



First Report on Spore to Spore Agar Culture of *Stemonitis axifera* (as per IUBN) T. Macbr. From Maharashtra, India

KEYWORDS

agar medium, lifecycle, sporulation, spore to spore

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ABSTRACT *The present paper describes the cultural details of Stemonitis axifera, a myxomyceteous member that successfully completed its life - cycle from spore to spore in 1.5 Water Agar Medium. The study gives an account of all the life events of the species from germination to sporulation and also describes the effect of major environmental factors i.e. temperature and humidity on growth of said species in agar medium*

Introduction:

Myxomycetes are the unique eukaryotic organisms comprising about 1000 species mainly found associated with decaying plant materials in almost all types of terrestrial ecosystems. It is one of the groups which are mostly neglected in modern research in terms of cultural studies. These forms have characters of both protozoan and fungus. Current classification of myxomycetes places them in Super class Amoebozoa and in the first rank Eumycetozoa (Adl et al 2005).

These are the effective experimental organisms used in many cytological, biochemical and biophysical laboratories to investigate fundamental biological problems such as cell differentiation, cell movement, nuclear division etc. Only 10% species have been cultured from spore to spore till now, mostly belonging to order Physarales and few to order Stemonitales such as *Stemonitis flavogenita*, *S. splendens* (Yang, 1968) and *S. fusca* (Collins, 1939 and Mac Manus, 1961)

Cohen (1939) first reported the axenic culture of *Stemonitis axifera*. Recently Kadam et al (2010) also reported the culture of same species from Marathwada region of Maharashtra but were unsuccessful to obtain both plasmodium and fruiting body. However, in the present work, successful spore to spore culture on agar medium of the said species has been obtained making it fourth species of *Stemonitis* in the order Stemonitales in cultural aspect besides *S. flavogenita*, *S. splendens* and *S. fusca*.

The subclass Stemonitomycetidae contains a single order Stemonitales consisting of approximately 100 species. The present class is distinguished from other subclasses by aphanoplasmodium type of plasmodium that develops into fruiting body (Ross 1973). *S. axifera* is the common species found associated with decaying wood and can be distinguished by its bright rusty brown coloured fruiting body.

Material and Method:

Collection, Preservation and Morphological studies: The study area selected for the study was nearby areas of Alibag Taluka, Raigad district of Maharashtra. The specimen was collected from Tala village in the year 2013 from living tree bark. The area was geo-referenced. Both external and internal morphology of the species were studied and the results were maintained in the form of photomicrographs.

Identification: To classify the specimen up to species level, the literature of M. C. Cooke (1877), Massee (1892), Lodhi (1934), Macbride and Martin (1934), Martin and Alexopoulos (1969), Lakhnupal and Mukerji (1981) and Lister A (1984) were used.

Agar Culture: Both hanging drop and agar plate method was employed to study the spore germination. The germination culture plates were set up on 1.5 water agar medium. In some plates the spores were directly deposited where as some plates were poured with spore suspension in distilled water in order to study the role of water in spore germination. The plates were continuously observed at regular interval for spore germination. Regular subculture was done to clean the cultures. The plates were regularly observed for plasmodial formation and the results were maintained in the form of photographs. All the petriplates were incubated at 22°C-25°C temperature and 95% humidity in stability chamber.

Results:

Germination of spore was by V shaped split in the spore wall. After 24 hrs of sowing, the cavity slides showed the presence of swarmer in hanging drop method, while the same process occurred after 4 days in 1.5 WA germination plates. Soon the population grew rapidly and found actively feeding on bacterial colonies developed in the petriplates as original spore inoculum. No food material was added to the plates.

All the germination plates showed active division of gametes while in some plates the myxamoebae retract pseudopodia and converted into microcysts. Formation of microcysts is the signal of adverse conditions. Plasmodium formation started after 12 days and soon the plasmodium showed flattened thinner plasmodial veins with regular cyclosis in both directions. The type plasmodium was aphanoplasmodium which also showed advancing fans, the character of phaneroplasmodium.

Subcultured 1.5 water agar plates also showed the formation of naked white coloured plasmodium in two patches in the petriplates. After 15 days, both the patches of plasmodium metamorphosed into three white milky fruiting bodies. The colour change observed during formation of fruiting bodies was from white-reddish pink to dark brown.

Thus spore to spore lifecycle of *Stemonitis axifera* completed in about 34 days in water agar plates.

Discussion:

The time period required for germination is from few hours to several days which also depend on the technique used for the germination. The reversion of the gametes into microcysts was observed during cultural studies which indicate the adverse conditions. 1.5 Water Agar medium was found to be best for spore germination, plasmodial formation and sporulation. Water also play a very important role in spore germination, as the spores sown in distilled water for half an hour showed early germination as compare to the direct inoculation of spores. The fruiting bodies formed in the agar plates were found to be somewhat smaller as compared to those found in nature. The time period required to complete life cycle may vary according to the favorable conditions. The best temperature and relative humidity found to be 25°C and 95% for the growth of said species. Thus the life cycle of *Stemonitis axifera* in water agar was completed in about 34 days.

Conclusion:

Cohen (1939) was the first worker who reported the axenic culture of *Stemonitis axifera* and suggested oat agar as satisfactory for pure culture of same species but in the present study weak agar i.e. 1.5 Water Agar was found best for germination, plasmodial formation and sporulation. Kadam et al (2010) also tried the cultural studies of *S. axifera* at 25°C but fail to develop the plasmodium and fruiting body while in the present study the whole life cycle of *S. axifera* i.e. germination, plasmodial formation and sporulation was successfully carried out at 25°C. Water, temperature and relative humidity are the major environmental factors which play very important role in growth of myxomycetes (Phate et al 2014).

Thus from the above reviewed literature, the present study proved to be the first report of spore to spore culture of *Stemonitis axifera* from Maharashtra, India.

TABLE – 1
Details of species habitat and Georeferenced locality

Name of the species	Habitat and Locality	GPS
<i>Stemonitis axifera</i>	Living tree bark, Tala village	18°26'21"N 73°71'54"E

TABLE – 2
Germination time required by the species to different methods used

Method used	Germination time required
Hanging drop	24 hrs
Agar plate	4 days

TABLE – 3
Details of plasmodium formation and Sporulation

Type of plasmodium	Plasmodial formation time required (in days)	Life cycle completed in days (spore to spore)
Aphanoplasmodium	12-15	about 34 days



Figure 1: *Stemonitis axifera* on tree bark.

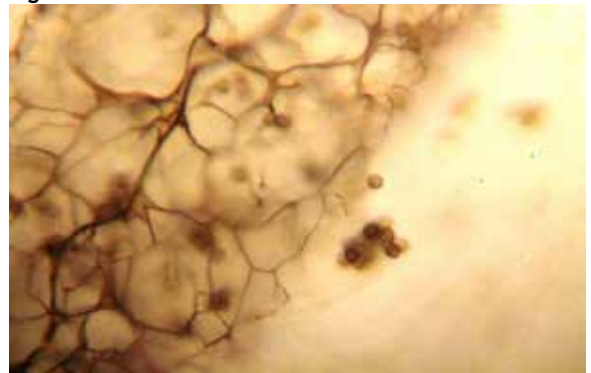
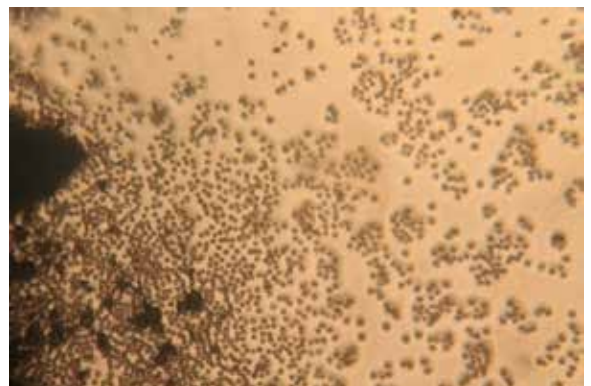


Figure 2: Photomicrographs of *Stemonitis axifera*. A. Capillitial net, B. Spores.



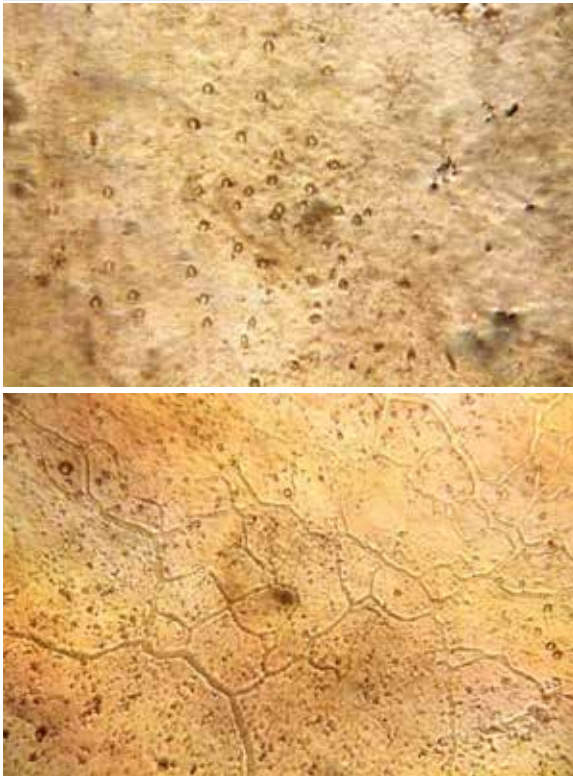


Figure 3: Cultural results on 1.5 Water Agar germination plates. A. Spore deposits on agar surface, B. Myxamoebae and swimmers, C. Aphanoplasmodium.



Figure 4: Cultural results on 1.5 Subculture Water Agar plates.

- A. Young white fruiting bodies,
- B. Reddish pink coloured sporangia.
- C. Dark Brown coloured mature sporangiate fruiting bodies.



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