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### **The Lichen Symbiosis: A Review**

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#### **ABSTRACT**

Organisms belonging to two different kingdoms are mutually associated to form a structure with particular morphology and anatomy. Lichens are not much eye-catching in its external morphology, but spectacular in its internal structure. Morphological studies don't reveal the mutualistic associations of individual partners while anatomical study reveals the beauty of its symbiotic association. The corroboration of recent studies on lichen and their symbiotic association suggests that, other micro communities such as algae, fungi and bacterial bionts are associated with many lichens in addition to the main photobiont and mycobiont. Microbial association of lichen helps them to develop a stable and successful symbiotic life, which can adapt in its natural habitat.

**KEYWORDS:** Lichen bionts, bacterial bionts, microbial community, symbiotic association

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## INTRODUCTION

### 1. *Lichen: A successful holobiont*

Lichens are holobionts with more than one participant in the association. They are usually comprised of a filamentous fungal partner called mycobiont and an algal partner called photobiont which can be either a eukaryotic chlorobiont (green algae) or a prokaryotic cyanobiont (cyanobacteria) and a very few are from other classes of algae. About 85% of lichens have chlorobionts, 10% have cyanobionts and less than 5% have other photobionts as their photosynthetic partner<sup>1,2</sup>. The presence of two partners, a fungus and an alga, makes them to be called as bipartite lichens and about 95% of lichens are bipartite. This dual nature of lichens is a widely accepted concept. Bipartite lichens can be of two, chlorolichens (chlorobiont as photobiont) and cyanolichens (cyanobiont as photobiont). More than 85% of the bipartite lichens are chlorolichens and only 13% have cyanobionts as their primary photobiont<sup>3</sup>.

About 4% of lichens are having more than two partners. Lichens with a mycobiont and two photobionts (a green alga and a cyanobacteria) are called tripartite lichens and if both the photobionts contribute to photosynthesis, then they are called co- primary photobionts<sup>1</sup>. Sometimes in tripartite lichens, the primary photobiont is the green alga and cyanobacteria (secondary photobiont) are restricted to specialized structures called cephalodia where they are more favoured for nitrogen fixation rather than contributing to photosynthesis<sup>4,5</sup>. Most of the lichenized fungi are members of the class, Ascomycetes and only a few belong to Basidiomycetes and the sterile species come under the artificial class, Deuteromycetes. Common photobiont partners are *Trebouxia*, *Trentepohlia* (chlorobionts) and *Nostoc* (cyanobiont)<sup>5-8</sup>.

#### 1.1. *Holobiont with multiple bionts*

Lichen as a mutualistic association between a fungal and an algal partner, is now, a traditional concept. Recent and advanced molecular level studies revealed the fact, that lichens harbour diverse microbial communities, both prokaryotic and eukaryotic, in addition to the mycobiont and photobiont<sup>9,10</sup>.

Lichens can host a wide variety of smaller biological entities such as heterotrophic bacteria, viruses, diatoms, green algae, free living cyanobacteria, epiphytic algae and epiphytic liverworts, which were found on large foliose cyanolichens in wet tropical forests. A total of 324 phyla with 236 bacteria, 74 viruses, 8 eukaryotes and 6 archaea were found associated with the lichen, *Peltigera hymenina*<sup>11</sup>.

### 1.1.1. Mycobiont

Not a single mycobiont, but many lichenicolous fungi are found associated with lichens as parasites, saprobes or commensals. They cause minimal damage to the host since they do not have direct nutrient exchange with the primary mycobiont, but exploit the host photobiont. In *Lecanora polytropa*, five different lichenicolous fungi were found associated. Most of them were melanized fungi, belonging to the classes, Dothideomycetes and Eurotiomycetes<sup>12</sup>.

### 1.1.2. Photobiont

Multiple green algal symbionts were found existing in a single lichen thallus. Several lineages of the green algal genus *Trebouxia*, was reported in *Lecanora muralis*<sup>13</sup>. Thallus outgrowths can associate with new algae, so that different algae could be detected in the lobes or areoles of a single lichen thallus<sup>14</sup>. Several *Nostoc* genotypes were reported from different cephalodia present on the same thallus of the lichen, *Peltigera venosa*. This might be because of its unusual development and lichenization patterns under various environmental conditions<sup>15</sup>.

About 50 internal transcribed spacer (ITS) algal (chlorobiont) sequences belonging to *Asterochloris* and *Trebouxia* lineages were obtained from *Cladonia* and *Stereocaulon* species collected from metal (Copper) polluted areas<sup>16</sup>. Constant co-existence of *Trebouxia* species TR9 and *Trebouxia jamesii* was reported in the lichen, *Ramalina farinacea*. A much higher chlorobiont diversity was also observed in this lichen with 31 OTUs representative of different microalgal genera, *Elliptochloris*, *Vulcanochloris* and *Diplosphaera* which were obtained with a 454-pyrosequencing method<sup>17</sup>. The co-existence of two *Trebouxia* species, TR9 and TR1 in *Ramalina farinacea* was found significant in the growth and photosynthesis of the lichen in various stressful conditions. It was found that TR9 thrived better in high temperature and irradiances, whereas TR1 in temperate and shady environments<sup>18</sup>. Seven different *Trebouxia* lineages belonging to two species, *T. decolorans* and *T. jamesii* were found associated with *Ramalina fraxinea*<sup>19</sup>.

Studies on genetic diversity of chlorobionts in different climates revealed that the lichen association with multiple bionts was frequently observed in cooler climate than in warmer conditions. The diversity was high in temperate regions than tropical regions. The mycobiont, *Protoparmelia* was found associated with multiple *Trebouxia* (chlorobiont) species in the arctic regions, whereas its association was limited to a single species of *Trebouxia* in the tropical climate. Due to less multiple associations, algal selectivity was high in the tropical areas<sup>20</sup>.

### 1.1.3. Lichenicolous lichen bionts

Existence of lichenicolous lichens have been reported by Rikkinen (2002). It has been found that *Toninia* species starts their growth on other cyanolichens and later become independent. *Lichinodium sirosiphoideum*, a cyanolichen was found growing on parmelioid chlorolichens. Some chlorolichens are found to be associated with free living cyanobacteria like *Stigonema* and *Gloeocapsa*, for additional nitrogen supply.

### 1.1.4. Bacterial bionts

Grube et al. (2009) analyzed the samples of *Cladonia arbuscula*, *Lecanora polytropa* and *Umbilicaria cylindrica* from alpine regions and found bacterial communities in abundance as highly structured and biofilm like assemblages on mycobiont surfaces. Fluorescence in situ hybridization (FISH) analysis revealed the dominance of *Alphaproteobacteria* in the community. Molecular fingerprints of the associated bacteria was performed using PCR- single strand conformation polymorphism using universal and group specific primers and showed distinct patterns of bacterial fingerprints for each lichen species. Cultivable bacterial strains were characterized and the presence of functional genes suggested the involvement of associated bacteria in nutrient cycling. The dominant members of *Alphaproteobacteria* and *Firmicutes* showed various lytic activities, antibiotic, vitamins and hormonal production, especially IAA (Indole-3- acetic acid), phosphate mobilization and antagonistic activity towards other microbes. *nif H* genes meant for symbiotic nitrogen fixation were also identified in the strains.

In the lung lichen, *Lobaria pulmonaria*, a lichenicolous fungus and a complex prokaryotic community, dominated by Rhizobiales, were found in addition to the chlorobiont and mycobiont. A new strategy of metaproteome analysis was conducted. Multifaceted interactions were observed within the lichen associations and the diversified functions of partners supported the longevity of *L. pulmonaria* under specific ecological circumstances<sup>21</sup>. It was found that one third of the overall bacterial community of *L. pulmonaria* was comprised of Rhizobiales. The endosymbiotic members of Rhizobiales belonged to Methylobacteriaceae, Bradyrhizobiaceae and Rhizobiaceae. Metagenomics study (SEED based functional analysis) of Rhizobiales revealed their functions in the symbiosis which included, vitamin and hormone production, nitrogen fixation and stress protection. Rhizobiales were located using a special probe by FISH- CLSM. Occurrence of exo and endosymbiotic members of Rhizobiales were confirmed in *L. pulmonaria*<sup>22</sup>. In *L. pulmonaria*, additional bacterial proteins of the members dominated by

*Proteobacteria* and also *Archaeobacteria*, were found together with the mycobiont and photobiont proteins.

Fungal proteins dominated with about 75.4% and the bacterial and green algal proteins were under the same spectrum of about 10%. Bacterial proteins of *L. pulmonaria* were studied and it was found that they were involved in post translational modifications, protein turnover and various other functions like transport of aminoacids, nucleotides and co- enzymes<sup>23</sup>.

213 *Actinomycetes* strains of Actinobacteria phylum, including novel species belonging to 38 genera, 19 families and 9 orders were isolated from 35 lichen samples belonging to 22 genera<sup>24</sup>. Several bacterial associates include representatives of the extremophilic *Acidobacteria* too. Members of Acetobacteraceae, Brucellaceae, many strains of the genus, *Methylobacterium* and a new lineage of Rhizobiales were also reported and the new lineage was found dominant in the microbial community. 16s ribosomal oligonucleotide primers were developed to target those bacteria<sup>25</sup>.

Abundance of *Alphaproteobacteria* was observed in lichens including *Cetraria islandica* and *Cladonia arbuscula*. After obtaining the total DNA extracts, Alphaproteobacterial fractions (16s rRNA genes) were amplified using group specific primers in PCR and the sequences were separated using single strand conformation polymorphism (SSCP). Sequencing of the separated bands revealed the dominance of *Alphaproteobacteria*<sup>10</sup>. Using fluorescence in situ hybridization, cells of *Alphaproteobacterium* species were found localized on the surface of *Trebouxia* cells isolated from the thallus of *Usnea hakonensis*. Physiological assays revealed that the *Alphaproteobacterium* species associated with *U. hakonensis* were able to use ribitol, mannitol and glucose that were abundant in the thallus. It was supposed that the bacterium would have been involved in the Carbon cycling of *U. hakonensis*<sup>26</sup>.

In the microbiota studies of *Parmelia*, *Rhizoplaca* and *Umbilicaria* species, the bacterial community was dominated by *Alphaproteobacteria* and numerous phylotypes closely related to Rhizobiales and other known nitrogen fixers like *Azospirillum*, *Bradyrhizobium* and *Frankia* were also observed<sup>27</sup>.

Bacterial communities associated with six *Cladonia* species, two *Hypogymnia* species, two *Roccella* species and *Pseudevernia furfuraceae* were investigated. Members of *Firmicutes*, species of *Actinobacteria*, *Proteobacteria*, *Paenibacillus*, *Burkholderia* and *Luteibactor rhizovicina* were found associated with the thalli. Thirty-four bacterial isolates were purified and grouped as phylotypes and representative isolates from each phylotype were characterized<sup>28</sup>.

Biosca et al. (2016) revealed the association of ecto and endolichenic bacteria with three lichen species, *Pseudevernia furfuracea*, *Ramalina farinacea* and *Parmotrema pseudotinctorium* and the improved recovery of those bacteria was possible with extended thalli washing with a surfactant, using an antioxidant buffer that helped to reduce the oxidative stress of endolichenic bacteria during thalli disruption and by using minimal media enriched with lichen extracts.

#### 1.1.5. Diverse bionts

In another study, heterotrophic eukaryotic protists (flagellates and non-flagellates) were found associated with lichens. The chrysophyte, *Ochromonas crenata* and the testate amoebae, *Corythion dubium* were found in association with the silica rich lichens, *Flavoparmelia carperata* and *Flavoparmelia baltimorensis* which help them in nitrogen cycling within the thallus and the building up of their cell walls<sup>30</sup>.

## 2. Bionts: Significant roles

Heterotrophic microbial bionts of lichens are having significant roles in the symbiosis. Prokaryotic and eukaryotic symbiotic associations helped them to survive in extreme conditions like outer space and Mars like environmental conditions<sup>31</sup>. Cyanobionts, mycobionts and bacterial bionts produce diverse secondary substances that have significant roles in the symbiosis. Mycobiont secretes specific lectins that recognize specific ligands on the compatible algal cells and determine the final success of association. An algal binding protein, arginase, secreted by the mycobiont, bound to the cell wall of isolated phycobionts of *Xanthorina parietina*<sup>32</sup>. Cyanobacterial hepatotoxins like nodularin and microcystin were found produced in *Sticta*, *Peltigera* and *Nephroma* species of humid and temperate regions, that help in defence against herbivores<sup>33</sup>. Surface alkanes and fatty acids were produced by the chlorobiont and mycobiont of the epiphytic lichen, *Xanthorina parietina*<sup>34</sup>.

Usnic acid and atranorin, the sunscreen lichen substances, produced by the mycobiont partners of *Cladonia foliacea* and *Pseudevernia furfuracea* were isolated from their acetonic extracts by Varol et al. (2015) and were evaluated for their photoprotective activities, and found cytotoxic to human skin. 3R-5-hydroxymellein, a secondary metabolite produced by the endolichenic fungus of *Parmotrema austrosinense* was found to have UV protective activities, even to human skin without any cytotoxicity<sup>36</sup>.

Hauck et al. (2009) reported that the presence of certain depsidones, depsides, fumarprotocetraric acid, perlatolic and thamnolic acids was found to be responsible for the outstanding tolerance of some

lichens to acidic air pollution. Lichens exhibit the mechanisms of metal accumulation and metal avoidance, in relation to their specific secondary metabolites. In a study conducted by Paukov et al. (2015), it was revealed that different metal concentrations were found in 4 different species of *Aspicilia*, which contained different depsidones. Dense growth of the epiphytic lichen, *Hypogymnia physodes* was found to affect the host, *Picea abies*, due to the penetration of its secondary metabolites to the host tissue<sup>39</sup>. Novel secondary metabolite production was detected from cultures of isolated mycobiont of lichen thalli of *Diorygma* species<sup>40</sup>.

## CONCLUSION

Recent studies of lichen symbiosis replace the old paradigm that, 'Lichens are symbiotic associations of a fungal and an algal partner' with the new fact that 'Lichens are micro ecosystems for multiple bionts including microspecies'. Lichens and their bionts are also sources of novel secondary metabolites, which help in the successful association and survival in various habitats. As shelter and food are provided to each other in a lichen association, many other microbes also harbour this association as there is an existing mutualistic association which is favourable for them. Thus, lichen creates a better environment for the microbial community to live in and get benefits from each other. Much attention should be given to the study of lichen biota and intensive works have to be carried out to study the lichen bionts and their significance.

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