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Research Article

Agar Culture of Myxomycetes *Fuligo septica* (L.) Wigg. from Alibag Raigad Maharashtra India

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Abstract

Myxomycetes being the promising source of secondary metabolites need to be evaluated for their potential in the development of the drugs. About 100 of novel secondary metabolites have been described till now and only 10% species are cultured so far, thus it is the need of the present to culture these species in laboratories so that they serve the society. The present study thus describes the agar culture of *Fuligo septica* which is apparently the first report from Alibag Raigad, Maharashtra.

Keywords: Agar; Alibag; *Fuligo*; Culture; India

Introduction

Myxomycetes are the small group of eukaryotic organisms that usually occur in association with decaying plant materials. About 1000 species [15] are known worldwide and are mostly overlooked due to the small size of fruiting bodies and the place they choose to grow. Current classification of myxomycetes places them in Super class Amoebozoa and in the first rank Eumycetozoa [1].

Various species of myxomycetes have been found as source of novel secondary metabolites with considerable potential in pharmaceutical industries and more than 100 secondary metabolites have been isolated from myxomycetes [10,37]. But small size of fruiting bodies makes it difficult to extract bioactive compounds [28] thus laboratory cultivation of these species is an effective way to overcome this, so that one can culture plasmodium and obtain secondary metabolites.

However only two species i.e., *Physarum* and *Didymium* among myxomycetes served as the model organisms in terms of agar culture. Out of known species only 10 % species have been cultured so far and about 60% in this is from order Physarales [14]. According to Collins [9] only 85 of the 500 species of myxomycetes have been cultured from spore to spore in laboratory. *Fuligo* from order Physarales have been found to be the source of some important secondary metabolites (Table 1).

Myxomycetes species	Secondary metabolites	References
Fuligo septica	Anthraquinonic acids (acyltetramic acids)	[21]
	Fuligorubin A (acyltetramic acids)	[4]
	Methanolic extract of fruiting	[6]
Fuligo cinerea	Fulicineroside (Activity against gram positive bacteria and crown gall tumors)	[33]
F. septica f. flava	Fuligoic acid (Yellow pigment)	[35]
Fuligo candida	Cycloanthranilylproline (Cytotoxicity against murine leukemia P388 cells)	[27]

Table 1: *Fuligo* as a source of secondary metabolites.

Only two species of *Fuligo* have been cultured in agar namely *Fuligo septica* and *Fuligo cinerea* [7]. Lazo [17] reported the bacterial free culture of *Fuligo septica* while Scholes [34] observed the cultivation, fruiting and germination of *Fuligo septica*.

In terms of laboratory cultivation, most of the studies report moist chamber culture technique while culture on agar is still lacking behind. In India most of the studies explain biodiversity and taxonomy of myxomycetes [5,16,19,20,25,26,32,38,39] while agar culture of myxomycetes is approximately negligible and in Maharashtra state it is totally lacking. This paper represents the first study of agar cultivation of *Fuligo septica* from Maharashtra state of India.

Material and Method

The specimen growing on decaying wood was collected from Alibag (18˚37'47"N 72˚53'22"E) near to Alibag. Alibag is a small coastal town located about 120 km south of Mumbai, Maharashtra. The specimen was immediately glued along with their substratum on moveable papers in plastic boxes. The specimens were air dried to prevent contamination.

To study external morphology the specimens were observed under stereomicroscopes and photographs were maintained. To study internal morphology like nature of peridium, columella, capillitium, spore colour, spore ornamentation etc slides were prepared the results were maintained in the form of photomicrographs.

To classify the specimen up to species level, the literature of M. C. Cooke [8], Massee [24], Lodhi [20], Macbride and Martin [22], Martin and Alexopoulos [23], Lakhanpal and Mukerji [16] and Lister A [18] were used.

For the composition of agar media and techniques, the paper published by Haskin and Wrigley de Basanta [14] was referred. The spores were collected from the fruiting bodies with the help of alcohol flamed forcep or needle. The bottom of the 1.5 water agar (WA) petriplates was divided into four quadrants with the help of fine marking pen. The spores were then inoculated in each of the quadrant by gently touching the forcep to the surface of the agar so that some spores remain submerged and some left on the surface. The areas of spores inoculated in each quadrant of the petriplates were marked in the form of small circles to check the germination easily.

All the petriplates were incubated at 25˚C temperature and 95% humidity in stability chamber. The plates were regularly observed for spore germination and plasmodial formation and the results were maintained in the form of photographs.

Results

Taxonomic details

Fuligo septica (L.) F.H. Wigg.

Fructifications aethaloid; aethalia small or large, pulvinate, sub-plasmodiocarpous, 2-10 cm long, grayish white, black when moist; cortex thick, calcareous, fragile; dehiscence irregular; capillitium reticulate, nodes large, fusiform calcareous, white, internodes small hyaline, non calcareous; hypothallus extensive, white, thick, calcareous; spore mass black, violaceous brown in transmitted light, globose, 7-8 µm in diameter, minutely verrucose.

Substratum

Decaying wood.

Agar culture

Spore germination took place within 24 hrs after sowing on 1.5 WA plates. The germination of spore was by V shaped split in the spore wall. 1.5 WA plates showed active division of myxamoebae and swarmers (Figure 2. A). After 5 days plasmodium appeared on one of the germination plate. The plasmodium was white in colour with prominent veins and small advancing fans (Figure 2. B and C).

Discussion

Germination occur by split method. 1.5 Water agar were found to be the best medium for spore germination and plasmodial formation. Temperature and relative humidity play very important role in life cycle of myxomycetes. The optimum temperature and relative humidity for the growth of *Fuligo septica* is found to be 25˚C and 95% respectively. The life cycle up to plasmodium completed in about 6 days.

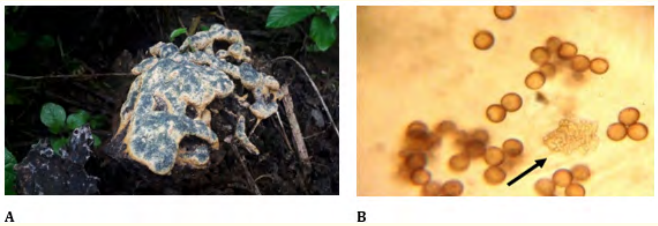

Figure 1: *Fuligo septica*. A. Fruiting bodies, B. Photomicrograph showing lime nodes and spores.

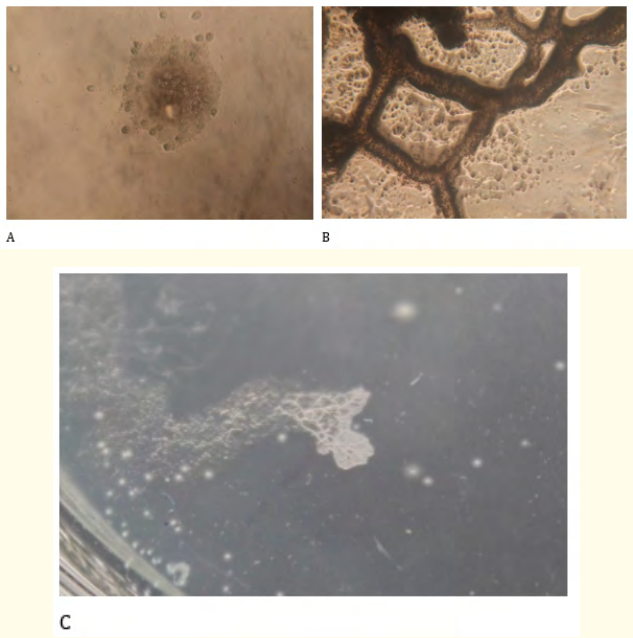

Figure 2: *Fuligo septica*. A. Spores and myxamoebae on 1.5 WA plate, B. Plasmodial veins under microscope, C. White plasmodium on 1.5 WA plate.

Conclusion

The genus *Fuligo* have been reported to show antibiotic activity against Bacillus subtilis and *Candida albicans* and showed cytotoxic activity against KB cells, a cell line derived from human carcinoma of nasopharynx [29]. Hence culture of such important species becomes the need of future.

Moreover, in India most of the studies reports taxonomy of myxomycetes, data regarding nutrition and laboratory culture of myxomycetes is totally lacking. Hence keeping the above fact in mind and knowing the difficulties of culturing these species, agar cultivation of the myxomycetes was taken into consideration. Alibag being a coastal town, the average environmental conditions provide suitable conditions for the growth of myxomycetes.

The spore germination period ranges from few hours to several days [14]. In *Fuligo septica* the spore germination occurs by split method [11] and took place within 24 hrs similar to the results obtained by Scholes [34] while Smith [36] observed spore germination after 30 hrs. Thus, the spore germination in *F. septica* may range from 1-2 days.

Natural flora of bacteria on the substate served as the food source for developing gametes. 1.5 water agar found to be the optimum medium for both spore germination and plasmodial formation. The results were up to plasmodial formation which took about 6 days. More such studies are necessary to know the biology and nutrition of myxomycetes which will enable the agar culture of myxomycetes.

Such agar cultures will not only provide material for DNA sequences to build phylogenies and to know more about their reproductive systems, but at the same time it would help to solve the various questions regarding the difficulties of culturing myxomycetes in laboratory. The present study of agar culture of *Fuligo septica* is thus apparently the first report from India and second report from world.

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