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Research Article

AN INTRODUCTORY STUDY ON A FOLIICOLOUS LICHEN FOUND ON COMMON TREES IN KERALA, INDIA: CHARACTERIZATION OF ITS SYMBIOTIC PARTNERS

Reshma. R. S* and Joseph Job

Department of Botany, St. Berchman's College, Changanacherry, Kottayam, Kerala - 686 101, India

A R T I C L E I N F O

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A B S T R A C T

Lichens are composite living organisms formed by the symbiotic relationship between the photobiont and the mycobiont. Foliicolous or epiphyllous lichens are those that grow on the leaves of vascular plants. In the present study we investigated the formation and reproduction of a *Phyllophiale* like foliicolous lichen which inhabited the leaves of almost all common trees in Kerala, India, especially *Mangifera indica*, *Ixora coccinea*, *Mesua ferrea, Syzygium cumini and Garcinia mangostana*. Morphological examination of the lichen peels revealed *Phycopeltis* as the major photobiont. *Trebouxia* cells were also found to be associating with the lichen. The study showed the presence of a fungal associate in all the collected lichen samples. We could culture the fungus *in vitro*. DNA barcoding using primers of ITS showed that fungal associate may be a member of Botryosphaeriaceae. *In vitro* culture of lichen seemed to produce many structures which were not observed during their natural development. The study also attempted to investigate the effect of the foliicolous lichen on the rate of photosynthesis of the host plant and found that that the presence of the lichen on the leaf surface has no retarding effect on the rate of photosynthesis.

INTRODUCTION

Lichen is a beautiful example of mutualistic symbiosis between fungi and algae or cyanobacteria. The fungal and algal partners were called mycobiont and photobiont respectively. The algal and/or cyanobacterial partners were photosynthetic. They provided carbohydrates and vitamins to the fungus. Cyanobacteria could fix atmospheric nitrogen. The fungus, in turn, protects its partners from desiccation and shades them from strong sunlight.

Development of new thallus takes place only when the environment is favorable. Even under such conditions, lichen thallus could develop only if both symbionts were present in the neighborhood. Lichens commonly reproduced when fragments of both algal and fungal tissues dispersed and grew to form a new lichen thallus (Brodo, 2001). In many lichens vegetative propagules such as soredia, isidia and thallus fragments were the major means of reproduction. Often the mycobiont was dispersed by means of ascospore, basidiospore, conidia or gemmae. The new mycelium developing from such propagules has to come across a suitable free living alga/cyanobacterium to initiate lichenization.

About 90% of all known lichens had a green alga as a symbiont. Among these, *Trebouxia* was reported to be the most common genus, occurring in majority of lichens.

Corresponding author:* **Reshma. R. S
Department of Botany, St. Berchman's College, Changanacherry, Kottayam, Kerala - 686 101, India

Several members of the Trentepohliales, including the genera *Cephaleuros, Trentepohlia, Phycopeltis, Physolinum* and *Printzina* are other common phycobionts in lichens. The most commonly occurring genus of symbiotic cyanobacteria were *Nostoc and Scytonema*. Almost all these phycobionts were capable of free living in the absence of a suitable fungal partner. About 99 % of lichen mycobionts reported were ascomycetes, whereas basidiomycetes comprised only less than 1 % (Brodo, 2001).

Foliicolous or epiphyllous lichens were those that grew on the leaves of vascular plants. Such lichens were widespread and were especially common in the tropical areas where the atmospheric humidity was high. Many lichenologists have attempted to culture whole lichens and the separate or recombined symbionts (Ahmadjian, 1967, 1973; Galun, 1988). In most cases, lichen tissue culture and cultures derived by recombining symbionts that have been cultured separately remained as undifferentiated cell aggregates, but such aggregates are very useful for many biological experiments (Ahmadjian, 1973). The impact of foliicolous lichens on the rate of photosynthesis of the host plant was a point of debate.

Although several lichen identification keys were available, there were several unidentified foliicolous lichens. In the present study we made an attempt to investigate in detail widespread foliicolous lichen found distributed on all most all the common trees in Kerala, India and to characterize its symbiotic partners.

MATERIALS AND METHODS

Plant Material

Leaves from different plants, *Mesua ferrea, Mangifera indica*, ***Ixora coccinea***, *Syzygium cumini* and *Garcinia mangostana,* harboring the particular foliicolous lichen were collected and observed (Fig 1).

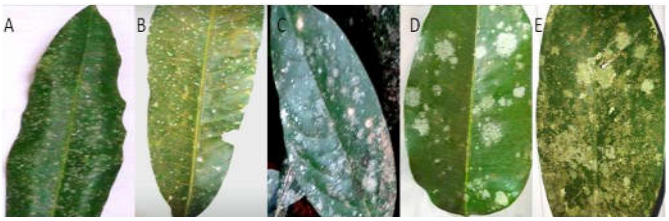

Fig 1 Lichen infected leaves of A. Mesua ferrea, B. Mangifera indica, C. *Ixora coccinea, D.* Syzygium cumini and E. Garcinia mangostana

Morphological observation

Microscopic observation

A strip of transparent universal adhesive film was gently applied on the infected surface of the leaf. The adhesive tape was then carefully pulled away from the surface, peeling away the epiphyllous lichen from the epidermis. Adhesive tape with attached lichen was immediately transferred to a glass slide and observed under a microscope. The images were captured using Olympus CH-20i Microscope camera.

Alkaline treatment

The developmental stages were determined by examining various discrete stages found in the field material. Leaf slices containing lichens were soaked overnight in NaOH (0.6M). Equal volume of HCl was used to neutralize the NaOH solution. From the treated leaves the lichen could be easily stripped. The separated lichen thallus was repeatedly rinsed with sterile water. The morphological traits were observed under microscope, with or without staining using lactophenol cotton blue. The images were captured using Olympus CH-20i Microscope camera.

In vitro culture

Sterilized Water agar medium and Bolds Basal media were used to culture the mycobiont and the lichen / photobiont respectively. Lichen thalli from the leaves were scraped using a sterile surgical blade and dissecting needle and inoculated onto the sterilized media. All the cultures were incubated under ambient conditions. The growth of culture aggregates was observed on every alternative day using a light microscope.

DNA barcoding using universal primers of ITS

DNA isolation was performed using NucleoSpin® Plant II Kit (Macherey-Nagel). The eluted DNA was stored at 4°C. The quality of the DNA isolated was checked using agarose gel electrophoresis. PCR Analysis was carried out with specific ITSprimers (Forward ITS-1F: 5'TCCGTAGGTGAACCTGCGG3' and Reverse ITS-4R: 5'TCCTCCGCTTATTGATATGC3'). The PCR amplification was carried out in a PCR thermal cycler (Gene Amp PCR System 9700, Applied Biosystems). The purity of PCR products were checked in 1.2% agarose gels by electrophoresis. Sequencing reaction was done in a PCR thermal cycler (Gene Amp PCR System 9700, Applied Biosystems) using the Big Dye Terminator v3.1 Cycle sequencing Kit (Applied Biosystems, USA) following manufactures protocol. The cleaned up air dried product was sequenced in ABI 3500 DNA Analyzer. The sequence quality was checked using Sequence Scanner Software v1 (Applied Biosystems). The sequence similarity was analyzed using the alignment tool BLAST.

Photosynthesis studies

Photosynthesis was measured by monitoring gas exchange systems using Infrared gas analyzers (IRGAs). In IRGAs, the reduction in IR transmission is a function of the concentration of CO2. A leaf was enclosed in a chamber, sealed to avoid gas exchange with the atmosphere, and the rate at which the CO_2 and H_2O concentration changes in the chamber were monitored. The sample and reference analyzers can be matched, without altering conditions in the leaf chamber.

RESULTS

Development of lichen in vivo

Epidermal peels observed under microscope revealed the presence of algal cells, fungal hyphae and lichens at different developmental stages. Two different types of algal cells were found to be associated with the lichen, discoid filamentous green alga *Phycopeltis* and unicellular green alga *Trebouxia* (Fig 2A and B). *Phycopeltis* was found distributed abundantly in the free-living state (Fig 2C). *Phyllophiale* like lichen thallus, comprised of the phycobiont *Phycopeltis*, with a multicellular thallus of closely packed, tightly branched radial filaments forming a disc like structure covered by a network of fungal hyphae were observed (Fig 2D). In some areas the hyphae surrounding coccoidal green algal cells forming distinctive structures could be seen (Fig 2E). The hyphae grew towards, between and around the filamentous branches of *Phycopeltis* algal cells producing lichenized hyphal branches which form the mat like fungal network (Fig 2F). It was not possible to find out the origin of association of lichenized fungal hyphae and the algal partner.

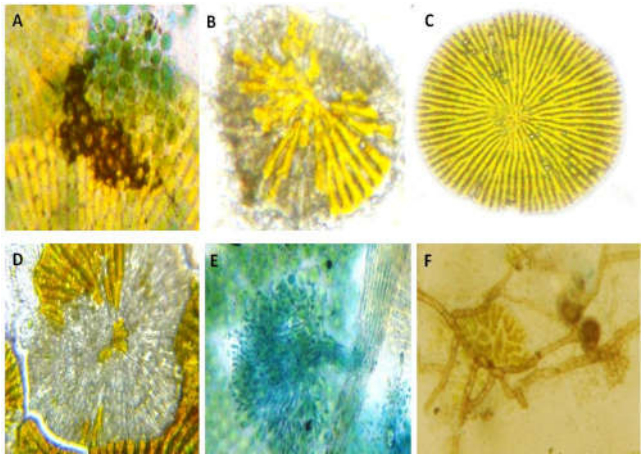

Fig 2A,B. Discoid filamentous green alga *Phycopeltis* and unicellular green algal cells scattered singly or in groups near the ends of fungal hyphae. **Fig2C**. *Phycopeltis* in the vicinity of lichen infection. **Fig 2D**. Lichen thallus comprised of the phycobiont *Phycopeltis,* covered by a network of fungal hyphae. **Fig 2E**. Distinctive structures formed by hyphal tips surrounded with coccoidal green algal cells. **Fig 2F**. Formation of lichenized hyphal branches

The functional presence of following reproductive structures on lichenized *Phycopeltis* thalli indicated that the alga was reproducing and dispersing independently, probably by both sexual and asexual means. Gametangia were often observed on

the lichenized algal thalli (Fig 3A,B) whereas stalked sporangia was found to be produced by both unlichenized and lichenized *Phycopeltis* (Fig 3C). Reproductive structures like isidia, soredia and thallus fragments that act as lichen propogules at various developmental stages were observed. The isidium always contained a single, central, radially symmetrical *Phycopeltis* thallus (Fig 3D,E,F). Soredia like propagules were also found to be giving rise to new thallus structures (Fig 3G,H,I). Branching of algal filaments was always observed to be apical and dichotomous. The dense radiating mass of mycelia formed by numerous hyphae might be developed from the observed fungal spores.

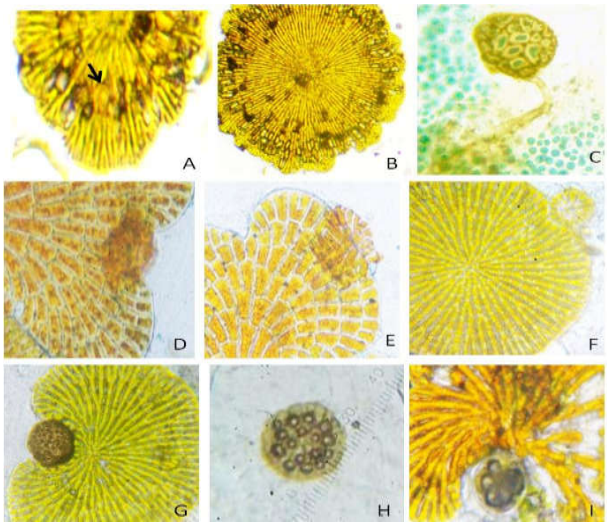

Fig 3 Reproductive strategies of lichen thallus. **Fig 3A,B**. Gametangia on the lichenized thalli surface giving rise to young thalli. **Fig 3C**. Stalked sporangial structure. Isidia **(Fig 3D,E,F)** and Soredia like propagules (**Fig 3G,H,I**) giving rise to new thalli structures.

Numerous free-living *Phycopeltis* thalli were frequently observed nearby the lichenized structures (Fig 4).

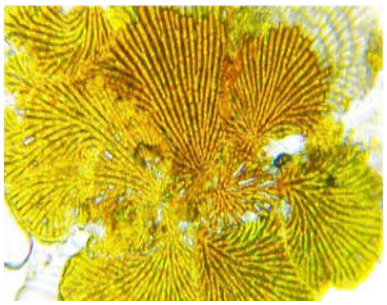

Fig 4 Free-living *Phycopeltis* thalli observed nearby the lichenized structures.

Many cells of the phycobiont of the lichen thalli appeared to be discolored and new small *Phycopeltis* thalli were found to emerge from near the ends of the outermost extremities of the algal filaments. They often gave rise to new, apparently unlichenized thalli of *Phycopeltis* near the margins of the degenerated thalli (Fig 5 A, B).

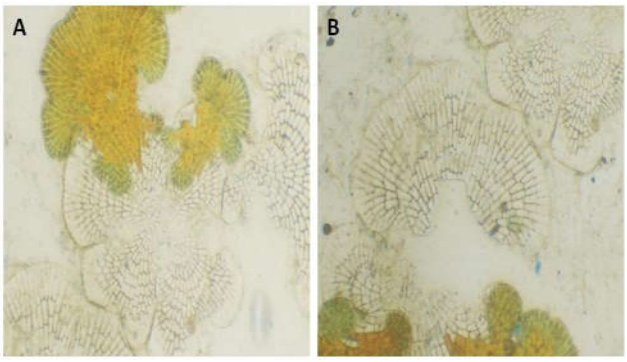

Fig 5 A,B Degeneration of lichen thallus and emergence of new *Phycopeltis* thallus

Development of lichen in vitro

When scraped lichen and leaf pieces containing lichen were cultured on water agar medium, profuse growth of fungal mycelia occurred (Fig 6A). The mycelia were formed of septate hyphae with specific branching pattern and growth. Later a green coloration thought to be of algal cells appeared in the medium. For the growth of algal cells, Bold Basal media was added to the media. At the regions that showed green colour, more algal cells were started to grow. Some multicellular aggregate structures were found to be formed by fungal hyphae (Fig 6B-E). Sporangia like stalked, coloured reproductive structures were grown on fungal hyphae (Fig 6F). Liberation of spore-like bodies from those structures was clearly observed (Fig 6G-I). Those spore-like bodies seemed to be adhered to the surface of modified aggregate structures as well as dispersed to fungal mycelia strands (Fig 6J-L). Lichen thalli like structures were found to be emerging from those structures. But a clear evidence of thallus developing from the spore-like structures was not obtained (Fig 6M, N).

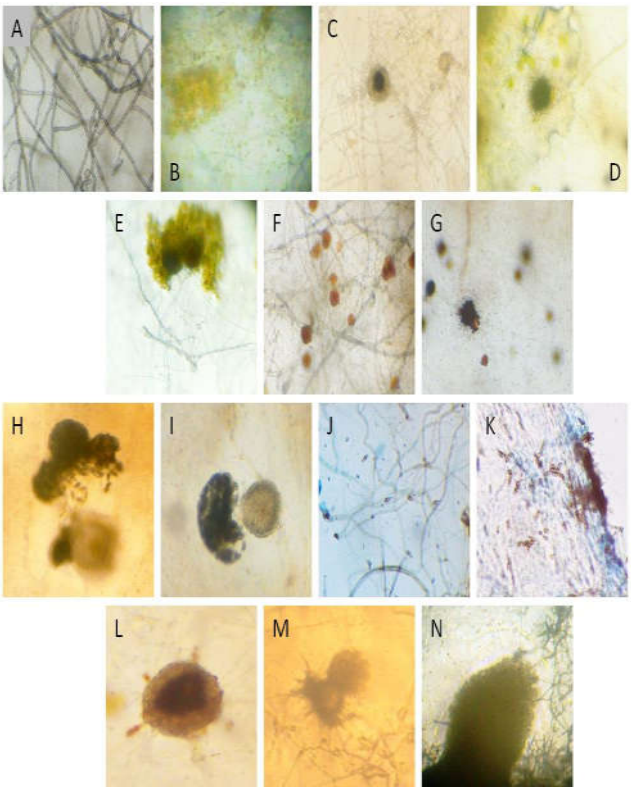

Fig 6A-N Various stages of development of lichen *in vitro*

In vitro Culture and Identification of Mycobiont

In water agar medium, from the scraped lichen pieces and leaf pieces containing lichen, a specific pattern of fungal growth was seen (Fig 7A). Gradually a green colouration appeared due to the formation of spores (Fig 7B). The microscopic studies showed the growth of highly branched, septate fungal hyphae (Fig 7C). The spores were also found to be formed in a peculiar pattern (Fig 7D-F). From normal uninfected leaves, used as control, no such growth occurred. This confirmed that the particular fungus was not an endophyte present in the leaf.

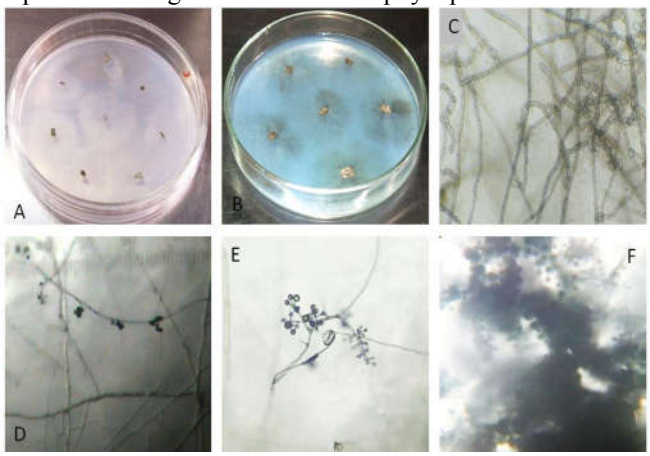

Fig 7 Fungal growth observed in water agar medium before sporulation (**Fig7A**) and after sporulation (**Fig 7B**). **Fig 7C**. Microscopical view of highly branched, septate fungal hyphae. **Fig 7D-F.** Specific pattern of sporulation

The fungus grown in water agar plates were subcultured in PDA medium. The pattern of mycelia growth was similar to that of growth in water agar medium, but no spore formation was observed. To identify the fungus, molecular biological studies were carried out. Pure DNA was isolated from the pure culture and the sequence was obtained by DNA Barcoding using universal primers of ITS. The sequence obtained was given in Box 1. The lineage report of the obtained sequence accessed by BLAST sequence alignment was given in Box 2. The taxonomy report showed that the isolated fungus may be a related species of *Lasiodiplodia*, a member of Botryosphaeriaceae (order Pleosporales).

>SR1234CAITSF_E01.ab1GTTGGAAGAATATCGGGGCTTCGGCTCGACTCTCCCACCCTTTGTGAACGTACCTCTCGTTGCTTTGGCGGCTTCG
GCCGCCAAAGGACCTTCAAACTCCAGTCAGTAAACGCAGACGTCTGATAAACAAGTTAATAAACTAAAACTTTCAACAACGGATCTCTTGGTTC
TGGCATCGATGAAGAACGCAGCGAAATGCGATAAGTAATGTGAATTGCAGAATTCAGTGAATCATCGAATCTTTGAACGCACATTGCGCCCCTT
GGTATTCCGGGGGGCATGCCTGTTCGAGCGTCATTACAACCCTCAAGCTCTGCTTGGAATTGGGCACCGTCCTCACTGCGGACGCGCCTCAAAG
ACCTCGGCGGTGGCTGTTCAGCCCTCAAGCGTAGTAGAATACACCTCGCTTTGGAGCGGTTGGCGTCGCCCGCCGGACGAACCTTCTGAACTTT
TCTCAAGGTTGACCTCGGATCAGGTAGGGATACCCGCTGAACTTAAGCATATCAATAAGCGGAGGAGGATCATTACCGAGTTTTCGGGCTTCGG
CTCGACTCTCCACCTTTGTGAACGTACCTCTGTTGCTTTGGCGGCGTCGGCGCCAAGGACTTCAACTCCGTCTTAAACGCAACCTCGGATGAAC
AGCCTATTAAACTAAAACTTTCACACGATCTTTGAATTCTGCATCATTAAAAGCACCATGGATAGTAATGTGAATTGCAAATCATGAATCATCA
ACTTTGAACGCATTGCGCCCTCGGGTATTCGGGAGGGCCAGCCGCTTTCCAGACGTTCATTCAACCTCCCTCTCTCTGGGGAAATTGGGGGACG
CTCTTCGGTCGGAAGACTCAAAACACTCTAGCGTGTCGTCGAGTATCACACCTCCTACACGAGAGATGTAATAACAACGTCAGTCTATTTGGAAG
CGAGTGGGGACGTCCGGCCCGGCAGCAACAACATCATTCATTCC

Box 1 DNA sequence of the fungus, sequenced by DNA Barcoding using ITS primers

Lineage Report

Organism Report Taxonomy Report

Organism	Blast Name	Score	Number of Hits	Description
Fungi	fungi		106	
.Ascomycota	ascomycetes		76	
..Botryosphaeriaceae	ascomycetes		72	
...Lasiodiplodia	ascomycetes		71	
....Lasiodiplodia theobromae	ascomycetes	1138	43	Lasiodiplodia theobromae hits
....Lasiodiplodia sp.	ascomycetes	1120	2	Lasiodiplodia sp. hits
....Lasiodiplodia crassispora	ascomycetes	1064	9	Lasiodiplodia crassispora hits
....Lasiodiplodia pseudotheobromae	ascomycetes	1035	6	Lasiodiplodia pseudotheobromae hits
....Lasiodiplodia brasiliensis	ascomycetes	1031	2	Lasiodiplodia brasiliensis hits
....Lasiodiplodia sp. BAB-4624	ascomycetes	963	1	Lasiodiplodia sp. BAB-4624 hits
....Lasiodiplodia rubropurpurea	ascomycetes	928	1	Lasiodiplodia rubropurpurea hits

Box 2 Taxonomy report of sequenced fungal DNA (https://blast.ncbi.nlm.nih.gov/Blast)

In vitro culture of phycobiont

The major phycobiont of the lichen under study was found to be *Phycopeltis* along with unicellular alga *Trebouxia*. Those unicellular algal cells were found to possess some unknown role during the lichenization – association of algal cells with fungal mycelia. For culturing the algal partner separately, scraped lichen pieces and leaf pieces containing lichen were inoculated in Bolds Basal medium. The major algal partner, *Phycopeltis* failed to grow *in vitro* and only the unicellular algal cells grew in the culture (Fig 8). The algal growth was found to be very slow and it took almost forty days for the algal cells to multiply.

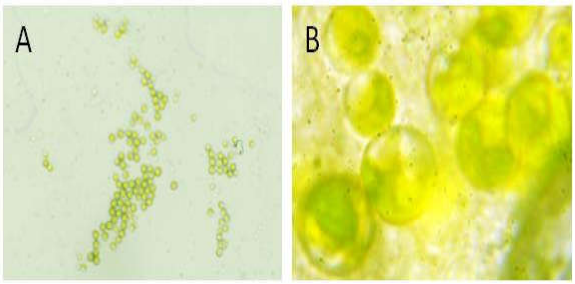

Fig 8 *Trebouxia* cells grown from the lichen in the algal specific Bolds Basal Media

Table 1 A comparison of various parameters influencing photosynthesis in uninfected and lichenized leaves using Infrared gas analyzer

Branch	Leaf	Photosynthetic rate (µmol $CO_2 m^{-2}s^{-2}$)	Stomatal conductance to water (mol $H_2Om^{-2}s^{-2}$)	Intercellular CO_2 concentration (µmol CO_2)	Photosynthetically Active Radiation (PAR)
I	Uninfected	0.8	0.03	384	500
	Lichenized	0.6	0.05	404	498
II	Uninfected	0.5	-0.05	397	499
	Lichenized	0.4	-0.04	400	498
III	Uninfected	4.4	0.01	985	498
	Lichenized	4.5	0.01	954	499

Effect of Foliicolous lichen on Photosynthesis of Host Leaves

The effect of the foliicolous lichen on the rate of photosynthesis of the host plant was studied using an infrared gas analyzer. Various parameters including photosynthetically active radiation (PAR), photosynthetic rate, stomatal conductance to H_2O gas and intercellular CO_2 concentration of lichenized and non lichenized leaf of the same branch were studied (Table 1). The results showed that there was no considerable variation in photosynthetic rate in infected and control leaves. Thus it can be interpreted that the presence of lichen on the leaf surface was not causing any significant negative effect on the photosynthetic rate. Further studies have

to be carried out to understand the physiological effect of these epiphytic lichens on host plants.

DISCUSSION

Sanders *et al*. (2002) reported that in *Phyllophiale*, the phycobiont is *Phycopeltis*, a member of the Trentepohliaceae. Our observation of distinctive structures formed by the postioning of phycobiont cells at the tips of a radially advancing fascicle of mycobiont hyphae were in conformity with *in situ* studies in *Phyllophiale* conducted earlier (Sanders *et al*., 2002). Positioning of chlorococcoid cells at hyphal tips appears to provide a very simple mechanism by which growth of the mycobiont directly distributes the dividing phycobiont cells to the youngest parts of the radially expanding thallus. Similar to our observation, growth of hyphae through filamentous branches of *Phycopeltis*, surrounding the alga and producing hyphal branches were also reported in *in situ* studies (Sanders *et al*., 2002).

Our results were in agreement with various previous reports about lichen reproduction. Lichens were reported to reproduce by means of vegetative propagules, such as soredia, which disperse both the symbionts together (Bailey, 1976; Schuster *et al*., 1985; Scheidegger, 1995), fungal ascospores or basidiospores, apparently resulting from sexual processes and/or asexual spores such as conidia. In some lichens, algal cells may be present within or upon the fungal spore-producing tissues, where they can be dispersed together with the liberated spores (Bertsch & Butin, 1967). In most cases, the fungal spores seem to be dispersed aposymbiotically; upon germination, the fungus would acquire a suitable algal symbiont and lichenize. Isidia represent thallus outgrowths with different developmental possibilities either direct growth or undergo a reorganizational phase. Sanders (2001a) reported some evidence of lichenization as well as symbiont codispersal in a preliminary study of foliicolous lichen colonization in *Phyllophiale.*

The probable reason of appearance of free living *Phycopeltis* in the vicinity of the lichenized form was explained in the previous studies (Sanders, 2001a). In *Phyllophiale,* special mechanism for maintaining the products of phycobiont sporogenesis within the confines of the lichen is not yet reported. Lateral extension ceased at points of contact between adjacent *Phycopeltis* thalli within the lichen. As a result, individual phycobiont thalli were easily distinguished within the *Phyllophiale* lichen. Also lichenized and non lichenized thalli can be distinguished by the thallus coloration due to difference in the pigments. Also, similar to our results, degeneration of germinated *Phyllophiale* isidia by discoloration was reported. Reports pointed that the fungal structure deteriorated by loosing cytoplasm from the cells while phycobiont disc die back, isolating peripheral lobes of growth and becoming colourless. It was also reported that new unlichenized thalli arise from the outermost extremities near the margins of the degenerated propagule (Sanders 2001b, 2002)

Some preliminary study of foliicolous lichen colonization *in situ* resulted in low diversity of lichens and thus it was suggested that the substratum and/or microenvironment were suboptimal for development of foliicolous lichens (Lucking, 2001). The algal and fungal partners cultured separately *in vitro* need not show the same morphological and physiological properties of naturally existing symbionts. In contrast Sanders *et al.* (2002) reported that same basic types of propagules occur in other lichens of different substrates and the development of those propagules presumably follow similar principles. The morphogenetic stimulus for the fungus might be provided by the photobiont, the fungus cannot develop into a thallus alone (Ahmadjian, 1980).

Some of the intermediate stages of our *in vitro* culture appeared to be similar to those reported by *in situ* culture grown on coverglass (Sanders *et al.,* 2002). Hyphae produced from the fungal spores, giving rise to a dense, radiating mat of mycelium were reported to surround trebouxioid algal cells giving rise to distinctive structures similar to the one we had observed. Our lichen showed the formation of three septate spores, which might be reported as three septate lichen spore belonging to *Porina rubentior* or a related taxon (Trichotheliaceae) by *in situ* studies. But the spores were reported to grow toward and contact already lichenized *Phycopeltis* within young *Phyllophiale* thalli, but not found in our *in vitro* culture. However, it was not possible to confirm whether any of the lichenizing hyphae associated with the alga originated from those germinated spores. Most of the foliicolous lichens produce thin-walled ascospores and which can be considered as an adaptation for rapid development (Santesson, 1952).

Reports showed that all developmental stages of the lichen *Phyllophiale*, from propagule germination to propagule production may take a long period of about one year. The developmental events in the lifecycles of those organisms and the subsequent processes involved in lichen thallus organization were poorly understood. Complications in the *in vitro* studies of lichen symbionts might be mainly because of the requirement of the long-term, time consuming techniques for their successful culture (Kranner, 2002). Moreover, combining lichen symbionts artificially or cultivating them from thallus fragments might not be representative of how lichen reproduction and ontogeny actually occured in nature (Sanders *et al*., 2002).

In vitro culture of mycobiont is reported to establish rapidly and spread in all directions due to germination from numerous peripheral points. The most abundant and diverse group of fungi occurring in foliicolous lichens were members of the Trichotheliaceae; those fungi appear to rely chiefly on aposymbiotically dispersed spores for reproduction (Santesson, 1952; Aptroot & Sipman, 1993; Lucking, 1996). It is reported that most abundant foliicolous lichen mycobionts include various members of Gomphillaceae and Trichotheliaceae (Vezda, 1979; Vezda & Poelt, 1987; Lucking, 1997; Ferraro *et al*., 2001).

Accurate identification of lichen phycobionts requires isolation and culture in the laboratory or molecular tools, and very few phycobionts of foliicolous lichens have been investigated (Tschermak-Woess, 1988). Preliminary molecular analyses indicate that *Trebouxia* and/or close relatives are the principal phycobionts in foliicolous members of the Gomphillaceae and Ectolechiaceae (Lucking, 1997).

Similar to our results, in previous *in situ* cultures, chlorococcalean green algal cells *Trebouxia*, were found scattered singly and in groups, free of association with fungi. Ahmadjian (1988) suggested that such reports did not

represent truly free-living *Trebouxia* populations but rather phycobiont cells liberated from degenerated propagules or thallus fragments. Encounter of a suitable alga for lichenization was often thought to be problematic because of the reportedly infrequent occurrence of such important phycobionts in the free-living state. Various investigators have considered the question of how unicellular phycobionts become distributed throughout the lichen thallus in the course of mycobiont growth (Sanders, 2002). However, the question of phycobiont availability in nature requires further study.

The algal distribution is suggested to be due to the repeated penetration of *Trebouxia* spore packets and subsequent separation of the daughter cells. A comparable process of separation and short-distance shifting of algal division products was proposed by Honegger (1987) to account for phycobiont distribution in complex lecanoralean lichen thalli. By contrast, in *Coenogonium*, where both the fungus and its algal symbiont were filamentous, no comparable means of mechanical distribution of the phycobiont was evident. The algal filaments were seemingly autonomous in their growth and branching, yet their direction of development rather strictly followed the rays of prothallic hyphae laid down by the mycobiont. Coordination of symbiont growth in this lichen might depend more closely on substances released by the mycobiont.

For many lichen fungi the symbiosis was interrupted and reacquired in the course of their life cycle. The existence of an aposymbiotic phase provides the opportunity for new nutritional experiments upon which natural selection may act. Selection may favor the germinating lichen fungal propagule which can take advantage of an alternative substrate when compatible algae are not available, leading to the eventual loss of the lichen mode of nutrition in some lineages.

The incorporation of additional external algae by hyphae of already lichenized propagules and thalli suggests that algal populations within lichen thalli might not be genetically uniform, or that their genetic composition might change in the course of development as reported in the lichen *Diploschistes muscorum* (Friedl *et al*., 1987) Other reports have suggested that the converse process, substitution of the mycobiont, may also occur; lichen fungi might associate with already lichenized algae, replacing the original lichenizing fungus of vegetative propagules (Ott *et al*., 1993) or mature thalli (Poelt, 1974; Hawksworth *et al*., 1980)

Effect of foliicolous lichens on photosynthetic rate was a controversial subject. The quantity of light transmitted through the lichens to the host leaf varies widely depending on the lichen species. Similar to our results, some researchers found that the host leaves compensated for the lichens and that total photosynthesis was not reduced. Anthony *et al*. (2002) reported that host leaves compensate the shading on leaf surface by epiphyllous lichen cover by photo-acclimatization from their studies on leaves of *Calamus australis* and *Lindsayomyrtus racemoide*. Some studies showed that in host plants the chlorophyll content in the regions of the leaves inhabited by lichens was significantly higher than in uncolonized leaf areas. This increase the photosynthetic efficiency may be due to various reasons like higher rate of Carbon assimilation per unit leaf area (Zhou *et al*., 2014), contribution of epiphylls for N-fixation for host leaves (Bentley *et al*., 1987) etc. Some researchers suggest that if part of a leaf was covered the total leaf photosynthesis was reduced so that there was less food production (Coley *et al*., 1993, Roskoski *et al*., 1981). In *Populus tremula* L., Solhaug *et al*. (1995) reported a considerable decrease in photosynthetic O_2 evolution by stem photosynthesis when with epiphytic lichens covered the bark surface. These results need not be similar for all species of lichens nor vascular host species.

The present work was a preliminary study that helped us to get a glimpse of the complexity in the morphology and reproductive strategies of the particular foliicolous lichen and to characterize its symbiotic partners. Morphology, the role of *Phycopeltis* as phycobiont, presence of *Trebouxia,* lichenization pattern etc. were found to have resemblances with that of *Phyllophiale*. But further studies have to be carried out for the identification and characterization of the lichen, role of unicellular algae in lichenization, developmental stages of the lichen *in vitro* and physiological influence of lichen and host on each other.

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