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Spore to spore agar culture of *Diachea subsessilis*: A new addition to the list of cultivated Myxomycetes

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ABSTRACT:

As myxomycetes were found to be the source of about 100 novel secondary metabolites, it becomes the need of the present to culture and explore them so that they can serve the society. They have also shown the potential for the development of drugs for clinical trials. From about 1000 described species, only 10 % species are cultured so far and of those more than 60% are from the order Physarales. This paper includes the description of spore to spore life cycle of ***Diachea subsessilis*** and apparently the only second know species in the genus cultured so far. The species was directly collected from the field and identified on the basis of its morphology and cultivated on 1.5 water agar.

KEYWORDS: agar cultivation, complete, *Diachea*, life cycle, myxomycetes.

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INTRODUCTION:

Myxomycetes (True slime molds) or Myxogastrids is a monophyletic group of about 1000 species[1], mostly associated with terrestrial habitat like decaying wood and leaves, aerial litter but some unusual habitats were also reported[2,3]. These are the overlooked eukaryotic organisms because of their small size fruiting bodies and the habitat they choose to live. Myxomycetes are currently classified as Myxogastrids and are placed in the Super Class Amoebozoa[4].

Since late 19th century the genus ***Diachea*** Fr. is known for its taxonomic controversy. ***Diachea***, a genus of about 12 species[5,6] was first described by Fries[7], who assigned the genus to family *Trichiacei*, between ***Arcyria*** and ***Stemonitis***. Later the genus was placed in family *Didymiaceae*[8] on the basis of plasmodium type, limy stalk, columella and hypothallus and subhypothallic stalk development. Gaither and Keller[9] suggested the transfer of genus either in *Stemonitidales* on the basis of iridescent peridium and non calcareous capillitium or *Physarales* on the basis of calcareous stalk and columella. Kalyansundaram and Mubarak Ali[10] suggested ***Diachea*** could be a connecting link between *Stemonitidales* and *Physarales* on the basis of biochemical studies of melanin extracted from spores of ***Diachea leucopodia***.

To date, only 10% of species where successfully cultured in laboratory setting mainly from order *Physarales*[11] and the most extensively studied genus are *Physarum* and *Didymium*[12] but the overall biology and the metabolism is still remained elusive (Table no. 1). The main contributing factors responsible for difficulty in culturing myxomycetes are environmental factors such as humidity and temperature, lack of nutritional requirements, improper approaches in culturing methods and insufficient sampling of the species. Indira[13] reported in vitro cultivation of ***Diachea splendens***, which was the only species cultured from the genus ***Diachea*** till now.

Table no. 1: List of cultured myxomycetes from order Physarales

Family	***Species***
Didymiaceae	*Diderma effusum* *Diderma hemisphaericum*
	Didymium annellus, D. annulisporum, D. atrichum, D. circumcissile, D. clavodecus, D. diiforme, D. dubium, D. ermophilum, D. iridis, D. karstenii, D. minus, D. nigripes, D. nullifilum, D. ovoideum, D. saturnas, D. squamulosum, D. trachysporum, D. vaccinum, D. wildprettii, D. operculatum
	Lepidoderma caresteanum
	Diachea splendens***
	Mucilago crustacean
	Physarina echinospora
	Squamuloderma nullifila
Physaraceae	*Badhamia affinia, B. foliicola, B. gracils, B. utricularia, B. rhytidosperma, B. semiannullata, B. spinisporum*
	Willkommlangea reticulate
	Fuligo cinerea, F. septica
	Physarella oblonga
	Physarum aeneum, P. apiculospermum, P. auriscalpium, P. cinereum, P. compressum, P. didermoides, P. flavicomum, P. globuliferum, P. gyrosum, P. leucophaeum, P. leucopus, P. melleus, P. nicaraguense, P. nucleatum, P. nudum, P. oblatum, P. polycephalum, P. pusillum, P. rigidum, P. serpula, P. tenerum, P. vernum, P. wingatense, P. borgoriense, P. bilgramii, P. notabile, P. roseum, P. stellatum, P. straminipes
	Protophysarum phloiogenum

***present paper**

MATERIAL AND METHODS:

The fruiting bodies were collected from decaying leaf in July 2012 from Kankeshwar forest trail (18˚44'66"N 72˚54'51"E), Alibag. Alibag is a small coastal town located about 120 km south of Mumbai, Maharashtra. Kankeshwar is a hill forest and is about 12 km from Alibag and is famous for its old temple of Lord Shiva. The average rainfall, temperature and humidity of this locality provide suitable climate for the growth of myxomycetes. Monsoons are extremely rainy in Alibag during the months of June to September. Frequent heavy downpours are common feature around this time. The maximum amount of rainfall in the month of July can reach up to 750 mm. The temperature ranges from 17-30°C while the humidity ranges between 70-90%.

For the composition of agar media and techniques, the paper published by Haskin and Wrigley de Basanta[14] was referred. The fruiting bodies were gently picked from the substratum and crushed by tapping them on the surface of 1.5 water agar plates in order to release spores. The spores

were placed on the agar surface (Fig. 2a) at each of the four quadrants of 1.5 water agar plates (Fig. 2a). The plates were incubated at 22-25° C at relative humidity 80-90%. The plates were regularly observed for germination and plasmodial formation.

RESULTS AND DISCUSSION:

Spore germination took place between 2 to 5 days after being seeded on 1.5 WA plates. After further 8 days, plasmodium appeared on one of the agar plate. The plasmodium was milky white (Fig. 2d) with prominent veins and advancing fans. The plasmodium often found growing beneath the agar surface. The milky white plasmodium then grew in size and started moving towards the edge of the petriplate. After reaching the edge, soon the whole plasmodium developed into numerous fruiting bodies. The fruiting bodies developed were at outer edge of the petriplate (Fig. 2e) away from the agar surface. The sporocarps thus were observed after 20-30 days from sowing of spores. The sporocarps were then air dried and slides were prepared to note down the taxonomic characters which were similar to those obtained from field.

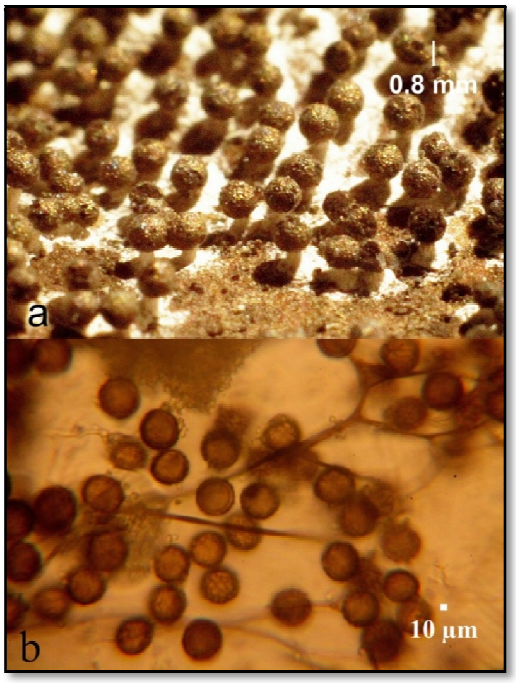

Figure 1: ***Diachea subsessilis*** **a. Sporangiate fruiting body b. Reticulate spores with capillitium**

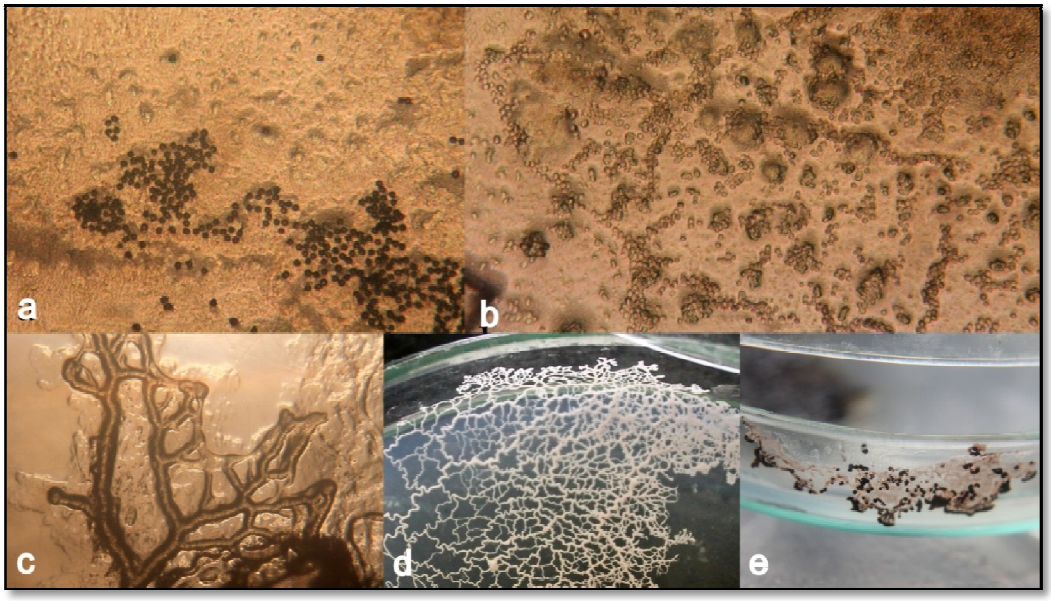

Figure 2: Life cycle of *Diachea subsessilis* on 1.5 Water Agar plates a. Spore deposit on agar surface b. Fusing gametes and young plasmodium c. Plasmodial tracks d. Milky white phaneroplasmodium e. Sporulation.

CONCLUSIONS:

Most of the studies related to myxomycetes in India were mostly taxonomy based[15,16,17,18,19,20,21,22] while the data regarding their laboratory culture and nutrition is negligible. Hence, considering the current situation of myxomycetes research in India and knowing the difficulties of maintaining theses species in laboratory, agar cultivation studies of myxomycetes were taken into consideration.

The average environmental conditions of Alibag provide suitable conditions for the growth of myxomycetes. Within the vicinity of Alibag, Kankeshwar forest was found to be best reservoir for the myxomycetes and thus can be added as a new locality for myxomycetes on Indian map.

In myxomycetes, spore germination varies from hours to days[14]. The technique used in germinating the spores generally affects the rate of germination. In ***D. subsessilis*** the spore germinated in 2 to 5 days after being inoculated on germination plates. Unfavorable condition causes the conversion of gametes to microcysts. The type and quality of food also play major role in myxomycetes growth but in the present study no food material was added and all the cultures were maintained on the bacteria that were grown in the petriplate accidently.

Indira[13] reported the culture of ***Diachea splendens*** on 3% carrot decoction agar and 3% oatmeal agar which is more nutrient rich and totally different from the 1.5 water agar medium used in the present study. The fruiting bodies developed were somewhat smaller and mostly sessile than the fruiting bodies that were obtained from the field. The whole plasmodium migrated away from the agar surface to sporulate. The reason of forming fruiting bodies away from agar surface in both the case is still under question. Both the species completed their life cycle on agar in nearly same time.

The earlier view of considering the genus ***Diachea*** as the connecting link between *Physarales* or *Stemonitidales* which was well supported by biochemical studies of melanin extract from spores, now can be well supported by the recent phylogenetic studies based on various molecular markers (18S rDNA and EF1α) by Fiore-Donno et al[23] where the first clade of dark spored myxomycetes (Columellidia) unites the traditional orders *Stemonitidales* and *Physarales*.

Comparison of nature of plasmodium of ***Diachea subsessilis*** and ***Diachea splendens***, revealed that in both cases the plasmodium was milky white with thin and thick plasmodial veins with small advancing fans. But the type of plasmodium in this case is found to be phaneroplasmodium in contrast to the plasmodium of ***D. splendens*** (intermediate between phanero and aphanoplasmodium). Thus the view of Indira of concluding ***Diachea*** as a connecting link between *Physarales* and *Stemonitidales* on the basis of nature of plasmodium in agar culture is still under question.

Thus cultivation of more species from this genus is encouraged so that the data obtained would throw some light on actual taxonomic position of genus ***Diachea***. 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 and will also help to solve the taxonomic placing of those genus which are still under question mark in phylogenetic tree.

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