Online: ISSN 2771-5868
Print: ISSN 2771-585X

Original Research Article

Agar Cultivation of Myxomycetes *Arcyria denudata* (L.) Wettst. -A potential source of secondary metabolites

Dr. Preeti Vinayak Phate*
J.S.M. College, Alibag, Raigad-402201 Maharashtra
***Corresponding author:** preetiphate.22@gmail.com
Received: January 29, 2023; revised: March 24, 2023; accepted: April 10, 2023
Available Online: May, 2023
https://doi.org/10.17605/OSF.IO/QR5TC

Abstract: Myxomycetes have proven to be the potential source of secondary metabolites but due to smaller size of fruiting bodies extraction of sufficient quantity of bioactive compounds become difficult thus laboratory culture of these species is important. The present paper describes the agar cultivation of *A. denudata* on 1.5 water agar medium. The species being a potential source of secondary metabolites was cultivated in laboratory from spore to spore in 20 days. Moreover, out of known species of myxomycetes only 10% species have been cultured so far and from those 60% species falls in order Physarales. *Arcyria denudata* belonging to order Trichiales is one of the three species cultured so far in the genus and thus the present study is apparently the first report from Maharashtra India.
Keywords: agar, Alibag, *Arcyria*, cultivation, India, spore to spore.

Introduction

Myxomycetes are the small group of eukaryotic organisms that usually occur in association with decaying plant materials. The nomenclatural database of Lado (2005-2019) includes about 1000 species which are worldwide. These species are mostly overlooked due to the small size of fruiting bodies and the place they choose to grow. The life cycle consists of mainly two stages i.e., fruiting bodies and free-living plasmodium. Current classification of myxomycetes places them in Super class Amoebozoa and in the first rank Eumycetozoa (Adl *et al.* 2005).

Various species of myxomycetes have been found as source of novel secondary metabolites with considerable potential in pharmaceutical industries. More than 100 secondary metabolites have been isolated from myxomycetes (Dembitsky *et al.* 2005), but smaller size of fruiting bodies makes one difficult to extract enough bioactive compounds for further screening (Nguyen TN *et al.* 2017). Laboratory cultivation of these species is thus an effective way to overcome this problem, as one can obtain plasmodium and fruiting bodies in large amount through culturing of these species and in turn enough secondary metabolites can be obtained.

According to literature, only 10% species have been cultured till now of which 60% belongs to order Physarales and only two species *Physarum* and *Didymium* among myxomycetes had served as the model organisms as most of the cultured species falls in this genus and found easy to cultivate (Haskin and Wrigley de Basanta 2008). According to Collins (1979) only 85 of the 500 species of myxomycetes have been cultured from spore to spore in laboratory i.e., completed their life cycle in laboratory. In terms of laboratory cultivation, most of the studies report moist chamber culture technique while culture on agar is still inadequate. The order Trichiales

represents about 200 species in nomenclatural database of Lado (2005-2019) of which only few species have been cultured on agar (Table No.1). In India most of the studies explain biodiversity and taxonomy of myxomycetes (Lister 1924, Lodhi 1934, Thite 1975, Thind 1977, Chavan and Kulkarni 1974, Ranade and Mishra 1977, Mishra and Ranade 1979, Lakhanpal and Mukerji 1981, Mishra et al 2013) while study reporting agar culture of myxomycetes is scarce. In Maharashtra state it is totally lacking except few reports (Phate 2019, Phate and Mishra 2014, Phate and Mishra 2014). The present paper thus represents the first study of agar cultivation of *Arcyria denudata* from Maharashtra state of India.

Table 1. List of cultured myxomycetes from order Trichiales.

Family	Species
Dianemaceae	*Calomyxa metallica*
Trichiaceae	*Arcyria cinerea* (Alexopoulos 1960)
	A. denudata (Gilbert 1929)
	A. elaterensis (Mulleavy, 1977)
	Metatrichia vesparium
	Perichaena depressa,
	P. quadrata (Keller and Brooks, 1971),
	P. vermicularis
	Trichia persimilis (Rammeloo, 1976b)
	Hemitrichia serpula (Phate and Mishra 2014)

Table 2. *Arcyria* as a source of secondary metabolites

Sample source	Compound name	Activities	References
Fruiting bodies of *Arcyria denudata*	Arcyriarubin B	Cytotoxic activity against Jurkat cells	Kamata *et al.,* 2006
		Medium inhibiting action against Bacillus brevis and Bacillus subtilisin	Steglich *et al.,* 1980
	Arcyriarubin C	Medium inhibiting action against Bacillus brevis and Bacillus subtilisin	Steglich *et al.,* 1980
	Aryrioxepin A		
Plasmodia of *Arcyria denudata*	Dihydroarcyriacyanin A	Cytotoxic activity against Jurkat cells	Kamata *et al.,* 2006
	Arcyroxocin B		
Fruiting bodies of *Arcyria ferruginea*	Arycriaflavin C	Inhibitor of GLI-mediated transcription	Nakatani *et al.,* 2003

Material and methods

The study area is Alibag which is a small coastal town located about 120 km south of Mumbai, Maharashtra. The specimen growing on decaying wood was collected from Aakshi village ($18^0 62' 40'' N$ $72^0 89' 07'' E$) near to Alibag. 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 and the results were maintained in the form of photomicrographs.

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), Lakhanpal and Mukerji (1981) and Lister A. (1894) were used.

For the composition of agar media and techniques, the paper published by Haskin and Wrigley de Basanta (2008) was referred.1.5 Water agar plates were prepared by adding 15 gm of Bacto agar in 1L of distilled water. 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 25^0C 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

Arcyria denudata (Linnaeus) Wettstein. (Fig.1)

Fructification sporangiate; sporangia stipitate, up to 5 mm in length; sporangia densely gregarious, appearing crowded when dehisced, cylindrical, tapering upward, brickred, fading to brownish with age, 2-3.5 mm long; stipe 1-1.5 mm long, rugose longitudinally, filled with spore like cells of 10-17 ìm width; hypothallus dark brown, rotate; peridium fugacious except for the basal calyculus; calyculus small and plicate; capillitium elastic, brick red or reddish brown, attached to the whole inner surface of the calyculus, basal threads almost smooth, the remaining threads marked with distant cogs, half rings and blunt spines, 3-4 ìm wide, branched and anastomosed to form a network without free ends; spore mass brick red, paler in transmitted light, globose, 6-8 ì m in diameter, marked with scattered warts.

Substratum: Decaying wood

Fig. 1. *Arcyria denudata*. A. Fruiting bodies on wood, B. Photomicrograph showing Capillitium (arrow) and spores (arrow).

Agar culture: Spore germination took place within 24 hrs after being seeded 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 (Fig. 2. A). After 8 days plasmodium appeared on one of the germinationplate. The plasmodium was white in colourwith prominent veins and small advancing fans (Fig. 2. B & C). The plasmodium often found growing beneath the agar surface. The white plasmodium grew and changed to yellow colour (Fig. 2. D) and started moving towards the edge of the petriplate. After 15 to 20 days sessile fruiting bodies were seen. The fruiting bodies were found to be stalkless (Fig. 2 E & F). and poorly

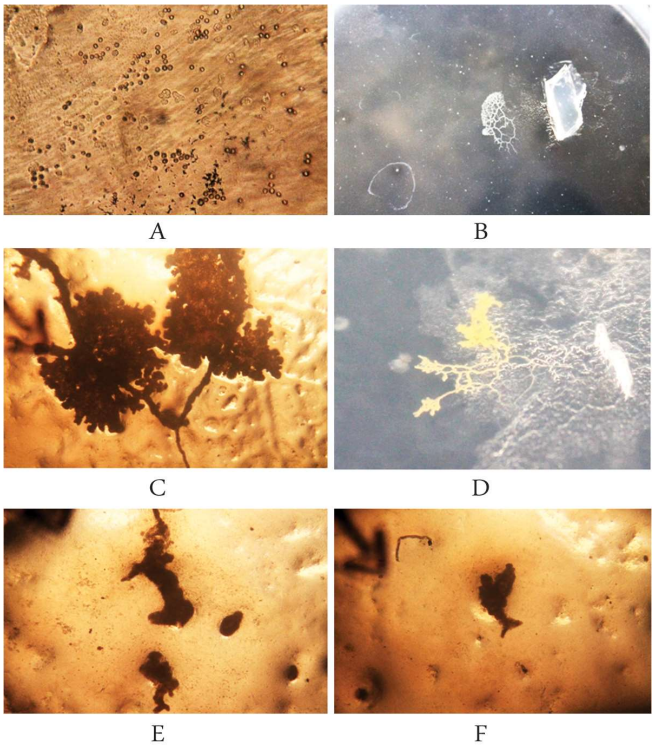

Fig. 2. *Arcyria denudata*. A. 1.5 WA plates showing Spores and feeding myxamoebae (100x), B. White plasmodium on 1.5 WA subculture plate, C. Advancing fans of plasmodium under microscope (100x), D. Colour change of plasmodium colour from white to yellow, E and F. Stalkless sporangia in 1.5 WA plates (100x).

developed. Thus, spore to spore lifecycle of *Arcyria denudata* completed in about 20 days in 1.5 water agar plates.

Discussion

The members of order Trichiales such as *Arcyria denudata* have been reported to produce some important secondary metabolites (Table 2) which have unusual structures and exhibit phosphorescence phenomena (Steglich, 1989). For instance, Arcyriarubin B isolated from fruiting bodies of *A. denudata* and Arcyroxocin B and Dihydroarcyriacyanin A isolated from plasmodium of *A. denudata* showed cytotoxic activity against Jurkat cells (Kamata *et al.,* 2006, Nakatani *et al.,* 2003). Hence culture of such important species becomes the need of future.

Germination occurs by split method. The spore germination period ranges from few hours to several days (Haskin and Wrigley de Basanta 2008). In *A. denudata* the

spore germination occurs by split method (Gilbert 1928) and took place within 24 hrs. 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.

Fruiting bodies obtained in 1.5 water agar were found to be sessile rather than stalked and poorly developed as those found in nature, which may be due to restricted environmental condition. Water, temperature and relative humidity play very important role in life cycle of myxomycetes. A thin film of water is found to be essential for development of myxamoebae and initial growth of plasmodium. The optimum temperature and relative humidity for the growth of *Arcyria denudata* is found to be 25^{0}C and 95% respectively. The spore-to-spore life cycle of *A. denudata* completed in 20 days on 1.5 water agar plates.

In India most of the studies reports taxonomy of myxomycetes andknowledge regarding nutrition and laboratory culture of myxomycetes is inadequate. Thus, 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.

Gilbert (1929) first reported the spore-to-spore agar culture of *A. denudata*. The present studies thus apparently the first report from India and second report from world. 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.

Acknowledgement

The author is grateful to the Principal Dr. A.K. Patil, J.S.M. College for constant support and guidance. The author is also thankful to Dr. R.L. Mishra former Head of Botany Department, J.S.M. College Alibag for introducing me to the world of myxomycetes.

References

Adl SM, Simpson AGB, Farmer MA, Andersen RA, Anderson OR, Barta JR *et al.,* 2005. The new higher level classification of eukaryotes with emphasis on the taxonomy of protists. Journal of Eukaryotic Microbiology. 52: 399-451.

Alexopoulos CJ. 1960a. Gross morphology of the plasmodium and its possible significance in the relationships among Myxomycetes. Mycologia. 52: 1-20.

Alexopoulos, CJ. 1960b. Morphology and laboratory cultivation of *Echinostelium minutum* de Bary. Am. J. Bot. 47: 37-43.

Chavan PD and Kulkarni UV. 1974. Additions to the fungi from Maharashtra, India. M.V.M. Patrika. 9: 132-139.

Clark J. 1995. Myxomycete reproductive systems: additional information. Mycologia. 87: 779-786.

Cooke MC. 1877. Myxomycetes of Great Britain.

Collins RO. 1979. Myxomycete Biosystematics. Some Recent Developments and Future Research Opportunities. Botanical Review. 45: 145-201.

Dembitsky VM, Øezanka T, Spížek J and Hanuš LO. 2005. Secondary metabolites of slime molds. Phytochemistry. 66: 747-769.

Gilbert FA. 1928. A study of the method of spore germination in Myxomycetes. Am J. Bot. 15: 345-352.

Gilbert FA. 1929. Spore germination in the Myxomycetes: A comparative study of spore germination by families. Am. J. Bot. 16: 421-432.

Gray WD and Alexopoulos CJ. 1968. Biology of the Myxomycetes. Ronald Press, New York. Pp.: 288.

Haskins EF and Wrigley de Basanta D. 2008. Methods of agar culture of Myxomycetes – an overview. Revista Mexicana de Micologia. 27: 1-7.

Hosoya T., Arai M., Koyano T., Kowithayakorn T. and M. Ishibashi M. 2008. Naturally occurring small-molecule inhibitors of hedgehog/GLI-mediated transcription. Chembiochem. 9: 1082-1092.

Kamata K, Suetsugu T, Yamamoto Y, Hayashi M, Komiyama K and Ishibashi M. 2006. Bisindole alkaloids from myxomycetes *Arcyria denudata* and *Arcyria obvelata*. J. Nat. Prod. 69: 1252-1254.

Keller and Brooks TE. 1971. A new species of *Perichaena* on decaying leaves. Mycologia. 63: 657-663.

Lado C. 2005-2019. An on-line nomenclatural information system of Eumycetozoa. Available at http://eumycetozoa.com/

Lakhanpal TN and Mukerji KG. 1981. Taxonomy of Indian Myxomycetes. J. Cramer. Bibliotheca Mycologia. 78: 1-531.

Lister A. 1894. A monograph of the Mycetozoa. London.

Lister G. 1924. Mycetozoa from North India. Journal of Botany. 62: 16-20.

Lodhi SA. 1934. Indian slime moulds (Myxomycetes). Univ. of Punjab, Lahore.

Macbride TH and Martin GW. 1934. The Myxomycetes. New York: Macmillan Co. Pp.: 339.

Martin GW and Alexopoulous CJ. 1969. The Myxomycetes. Iowa City: University of Iowa Press.

Massee G. 1892. A monograph of the Myxomycetes, London.

Mishra RL and Ranade VD. 1979. Myxomycetes of Maharashtra. II. The genus *Diderma* Pers. Maharashtra Vidnyan Mandir Patrika. 14: 33-41.

Mishra RL, Phate PV and Ranade VD. 2013. Myxomycetes of Maharashtra: two new species of *Diderma* Pers. Mycosphere. 4(5): 865-869.

Mulleavy P. 1977. The description and laboratory cultivation of *Arcyria elaterensis*, a new species of Myxomycetes. Mycologia. 69: 693-700.

Nakatani S, Naoe A, Yamamoto Y, Yamauchi T, Yamaguchi N and Ishibashi M. 2003. Isolation of bisindole alkaloids that inhibit the cell cycle from myxomycetes Arcyria ferruginea and Tubiferacasparyi. Bioorg. Med. Chem. Lett. 13, 2879-2881.

Nguyen TN Nguyen, Tuyen TM Huynh, Steven L. Stephenson and Hanh TM Tran. 2017. Study of the biological activities of *Physarum polycephalum* and *Physarella oblonga* plasmodial extracts, Mycosphere. 8: 968-976.

Phate PV. and Mishra RL. 2014. Culture of *Hemitrichia serpula* on wide range of agar medium, Int. J. Curr. Microbiol. Appl. Sci. 3: 480-488.

Phate PV and Mishra RL. 2014. First report on spore to spore agar culture of *Stemoniti saxifera* T. Macbr. From Maharashtra India. Indian Journal of Applied Research. 4(11): 82-84.

Phate PV. 2019. Spore to spore agar culture of *Diachea subsessilis*: A new addition to the list of cultivated myxomycetes. International Journal of Scientific Research and Reviews. 8(3): 505-512.

Rammeioo J. 1976b. Spore to spore culture of *Trichia persimilis* Karst. Bull. Jard. Bot. Nat. Belg. 46: 403-406.

Ranade VD and Mishra RL. 1977. Myxomycetes of Maharashtra-III. M.V.M. Patrika. 12: 25-27.

Steglich W, Steffan B, Kopanski L and Eckhardt G. 1980. Pilzpigmente, 36. Indolfarbstoffeaus Fruchtko¨rpern des Schleimpilzes *Arcyria denudata*. Angew. Chem., Int. Ed. Engl. 19, 459-460.

Steglich W. 1989. Slime moulds (Myxomycetes) as a source of new biologically active metabolites. Pure and Applied Chemistry. 61(3): 281-288.

Thite AN. 1975. New and noteworthy fungi from Maharashtra. M.V.M. Patrika. 9: 117-120.

Thind KS. 1977. The Myxomycetes of India. Indian Council of Agricultural Research, New Delhi. 1-452.