

The lichen thallus as a microbial habitat

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Abstract: Lichenized fungi associate with their photobionts to develop characteristic thallus structures. A wide range of other microbial organisms colonizes these structures. Other fungi growing on the lichen thalli are commonly known as lichenicolous fungi. Some of them are lichenized to develop their own lichen thalli on the hosts. Most of these lichenized and non-lichenized lichenicolous fungi are highly specific for their hosts. Beside these morphologically recognizable phenotypes, many other fungi are also detected in lichens using culture-dependent and culture-independent approaches. The biology of these fungi and their specificity patterns are not well understood. In addition to the lichen-associated fungi, surface colonizing and host-specific bacterial communities have also been characterized in great detail recently. The lichen-associated bacterial communities are structured by geography, thallus age, as well as biotic and abiotic factors. Bacteria can be transmitted vertically across generations by vegetative propagules of lichens. Metagenomic and metaproteomic data suggest that the bacterial communities are functional components in the lichen symbiosis. Lichens also provide diversified habitats for numerous other associated microorganisms such as protists and algae. To study interactions among the entire microbial cosmos in the lichen symbiosis becomes a varied and multidisciplinary research challenge.

1. Introduction

The lichen thallus results from the self-supporting interaction of fungi with associated symbiotic algal (and/or cyanobacterial) partners. Usually, the fungal partners dominate biomass and dictate the shape of lichen thalli. The fungi shelter the algal/cyanobacterial partners beneath protective peripheric layers, which are produced by the conglutination of fungal cell walls. Conglutination and compaction of the vegetative mycelium are among the key evolutionary innovations that made lichen thallus evolution possible. Approximately 20,000 fungal species evolved in the lichenized stage, mostly in unrelated main lineages of Ascomycota. The diverse fungi associate with only about 120 known species of cyanobacteria or algae (HONEGGER 2012). The photosynthetic partners, green algae in about 90 % of lichen species, generally proliferate under the fungal 'protectorate', but at the cost of their sexual functions. On the other hand, the mycobionts need the algal partners to produce their characteristic thallus morphologies (GRUBE & HAWKSWORTH 2007). The latter are in most fungal species also required for the development of sexual and asexual reproduction structures.

The frequent ability to survive in a cryptobiotic stage and the fine-tuned balance of respiration versus photosynthesis helps the lichens to tolerate environmental fluctuations as well as extremes of desiccation and temperatures. Lichens are thus prominent organisms at high altitudes and latitudes where conditions often become too adverse and nutrient-poor for most other multi-

cellular organisms. Without competition of plants, individual thalli can reach very high ages in these habitats, occasionally in the range of thousands of years. The extraordinary persistence of lichen thalli and their morphological variation provide richly diversified and narrow-scaled niches for microorganisms, in particular fungi and bacteria. While lichen-associated fungi have been studied thoroughly, and even before the symbiotic nature of lichens was discovered, bacteria in lichens received only little attention until more intensive research was conducted in the last decade. The following review presents the current knowledge about the associated microorganisms in lichens, with a short introduction about the variation of algal partners.

2. Photobiont diversity and selectivity

The knowledge about lichen photobiont diversity has substantially improved over the past two decades by the broad application of refined molecular approaches. Patterns of photobiont association became apparent at various taxonomic levels. At higher taxonomic levels of fungi, lineages differ by their overall preference for main groups of algae (such as trentepohlioid and chlorococcoid algae, or cyanobacteria; MIADLIKOWSKA et al. 2006), whereas fungi within these lineages may have varied patterns of selectivity within these main groups of algae. This might be correlated with the adaptation of lichens to wide geographic ranges and climatic variation. For example, a wider spectrum of photobionts of the genus *Trebouxia* has been correlated with the geographic range (BLAHA et al. 2006, FERNANDEZ-MENDOZA et al. 2011, DAL GRANDE et al. 2018). The flexibility in accepting algal partners – depending on local factors – recalls the hypothesis of ‘habitat-adapted symbioses’ by RODRIGUEZ et al. (2008).

Sequence data and microscopic approaches also showed that more than one strain of related algae can co-occur in the same lichen thallus, which confirms earlier observations made with culturing approaches (FRIEDL 1989). It was suggested that this type of algal plurality could increase the ecological amplitude of lichens and their adaptation to environmental variation (CASANO et al. 2011). Irrespective of functional divergence, closely related algae might be under common symbiotic control (“symbiotic reliance”). The occasional co-occurrence of unrelated photobionts e.g. green algae and cyanobacteria, requires their spatial separation (e.g. cephalodia as specific organs to host cyanobacteria). Only one publication so far reports the presence of cyanobacteria directly among green algae (HENSKENS et al 2012), while other mixed occurrences of unrelated algae can sometimes be associated with the presence of lichenicolous fungi (e.g. co-occurrence *Trentepohlia* and *Trebouxia* in thalli of *Cladonia* infected by *Arthonia* species).

Depending on ecological circumstances (nutrients, humidity), diverse algae can colonize the surfaces of lichens, and compatible strains of these algae may possibly be integrated in a pre-existing thallus (MUGGIA et al. 2013b), analogous to the incorporation of attached cyanobacteria into internal cephalodia (CORNEJO & SCHEIDEGGER 2013). In a wider ecological context, epithalline algae might serve as nutritional sources for other epithalline colonizers, or for feeding invertebrates, or for the lichen thallus itself. Interestingly, also terrestrial diatoms have been found between the thallus filaments of *Coenogonium linkii* in the understorey of tropical rain forests (LAKATOS et al. 2004), where epithalline algal diversity of lichens is generally understudied.

3. Life strategies and diversity of lichen-associated fungi

Lichenicolous fungi, comprising all fungi living with lichens beside the thallus-forming mycobiont, were recognized even before the symbiotic nature of their hosts was discovered in the second half of the 19th century. Till now, more than 1,800 species of lichenicolous fungi have been described but their precise number is still not clear (<http://www.lichenicolous.net>). Morphological characters are still the basis for species recognition of these fungi and the annual rate of new species descriptions is still high. According to phylogenetic studies, the evolutionary origins of the lichenicolous life style are diverse, but a substantial fraction seems to stem from originally lichenized lineages (e.g. FRISCH et al. 2014). Most lichenicolous fungi seem to live together with their hosts as commensals rather than being destructive parasites. This behaviour correlates with a high specificity for their hosts. Using histochemical staining it can be shown that fungi have preferences either for algal cells or the living fungal structures of the host, indicating clear cases of biotrophic relationships. Infectious hyphal structures of mycobiont-parasitic species were described by DE LOS RIOS and GRUBE (2000). These structures comprise simple or complex haustoria with finger-like projections into the host hyphae (in *Pyrenidium actinellum* infecting the *Peltigera venosa*). Even the growth of hyphae inside of host hyphae has been observed, such as in *Dacampia engeliana* infections of *Solorina saccata*. Simple haustoria are also present, for example in species that exploit the algae of their hosts such as known from the genus *Zwackhiomyces* (GRUBE & HAFELLNER 1990; Fig 1). Other algal exploiters establish contacts with the photobionts of their host lichens using appressoria (e.g. lichenicolous species in the genus *Arthonia*). But there are also numerous lichenicolous species in which specificity for the host's partners is unclear, or where lichenicolous hyphae are present in the extracellular matrix formed by one or both symbiotic partners.

Because the vitality of lichen thalli ranges from actively growing young to decaying parts, the delimitation of biotrophic, necrotrophic or even saprotrophic life styles is not clear in all cases. For example, certain lichen-associated

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fungi have specialized to grow in epinecral layers or epicortices, i.e. structures lacking living host cells and formed by extracellular polysaccharides shed from the living part of the upper surface. In Parmeliaceae, SPRIBILLE (2016) discovered that the epicortices are commonly associated with basidiomyceteous yeasts. Other lichen-associated fungi have a preference for decaying parts of lichens and thereby link with necrotic to saprobic life styles (APTROOT & ALSTRUP 1999). Possibly, the biological gradients within a lichen thallus contribute soft constraints, which could promote the diversification of fungal life styles. In fact, ARNOLD et al. (2009) suggested that lichens represent a ‘cradle’ of fungal evolution.

Numerous lichenicolous fungi are able to cause hypertrophic deformations in lichen thalli. Within these galls, the parasites usually develop their reproductive organs. Anatomies of galls and interactions between host and parasite differ significantly among gall-forming lichenicolous fungi (GRUBE & DE LOS RIOS 2001). Galls of lichens can be devoid of photobionts but usually contain hyphae of both fungal symbionts. The infections on *Usnea* thalli caused by the heterobasidiomycete *Biatoropsis usnearum*, studied in detail by histochemical staining, initiate in the cortical layer of the host. Later on, this parasite forms tremelloid haustoria primarily in the central part of mature galls. Fully developed galls provide a microhabitat for other lichen colonizers, such as *Cyphobasidium* (MILLANES et al. 2016), one of the few so far known genera of Pucciniomycotina in lichens. *Cyphobasidium*, characterized by its distinctive probasidia, is also known to produce independent galls on Parmeliaceae. SPRIBILLE et al. (2016) described a new order to comprise this lineage of basidiomycetes and demonstrated its presence in a wide range of parmelioid lichens by screening of a large number of host lichens by PCR using specific probes.

Lichenicolous lichens

Species able to develop their own symbiotic thalli on lichenized hosts are known as lichenicolous lichens (POELT & DOPPELBAUER 1956, POELT 1990) All lichenicolous lichens evolved within genera of lichens and their development requires the pre-formed thallus of their specific hosts (RAMBOLD & TRIEBEL 1992). Species can either specifically develop their thalli on the surface of other lichens (epikapylic development) or in the internal structures of the host (endokapylic development). The specific invasion into a host thallus needs to be distinguished from cases of unspecific overgrowing by neighboring thalli. So-called juvenile parasites exploit their lichen host only in early stages (e.g. *Arthrurhaphis citrinella* on *Baeomyces rufus*, *Diploschistes muscorum* on *Cladonia* species) until they grow independently from their hosts in later stages. It cannot always be proven whether mature independent thalli were initially parasitic, though this type of parasite apparently exceeds the growth rate

of the host and is not necessarily delimited by the size of the host. In contrast infections of other lichenicolous lichens remain localized on their host.

Propagation inside the host thallus is often followed by changes in morphology and eventually the production of secondary metabolites by the parasite, and may also involve a switch of algal partners. For example, FRIEDL (1987) demonstrated that the original *Asterochloris* species is replaced by *Trebouxia* species after the thalli of *Cladonia* are transformed by *Diploschistes muscorum*. Recent data show that algal switches are not generally required, as mature *D. muscorum* specimens may maintain the photobionts of the host lichen, or even host multiple algal types (WEDIN et al. 2015). While a switching of the algal partner is also seen when certain epiphyllous *Porina* species are infected by lichen-forming *Chroodiscus* species (LÜCKING & GRUBE 2002), algal partners of the hosts seem to be retained in other lichenicolous lichens (DE LOS RIOS et al. 2002).

The non-lichenized life style of lichenicolous species without is not clearly separable from a lichenized parasitic life style, when the parasitic thallus organization is not distinct. The fungus *Tetramelas pulverulentus* (on *Physconia muscigena*) slightly modifies the host morphology and develops externally recognizable infection symptoms. However, this species actually develops peculiar endokapylic (i.e. mycelia covered by strata of the host) lichenized thalli.

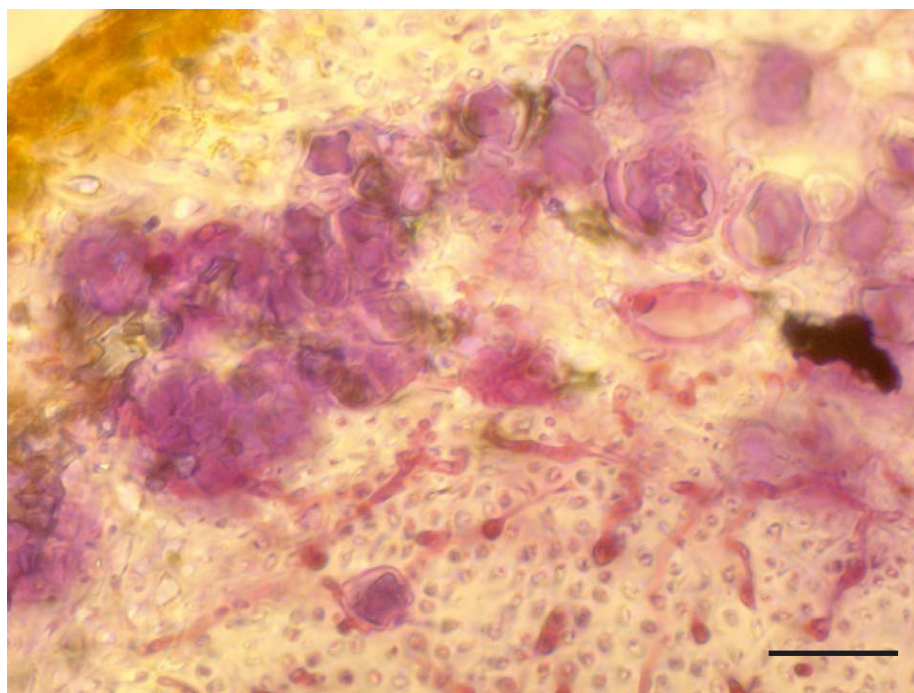


Fig. 1: *Zwackhiomyces coepulonus* parasitizing *Xanthoria elegans*. Scale bar = 15 μ m.

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The phenomenon of lichen hyperparasitism comprises even more complex cases of interactions in lichen symbioses. For example, non-lichenized fungi (e.g. *Stigmidium arthrorhaphidis*, *Cercidospora trypetheliza*, *C. soror*) can infect the lichenicolous lichen *Arthrorhaphis citrinella*, which grows on *Baeomyces*, *Cladonia*-squamules, or decaying lichens. Also lichens develop hyperparasitically on lichenicolous lichens: the lichenicolous lichen *Rhizocarpon diploschistidina* parasitizes the lichen *Diploschistes muscorum*, which grows as a juvenile parasite of *Cladonia* species.

Culture-dependent and independent assessments of lichen-associated fungal diversity

In addition to the phenotypically recognizable lichenicolous fungi, an uncountable number of fungal species associates with lichens but remain undetected by the eye. Isolation techniques and axenic cultivation revealed that these species either reside on the surfaces of the thalli (PETRINI et al. 1990, GIRLANDA et al. 1997, PRILLINGER et al. 1997, PERSOH & RAMBOLD 2012), or occur internally in lichen host thalli (also known as “endolichenic fungi”, ARNOLD et al. 2009). Microscopic evidence already demonstrates the hyphal growth of endolichenic fungi in internal part or the significant abundance of yeasts in the upper cortex (HONEGGER 2012, SPRIBILLE et al. 2016). Given the abundance and the potential of culturable endolichenic fungi to produce secondary metabolites, or to induce the production of compounds (SPRIBILLE et al. 2016), it will be of interest to study what other biological effects, (including influences on the phenotype) these fungi might have to their hosts.

Culture techniques already retrieved a surprising number of fungi from lichens, but these numbers only represent a subset of the total fungal diversity present in environmental samples. Therefore, culture-independent methods are now used to characterize the complete mycobiome of lichen thalli. Using DNA-fingerprinting techniques, FLEISCHHACKER et al. (2015) found a high diversity of lichen-associated fungi but without correlation with the presence of externally visible lichenicolous fungi. ZHANG et al. (2015) provided an overview of diversity and distribution of fungi in lichens from an Arctic habitat using next generation sequencing. Their study of the lichen mycobiome indicates that lichens harbor fungi, which are known from diverse other ecological niches. These observations were confirmed by results of FERNÁNDEZ-MENDOZA et al. (2016), who studied mycobiomes of different alpine lichen species. It became also clear that the diversity of co-occurring fungi varies substantially in different lichen species. However, the analyses revealed the presence of Tremellomycetes in almost every studied lichen thallus, even if characteristic galls that are formed by tremelloid fungi were missing. Clearly, the specificity of these basidiomycetes remains to be studied in greater detail, as other studies already revealed a high specificity (WERTH et al. 2013). Novel microscopic

techniques, such as *in situ* hybridization as used by SPRIBILLE et al. (2016) may show the location of tremelloid fungi in thalli and eventually if their presence without gall-formation might anyway have an influence on the morphology of the host.

The mycobiont-accompanying presence of fungi, most of which are not detrimental to their hosts, is a general phenomenon of lichen symbioses. A significant fraction of endolichenic communities is also shared with the fungal communities of bryophytes (U'REN et al. 2012), suggesting a shared environmental pool. Lichenicolous fungi in the narrow sense, i.e. those, which are recognizable on lichens by their fertile structures, might have a long evolutionary history with their host lichens.

4. Lichens and their association with bacteria

Cyanobacteria, as primary bacterial photosynthetic partners, are present in approximately one tenth of lichenized fungi. About 2 % of the lichens contain cyanobacteria in addition to the primary green algal partner, usually in special organs, cephalodia, which develop internally of the thallus or externally in the surface of the thalli. Due to the presence of both, green algae and cyanobacteria in lichens, already CENGIA-SAMBO (1924) suggested the term polysymbiosis for these cases. HYVÄRINEN et al. (2002) showed that the ratio of heterocysts is higher in cephalodia than in the algal layer of cyanobacterial lichens, which confirms their specific role in nitrogen fixation. A clear functional segregation is thus emerging in lichen thalli with the involvement of these unrelated partners. In addition, lichens can regularly form associations with cyanobacteria growing adjacent to the thalli, usually *Stigonema* species. This phenomenon, which indicates that certain cyanobacterial consortia may facilitate the development of lichens, is known as cyanotrophy (PÖELT & MAYRHOFER 1988).

Only few studies in the 20th century reported about the presence of other bacteria in bacteria. An early report about bacteria in the tropical lichen *Herpothallon sanguineum* by UPHOF (1925) was controversial, since SUESSENGUTH (1926) suspected that bacteria actually represented crystallized secondary compounds. Ten years later, Russian-Armenian and other researcher, confirmed the presence of nitrogen-fixing bacteria in lichens by enrichment cultures (HENKEL & YUZHAKOVA 1936, ISKINA 1938, SCOTT 1956). Other bacteria were isolated from lichens as well (*Bacillus*: HENKEL & PLOTNIKOVA 1973, *Beijerinckia*: PANOSYAN & NIKOGOSYAN 1966, *Clostridium*: ISKINA 1938, *Pseudomonas*: HENKEL & PLOTNIKOVA 1973). The finding of Actinobacteria suggested a defensive role for bacteria in lichens (ZOOK 1983). LENOVA and BLUM (1983) realized that up to millions of bacterial cells could be present per gram of a lichen thallus. Despite these convincing reports, the bacterial associations played hardly any role in lichenological research, perhaps also because tools

were not sufficient to find any meaningful information with the culture dependent data and because lichenologists were unfamiliar with bacterial research. This situation has now dramatically changed with the availability of modern sequencing approaches and with revised concepts of bacterial classification.

Sequencing of lichen-associated bacterial communities revealed information about their diversity on lichen thalli (CARDINALE et al. 2006, GRUBE & BERG 2009, GRUBE et al. 2009, HODKINSON & LUTZONI 2009, SELBMANN et al. 2010, BATES et al. 2011, BJELLAND et al. 2011, MUSHEGIAN et al. 2011, HODKINSON et al. 2012). According to these pioneering works Alphaproteobacteria are commonly present and usually dominating the bacterial communities, while other bacterial groups are also found in significant amounts. The sequencing data are complemented with microscopic information, in particular employing fluorescence *in situ* hybridization (FISH; CARDINALE et al. 2008). Using specific probes, the distribution of bacteria belonging to certain groups can be visualized under the microscope (particularly confocal laser scanning microscopy, CLSM). The microscopic data show that bacteria often form biofilm-like communities on the lichen thalli. Bacteria are usually tightly connected with the fungal structures, and preferentially with hydrophilic surfaces of the lichens. The bacteria can also intrude to various depths in the intercellular matrix of the upper cortex, and were occasionally also found inside the hyphae of the fungal hosts (CARDINALE et al. 2008, ERLACHER et al. 2015).

Bacterial habitats

Lichens with their distinctive thallus shapes provide an enormous diversity of habitats for associated bacteria. Light microscopy and epifluorescence microscopy can be used to visualize the spatial distribution of microbes in different parts of lichens (Fig 2.), and the use of confocal laser scanning microscopy together with fluorescence *in situ* hybridization proved ideal to localize the taxonomic composition of bacteria more specifically (CARDINALE et al. 2008). Previous comparisons of reindeer lichens revealed clear differences, which seem to correlate with both the exposition of the lichens to the sun but also to the age of the analysed thallus parts (CARDINALE et al. 2012). Since bacteria attach primarily to the surfaces of lichen thalli, the larger morphology of lichen thalli plays a crucial role for bacterial colonization, ranging from the interfaces with soil to the exposed surfaces of the thalli. Apart from the upper surface, additional niches were already compared by GRUBE et al. (2009), for example the lower surface of the *Umbilicaria cylindrica*, or the lateral margins of the areoles in *Lecanora rupicola*. Using wide-field transmission microscopy, bacterial colonization can be readily detected for examples on the aerial hyphae of *Peltigera*, apothecial flanks, or in dead cells in the upper cortex of *Catapyrenium* species. As bacteria can colonize niches between areoles and squamules of crustose lichens, surface increase by forming cracks

increases the habitat for associated bacteria. Bacteria do not only colonize aerial surfaces of lichens, but also the substrate interface. The attachment of lichen to the soil is accomplished by characteristic organs, which have been studied by light and electron microscopy (ASTA et al. 2001). Attachment structures range from mycelial felts of narrowly spaced hyphae (rhizohyphae) to root-like rhizines that are composed of conglutinated hyphae (POELT & GRUBE 1993). However, it still needs to be evaluated to what extent these structures also influence the microbial colonization of the soil beneath. MAIER et al. (2014) demonstrated that bacteria colonized *Psora decipiens* and *Toninia sedifolia* on the hyphal net at the thallus-soil interface of *P. decipiens*. This pattern agrees with results of MUGGIA et al. (2013a) for other biocrust lichens. In that study, bacterial cells were intermixed with photobiont cells in the lower parts of the lichen thalli and on the surface of the squamules of *Baeomyces placophyllus* and *P. decipiens* (MUGGIA et al. 2013a). In the inconspicuous thallus structures of *Arthrorhaphis citrinella*, *Baeomyces rufus*, *Icmadophila ericetorum* and *Trapeliopsis granulosa*, we also observed association of bacteria with hyphae and algae, as well as bacteria on the outer surface of the mycobiont-photobiont aggregates. Due to the small-scaled variation in ecological parameters in the stratified thallus and soil beneath, the composition of bacteria in lichens may vary at very small scales. The host-specific enrichment of bacteria in lichens and in the subjacent layers of the substrate (the hypothallosphere) has also been demonstrated earlier for rock-inhabiting biocrust-forming lichens (BJELLAND et al. 2011).

Bacteria are potentially involved in complex interactions in the lichen symbiosis, which rather represents a symbiotic network 'holobiont', rather than a mere fungal-algal relationship. GRUBE et al. (2015) comparatively assessed metagenomic and proteomic data, and complemented this comparison with information from molecular, microscopic and physiological assays. More than 800 bacterial species were estimated to occur on *Lobaria pulmonaria*. The community has the ability to contribu-

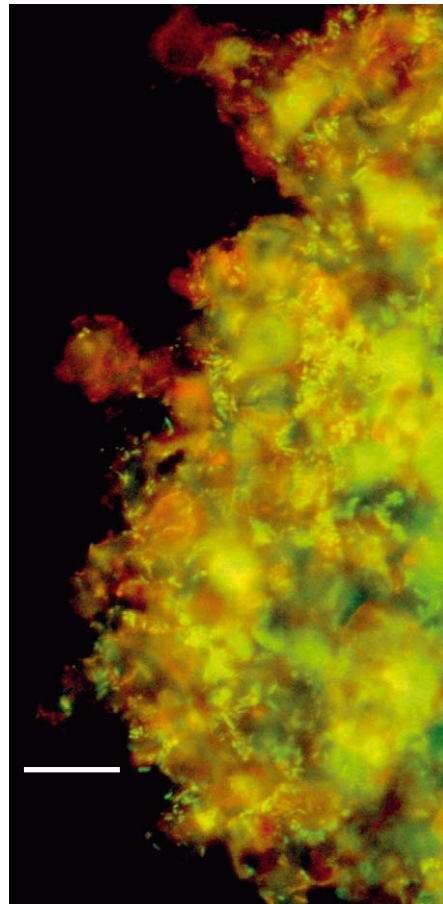


Fig. 2: *Peltigera praetextata* with abundant bacterial colonization. Scale bar = 10 μm .

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te multiple aspects to the symbiotic system, which includes nutrient supply, especially of nitrogen, phosphorus and sulfur. Genomic data suggest a potential in pathogen defense, resistance against abiotic factors, as well as fungal and algal growth support by provision of hormones and vitamins, detoxification of metabolites, and degradation of older parts of the lichen thallus. Hence it is likely that lichen-associated bacteria support the longevity and persistence of lichens under extreme and nutrient deprived ecological conditions.

5. Interactions between lichen-associated fungi and bacteria

As the symbiotic integrity of lichens can be affected by the fungal parasites, a shift in the composition of the bacterial associates of these lichens might be a consequence of lichenicolous fungal infections. This idea was studied with the soil lichen *Solorina crocea* (GRUBE et al. 2012). This lichen is frequently infected by a lichenicolous fungus, *Rhagadostoma lichenicola* (Sordariomycetes), which develops black-colored perithecia on the living lichen host. The grouped blackish ascomata emerge from the upper surface of the host thallus. Microscopy reveals a richly branched, dark mycelium beneath the parasite's fruitbodies in the host plectenchyma. The mycelium extends locally into the internal layers of the nearby regions of the lichen thallus. No specific infection structures with algal or fungal host cells are observed. Apparently, these infections do not immediately impair the host's fruiting body formation nor do they kill the host. Nevertheless, GRUBE et al. (2012) found evidence for a shift in the bacterial communities at the strain level. Strain-specific abundance shifts occurred particularly in Acidobacteria. The consequences for the bacterial community are clearly more pronounced with infections by lichenicolous lichens, which completely restructure the thallus of the host lichen. By analyses of different infection stages of *Diploschistes muscorum* on the host *Cladonia symphylicarpa*, WEDIN et al. (2015) found a decrease in relative abundance of Alphaproteobacteria. Also, Armatimonadia, Spartobacteria and Acidobacteria were more abundant in *Cladonia*, but Betaproteobacteria increased gradually in relative abundance during the transition to mature *Diploschistes muscorum*. These results from few selected examples offer only a glimpse of the enormous complexity of fungal-bacterial interactions, which might also occur between asymptomatic fungi and bacteria on lichens, as they often occur in close proximity (Fig. 3). As bacteria often have a substantial influence on germination and behaviour of other fungi, it might be a topic of future study which bacteria could be either antagonists or growth-promoters of lichen-associated fungi.

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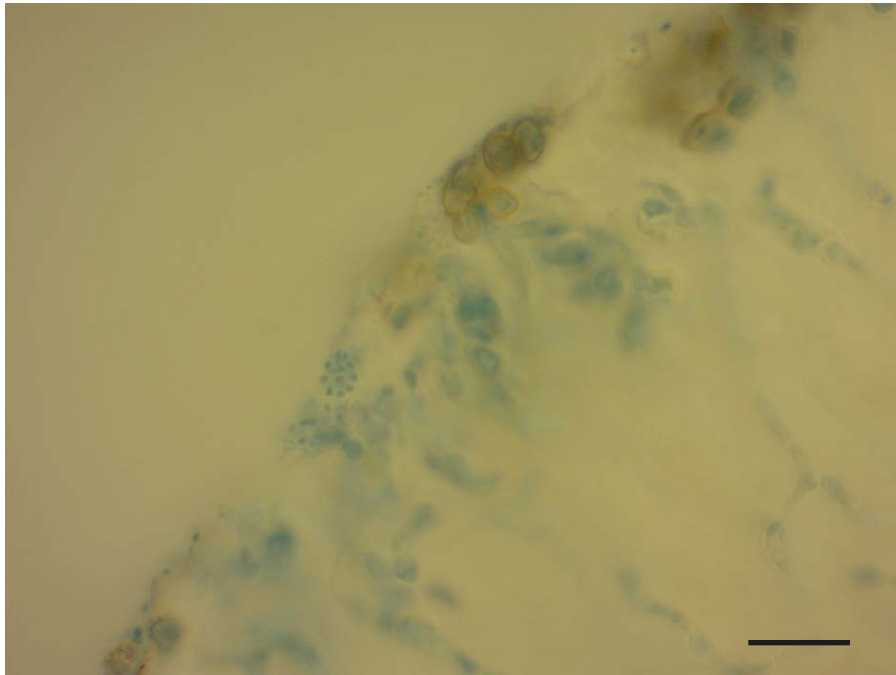


Fig. 3: *Chondropsis semiviridis* with bacterial and fungal colonization in the upper cortex. Scale bar = 10 μ m.



Fig. 4: *Rhagadostoma lichenicola* infecting *Solorina crocea*. Scale bar = 1 mm.

6. Other microorganisms

Beside bacteria, fungi and algae, further microbial organisms seem to be regularly present in lichens, including plasmodial myxomycete amoebae, heterotrophic nanoflagellates, and naked and testate amoebas. Protists have apparently no specific relationships with lichens according to present knowledge (BATES et al. 2012, ANDERSON 2015, BOCK & ANDERSON 2015). However, WILKINSON et al. (2014) speculated that amoeba could play a more important role in lichens. Interestingly plasmodial slime molds have been isolated preferentially from young lobes of thalli, after previous moistening of thalli (which actually represents an enrichment procedure). It is possible that these studies, so far conducted with only few selected lichens, hardly scratch the surface of protist diversity on lichens. Their diversity might perhaps be higher in humid to wet habitats.

7. Conclusions

As already put forward by HONEGGER (1992), the lichen thalli are consortia with unknown numbers of participants. Recent work demonstrated the high diversity of microbial colonizers and confirmed that lichen thalli are complex habitats for many species beside the primary fungal and algal partners. The association with the internalized photobiont partners apparently provides primary functions in the symbiotic interplay, whereas the associated microbial consortium may contribute in various further ways to the fitness of the holobiont. The influence on the biology of the lichen holobiont still remains to be studied with experimental approaches combining molecular analyses and microscopy. Yet, the microbial partners inhabiting lichen thalli may also play a role for other organisms. It is still completely unknown to what extent lichen-associated micro- and mesofauna influence or benefit from epithalline microbial communities (MEIER et al. 2002, FRÖBERG et al. 2003). Observations suggest that springtails and other arthropods graze microbial biofilms on the surfaces of lichens, especially in humid and wet situations (Grube, personal observations; Fig. 5). We are certainly facing an exciting future of research about of the microbial ecology of lichens and its role in ecosystems.



Fig. 5. *Isotoma* cf. *saltans* (Collembola) feeding on epithalline microbial communities of *Solorina saccata*. Scale bar = 1 mm.

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