IN VIVO CULTURING OF A SPECIES OF MYXOMYCETES [STEMONITIS AXIFERA BULL]

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ABSTRACT

Myxomycetes have become very important in current research in the field of medicine, biological control of both plant and animal diseases, genetics and pharmaceutical industries. The need to produce these organisms at an industrial level becomes very crucial. This study was aimed at the realization of this objective. In this study, a compost of saw dust from Brachystagia nigerica [780 g or 78% of compost], wheat bran [200g or 20% of compost], and calcium carbonate [20g or 2% of compost] was mixed in tap water to achieve approximately 65% moisture content. A spawn of Ganoderma lucidum [a member of the mushrooms] was inoculated into the compost block and allowed to cool down for 2 hours. After 21 days Stemonitis axifera [the myxomycete] fructifications grew out from the compost.

Key words: Myxomycetes, compost, sawdust, wheat bran, spawn.

INTRODUCTION

Myxomycetes are fungus-like eukaryotes that are the largest subclass of mycetozoa. Traditionally, they are studied by botanists especially the mycologists. They are said to have evolved independently of the fungi from simple flagellate organisms (De Bary, 1887; Macbrille and Martin, 1934). Recently, biochemists, biophysicists and geneticists have recognized the importance of the myxomycetes as a tool in the study of fundamental biological processes, because of their ability to divide in natural synchrony, every eight to ten hours. The vigorous protoplasmic streaming exhibited by the plasmodium of myxomycetes presents an ideal situation for studying cellular motility.

Myxomycetes have also been used as bio control agents (Ejale and Orunwense, 2006). Biological control is an environmentally friendly and effective means of reducing
the amount of inoculums of a pathogen through one or more organisms other than man (Cook and Baker, 1983). It does not constitute health hazards in the environment. Also, biological control frequently implies the exploitation of organisms usually called antagonists in the environment to decrease the capacity of the pathogenic organisms to cause diseases (Baker 1985; David 1990). Biological control is preferred to other control methods because of the increased awareness of safety and toxicity problems associated with fungicides and insecticides.

The use of the plasmodium of a myxomycetes as possible biological control agents of plant pathogenic organisms is as a result of their ability to feed on fungal and bacterial spores. As a group of organisms, the myxomycetes have not been implicated as disease causing agents which is in agreement with the aim of biological control methods to promote safety.

To be able to utilize the myxomycetes as research materials or biological control agents, they must be produced in large quantities. This is what this study was intended to archive.

**MATERIALS AND METHODS**

Plate 1: *Brachystagia nigerica*
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Plate 2: Wheat bran
Plate 3: Calcium carbonate

Plate 4: *Ganoderma lucidum*
Plate 5: Camera lucida

Plate 6: Mycological colour chart
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Treatment:
The culture was prepared by selecting a young and healthy mushroom that was disinfected with 70% alcohol by rubbing a cotton swab. With a sterilized scalpel, approximately one centimeter cube of the tissue was cut and placed at the center of the Petri dish with agar using a sterilized scalpel. The plates were incubated at room temperature (28°C-30°C) for 14 days until the media were fully impregnated with the mycelia.

Spawn production: - The grains were picked to remove extraneous particles, washed in tap water, transferred into clean casserole, and water was added till one inch above the level of grains. One hundred grams of each grain was weighed out washed, while dead and floating grains were removed. The grains were then soaked overnight in water and then washed again. The grains were heated at 100°C with an equal volume of water for twenty minutes after which they were spread on an inclined surface to drain off excess water, leaving the grains just damp (65 to 70% moisture). The bottles were filled up to 1/3 capacity, plugged with cotton wool and covered with aluminium foil. They were autoclaved at 1.1kgcm⁻² for 20 minutes. The bottles were cooled at room temperature and aseptically inoculated with young culture of the mushroom mould. The grains were incubated at room temperature until they were fully impregnated with the mushroom molds. (Fang et al., 2002, Smith et al., 2002).

The sawdust medium used was composed of 780g (78%) sawdust (Brachystagia nigerica), 200g (20%) wheat bran, 20g (2%) percent calcium carbonate or lime. Tap water was added until the mixture attained 65-70% moisture, which was determined by pressing a handful of mixture in the hand until no water ran off in between fingers.

The substrate or mixture was piled in a pyramidal form, covered with plastic sheet for a period of five days, while turning it around at two days intervals, refilling and returning the plastic cover each time. On the fifth day or after the second turning, the piled material was aerated by spreading it thinly in a shaded area to remove the toxic gases that may have been produced during the period of composting. After the acidic smell had stopped, the moisture content was checked and adjusted making sure that it was 65 to 70% (Chang and Miles, 2004).

Compact transparent bags [loosely packed] were used as containers. The substrate was measured into each bag at 10g per bag. A plastic ring was placed at the upper part of the bag to act as a mouth which was plugged with cotton wool, covered with aluminium foil, and finally tied with a rubber band. They were sterilized for an hour and a half by subjecting them to steaming in a container using a big drum.

To propagate the mushrooms, the sterilized and cooled bags were transferred into the inoculation room, which had been previously disinfected. Each bag was aseptically inoculated with the mother spawn earlier prepared in the incubation.
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house, and incubate at 25-28 °C for a month or until fully impregnated with the mushroom mold (Chang and Buswell, 1999, Stamets, 2000).

The line drawings of the various parts of the fructification of the myxomycete was done with the camera lucida, while the mycological colour chart was used to describe the parts. Identification was in accordance with the method of Farr (1976).

RESULTS

On full colonization of fruit bags with mycelium, the colour of the compost block started changing gradually to pale brown. The bags were watered aerially as other bags, but 14 days after the appearance of a pale brown colour, just prior to primordia appearance, the fruiting bags were fully colonized by *Stemonitis axifera*.

**Taxonomic description:**

*Stemonitis axifera* (Bull.) Macbr


Sporangia is cylindric, acuminated, bright rusty brown (RAL 2000), becoming pale brown (RAL 1017), 7.7mm long,

**Plate 7:** Fructifications of *Stemonitis axifera* (Bull.) Macbr that grew from the medium.
fasciculate in small or medium–sized clusters, occasionally in large fruiting arising from membranous hypothallus; stalk black (RAL 9005), shining, 2.7mm long; columella branching freely and evenly, dissipated below the apex; surface net delicate, small-meshed, persistent; spores pale brown (RAL 1017) by transmitted light, nearly smooth or minutely punctuate, 8.4um in diameter.

DISCUSSION
Myxomycetes have been recorded to be seen in various habitats including decaying substrata, on fruits, insects and soil. Cultivation of Myxomycetes is not widespread. This study showed its emergence on the compost block of a medicinal mushroom *Ganoderma lucidum* indicating its close resemblance to fungi.

Fig 1: *Stemonitis axifera* (Bull.) Macbr.

a. fructification before dehiscence (x30); b. fructifications (x2) on habitat and capillitium threads and spores (x500); c. part of columella capillitium and spores(x3000); i. columella; ii. Spores; iii. Peridium; aiv & ivb. Stipe; v. part of columella; vi. 1º capillitium; vii. Spores; viii. 2º capillitium in terms of nutrient needs. The presence of *S. axifera* in the
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Fruiting bags prevented the further growth of *Ganoderma lucidum* mycelium which seem to be completely colonized by the myxomycetes.

This Nigerian collection of *S. axifera* closely resembles the type description except in spore size which is slightly larger (8.4um in diam.) than the type description (5.0um-7.5um in diam.). It is characterized by fasciculate bright grayish brown (RAL 1019) to brown (RAL 8003) sporangia with a black (RAL 9005) stipe. This species is common in West Africa. It was described by Ing and McHugh (1968) from Nigeria and Farr (1959) from Liberia and Canary Islands.

This study supports others that found the most important environmental parameter that affects myxomycete species distribution to be the pH of the substratum (Schnittler and Stephenson 2002; Schnittler et al., 2006; Wrigley de Basanta et al., 2008). Above this, the result obtained in this study gives a strong hope of the possibility of producing myxomycetes in large quantities for both research and other uses of benefit to man.

REFERENCES


