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Research Article

Isolation, Growth and Culture Morphology of *Polyporus grammocephalus*

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Abstract

Cultural studies have played very important role in identification of fungi. The higher fungi show different growth pattern when they are grown in different culture medium. However, considerable work on cultural studies was not carried out in Basidiomycetous mushroom. Therefore, in the present investigation isolation of *Polyporus grammocephalus* culture, its growth, culture morphology both in solid and submerged state condition were studied. The culture was isolated from the fresh fruit bodies of *Polyporus grammocephalus* by carefully removing a small piece (1x1 cm) of the inner tissue and placed on Potato Dextrose Agar medium (PDA) both on test tube slants and on plates that had previously been prepared and incubated at 4°C. Growth studies were done on Potato dextrose agar/broth medium. Changes on the abaxial and adaxial surface were observed in plate culture with PDA medium and recorded.

Keywords: Higher fungi, Basidiomycetes, *Polyporus grammocephalus,* cultural characteristics, growth morphology.

Introduction

Basidiomycetes fungi can be easily grown in artificial medium. It is very important to grow them in medium in order to understand various characteristics of fungi. The higher fungi show different growth patterns when they are grown in different culture mediums. Cultural studies have played a very important role in identification of sporulating fungi. However, in higher fungi, especially in non-sporulating fungi it is not being used widely because of its variations in the culture morphology which is very less so as to distinguish them to different species. The early work on identifying non-sporulating higher fungi was done by Long and Harsch (De and Anjali Roy, 1981). Nobles made an extensive study of the various methods for identification using cultural characteristics. But later on such work was not given importance and is less concentrated. *Polyporus grammocephalus* is a wild edible mushroom distributed widely in tropics including India (Bakshi, 1971). Although distributed very well, less work has been done on this mushroom (Krüger and Gargas 2004; Roy *et al*., 2010; Huang *et al*., 2011; Selvam *et al*., 2012; Giri *et al*., 2012). Keeping these in mind our laboratory has initiated investigation on the species thoroughly, which include the present work on its growth and cultural morphology.

Materials and methods

Isolation: The culture was isolated from the fresh fruit bodies of *Polyporus grammocephalus* by carefully removing a small piece (1x1 cm) of the inner tissue and placed on Potato Dextrose Agar medium (PDA) both on test tube slants and on plates that had previously been prepared and incubated at 4°C. Several such sub-culturing was done until pure cultures were obtained. The pure culture was deposited in mycology lab culture collection (FBFBL 1301C).

Growth studies: Growth studies were done on Potato dextrose agar/broth medium. The agar block was cut by means of sterile cork borer. The inoculum was placed with mycelial face contacting the growth medium. The cultures were kept at dark in the incubator maintained at 28±2°C and were brought to light at the time of examination only. The Linear Downward Growth (LDG) of basidiomycetes mushroom fungi and their mycelia proliferation was studied following the procedure of Rafique (1998). Single agar block (8 mm dia) of 7 d old culture of *P. grammocephalus* was aseptically inoculated into the individual tubes. The time taken for linear downward growth of fungi to reach the bottom of the tube was noted. Mycelial agar blocks (8 mm dia), taken from the margin of 7 d old fungal colonies were inoculated in the center of plates containing PDA medium. After 7 d, the radial growth was measured by noting the radius of the colony from 5th d of growth onwards (Soccol *et al.,* 1994).

Culture morphology: Culture morphology of the mat on the solid medium was studied through observation on different day intervals. Changes on the abaxial and adaxial surface were observed in plate culture with PDA medium and recorded. The same was also grown in test tube slants and the data recorded.

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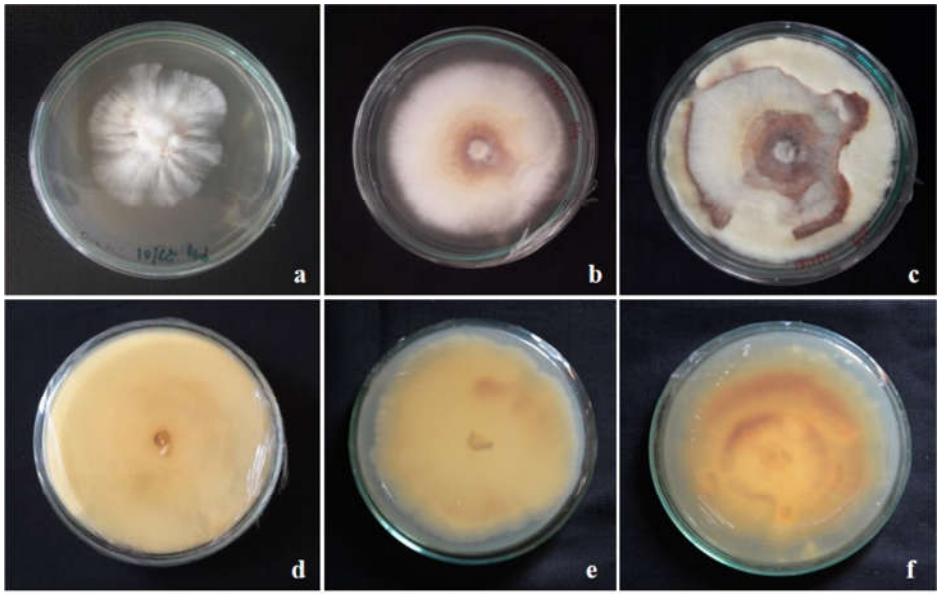
Fig. 1. Radial growth and culture morphology of *P. grammocephalus* on adaxial (a-c) and abaxial surface (d-f).



Morphology of mycelium under submerged culture was also studied through observation on different day intervals both using static and agitated condition. The flasks were incubated as static cultures as well as agitated condition for a period of 21 d and the observations were made at an interval of 7 d. Changes on the culture was observed and recorded in liquid medium (Potato Dextrose Broth).

Results and discussion

The linear downward growth of basidiomycetes mushroom fungi was studied at 28±2°C and the time taken for linear downward growth of fungi to reach the bottom of the tube was noted and the extent of mycelial proliferation which was on the 9th d. The radial growth was measured by noting the radius of the colony from 5th d of growth onwards (Soccol *et al.*, 1994). The full plate coverage was achieved on 11th d of inoculation. The presented data is the result obtained from the triplicate outcome. Changes on the culture surface both on adaxial and abaxial surface was observed. Initially the culture appeared in pure white later it develops tough brown patches arising from the older portions. The culture is cottony white, spreading like cotton threads initially but later becomes leathery, pelliculose and when completely grown on plates having uneven, wrinkled or wavy surface.

The plate culture developed chestnut brown or wood brown patches at maturity usually at the center on the adaxial surface. The browny patches also have uneven surface showing nodular and wavy pattern on the surface with thin film of white mycelium dispersed on the surface of brown patches.

The abaxial surface showed no colour change in the medium but the brown patches on the adaxial surface are visible on the adaxial surface as concentric rings alternating with white colour at maturity (Fig. 1). The test tube slants also showed cottony mycelium but the brown leathery patches appeared in the margins of the test tube slants after 12-15 d of sub-culturing (Fig. 2). The hyphae on the plates are hyaline, thin walled, septate with narrow lumen with sparse branching. The growth characteristics of this isolate is similar to that of the isolate *P. grammocephalus* 7132 reported by Bakshi *et al.* (1969).

Morphology of mycelium in surface and submerged culture was studied through observation on different day intervals. The changes on the culture surface were recorded. The culture in static condition in surface culture showed similar pattern as that of the solid medium but the brown patches are reduced in diameter. But the submerged culture under agitated condition in room temperature showed slight pale yellow color and the parent colony mat is broken into numerous smaller colonies which survive as daughter colonies. But the brown patch development was not observed in agitated condition (Fig. 3). The species showed mycelial branching clearly in the daughter colonies which are lengthy and getting narrow towards the tip with very few mycelia. When the mycelial branches were observed under microscope, the mycelial hairs were found to be loosely arranged and not intact, can be easily separable (Fig. 4). Not much information was found on the submerged mycelium.

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Fig. 2a & b. Linear downward growth of *P. grammocephalus.*

Fig. 3. Growth of *P. grammocephalus* under liquid medium (PDB). a. static condition; b. agitated condition

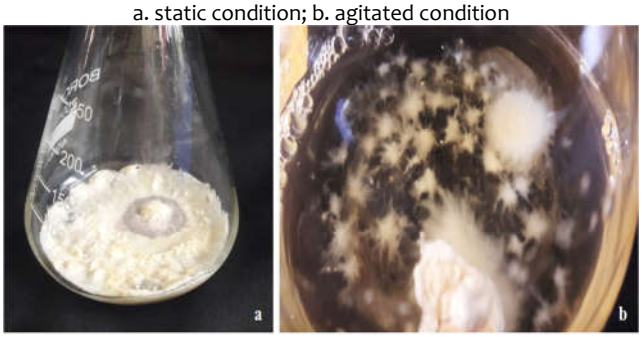
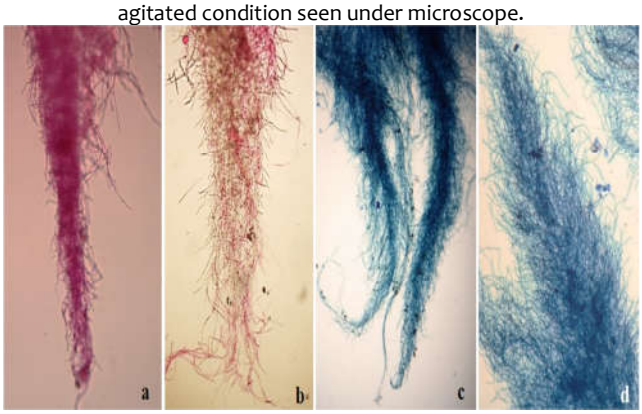


Fig. 4a-d. Pellet morphology of *P. grammocephalus* under agitated condition seen under microscope.



Conclusion

Isolation of *Polyporus grammocephalus* culture, its growth, culture morphology both in solid and submerged state condition was studied. The culture is cottony white, spreading like cotton threads initially but later becomes leathery, pelliculose and when completely grown on plates having uneven, wrinkled or wavy surface. The hyphae on the plates are hyaline, thin walled, septate with narrow lumen with sparse branching. When the mycelial branches were observed under microscope, the mycelial hairs were found to be loosely arranged and not intact, can be easily separable. Further studies may be carried out in detail to study the beneficial effects of this fungus.

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