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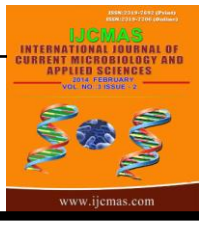

Original Research Article

Culture of *Hemitrichia serpula* on wide range of agar medium

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K e y w o r d s

Indigenous corn flour agar; myxomycetes; relative humidity;

A B S T R A C T

Among the known species of myxomycetes, *Hemitrichia serpula* is one of the most distinctive myxomyceteous genus that do not fall in the list of 10 % spore to spore cultured species. This is the first attempt to culture the selected specie on a wide range of agar medium. The aim is to study the life cycle on culture plates and also the impact of temperature and relative humidity on the growth. In the present study a new media named Indigenous Corn flour agar was formulated and found to be excellent for the growth of said species.

Introduction

Myxomycetes are the small, homogenous group of fungus-like organisms, with approximately 700 species known worldwide. They are the nature's most extraordinary creatures and are often known as True slime molds or Acellular Slime Molds. Myxomycetes are typically collected as fruiting bodies and plasmodia and occur wherever the conditions are suitable for them on earth's surface.

Only *Physarum* and *Didymium* (Aldrich & Daniel, 1982) serves as an excellent model system for study but give only a partial view of the biology of the Myxomycetes and hence aim is to get a wide range of Myxomycetes into agar culture which can be further used as material for student research project and such material will also serve as primary research organisms in laboratories around the world.

The main aim of the present study is to discuss the cultural aspect of *Hemitrichia serpula* on different agar medium and also the effect of relative humidity and temperature. Most of the easily cultured species are of the order Physarales and no extensive studies have been carried out on the nutritional requirements of the slime molds, so aim is to find out the nutritional requirement of the selected species i.e., *Hemitrichia serpula*.

Materials and Methods

Collection

The sampling was done in the period 2010-2013 from different localities like Alibag, Aakshi and Jirad of Raigad District (Maharashtra) throughout the year. All the localities were geo referenced. The

specimens were immediately glued along with substratum in plastic boxes, match boxes or cardboard boxes but plastic boxes were found to be best for storage purpose. The specimens were air dried to prevent contamination with other organisms and unique numbers were given to the specimens to carry out the work with ease. A paper containing the details like name of specimen, date of collection, place of collection, habitat etc. was mounted on each plastic box. To study the external morphology the specimens were observed under stereomicroscope and photographs were taken.

Slide preparation

Temporary slides were prepared to study the internal morphology like nature of peridium, columella, capillitium, spore colour, spore ornamentation etc. of the specimens. For semi permanent preparation of slides, mounting media like Amann's lactophenol medium and Hantsch's fluid (Martin and Alexopolous, 1969) were used. A microscope equipped with an ocular micrometer and oil immersion objective was used for measurement of spore size, capillitium diameter etc. Photomicrographs were taken showing the details of internal structure.

Identification

For identification of material up to species level, the literature of Lakhanpal and Mukerji (1981) was referred.

Culture

The spores from the plasmodiocarpus type of fruiting body were collected with the help of alcohol flamed forcep or needle. Wide range of agar media like 0.75 Water Agar (0.75 WA), 1.5% Water Agar (1.5 WA), Asparagine Mannitol Agar, weak Yeast Extract Agar, Carrot Agar, Indigenous Corn flour Agar, Oat Meal Agar and Extract Agar were tried to observe the details of growth of species. Extract agar media was prepared by soaking the natural substrate of *H. serpula* (50 gm in 1liter DW) for 24 hrs and filtering the supernatant through cheese cloth. The volume was then makeup with DW up to 1 liter and then agar was added. A new Indigenous Corn flour agar recipe was formulated in the present study and tried to observe the growth of the species.

The bottom of the 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 regularly and easily. The plates were incubated in stability chamber at various ranges of temperature and relative humidity.

Spore germination was found in days to few months and was observed by inverting the petriplates, still closed, on the stage of a compound microscope from which the clips had been removed. The petriplates were generally found associated with filamentous fungi. In order to clean the culture, sub - culturing was carried several times by transferring the clear blocks of agar containing the myxoamoebae and swarmers to a new agar plate. Another method used for sub-culture was to find the clear area of agar containing the myxoamoebae and swarmers, adding a drop of water on that area and transferring them with the help of capillary tube and touching the same tube in new petriplates repeatedly releasing the same.

Some 1.5 WA culture plates with spores were flooded with 2ml DW on the surface of agar to observe the role of distilled water in spore germination and plasmodial formation.

Hanging drop method was also used to get the successful germination of spores in cavity slides. The spores germinated in cavity slides were then transferred to the agar plates of different media.

Different methods were tried to get the successful germination of spores in hanging drop and on culture plates. Some agar plates were inoculated with spores soaked in distilled water for half an hour, some with spores treated with 1% bile salts for 30 minutes and 0.001% Tween 80 for 7-10 min respectively. Similar treated spores were also tried to germinate in hanging drop method.

The above said media were continuously tried to get the successful culture. Some agar plates containing the swarmers and myxoamoebae were also sprinkled with sterilized oat to get the results.

The variations in temperature and relative humidity were done to note down the cultural changes. All the results obtained were maintained in the form of photographs.

Results and Discussion

The observation and results were tabulated (tables 1-5).

After trying the various ranges of temperature and relative humidity, it is found that the standard temperature and relative humidity for germination of spores and young plasmodium development in *Hemitrichia serpula* is 25˚C and 95% respectively which also suggest that temperature and relative humidity are the major environmental factors which play very important role in the life cycle.

Successful germination of spores of *H. serpula* was found to be in Distilled water in both Hanging drop method and Culture plates. The spores treated with 1% Bile salts (Elliott, 1948) germinated in 5-10 days in 1.5 WA plates. The treatment with 0.001% Tween 80 (Indira, 1969) was found to be ineffective in this case.

It was found that the germination rate was more in plates with spore suspension (spores soaked in DW for half an hour) than plates where the spores were direct inoculated in four quadrants which suggest the role of water in germination of spores of *H. serpula*.

From the wide range of media used in the study 1.5% Water Agar, Oat Agar and Indigenous Corn flour Agar were found to be best for the germination, fusion of gametes and formation of plasmodium. The Carrot Agar medium strict to be good for subculture. Asparagine Mannitol agar and weak Yeast extract agar remain ineffective. Degree of growth on Extract agar was found to be less during the studies. The new Indigenous Corn flour Agar media used in the study shows excellent result for both spore germination and plasmodial formation. The specie showed germination in 2-4 days and plasmodium tracks arises in 20 days on said culture plates.

Table.1 Details of the Georeferenced localities

Name of the specie	Habitat	Year of collection	Locality	GPS
Hemitrichia serpula	Dried leaves of *Cocos nucifera*	2010-2013	Alibag	18˚38'35" N,72˚52'14"E
			Aakshi	18˚37'47" N,72˚73'22"E
			Jirad	18˚45'25" N,72˚54'14"E

Table.2 Spore germination (Temperature - 25˚C and Relative Humidity - 95%)

Spore suspension in	Spore germination	
	Hanging drop	Culture plate
Distilled water	+	+
Tween 80	-	-
Bile salts	+	+

Germination: Absent (-) & Present (+)

Table.3 Degree of growth on different culture media at given temperature and relative humidity

Medium used	Growth			
	T- 22˚C and RH 85%		T-25˚C and RH 95%	
	Spore germination	Formation of plasmodium	Spore germination	Formation of plasmodium
1% Water Agar	-	-	+	+
1.5% Water Agar	-	-	+++	+++
Asparagine Mannitol Agar	-	-	-	-
Weak Yeast Extract Agar	-	-	-	-
Indigenous Corn flour Agar	-	-	+++	+++
Oat Agar	-	-	+++	+++
Carrot Agar	-	-	-	+++
Extract Agar	-	-	+	+

Degree of growth: Absent (-), Medium (+), Good (++), Very good (+++)
T- Temperature and RH- Relative Humidity

Table.4 Comparison of spore germination time period (days) on different agar

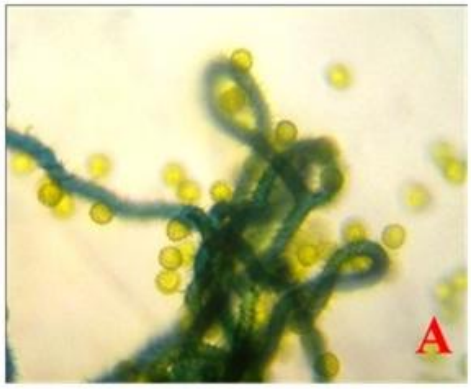
Medium used	Germination time required (days)
1.5 % Water agar	1-4 days
Indigenous Corn flour agar	2-4 days
Oat agar	2-4 days

Table.5 Time period required (days) for plasmodial formation in *Hemitrichia serpula* on 1.5 Water agar media and Indigenous Corn flour agar

Medium	Germination time required (days)	Plasmodium formation (days)
1.5% Water Agar	1-4 days	15days
Indigenous Corn flour agar	2-4 days	20 days

Figure.1 Plasmodiocarpus fruiting body of *Hemitrichia serpula* on dried leaves of *Cocus nucifera*.

Figure.2 Photomicrographs of ***H. serpula*** A- Capillitium and Spores. B- Reticulate spores.



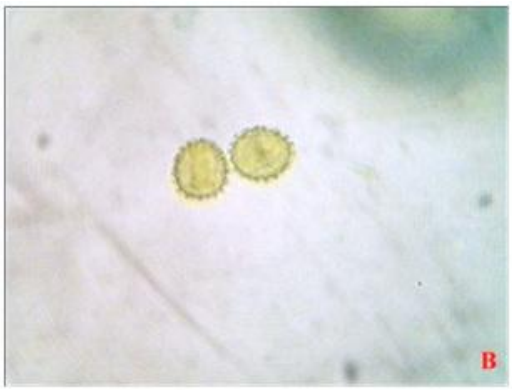


Figure.3 Spore germination on 1.5 WA plates at pH 5.6, 25˚C and RH 95% spores treated with 1% Bile salts.

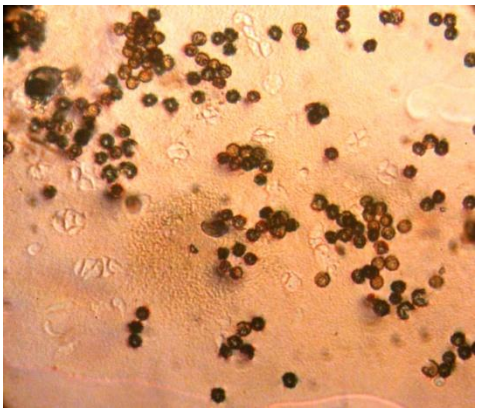


Figure.4 Culture of ***H. serpula*** on 1.5 Water Agar plates at pH 5.6, 25˚C and RH 95%, A- Spore germination and wandering myxoamoebae and swarmers. B – Myxoamoebae and swarmers trying to fuse. C – Fusion of gametes to form plasmodium. D - Initiation of plasmodium.

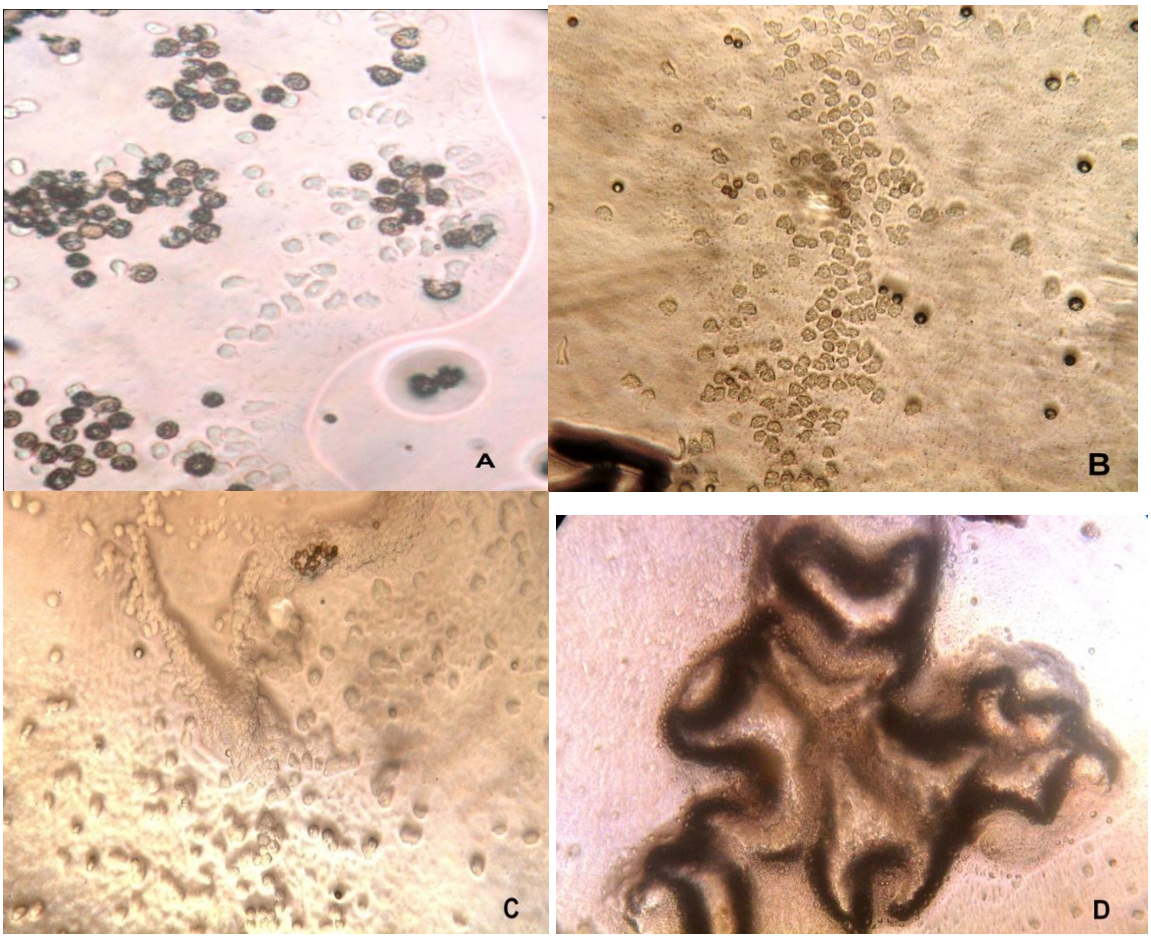


Figure.5 Subculture results on Carrot agar plates at pH 6.5, 25˚C and RH 95%, A- Myxoamoebae and swarmers. B - Young plasmodium on bacterial colonies.

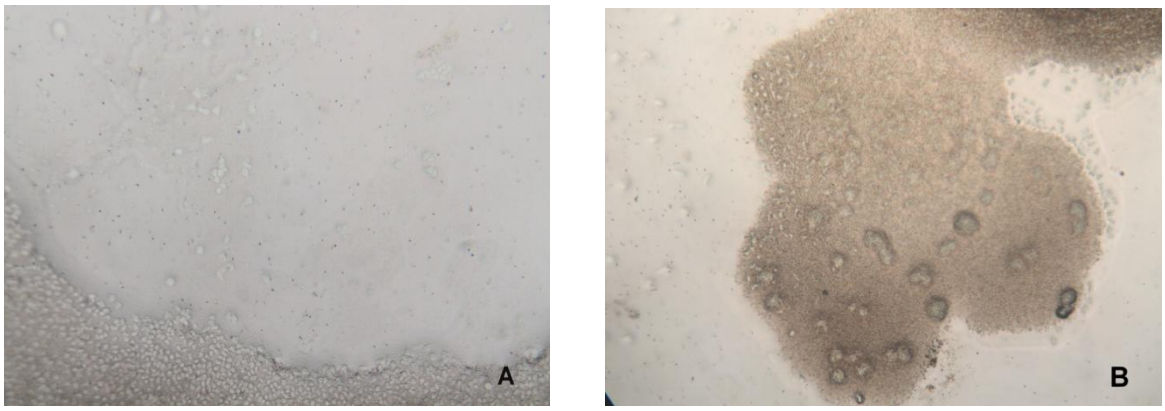


Figure.6 Culture of ***H. serpula*** on Indigenous Corn flour agar at pH 6.5, 25˚C and RH 95%, A- Myxoamoebae and swarmers feeding on the agar plate. B- Formation of young plasmodium. C – Plasmodium D – Plasmodial tracks on agar plates

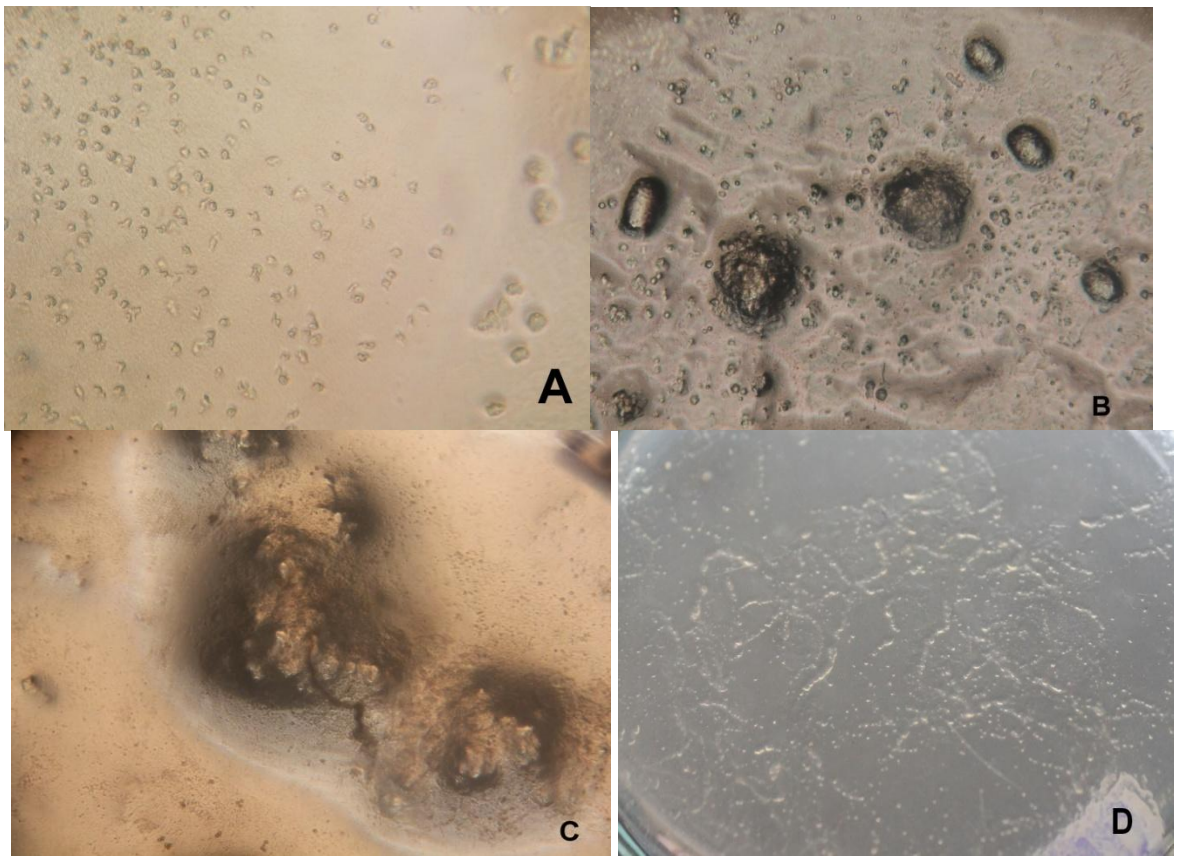
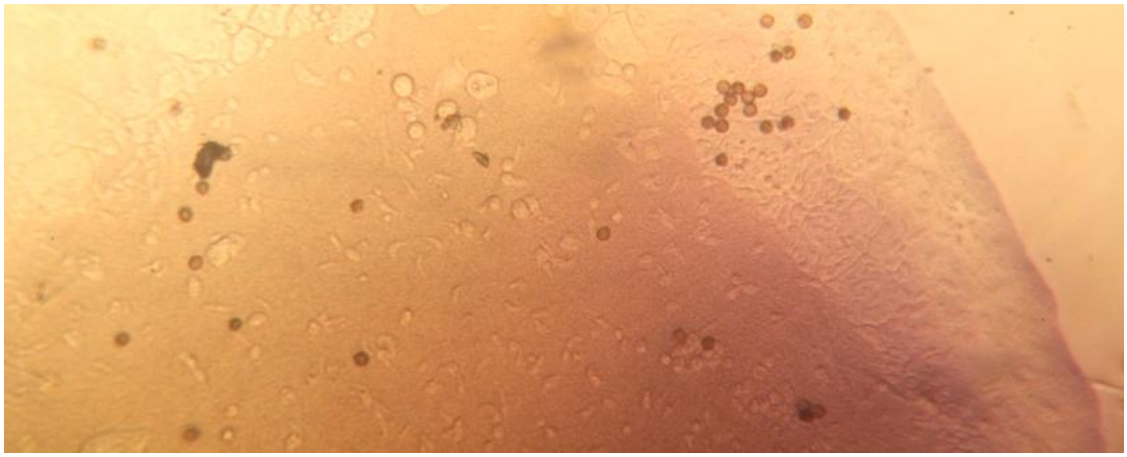


Figure.7 Spore germination on Oat agar plates at pH 6.5, T- 25˚C and RH 95%.



During study it was found that, 1.5 WA plates containing the gametes when flooded with distilled water give rise to early plasmodium as compare to the 1.5 WA plates without distilled water which again suggest the role of distilled water in formation of plasmodium in *Hemitrichia serpula*. No bacterial food material was added to the plates, all the cultures were grown on the original food inoculums grown along with the culture. The above results clear that the one can use high nutrient medium like Indigenous Corn flour Agar and Oat Agar for spore germination instead of routinely using 1.5 Water Agar and 1 % Water Agar. Hence the study somewhat had broadened the range of nutrition of *Hemitrichia serpula*. Once such nutritional range is available, it can be said that the percentage of cultured species among known Myxomycetes had increased to some extent.

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References

Alexopoulos CJ, Mims CW, and Blackwell M. 1996 - Introductory Mycology, 4th edition, John Wiley & Sons, Inc.

Aldrich HC and Daniel JW. 1982 - Cell biology of *Physarum* and *Didymium*. Academic Press, New York. 444p, 373p.

Balaji S, Sujatha A and Kalyanasundaram I. 1999 - A simple rapid procedure for obtaining axenic cultures from monoxenic cultures of myxomycete plasmodia. Can. J. Microbiol. 45, 865-870.

Bastana W, Lado and Estrada T. 2011 - Spore to spore culture of *Didymium operculatum*, a new myxomycete from the Atacama Desert of Chile. Mycologia, 103 (4), 895-903.

Chin LH and Chang JH. 2012 - Six genera of Physaraceae in Taiwan. Taiwania, 57(3), 263-270.

Davison EM, Davison PJN and Brims MH. 2008 - Moist chamber and field collections of myxomycetes from the Northern Simpson Desert, Australia. Australasian Mycologist 27(3), 129-135

Gray WD and Alexopoulos CJ. 1968 - Biology of the Myxomycetes. Ronald Press Company, New York. 288p.

Gray WD. 1955 - The laboratory cultivation and development of the myxomycetes *Physarella oblonga* and *Physarum didermoides*. The Ohio Journal of Science 55(4): 212.

Gray WD. 1955 - Riboflavin synthesis in cultures of *Physarum polycephalum*. The Ohio Journal of Science 55(4): 212.

Haskin, Edward F, Wrigley B, Diana. 2008 - Methods of agar culture of myxomycetes: an overview. Revista Mexicana de Micologia, Vol. 27, pp 1-7.

Harold WK. 2008 - Myxomycete plasmodia and fruiting bodies: Unusual occurrences and user friendly study techniques. Spring Vol 1:1, 24-37.

Henney HR and Henney MR. 1968 - Nutritional requirements for the growth in pure culture of the myxomycete *Physarum rigidum* and related species. J. Gen Microbiol. 53, 333-339.

Keller HW and Everhart ES. 2010 - Importance of myxomycetes in Biological research and teaching. Fungi. Vol 3:1, 13-27.

Rakoczy L. 1998 - Plasmodial pigmentation of the acellular slime mould *Physarum polycephalum* in relation to the irradiation period. Polish Journal of Environmental Studies. Vol 7, 337-342.

Spiegel FW, Haskin EF, Cavender JC, Landolt JC, Lindley LA, Edwards SM, Nderitu G and Shadwick JD. A beginner's guide to isolating and culturing Eumycetozoans. Online at http://slimemold.uark.edu/pdfs/isohandbook.pdf

Scholes PM. 1961 - Some observations on the cultivation, fruiting and germination of *Fuligo septica*. J. Gen. Microbiol. 29, 137-148.

Winsett KE, Stephenson SL. 2011 - Global distribution and molecular diversity of *Didymium difforme*. Mycosphere 2(2), 135-146.

Wolfgang S. 1989 - Slime moulds as a source of new biologically active metabolites. Pure and Appl. Chem, Vol 61. 281-288.

Wrigley B, Lado E. 2011 - Spore to spore culture of Didymium operculatum, a new myxomycete from the atacama desert of chile. Mycologia 103(4) pp 895-903