World J Microbiol Biotechnol (2016) 32:68
DOI 10.1007/s11274-016-2019-2

REVIEW

Nutrient scavenging activity and antagonistic factors of non-photobiont lichen-associated bacteria: a review

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Received: 8 December 2015 / Accepted: 28 January 2016 / Published online: 1 March 2016

Abstract Lichens are defined as the specific symbiotic structure comprising a fungus and a green alga and/or cyanobacterium. Up until recently, non-photobiont endothallic bacteria, while known to be present in large numbers, have generally been dismissed as functionally irrelevant cohabitants of the lichen thallus, or even environmental contaminants. Recent analyses of lichen metagenomes and innovative co-culture experiments have uncovered a functionally complex community that appears to contribute to a healthy lichen thallus in several ways. Lichen-associated bacteriomes are typically dominated by several lineages of *Proteobacteria*, some of which may be specific for lichen species. Recent work has implicated members of these lineages in several important ecophysiological roles. These include nutrient scavenging, including mobilization of iron and phosphate, nitrogen fixation, cellulase, xylanase and amylase activities, and oxidation of recalcitrant compounds, e.g. aromatics and aliphatics. Production of volatile organic compounds, conferring antibacterial and antifungal activity, has also been demonstrated for several lichen-associated isolates. In the present paper we review the nature of non-phototrophic endolichenic bacteria associated with lichens, and give insight into the current state of knowledge on their importance the lichen symbiotic association.

Keywords Lichen · Bacteria · Symbiosis · Endothallic · Microbiome

Introduction

Lichens

Lichens form fascinating symbiotic relationships, where two or three organisms associate into unique and easily recognizable structures, the vegetative thalli. The symbiosis yields a biotope that can be considered a small ecosystem and is usually composed of a fungal partner, the mycobiont, and one or more photosynthetic partners, the photobiont, which generally is either a green alga or a cyanobacterium (Nash 2008a). Lichens can thrive in practically any terrestrial environment, they are among the earliest colonizers of severe habitats and recently exposed land (Gadd 2007), and are often major components of widespread communities such as biocrusts (Sancho et al. 2014). Lichens are dominant in some parts of the planet's landscape and have been estimated to cover up to 8 % of the total land surface (Ahmadjian 1995). Lichens are usually highly tolerant of harsh environmental conditions such as extremes in temperature, low water content, low nutrient availability and high UV light intensities. They are thus often found in environments where few other macroscopic organisms would thrive (Beckett et al. 2008), including extreme environments such as the Antarctic (Øvstedal and Smith 2001), the Arctic (Printzen 2008), on mountain summits, as components of desert crusts (Büdel et al. 2009) and tropic regions (Morley and Gibson 2004). Lichens are perennial and slow-growing organisms that present a variety of colorful forms and maintain fairly uniform, long-lived morphologies, which can be maintained up to several

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thousand years. Despite their resistance to abiotic stresses in their dormant stage, lichens can be vulnerable to even slight changes in environmental conditions that can disrupt the integrity of the symbiotic association (Erlacher et al. 2015).

General description of the lichen symbiosis

Lichens represent a classic example of self-sustaining symbiotic interactions that today are recognized to be widespread in nature, and involving different kingdoms of life (Nash 2008a). As a heterotrophic organism, the mycobiont requires carbohydrates, which are produced by the photobiont; sugar alcohols by most algal photobionts and glucose by cyanobionts (Nash 2008a). The majority, or approximately 85 % of lichens, contain green microalgae as photobionts. In addition to the principal photobiont, tripartite lichens are characterized by cyanobacteria associated within their thalli, usually in specific structures called cephalodia. In tripartite lichens, the cyanobacteria are apparently focused on supplying fixed nitrogen used by other members of the symbiosis (Rikkinen 2002). The nitrogen fixation has important effects on the lichen ecology, promoting colonization of special ecological niches, such as extremely oligotrophic habitats (Büdel and Scheidegger 2008).

Lichen growth and resources

Individual lichen growth is dependent on resource acquisition, followed by biosynthesis of cellular compounds, minus losses of thallus material through dispersal, fragmentation, grazing and necrosis (Palmqvist 2000). Being poikilohydric organisms, unable to control their water status, environmental conditions have a large effect on lichen growth. However, when wet and metabolically active, lichens can convert incident light energy into new biomass just as efficiently as vascular plants. Successful lichen growth requires a balance between the carbon acquisition and mineral availability. Mineral nutrients, particularly nitrogen and phosphorous, are essential for thallus growth (Palmqvist et al. 2008).

About 40–50 % of a lichen's dry mass is composed of carbon, which they mainly gain from photobiont photosynthesis (Green et al. 2008). The poikilohydric characteristic of lichens in general dominates their CO_2 exchange behavior. The rate of gas exchange dramatically changes in contrast to the thallus water content; when the water content is low, CO_2 diffuses but when hydrated, diffusion of CO_2 is blocked (Honegger 1991). The CO_2 exchange rates can vary among lichen species or lichen type and environmental conditions can also affect the exchange rates (Green et al. 2008). Nitrogen is involved in many processes for all symbionts and although it is an important nutrient, fixed nitrogen can cause stress if supplied in excess (Johansson et al. 2010). The nitrogen acquisition mode can vary among lichen species, depending on the associated photobiont. Green algal lichens mostly depend on direct nitrogen deposition on the thallus surface whereas cyanobacterial lichens (either bi- or tripartite) carry out biological nitrogen fixation of atmospheric nitrogen (Dahlmann et al. 2004). Lichens can assimilate nitrogenous compounds as nitrate (NO_3^-), ammonium (NH_4^+) or organic N (Nash 2008b; Johansson et al. 2011). The effects of nitrogen limitation on lichen productivity have been relatively well studied (Palmqvist et al. 2008) but the effect of phosphorus limitation is not fully known. However, recent studies have e.g. shown that addition of phosphorus may increase growth rate, abundance and species richness of lichens, especially N_2-fixing species (McCune et al. 2009; Benner et al. 2007; Benner and Vitousek 2007). Only trace concentrations of phosphorus are present in the atmosphere as dust from soil erosion and rock weathering, and thus lichens have developed uptake pathways for their phosphate capture (Smith 1960; Farrar 1976).

Bacterial communities

Although lichens are generally defined only as bipartite or tripartite mycobiont–photobiont symbioses, the presence of other microorganisms in the lichen thalli has long been known. Following Uphof's (1925) early report on "purple bacteria" in lichens, suggesting that non-cyanobacterial prokaryotes were present, a series of papers were published in the following years (Henkel and Plotnikova 1936; Iskina 1938; Panosyan and Nikogosyan 1966). Throughout most of the twentieth century, however, the focus was primarily on the symbiotic components of lichens and their ability to produce secondary metabolites with biological activities (Huneck 1999; Müller 2001) and little was known about the endolichenic microbial communities, their function or diversity.

Bacterial diversity of the culturable biota

Some 10 years ago, the first modern-era study on the non-phototrophic lichen-associated microbiota was published (González et al. 2005). That study focused on *Actinobacteria*, isolated from 25 terrestrial lichen samples collected in Alaska, Hawaii and Reunion. The lichens sampled were not identified in the paper but were described as saxicolous (rock-dwelling) and arboricolous (tree-dwelling). A standard medium (YME agar) was used for actinobacterial cultivation from all lichen samples, resulting in a large number (337) of strains which were identified on the basis of DNA fingerprinting and fatty acid analysis. The majority of isolates belonged to the families *Micromonosporaceae* (142) and *Streptomycetaceae* (110), although *Pseudonocardiaceae* (30), *Nocardiaceaei*(4), *Streptosporangiaceae* (2), *Thermomonosporaceae* (7) and *Geodermatophilaceae* (1) were also detected. The isolates were further screened for the presence of genes involved in polyketide, polypeptide and isoprenoid biosynthesis, and antimicrobial activity against *Escherichia coli*, *Staphylococcus aures* and *Candida albicans*. At least one biosynthetic cluster was observed in a large part (over 60 %) of the isolates and 27 % showed antimicrobial activity against at least one of the targeted microorganisms.

The following year, two papers were published on lichen-associated bacterial communities (Cardinale et al. 2006; Liba et al. 2006). Cardinale and coworkers analyzed 11 different lichens, belonging to eight species and sampled at five different sites in Austria and France. The lichens sampled belonged to the genera *Cladonia*, *Pseudevernia*, *Hypogymnia* and *Roccella*. Bacteria from external and internal surfaces of the lichen thalli were isolated, using TY and sugar-rich/N-free medium, resulting in 34 morphologically distinct bacteria. Liba et al. (2006) sampled 5 foliose cyanolichens from rainforests in Brazil and isolated acetylene-reduction positive strains on nitrogen-free medium. The presence of *nifH* genes, involved in nitrogen fixation, was confirmed by dot-blot hybridization of genomic DNA and the microbiota was suggested to be involved in nitrogen fixation for the lichen symbiosis. The isolates (17) all belonged to different genera of *Gammaproteobacteria* and further analysis of their functions showed them to be involved in phosphate solubilization, indole acetic acid (IAA) production and amino acid secretion.

Since these studies, a number similar publications have appeared. While many of them have targeted individual isolates or species, others have focused on special interests or the biotechnological potential of the microbiota. Overall, the taxonomic bacterial diversity has been fairly well established and in some studies, possible roles of the associated microbiota have been suggested (see Table 1 for overview).

In the first study published on bacterial associates of Antarctic lichens, a number of psychrotolerant strains were revealed (Selbmann et al. 2010). Of the thirty bacterial isolates, a new species of *Deinococcus–Thermus* was reported and several other strains represented potential new taxa. The bacterial diversity of Antarctic and Arctic lichens was further studied by Kim et al. (2014) and Lee et al. (2014). Although no specific roles was suggested for the microbiota within the symbiosis, the strains isolated from the crustose lichen *Ochrolechia* sp. had antimicrobial and antioxidant activity (Kim et al. 2014). The isolates were found to belong to *Sphingomonas* and *Burkholderia*, genera abundantly found in Antarctic and Arctic lichens (Lee et al. 2014). Lee et al. studied the microbiota of nine different lichens, recovering isolates affiliated with *Actinobacteria*, *Bacteroidetes*, *Deinococcus–Thermus*, *Firmicutes*, *Alphaproteobacteria*, *Betaproteobacteria* and *Gammaproteobacteria*. Although previous studies using cultivation methods had poor representation of *Alphaproteobacteria* (Cardinale et al. 2006; Selbmann et al. 2010), they were found to be predominant in Lee's study. The majority of the alphaproteobacterial isolates were affiliated with the genus *Sphingomonas*. In addition to *Sphingomonas*, members of the genera *Frondihabitans*, *Hymenobacter* and *Burkholderia* were isolated across lichen samples from both the Arctic and Antarctica and members of *Nakamurella*, *Streptomyces*, *Deinococcus*, *Paenibacillus*, *Aurantimonas*, *Methyloferula*, *Psychrobacter*, *Pseudomonas* and *Rhodanobacter* were recovered with lower frequency. Based on functional tests, the microbiota of the Arctic and Antarctic lichens were commonly found to have protease and lipase activity and were suggested to be involved in nutrient supply for the symbiosis as a whole. In a recent study on crustose lichens on the Northern Iceland seashore, at total of 93 bacterial strains were isolated from four different lichens. Based on 16S rDNA sequencing, the collection was found to contain members belonging to *Alphaproteobacteria*, *Bacilli*, *Actinobacteria*, *Flavobacteria*, *Cytophagia*, *Sphingobacteria* and *Gammaproteobacteria*. Selected isolates were further tested for secretion of several extracellular enzymes and were found to be proteolytic, glucanolytic and amylolytic. Furthermore, a number of the tested isolates were phosphate-solubilizing and nitrogen fixing (Sigurbjörnsdóttir et al. 2014).

In summary, cultivation-based studies have revealed that the culturable non-phototrophic microbiota of lichens tends to be dominated by members of the phyla *Actinobacteria*, *Firmicutes* and *Proteobacteria*. Some studies also yielded members of *Deinococcus–Thermus*, *Bacteroidetes* and *Acidobacteria*. While some of the studies did not consider potential roles of the lichen associates, we can surmise that the culturable fraction of lichen-associated bacteriomes are involved in nitrogen fixation, phosphate solubilization, hormone production, nutrient supply via various lytic activities and that they play roles in defense mechanisms through antagonistic activities.

Bacterial diversity via culture-independent methods

While many studies have described the diversity of bacterial lichen-associates and their production of bioactive compounds (Davies et al. 2005; Yamamura et al. 2011), the culturable fraction is nevertheless believed to represent only a small part of the total bacterial biota in environmental samples (Amann et al. 1995). Several methods can

Table 1 A summary of culture dependent studies of lichen associates

Lichen species	Lichen habitat	Taxonomic diversity of the isolated microbiota	Roles of microbiota within the symbiosis/potential biotechnological applications	References
Unidentified	Terrestrial	*Actinobacteria*	None suggested/ Bioactive	González et al. (2005)
Cladonia digitata, *C. rangiferina*, *C. coniocraea*, *C. pyxidata*, *C. coccifera*, *Pseudevernia furfuracea*, *Hypogymnia physodes*, *Roccella psychopsis*, *R. fuciformis*	Terrestrial	*Firmicutes*, *Actinobacteria*, *Alphaproteobacteria*, *Betaproteobacteria*, *Gammaproteobacteria*	N-fixing/ Diazotrophic bacteria	Cardinale et al. (2006)
Canoparmelia caroliniana, *C. crozalsiana*, *C. texana*, *Parmotrema sanctiangeli*, *P. tinctorum*	Cyanolichens from rain forest in Brazil	*Gammaproteobacteria*	N-fixing, phosphate solubilization, IAA production, nutrition contribution via amino acid release	Liba et al. (2006)
Cladonia arbuscula, *Lecanora polytropa*, *Umbilicaria cylindrica*	Saxicolous above tree line in Austria	*Alphaproteobacteria*, *Actinobacteria*, *Firmicutes*, *Betaproteobacteria*, *Gammaproteobacteria*	N-fixing, proteolysis, chitionlysis, glucanolysis, phosphate solubilization, IAA production, antagonistic	Grube et al. (2009)
Acarospora flavocordia, *Lecanora fuscobrunnea*, *Lecidea cancriformis*, *Rhyzocarpon* sp., *Umbilicaria decussata*, *Usnea antarctica*, *Xanthoria elegans*	Antarctic	*Actinobacteria*, *Gammaproteobacteria*, *Deinococcus-Thermus*, *Firmicutes*	None suggested	Selbmann et al. (2010)
Cladonia sp. *Cladonia rangifernia*, *Sphaerophorus globose*	Epigeal bog in N-Russia (Karelia and Arctic tundra)	*Actinobacteria*, *Acidobacteria*, *Alphaproteobacteria*, *Betaproteobacteria*	None suggested	Pankratov (2012)
Ochrolechia sp.	Arctic region	*Alphaproteobacteria*, *Betaproteobacteria*	None suggested/ antibacterial and antioxidant activity	Kim et al. (2014)
Usnea sp., *Cladonia borealis*, *Psoroma* sp., *Stereocaulon* sp., *Umbilicaria* sp., *Cetraria* sp., *Cladonia* sp., *Ochrolechia* sp.	Antarctic and Arctic regions	*Actinobacteria*, *Bacteroidetes*, *Deinococcus-Thermus*, *Firmicutes*, *Alphaproteobacteria*, *Betaproteobacteria*, *Gammaproteobacteria*	Nutrient supply (proteolysis and lipase activity)	Lee et al. (2014)
Lecanora helicopis, *Verrucaria ceuthocarpa*, *Hydropunctaria maura*, *Caloplaca verruculifera*	Seashore lichens, Northern Iceland	*Alphaproteobacteria*, *Bacilli*, *Actinobacteria*, *Flavobacteria*, *Cytophagia*, *Sphingobacteria*, *Gammaproteobacteria*	Proteolysis, glucanolysis, amylolysis, phosphate solubilisation, nitrogen fixation	Sigurbjörnsdóttir et al. (2014)
Lobaria pulmonaria	Maple trees in the Alps		Nutrient supply phosphate solubilization, antagonistic activity	Grube et al. (2015)
Lobaria pulmonaria	Three different locations in Austria	*Betaproteobacteria*, *Gammaproteobacteria*, *Actinobacteria*, *Firmicutes*	Antagonistic activity	Cernava et al. (2015a)
Lobaria pulmonaria	Three different locations in Austria		Volatile organic compound production, spermidine production, hydrogen cyanide production	Cernava et al. (2015b)

be used to expose the unculturable bacterial fraction, including microbial fingerprinting where PCR products yield analyzable banding patterns (Portillo et al. 2011). Different primers can be used, either universal or group specific, and the bands can further be excised from the gel matrix and characterized by sequencing. Such fingerprinting methods have been used in a number of studies, revealing the taxonomic diversity of the total bacterial biota of lichens (see Table 2 for overview). Although fingerprinting methods have in general many advantages, e.g. comparatively low cost, are not time consuming and banding patterns can directly be compared between samples, these methods generally lack the resolution required for thorough phylogenetic inference (Grube and Berg 2009). Instead, the analysis of 16S rDNA libraries, often supplanted by other conserved gene markers, is now commonly used to identify the total bacterial community in lichens (Grube et al. 2012, 2015; Aschenbrenner et al. 2014).

The location of bacteria in the symbiotic structure has been established in a number of studies, using fluorescent in situ hybridization (FISH) and confocal laser scanning microscopy (CLSM). In the first published study where FISH-CLSM analysis of lichen-associated bacteria was performed, a protocol for cryosectioning of lichen fragments was optimized, and dense colonization of bacteria was mainly found on the internal thallus surfaces of *Cladonia arbuscula* (Cardinale et al. 2008). In the same study, group-specific FISH probes revealed the prominence of *Alphaproteobacteria* (>60 % of all bacteria). *Actinobacteria* and *Betaproteobacteria* were also detected although at a much lower abundance. Few members of *Firmicutes* were found but *Gammaproteobacteria* were not detected. In this pioneering study, lichens were found to host more bacteria than previously thought and the method has since been used in several studies. Grube et al. (2009) studied the bacterial diversity of three lichen species, *C. arbuscula*, *Lecanora polytropa* and *Umbilicaria cylindrica*, collected from alpine environments. The number of bacteria per gram of *C. arbuscula* specimen were comparable to the previous study of Cardinale et al. (2008) but lower numbers were obtained for *L. polytropa* and *U. cylindrica*, 7×10^5 and 9×10^5 mm^{-3} of lichen volume, respectively. Using FISH group-specific probes, *Alphaproteobacteria* were estimated to form 45 to 75 % of the total bacterial biota but other groups much less. In this work, bacterial fingerprints from the three lichen species were compared for universal bacterial and group-specific fingerprints (*Alphaproteobacteria*, *Pseudomonas* and *Burkholderia*) and the bacterial biota found to be species-specific (Grube et al. 2009).

Interestingly, relatively few studies thus far have focused on the non-phototrophic bacteria of crustose lichens. In one of the few published studies, samples of *Hydropunctaria maura*, *Ophioparma ventosa*, *Pertusaria corallina* and *Rhizocarpon geographicum* were analysed (Bjelland et al. 2011). In this study, DGGE and clone library sequencing were used to determine the composition and abundance of bacteria. The classes *Acidobacteria*, *Proteobacteria* (*Alpha-* and *Betaproteobacteria*) and *Chloroflexi* dominated the bacterial biota in *O. ventosa*, *P. corallina* and *R. geographicum* although *O. ventosa* was slightly different and more often associated with *Beta-* and *Gammaproteobacteria*. In terms of abundance, the samples in this study had similar numbers of bacteria per cell of lichen thallus as previously reported by Cardinale et al. (2008) and Grube et al. (2009). *H. maura*, however, had a higher number, 1.8×10^{11} g^{-1} and the bacterial community reflected the marine influence, not detected in the other three lichen samples. Moreover, *Archaea* were, for the first time, shown to be associated with lichens.

Many studies have used culture independent methods to elucidate the functional role of the lichen-associates. In Grube's recent work (Grube et al. 2015) the functional gene composition of the lung lichen *Lobaria pulmonaria* was revealed as well as the taxonomic structure. *Proteobacteria* were found to be dominant, with *Alphaproteobacteria* the prominent taxon, but members of *Beta-*, *Gamma-* and *Deltaproteobacteria* were also present. Greatest homology to members of the taxa *Actinobacteria*, *Acidobacteria* and *Bacteroidetes* were found in 2, 2 and 1 % of the total bacterial contigs, respectively. When the lichen metagenome was subjected to functional analyses with SEED and KEGG, most contigs were found to be involved in primary metabolism such as carbohydrate, energy, lipid, nucleotide and amino acid metabolism, glycan biosynthesis and genetic information processing. When the metagenome was further screened for selected functions genes involved in e.g. nutrient scavenging, resistance against biotic and abiotic stress factors, hormone production and various lytic activities were detected. In another recent study, the bacterial microbiota associated with *Peltigera membranacea* was analyzed in terms of taxonomic structure and functional gene composition. *Proteobacteria* composed the majority of the bacterial microbiota but *Acidobacteria*, *Actinobacteria*, *Bacteroidetes* and *Verrucomicrobia* were also detected. Genes involved in phosphate scavenging were commonly found in the dataset, as were enzymes involved in various lytic activities (Sigurbjörnsdóttir et al. 2015).

Functional roles of lichen associates

Nitrogen is one of the essential elements lichens need for growth. In cyanolichens, the photobiont can gain nitrogen from the atmosphere but nitrogen fixation has been

Table 2 A summary of culture independent studies of lichen associates

Lichen species	Lichen habitat	Technique(s) applied	Taxonomic diversity of the associated microbiota	Potential activity of the associated microbiota	References
Cladonia digitata, *C. rangiferina*, *C. coniocraea*, *C. pyxidata*, *C. coccifera*, *Pseudevernia furfuracea*, *Hypogymnia physodes*, *Roccella psychopsis*, *R. fuciformis*		ITS fingerprinting, sequencing of bands	*Betaproteobacteria*, *Gammaproteobacteria*, Actinobacteria	N-fixing	Cardinale et al. (2006)
Cladonia arbuscula	Mountain ridge in Styria, Austria	FISH, SSCP	*Alphaproteobacteria*, Actinobacteria, *Betaproteobacteria*	None suggested	Cardinale et al. (2008)
Cladonia cristatella, *C. cryptochlorophaea*, *C. sobolescens*, *C. peziziformis*, *C. subtenuis*, *Flavoparmelia caperata*, *Parmotrema perforatum*, *Peltigera phyllidiosa*	Virginia and N-Carolina, USA	Sanger sequencing (direct of PCR products using universal primers)	*Alphaproteobacteria*, Acidobacteria, *Gammaproteobacteria*	None suggested	Hodkinson and Lutzoni (2009)
Cladonia arbuscula, *Lecanora polytropa*, *Umbilicaria cylindrica*	Saxicolous above tree line in Styria, Austria	FISH, SSCP	*Alphaproteobacteria*, Actinobacteria, *Betaproteobacteria*	High abundance of *nifH* genes, diazotrophic bacteria	Grube et al. (2009)
Hydropunctaria maura, *Ophioparma ventosa*, *Pertusaria corallina*, *Rhizocarpon geographicum*	Saxicolous lichens from SW-Norway	DGGE, clone libraries	*Alphaproteobacteria*, *Betaproteobacteria*, Actinobacteria, Acidobacteria, Firmicutes, Chloroflexi, Bacteroidetes	None suggested	Bjelland et al. (2011)
Xanthoparmelia plittii, *X. somloensis*	Foliose lichens sampled in Massachusetts, USA	Pyrosequencing of 16S rRNAs	*Alphaproteobacteria*, Acidobacteria, Bacteroidetes, *Gammaproteobacteria*, *Deltaproteobacteria*	None suggested	Mushegian et al. (2011)
Lobaria pulmonaria	Foliose lichen, arboicolous	FISH, pyrosequencing	*Alpaproteobacteria*, *Betaproteobacteria*, *Gammaproteobacteria*, *Deltaproteobacteria*, Bacteroidetes, Actinobacteria, Firmicutes, Verrucomicrobia	Nutrient availability (various lytic activity), resource reallocation (amino acid release)	Schneider et al. (2011)
Parmelia sulcata, *Rhizoplaca chrysoleuca*, *Umbilicaria americana*, *U. phaea*	Foliose lichens from granite rock outcrops, Colorado, USA	Pyrosequencing of 16S rRNAs	*Alphaproteobacteria*, Acidobacteria, *Gammaproteobacteria*, Firmicutes, Verrucomicrobia, Planctomycetes, Actinobacteria, *Betaproteobacteria*, Bacteroidetes, *Deltaproteobacteria*	N-fixing, phosphate solubilisation	Bates et al. (2011)

Table 2 continued

Lichen species	Lichen habitat	Technique(s) applied	Taxonomic diversity of the associated microbiota	Potential activity of the associated microbiota	References
Lobaria pulmonaria	Styria, Austria	FISH, SSCP	*Alphaproteobacteria*	N-fixing	Cardinale et al. (2012a)
Cetraria islandica, *Lobaria pulmonaria*, *Lecanora polytropa*, *Cladonia arbuscula*, *Umbilicaria cylindrical*, *Cladonia coccifera*	Different parts of thalli sampled (e.g. old, whole, young), Styria, Austria	FISH, SSCP	*Alphaproteobacteria*, *Betaproteobacteria*, *Gammaproteobacteria*, Actinobacteria	None suggested	Cardinale et al. (2012b,)
Solorina crocea	Styria, Austria	Pyrosequencing of 16S rRNAs	Acidobacteria, Proteobacteria, Planctomycetes, Actinobacteria	N-fixing	Grube et al. (2012)
Cladonia sp., *Flavocetraria* sp., *Ophioparma* sp., *Umbilicaria* sp., *Usnea* sp., *Dictyonema* sp., *Leptogium* sp., *Peltigera* sp., *Sticta* sp.	Tropical or arctic latitudes	Pyrosequencing of 16S rRNAs	*Alphaproteobacteria*, Acidobacteria, *Betaproteobacteria*, *Gammaproteobacteria*, Actinobacteria, Verrucomicrobia, Planctomycetes, Bacteroidetes, Deltaproteobacteria, Firmicutes	None suggested	Hodkinson et al. (2012)
Cetraria aculeata	Collected from different places; Antarctica, Spain, Germany and Iceland	PCR with group specific primers, clone libraries	*Alphaproteobacteria*	None suggested	Printzen et al. (2012)
Arthrographis citronella, *Baeomyces placophyllus*, *B. rufus*, *Icmadophila ericetorum*, *Psora decipiens*, *Trapeliopsis granulosa*	Styria, Austria	FISH	*Alphaproteobacteria*, Acidobacteria	None suggested	Muggia et al. (2013)
Lobaria pulmonaria	Maple trees in the Alps	Pyrosequencing of 16S rRNAs	*Alphaproteobacteria*, *Betaproteobacteria*, *Gammaproteobacteria*, *Deltaproteobacteria*, *Epsilonproteobacteria*, Actinobacteria, Acidobacteria, Bacteroidetes	Nutrient supply, pathogen defence, abiotic stress resistance, detoxification of metabolites, lytic activity	Grube et al. (2015)
Lobaria pulmonaria	Styria, Austria	SSCP, pyrosequencing of 16S rRNAs, FISH	*Alphaproteobacteria*, Bacteroidetes, Verrucomicrobia, *Deltaproteobacteria*, Actinobacteria, *Betaproteobacteria*, *Gammaproteobacteria*, Acidobacteria, Planctomycetes	None suggested	Aschenbrenner et al. (2014)
Lobaria pulmonaria	Three different locations in Austria	Pyrosequencing of 16S rRNAs	*Betaproteobacteria*, *Gammaproteobacteria*, Actinobacteria, Firmicutes	Antagonistic activity	Cernava et al. (2015a)

Table 2 continued

Lichen species	Lichen habitat	Technique(s) applied	Taxonomic diversity of the associated microbiota	Potential activity of the associated microbiota	References
Lobaria pulmonaria	Two mountain forests	Pyrosequencing of 16S rRNAs, FISH	*Alphaproteobacteria*	N-fixing, auxin and vitamin production, stress protection	Erlacher et al. (2015)
Peltigera membranacea	Sub-Arctic heathland, Iceland	Shotgun pyrosequencing, rRNA analysis	*Alphaproteobacteria*, *Bacteroidetes*, *Actinobacteria*, *Betaproteobacteria*, *Verrucomicrobia*, *Gammaproteobacteria*, *Deltaproteobacteria*	Phosphate solubilization, nutrient availability (cellulolytic, glucanolytic), defence mechanism within the symbiosis.	Sigurbjörnsdóttir et al. (2015)

suggested to be one of the important roles of the non-cyanobacterial prokaryotes of lichens. Nitrogen is abundant in Nature, however, it is not always available in form which organisms can utilize. Atmospheric nitrogen must thus be fixed to usable form, in an energy-intensive transformation mediated by nitrogenase enzymes (Shridhar 2012). Nitrogen fixing bacteria, often referred to as diazotrophs, might deliver nitrogen suitable for the symbiotic partners of lichens (Kneip et al. 2007) a role of special importance in non-cyanobacterial lichens and in the case of nitrogen limiting conditions (Grube and Berg 2009).

Phosphorus (P) is another essential macronutrient for plants and lichens and is involved in all major metabolic pathways (Hayat et al. 2010; Sharma et al. 2013; Zhao et al. 2014). In soil, P exists in two forms, as organic and inorganic phosphates, and needs to be converted to soluble form for plants (Richardson et al. 2001). Phosphate solubilizing bacteria (PSB) have the ability to release P and make it available to plants (Richardson et al. 2001; Sashidhar and Podile 2010; Sharma et al. 2013; Chhabra et al. 2013; Zhao et al. 2014). The pathways involved in phosphate solubilization are complex and several key enzymes are needed, including alkaline and acid phosphatases, phytases and phosphatases (Rodríguez et al. 2006; Sharma et al. 2013). Organic acids, especially gluconic acid, are essential for acidification, a key step in the dissolution of many poorly soluble mineral phosphates. Goldstein (1995) proposed the direct oxidation of glucose to gluconic acid (GA), and in some cases 2-ketogluconic acid, to be a key step of the mineral phosphate solubilization (MPS) in some Gram-negative bacteria. Membrane-bound glucose dehydrogenase mediates the pathway and requires pyrroloquinoline quinone (PQQ) as a cofactor (Goldstein 1995). Bacteria of various genera have been reported to be efficient phosphate solubilizers, e.g. members of *Pseudomonas*, *Bacillus*, *Rhizobium*, *Burkholderia*, *Achromobacter*, *Agrobacterium*, *Micrococcus*, *Aerobacter*, *Flavobacterium* and *Erwinia*. The bacteria can solubilize mineral phosphate (inorganic compounds), including tricalcium phosphate, hydroxyl apatite and rock phosphate (Rodríguez and Fraga 1999; Rodríguez et al. 2006).

In many of the recent functional and metagenomic studies, one of the suggested traits of the bacterial microbiota of lichens is their wide range of lytic activities; chitinolysis, proteolysis and glucanolysis (Grube et al. 2009, 2015; Lee et al. 2014; Schneider et al. 2011). These extracellular enzymes are relatively abundant among lichen associates and are suggested to be involved in nutrient mobilization within the lichen symbiosis and contribute to the hydrolysis of organic compounds. The mycobiont comprises the major part of a lichen thallus and as fungal cells are mixtures of polysaccharides and proteins, bacterial strains with lytic activity are likely to degrade older parts of the thallus where antibacterial compounds are no longer present. Many foliose and fruticose lichens grow in a cellulose-rich environment and often in close proximity to mosses and other vegetation, thus cellulolysis might be particularly important for many lichen symbioses. Albeit lichens generally obtain their carbon from the photosynthetic partner (Palmqvist et al. 2008) it has been suggested that cellulase systems, found within lichens, are used for saprophytic activity which could be beneficial for the symbiosis, e.g. when lichens are covered by snow (Beckett et al. 2013).

Plant growth-promoting rhizobacteria (PGPR) can influence plant growth by synthesizing and exporting phytohormones which may act as regulators in plant growth and development (Hayat et al. 2010). One of the physiologically most active phytohormones in plants is indole-3-acetic acid (IAA) and it is produced by several lichen associates (Liba et al. 2006; Grube et al. 2009). In plants, production of IAA is known to stimulate root elongation and long-term (e.g. cell division and differentiation) responses (Spaepen et al. 2007; Hayat et al. 2010).

IAA has been shown to effect fungal growth (Gryndler et al. 1998) and cell division in unicellular algae (Lau et al. 2009). Therefore, IAA producing bacteria have been suggested to alter morphogenetic processes of lichen symbioses and influence both the mycobiont and algal partners (Grube and Berg 2009).

Bacteria of the phylum *Actinobacteria* are known for their antimicrobial properties and biosynthetic potential (Arul Jose and Jebakumar 2013) and have commonly been cultured from lichen species. It has thus been suggested that such activities are involved in the defense mechanism of lichens (Grube et al. 2009, 2015; Cernava et al. 2015b).

Although the presence of other microorganisms within the lichen thallus has long been recognized, the first conclusive studies were only published in the last decade. Culturing and culture independent methods have now been used to elucidate the taxonomic structure and functional gene composition of lichen associates. In most lichens studied thus far, *Proteobacteria* are most abundant although members from many other classes have also been found, including *Archaea*. Studies now indicate that lichen associates have various roles within the lichen symbiosis, including nitrogen fixation, phosphate solubilization and nutrient mobilization through various lytic activities.

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