronic gastritis. A good example is *Hericium erinacius* (Oei, 1991, 1996). Some mushrooms such as *Tremella fulciformis* are used for curing leukaemia, coughing, phlegm and asthma of patients suffering from chronic bronchitis (Oei, 1991, 1996). According to Gao *et al*. (1997) mushrooms like *T. fulciformis* also possesses antitumour, hypoglycaemic and cytosine stimulating activities. Antinoceptive components have been isolated from *Ganoderma lucidum* (Koyama *et al.,* 1997). This fungus (*G. lucidum*) has been used in curing neurasthenia, chronic bronchitis and coronary heart disease. Lanostanes triterpenes is another compound from mushroom extracts that have been found to be of medicinal value. The compound has been isolated from *G. lucidum* and it has cytotoxic, antineoplastic and hypotensive activities. The fungus is widely used in Asian medicine to treat all types of diseases (Keller *et al*., 1997). Another collagenase inhibitor

considered as candidate for medicine for the treatment of rheumatism, metastasis or periodontal disease has been isolated from *Daedalea dickinsii* (Kawagishi *et al*., 1997). Oyster mushrooms (*Pleurotus ostreatus*) decrease serum and liver cholesterol and increases cholesterol 7-hydroxylase activities and faecal excretion of neutral sterols and bile acids in hypercholesterolomic rats (Bobek *et al.,* 1994). Engler *et al*. (1998) obtained antibiotic metabolites named oudemasin A and D, illudin S and pterulone B from *Collybia nivalis, Omphalotus olearius,* a *Flavolaschia and a Pterula sp.*

A strain of *Cyathus striatus* has been found to produce striatins A, B, C during mycelial growth and these crystalline antibiotics were highly active against *Fungi* imperfecti and a variety of Gram-positive bacteria, as well as some Gram negative bacteria (Anke *et al*., 1976). *Lactarii vellutinus* produces an extremely labile compound, identified as stearyl-vellutinal when injured. This biologically inactive precursor which has been shown to be responsible for the dark blue color that develops when the mushroom is treated with sulphovanillin (Favre-Bonvin *et al*., 1982) is rapidly converted to a strongly antibiotic and pungent sesquiterpenoids dialdehydes such as isovelleral [2]. (Sterner *et al*., 1983; Stadler and Sterner, 1998).

Various bioactive compounds isolated from culture extracts of Ethiopian higher fungi showed other biological properties such as antiprotozoal, anthelmintic, phytotoxic and brine shrimp lethality activities (Dagne and Abate, 1995). Also, Inchausti *et al*. (1997) investigated Leishmanicidal and Trypanocidal activity of the extracts and secondary metabolites of some Basidiomycetes. A naturally occurring purine nucleoside found in some mushrooms showed high degree of activity against Mycobacterium (Gupta and Bhakum, 1982). Aqueous extracts of spent mushroom substrate are used in foliar disease control (Yokalem *et al*., 1994).

Wild mushrooms are seasonal, and a particular mushroom may disappear from the initial place of collection for a number of years, appearing in another place beyond reach (Harding, 1996; Laessoe, 1998).

Russula sp. (gilled mushroom) and *Pycnoporus cinnabarinus (polypore)* are wild mushrooms. Their fruitbodies were screened and found to possess antibacterial properties in previous work (Fajana *et al*., 1999). This study examined the two wild mushrooms in laboratory cultivation for growth and bioactive compound production, in submerged fermentation.

MATERIALS AND METHODS

Cultivation experiments

Mycelial cultivation through spore germination

Sabouraud dextrose agar slant was heavily inoculated with spores collected from the gilled mushroom, *Russula sp.* Incubation was carried out at ambient temperature for 7 days. Several subculturing exercises were carried out until a pure culture was obtained. The mycelial culture thus obtained was used as inoculum in subsequent experiments.

Mycelial cultivation through tissue culturing

The fruitbody of the polypore mushroom *Pycnoporus cinnabarinus* was collected at a young stage with the aid of sterile forceps, wrapped with sterile foil paper and transported to the laboratory. It was washed thoroughly with several changes of sterile distilled water and was, thereafter, aseptically broken lengthwise exposing the inner tissue (trama) with the aid of a sterile blade. A small piece of 2 x 2mm of the sterile tissue was then aseptically transferred onto plates of solid acidified Sabouraud dextrose agar. Four replicates were made and the plates were incubated at ambient temperature for 72 hours. Subculturing for pure tissue mycelial production was prepared by transferring a small square of 5 x 5mm from the mother plate onto a fresh solid media plates. All transfers were made aseptically. The tissue culture obtained thus was used in subsequent experiments.

Measurement of linear growth

Colonies were grown at ambient temperature. The colony diameters were measured every 24 hours to the nearest 0.5mm. Mean diameter of six colonies at 24 hourly for 7days were fitted to the best straight line by regression analysis and the slope of the best fit line was taken as the rate.

Cultivation for metabolite production

Growth conditions for metabolite production

Fungal cultures were grown in 100ml conical flasks each of which contained 50ml of sterile Sabouraud dextrose broth. The cultures were then incubated at room temperature for 7days by shaking on a rotatory shaker (Gallenkhamp) at 120 rev/min.

Measurements of growth for metabolite production

Cultures of each of the organisms were withdrawn 24 hourly in triplicates and filtered using No.1 filter

paper. The mycelia on the pre-weighted filter paper were oven-dried at 80^{o}C to a constant weight. The weights were used to plot the growth characteristics.

Isolation of the crude extracts

The filtrates obtained 24 hourly in triplicates were pooled together and partitioned in ethyl acetate using a separating funnel. The ethyl acetate fractions were evaporated to dryness *in vacuo*. For zero hour extracts, a negative control flask (not inoculated with fungal culture) and a positive control flask (inoculated with fungal culture) were extracted within an hour of inoculation.

Bioassay monitoring of antibacterial metabolite production by agar diffusion method.

The extracts were reconstituted in 0.5ml of 50% aqueous methanol and 100µl of each test solution was assayed using agar cup plate diffusion method earlier described in Fajana *et al*. (1999). The culture plates were then sprayed with methyl thiazoyl tetrazolium chloride (MTT), and further incubated for 15 minutes. The diameters of zones of inhibition were then measured with a transparent ruler.

RESULTS

Measurement of linear growth on solid media

The results obtained for the linear growth of *Russula sp.* and *Pycnoporus cinnabarinus* are shown in fig.1. The rate of growth as shown from the slope is 1.23mm per hour for *Russula sp* and 0.54mm per hour for *Pycnoporus cinnabarinus.* The growth on Sabouraud dextrose agar is shown in plate 1.

Plate 1:
Cultures of *Russula sp.* and *Pycnoporus cinnabarinus* grown at ambient temperature for 7days on Sabouraud Dextrose Agar. A = *Russula sp.* ; B = *Pycnoporus cinnabarinus*

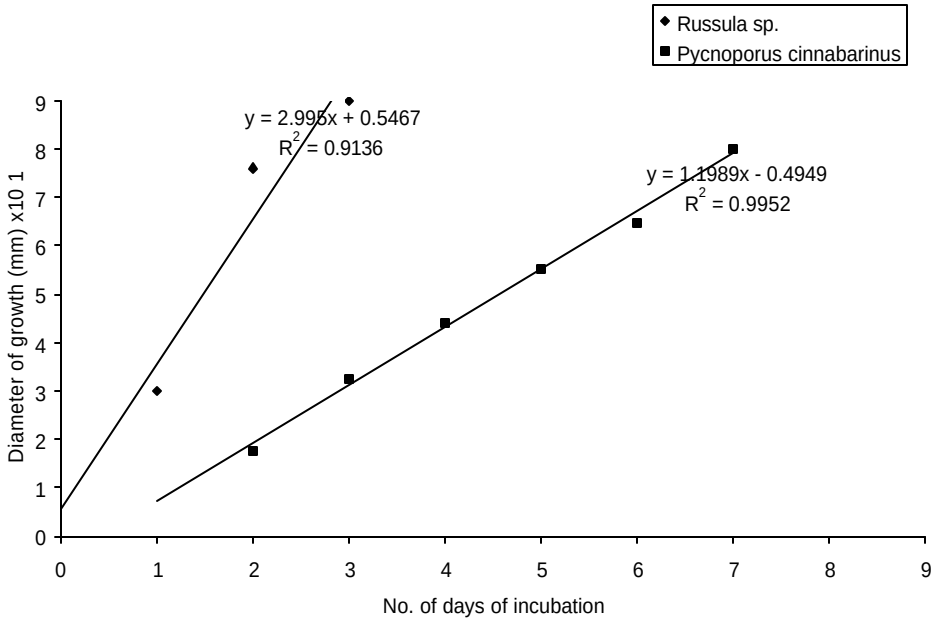

Fig. 1. Linear growth of mushroom on sabouraud dextrose agar

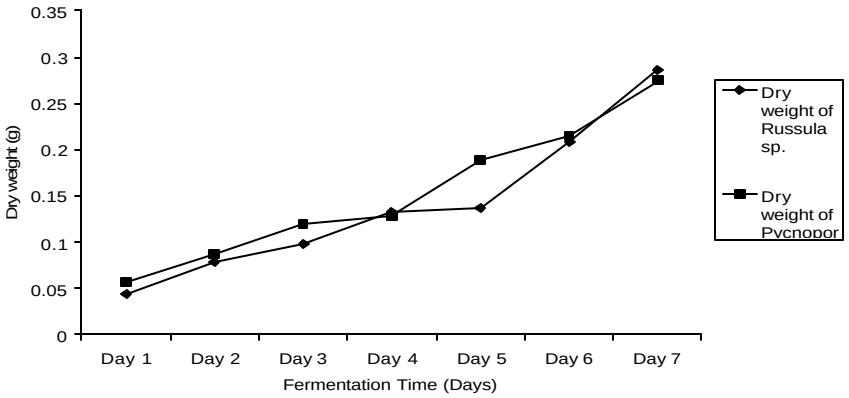

Fig. 2. Growth characteristics of Mushrooms

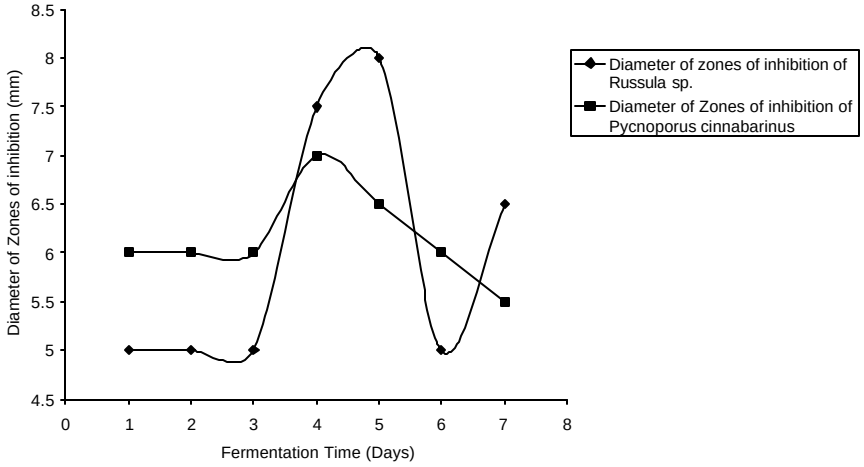

Fig. 3. Production of Bioactive compound by mushroom

***Growth characteristics on* Sabouraud *dextrose liquid medium*.**

The inocula of both mushrooms formed filamentous strands and numerous pellets that were of different sizes. The pellets were covered with tiny, short, and pointed hairs.

Antibacterial test of aqueous fraction of culture filtrate

The antibacterial test of the aqueous fraction obtained after extraction of the culture filterate with ethyl acetate revealed no activity.

Measurement of growth and antibacterial metabolite production

Growth characteristics by dry weight

The growth characteristics by dry weight of *Russula sp.* and *Pycnoporus cinnabarinus* as shown in Fig. 2 showed relative increase in mycelial mass in relation to fermentation time.

Bioassay monitoring of antibacterial metabolite production

The results of the monitoring of the production of bioactive compound in the culture filtrate of the extracts of submerged fermentation by agar diffusion method for *Russula sp.* and *Pycnoporus cinnabarinus* are shown in Figs. 3 respectively. The peak of metabolite production as detected by the activity in *Russula sp* was on the fifth day. The actual production, as deduced from the graph, was between the fourth and the fifth day, subsequently followed by a decrease and then a rise again in activity. For *Pycnoporus cinnabarinus*, the peak of the activity was on the fourth day, with the actual production between the third and fourth day. This was followed by gradual decrease in activity.

DISCUSSION

The mycelia of *Russula sp.* and *Pycnoporus cinnabarinus* were successfully grown on conventional laboratory media. *Russula sp.* was cultivated by spore germination method while *Pycnoporus cinnabarinus* was cultivated through tissue culturing method. In practice, tissue culturing is regarded as the best method of mushroom cultivation (Oei, 1996). It has been reported that spore germination has the disadvantage of taking longer period, with the minute spore size making it relatively difficult to handle (Yu et *al*., 1984; Oei, 1996). However, from this study, it was observed that the spores of *Russula sp.* germinated within the seven days of incubation. The mycelia of *Russula sp.* had a rate of growth of 1.23mm/hour while that of *Pycnoporus cinnabarinus* had a rate of growth of 0.53mm/hour (Fig.1) indicating a higher rate of growth for the *Russula sp.* Spore germination and growth measurement are biological assay indicating the viability of the fungal spores and hyphae (Yu *et al.,* 1984).

The growth and production of antibacterial metabolite of *Russula sp.* and *Pycnoporus cinnabarinus* as shown in Figs. 2 and 3 revealed that dry weight increases with fermentation time while activity was detected in all the fermentation extract. The activity resulting from the first three days could be attributed to an inhibitory effect of the fermentation broth as indicated from the zero day (control) activities. This also revealed that the culture media extract itself exhibited an antimicrobial activity against *B. subtilis* NCTC 8236, which is another form of interference in the activity being measured. The interference could be suggested to have arisen from certain ethyl acetate extractable organic component(s) of the broth, which had become concentrated by extraction, to possibly a growth inhibitory concentration.

For both *Russula sp.* and *Pycnoporus cinnabarinus,* the results observed on the production of antibacterial metabolite showed that, by the fourth day, activity was at the peak or very near the peak (Figs. 3). The fall in activity in both species suggests that the active component is chemically unstable or is being used up by degradation or further converted to other metabolites. The subsequent rise in activity of *Russula sp.* suggests that other metabolite, which is also active, is being formed.

This study had shown the pattern of growth and antibacterial metabolite production by mushrooms collected from the wild. Such a study should be a preliminary experiment, which will help in the location of the appropriate harvest time in submerged fermentation studies.

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