

Chapter 9

Lichen Microbiome: Diversity Biological Role and Biotechnological Application



R. R. Sargsyan, A. Tsurykau, and Hovik Panosyan

Abstract Lichens were traditionally considered as a remarkable assemblage of fungi with unicellular phototroph (algae or cyanobacteria) that have converged on similar symbiotic strategies. However, this view of lichens has recently been reconsidered by findings of miscellaneous associated microbes colonizing on or within the thallus causing no apparent effect. This hidden diversity includes filamentous fungi, lichen-inhabiting yeasts, as well as various prokaryotic bacteria. Despite these endothallic and exothallic organisms do not belong to constant lichen symbionts, they usually play important roles in lichen biology by participating in the lichen metabolism, regulating water relations, affecting thallus architecture, and being involved in the degradation processes. However, it is often difficult to understand microorganismal input and uptake and therefore determine their symbiotic outcome due to the complexity of lichen symbiosis. In this chapter, diversity and biological role of usually neglected or overlooked lichen microbial consortia are reviewed and their possible biotechnological application is discussed.

Keywords Lichen · Colonization · Symbiosis · Metabolism · Consortia

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9.1 Introduction

Traditionally lichens are considered to be mutualistic associations encompassed of fungus as a mycobiont and either an algae or a cyanobacteria as a photobiont (Honegger 1991). About 20,000 species of lichens growing on rock stones (saxicolous lichens) and on the tree barks and branches (corticolous lichens or epiphytes) have been recorded worldwide (Ellis 2012). Lichens are found in various habitats from polar to equatorial regions. They are found in various geographical zones, from lowland layers to alpine levels. To withstand extreme conditions characterized by abnormal temperatures, periodic desiccation, high levels of UV radiation, and high concentrations of salts, lichens synthesize secondary metabolites (e.g., radioprotectants, cryoprotectants, compatible solutes) widely used in various biotechnologies (Suzuki et al. 2016; Subhashini et al. 2017; Sargsyan et al. 2021).

Since the recognition that a diverse microbial community is integral part to the traditionally recognized mycobiont and photobiont mutualistic association, lichens have progressively become a subject of research in ecological microbiology (Bates et al. 2011; Pankratov et al. 2017; Yang et al. 2020). It was revealed that lichens usually provide habitats for bacteria being different from those of nearby substrates. Moreover, lichens adapted to grow in different habitats usually host appropriate bacterial batch (Bates et al. 2011; Mushegian et al. 2011). These microbiomes often involve of non-photosynthetic diazotrophs, which usually provide benefits to the host lichen by their metabolic activities. There are many reports confirming that different groups of the lichen–bacterial associations are highly structured. Bacteria are not distributed only across the lichen thallus. More-consistent bacterial communities with different species, it is supposed to be in central parts of thalli (Kumar et al. 2014; Mushegian et al. 2011). In consequence, the long-established concept of mutualistic relationship between lichenized fungi and algae or cyano-bacteria is in need of revision and should also encompass bacterial component. It has been shown important role of lichenized bacterial community in the nutrient cycling of lichens (Grube and Berg 2009; Bates et al. 2011; Hawksworth and Grube 2020; Singh et al. 2020).

9.2 Microbial Diversity in Lichens

Lichen bacterial associations were first mentioned in the last century (Uphof 1925; Henckel and Yuzhakova 1936; Iskina 1938). Those studies were mainly based on traditional cultivation techniques and biochemical and morphological identification. The dominating genera were *Azotobacter*, *Pseudomonas* (Gammaproteobacteria), *Beijerinckia* (Alphaproteobacteria), *Bacillus* and *Clostridium* (Firmicutes) (Iskina 1938; Panosyan and Nikogosyan 1966).

Still only less than 1% of the microorganisms found in natural habitats have been cultivated and subsequently isolated so far. The development of molecular biology

methodology largely promoted to expand our knowledge of environmental microbial diversity (Yang et al. 2019). The culture-independent studies uncovered a vast biodiversity of endothallic and exothallic bacteria (see Table 9.1) (González et al. 2005; Cardinale et al. 2006; Liba et al. 2006; Selbmann et al. 2010; Pankratov et al. 2017).

In the beginning to study lichen-associated bacteria, several culture-independent methods including denaturing gradient gel electrophoresis (DGGE), terminal restriction fragment length polymorphism (T-RFLP), and single-strand conformation polymorphism (SSCP) have been used (Liu et al. 1997; Muyzer and Smalla 1998; Schwieger and Tebbe 1998). Apart from genetic techniques, specific fluorescence in situ hybridization and confocal laser scanning microscopy (FISH-CLSM) methods were also used to uncover the lichen microbial content (Cardinale et al. 2008; Grube and Berg 2009; Muggia et al. 2013; Aschenbrenner 2015).

Currently, the molecular analyses (e.g., 16S rRNA gene clone library construction, pyrosequencing, metagenomics) are the most commonly used (Singh et al. 2016). Combination of classical microbiology with new molecular biology techniques has considerably enlarged our apprehension of the taxonomic and metabolic diversity of lichen microbiota.

CTAB method still remains to be one of the most available methods to extract the total DNA of lichens (Cardinale et al. 2006; Singh et al. 2016). Prior to the main extraction process, lichen thalli are usually washed by distilled sterile water, 5–9% H₂O₂, or ethanol. After that, it is required to clean the sterilized lichen thalli from the washing solution residues, usually distilled water is used in this step. After the “sterilization” of lichen thalli, the main DNA extraction process is initiated (Fig. 9.1).

Cardinale et al. (2012) revealed curious information regarding the lichen-inhabiting bacteria and age of the lichen thalli, its substrate, and growth conditions (e.g., solar irradiation, humidity). The youngest and therefore the most physiologically active lichen thalli were dominated by Alphaproteobacteria. In contrast, older parts of lichen thalli were associated with Actinobacteria. Density of Actinobacteria and Betaproteobacteria was also higher in shaded places while Alphaproteobacteria were highly predominant in the sunny sides regardless lichen age. The author noted that no members of Alphaproteobacteria were cultivated, which is possibly connected with special requirements for growth, e.g., the substances produced by the lichen thalli.

A core lichen microbiome was discovered by Sierra et al. (2020). Based on 16S rRNA gene amplicon sequence analysis, microbiome of representatives belonging to seven lichen genera (*Cora*, *Hypotrachyna*, *Usnea*, *Cladonia*, *Peltigera*, *Stereocaulon*, and *Sticta*) was screened. Phyla Proteobacteria, Acidobacteria, and Cyanobacteria were shown to be abundant in all studied lichens. The other microbiome members were also present but were varying from genera to genera.

Localization of bacteria in lichen thallus and possible dispersion of bacterial fraction were studied by Aschenbrenner et al. (2014). It was established that bacteria colonize also symbiotic propagules, which are intended for short-distance transmission of the lichen. *Cystobacterineae* (*Deltaproteobacteria*) prevailed in both the

Table 9.1 Some identified bacteria from different lichen species

Lichen species	Isolates			Genus	Identification method	Author
	Phylum	Order				
<i>wl</i>	Firmicutes	Caryophanales		<i>Bacillus Paenibacillus</i>	Molecular genetic identification	Cardinale et al. (2006)
	Proteobacteria	Burkholderiaceae		<i>Burkholderia</i>		
	Gammaproteobacteria	Xanthomonadaceae		<i>Luteibacter</i>	Molecular genetic identification	Cardinale et al. (2006)
<i>Cladonia coniocraea</i>	Alphaproteobacteria	–		–	FISH method	Pankratov et al. (2017)
	Actinobacteria					
	Acidobacteria					
	Firmicutes	Caryophanales		<i>Paenibacillus</i>	Molecular genetic identification	Cardinale et al. (2006)
	Proteobacteria	Burkholderiales		<i>Burkholderia</i>		
<i>Hypogymnia physodes</i> (on moss)		Rhodospirillales		<i>Inquilinus</i>		
	Firmicutes	Caryophanales		<i>Paenibacillus</i>	Molecular genetic identification	Cardinale et al. (2006)
	Actinobacteria	Cellulomonadaceae		Agricultural soil bacterium isolate SI-14 (AJ252581)	Molecular genetic identification	Cardinale et al. (2006)
<i>Hypogymnia physodes</i> (on bark)		Micromonosporales		<i>Micromonospora</i>		
		Streptomyetales		<i>Streptomyces</i>		
	Firmicutes	–		Uncultured bacterium clone X20 (DQ083105)		
	Actinobacteria	Nakamurellales		<i>Saxebacter</i>	Isolation by cultivation	Selbmann et al. (2010)
Lecanora fuscobrunnea				Unclassified <i>Actinobacteria</i>		
<i>Lobaria pulmonaria</i>	Proteobacteria	Hyphomicrobiales		<i>Beijerinckia</i>	Molecular genetic identification	Erlacher et al. (2015)
				<i>Bradyrhizobium</i>		
				<i>Brucellaea</i>		
				<i>Methylobacterium</i>		
				<i>Nitrobacter</i>		

<i>Lobaria reitgera</i>	Actinobacteria			<i>Phyllobacterium</i>	Molecular genetic identification	Jiang et al. (2017)
				<i>Rhodopseudomonas</i>		
				<i>Xanthobacter</i>		
	Deinococcus-Thermus	Micrococcales		<i>Micrococcus</i>	Molecular genetic identification	Jiang et al. (2017)
		Mycobacteriales		<i>Subtercola</i>		
				<i>Rhodococcus</i>		
	Proteobacteria	Deinococcales		<i>Mycobacterium</i>		
				<i>Deinococcus</i>		
		Burkholderiales		<i>Burkholderia</i>		
		Hyphomicrobiales		<i>Variovorax</i>		
				<i>Beijerinckia</i>		
				<i>Methylobacterium</i>		
<i>Parmelia omphalodes</i>	Actinobacteria	Lysobacterales		<i>Luteibacter</i>	Molecular genetic identification	Jiang et al. (2017)
		Rhodobacterales		<i>Paracoccus</i>		
		Sphingomonadales		<i>Porphyrobacter</i>		
				<i>Sphingomonas</i>		
		Frankiales		<i>Motilbacter</i>		
		Geodermatophilales		<i>Modestobacter</i>		
		Kineosporiales		<i>Kineococcus</i>		
		Micrococcales		<i>Amnibacterium</i>		
				<i>Curtobacterium</i>		
				<i>Microbacterium</i>		
	Mycobacteriales			<i>Micrococcus</i>		
				<i>Corynebacterium</i>		
				<i>Mycobacterium</i>		
		Nakamurellales		<i>Nakamurella</i>		
				<i>Friedmanniella</i>		
		Propionibacteriales				

(continued)

Table 9.1 (continued)

Lichen species	Isolates		Order	Genus	Identification method	Author
	Phylum					
				<i>Microclunatus</i>		
				<i>Nocardioidea</i>		
			Streptomycetales	<i>Streptomyces</i>		
	Firmicutes		Caryophanales	<i>Bacillus</i>		
			Eubacteriales	<i>Clostridium</i>		
	Proteobacteria		Burkholderiales	<i>Burkholderia</i>		
			Hyphomicrobiales	<i>Beijerinckia</i>		
				<i>Methylobacterium</i>		
			Pseudomonadales	<i>Acinetobacter</i>		
			Rhodospirillales	<i>Roseomonas</i>		
<i>Pseudevernia furfuracea</i> Rusavskia elegans	Firmicutes		Sphingomonadales	<i>Sandaracinorhabdus</i>		
				<i>Sphingomonas</i>		
	Actinobacteria		Caryophanales	<i>Paenibacillus</i>	Molecular genetic identification	Cardinale et al. (2006)
			Mycobacteriales	<i>Mycobacterium</i>	Isolation by cultivation	Selbmann et al. (2010)
	Firmicutes		Caryophanales	<i>Bacillus</i>		
				<i>Paenibacillus</i>		
	Proteobacteria		Pseudomonadales	<i>Pseudomonas</i>		
	Actinobacteria		Nakamurellales	<i>Nakamurella</i>		
	Planctomycetes		Gemmatales	<i>Gemmata</i>		
			Isosphaerales	<i>Isosphaera</i>		
Solorina crocea	Proteobacteria		Lysobacteriales	<i>Dyella</i>		
			Myxococcales	<i>Byssovoxax</i>		
			Sphingomonadales	<i>Novosphingobium</i>		
				<i>Sphingomonas</i>		
					Molecular genetic identification	Grube et al. (2015)

Umbilicaria cylindrica	Proteobacteria	Burkholderiales	<i>Burkholderia</i>	Isolation by cultivation	Grube and Berg (2009)
		Caryophanales	<i>Bacillus</i>		
		Pseudomonadales	<i>Acinetobacter</i>		
		Hyphomicrobiales	<i>Methylocystis</i>		
		Rhodospirillales	<i>Acidisoma</i>		
		Sphingomonadales	<i>Gluconoacetobacter</i>		
		–	<i>Sphingomonas</i>		
		–	–	FISH method	
Umbilicaria decussata	Actinobacteria	Micrococcales	<i>Knoellia</i>	Isolation by cultivation	Selbmann et al. (2010)
	Deinococcus- Thermus	Deinococcales	<i>Deinococcus</i>		
	Firmicutes	Caryophanales	<i>Paenibacillus</i>		
	Actinobacteria	Mycobacteriales	<i>Rhodococcus</i>		
Umbilicaria esculenta	Deinococcus- Thermus	Micrococcales	<i>Arthrobacter</i>	Molecular genetic identification	Jiang et al. (2017)
			<i>Clavibacter</i>		
			<i>Microbacterium</i>		
			<i>Micrococcus</i>		
		Micromonosporales	<i>Micromonospora</i>		
		Nakamurellales	<i>Nakamurella</i>		
		Deinococcales	<i>Deinococcus</i>		
	Firmicutes	Caryophanales	<i>Bacillus</i>		
		Burkholderiales	<i>Burkholderia</i>		
			<i>Massilia</i>		
	Proteobacteria	Enterobacterales	<i>Klebsiella</i>		
		Hyphomicrobiales	<i>Aureimonas</i>		
			<i>Beijerinckia</i>		
			<i>Methylobacterium</i>		

(continued)

Table 9.1 (continued)

Lichen species	Isolates		Order	Genus	Identification method	Author
	Phylum					
<i>Umbilicaria pustulata</i>		Pseudomonadales	<i>Pseudomonas</i>	Molecular genetic identification	Greshake Tzovaras et al. (2020)	
		Sphingomonadales	<i>Novosphingobium</i>			
			<i>Sphingomonas</i>			
	Actinobacteria	Actinomycetales	<i>Cellulomonas</i>			
		Kineosporiales	<i>Kineococcus</i>			
			<i>Kineosporia</i>			
			<i>Quadrifidraera</i>			
			<i>Thalassiaella</i>			
		Micrococcales	<i>Alpinimonas</i>			
		Acidobacteria	Acidobacteriales			<i>Acidipila</i>
	<i>Acidobacterium</i>					
	<i>Edaphobacter</i>					
	<i>Granulicella</i>					
	<i>Occallatibacter</i>					
<i>Terriglobus</i>						
Bryobacterales	<i>Bryobacter</i>					
Armatimonadetes	Holophagales	<i>Paludibaculum</i>				
		<i>Geothrix</i>				
		<i>Armatimonas</i>				
Bacteroidetes	Armatimonadales	<i>Fimbrimonas</i>				
	Fimbrimonadales	<i>Ferruginibacter</i>				
	Chitinophagales	<i>Flavisolibacter</i>				
		<i>Pseudoflavitalea</i>				
			<i>Puia</i>			

	Firmicutes	Eubacteriales	<i>Terrimonas</i>		
			<i>Desulfotomaculum</i>		
Proteobacteria	Proteobacteria	Hyphomicrobiales	<i>Hellobacterium</i>		
			<i>Beijerinckia</i>		
			<i>Bradyrhizobium</i>		
			<i>Lichenibacter</i>		
			<i>Lichenihabitans</i>		
			<i>Methylocella</i>		
			<i>Methylocys</i>		
			<i>Rhodoblastus</i>		
			<i>Rhodomicrobium</i>		
		Rhodospirillales	<i>Acidiphilium</i>		
			<i>Acidisphaera</i>		
			<i>Acidomonas</i>		
			<i>Azospirillum</i>		
			<i>Endobacter</i>		
			<i>Gluconacetobacter</i>		
			<i>Gluconacetobacter</i>		
			<i>Granulibacter</i>		
			<i>Komagataeibacter</i>		
			<i>Rhodopila</i>		
			<i>Rhodovastum</i>		
Usnea antarctica	Actinobacteria	Micrococcales	<i>Arthrobacter</i>	Isolation by cultivation	Selbmann et al. (2010)
	Proteobacteria	Pseudomonadales	<i>Pseudomonas</i>		
	Proteobacteria	Pseudomonadales	<i>Acinetobacter</i>		
Not identified saxicolous lichen				Molecular genetic identification	Hunanyan and Sargsyan (2019)

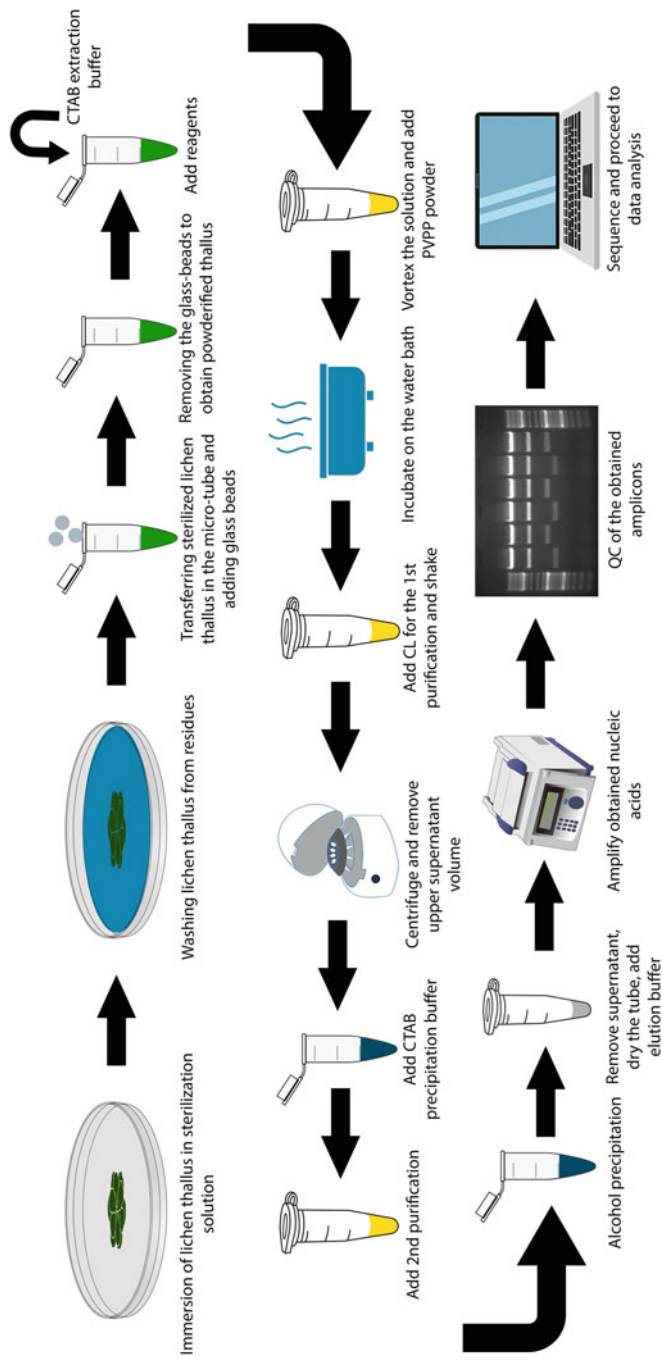


Fig. 9.1 Step-by-step guide of DNA isolation using CTAB method (Cubero and Crespo 2002)

lichen thalli and isidioid soredia, making up to 42% of all investigated microbes. In contrast, Alphaproteobacteria predominated within propagules. The presence of bacteria in vegetative propagules may indicate the need for the former for the functioning of lichen symbiosis. The microbial cargo is also confirmed by geographical shift: bacterial composition of lichen thalli sampled from the same region demonstrated closer similarity than those of distant populations (Aschenbrenner et al. 2014).

Except prokaryotes, yeasts seem to be another part of lichen symbiosis being mainly neglected until the recent discovery by Spribille et al. (2016). Correlation between yeast abundance and variations in lichen phenotype indicates their role as a potential symbiotic partner in the lichen mutualistic association (Palmqvist et al. 2017; Suryanarayanan and Thirunavukkarasu 2017; Zúñiga et al. 2017).

Up to now, a limited number of yeasts were detected in lichen thalli, mainly belonging to Cyphobasidiales and Microsporomycetaceae (Cystobasidiales), both Cystobasidiomycetes, Pucciniomycotina, Basidiomycota (Table 9.2).

Subsequent studies provided contradictory data. Obviously, the yeast-like fungi are not as ubiquitous as it was suggested. Despite Cernava et al. (2015) found these yeasts in 95% of the studied specimens of lichen genus *Cladonia* collected in various climatic conditions and habitats, other studies have not been as promising. Smith et al. (2020) detected Cystobasidiales yeasts in five of the 35 samples while Lendemer et al. (2019) confirmed Cyphobasidium or other Cystobasidiomycete yeasts only in nine of the 413 samples, and in nine of the 339 investigated lichen species. Furthermore, the study by Mark et al. (2020) contradicted previous findings of high mycobiont specificity of basidiomycete yeasts. This corresponds to the data presented in Table 9.2.

9.3 Microbiome Functions

The role of lichen-inhabiting bacterial communities remains largely elusive although its revealing may promise us high practical potential. The role of the bacterial composition in the symbiotic associations was stated by Schneider et al. (2011) who found correlations between microorganism composition and protein profile. The studies of the protein spectrum highlighted that *Bacteria* and *Archea* contribution was even more than input stated for the green algae. The main functional categories of *Bacteria* and *Archea* were the posttranslational modification, protein turnover and supply of chaperones (Fig. 9.2).

The antagonistic properties of bacterial community were investigated by Cernava et al. (2015). The isolated bacteria of *Lobaria pulmanoria* were most active against the lichenicolous fungus *Rhinoeclecladoniella* sp., while the antibacterial activity was low.

Some progress has been made by applying the metaproteomic approaches which helped to reveal the involvement of lichen microbe communities in functions such as nutrient supply, resistance against stress factors, support of photosynthesis,

Table 9.2 Some basidiomycete yeasts detected in lichen thalli. Lichen nomenclature follows Wijayawardene et al. (2020)

Yeast taxa	Host lichen	Author
<i>Buckleyzyma aurantiaca</i> (Cystobasidiomycetes)	<i>Lecanora carpinea</i> s. lat., <i>Lecanora chlarotera</i> , <i>Lecanora pulicaris</i> , <i>Pseudevernia furfuracea</i>	Mark et al. (2020)
<i>Cyphobasidium hypogymniicola</i> (Cyphobasidiomycetes)	<i>Hypogymnia hultenii</i> , <i>Hypogymnia imshaugii</i> , <i>Hypogymnia incurvoides</i> , <i>Hypogymnia krogiae</i> , <i>Hypogymnia physodes</i> , <i>Hypogymnia vittata</i> , <i>Lecanora pulicaris</i> , <i>Parmelia sulcata</i> , <i>Pseudevernia furfuracea</i>	Diederich (1996, 2003, 2007), Holien (2005), Urbanavichene and Urbanavichus (2005), Hodkinson et al. (2009), Millanes et al. (2016), Mark et al. (2020)
<i>Cyphobasidium usneicola</i> (Cyphobasidiomycetes)	<i>Hypogymnia physodes</i> , <i>Hypogymnia tubulosa</i> , <i>Lecanora chlarotera</i> , <i>Lecanora pulicaris</i> , <i>Parmelia sulcata</i> , <i>Physcia adscendens/tenella</i> , <i>Pseudevernia furfuracea</i> , <i>Usnea articulata</i> , <i>Usnea brasiliensis</i> , <i>Usnea cornuta</i> s. lat., <i>Usnea galapagona</i> , <i>Usnea hirta</i> , <i>Usnea madeirensis</i> , <i>Usnea</i> cf. <i>praetervisa</i> , <i>Usnea silesiaca</i> , <i>Usnea subfloridana</i> s. lat., <i>Usnea subscabrosa</i>	Diederich (1996, 2003), Millanes et al. (2016), Mark et al. (2020)
<i>Cyphobasidium</i> spp. (Cyphobasidiomycetes)	<i>Bryoria nadvornikiana</i> , <i>Heterodermia leucomelos</i> , <i>Lecidea roseotincta</i> , <i>Opegrapha vulgata</i> , <i>Parmotrema hypotropum</i> , <i>Parmotrema subsumptum</i> , <i>Usnea cornuta</i> , <i>Usnea strigosa</i> , <i>Usnea subgracilis</i>	Lendemer et al. (2019)
Cyphobasidiales spp. (Cyphobasidiomycetes)	<i>Alectoria</i> sp., <i>Anzia</i> sp., <i>Asahinea</i> sp., <i>Brodoa</i> sp., <i>Bryoria</i> spp., <i>Bulbothrix</i> sp., <i>Cetraria</i> sp., <i>Cetrelia</i> sp., <i>Esslingeriana</i> sp., <i>Evernia</i> sp., <i>Flavopunctelia</i> sp., <i>Hypogymnia</i> sp., <i>Hypotrachyna</i> sp., <i>Imshaugia</i> sp., <i>Letharia</i> sp., <i>Melanelia</i> sp., <i>Menegazzia</i> sp., <i>Montanelia</i> sp., <i>Myelochroa</i> sp., <i>Nephromopsis</i> sp., <i>Nodobryoria</i> sp., <i>Omphalora</i> sp., <i>Oropogon</i> sp., <i>Parmelia</i> sp., <i>Parmelina</i> sp., <i>Parmotrema</i> sp., <i>Platismatia</i> sp., <i>Pseudevernia</i> sp., <i>Pseudoparmelia</i> sp., <i>Usnea</i> sp., <i>Xanthoparmelia</i> sp.	Spribille et al. (2016)
<i>Hasegawazyma</i> spp. (Cystobasidiomycetes)	<i>Hypogymnia tubulosa</i> , <i>Lecanora argentata</i> , <i>Lecanora carpinea</i> s. lat., <i>Lecanora chlarotera</i> ,	Mark et al. (2020)

(continued)

Table 9.2 (continued)

Yeast taxa	Host lichen	Author
	<i>Lecanora pulicaris</i> , <i>Parmelia sulcata</i> , <i>Pseudevernia furfuracea</i>	
<i>Lichenzyma pisutiana</i> (Cystobasidiomycetes)	<i>Cladonia arbuscula</i> , <i>Cladonia cariosa</i> , <i>Cladonia chlorophaea</i> s. lat., <i>Cladonia cornuta</i> , <i>Cladonia deformis</i> , <i>Cladonia diversa</i> , <i>Cladonia floerkeana</i> , <i>Cladonia furcata</i> , <i>Cladonia gracilis</i> , <i>Cladonia merochlorophaea</i> , <i>Cladonia phyllophora</i> , <i>Cladonia pocillum</i> , <i>Cladonia polycarpoides</i> , <i>Cladonia pyxidata</i> , <i>Cladonia rangiferina</i> , <i>Cladonia rangiformis</i> , <i>Cladonia rei</i> , <i>Cladonia</i> cf. <i>subulata</i> , <i>Cladonia verticillata</i> , <i>Lecanora argentata</i> , <i>Lecanora carpineae</i> s. lat., <i>Lecanora chlarotera</i>	Cernava et al. (2015), Mark et al. (2020)
Microsporomycetaceae spp. (Cystobasidiomycetes)	<i>Cladonia cornuta</i> , <i>Cladonia foliacea</i> , <i>Cladonia furcata</i> , <i>Cladonia humilis</i> , <i>Cladonia</i> cf. <i>macroceras</i> , <i>Cladonia pocillum</i> , <i>Cladonia rangiformis</i> , <i>Cladonia rei</i> , <i>Cladonia subulata</i>	Cernava et al. (2015)
<i>Microsporomyces</i> cf. <i>pini</i> (Cystobasidiomycetes)	<i>Lecanora carpineae</i> s. lat., <i>Lecanora pulicaris</i>	Mark et al. (2020)
<i>Microsporomyces</i> spp. (Cystobasidiomycetes)	<i>Hypogymnia physodes</i> , <i>Hypogymnia tubulosa</i> , <i>Lecanora carpineae</i> s. lat., <i>Lecanora pulicaris</i> , <i>Parmelia sulcata</i> , <i>Physcia adscendens/tenella</i>	Mark et al. (2020)

production of hormone, detoxification of end metabolites, and lytic activity (Grube et al. 2015) (Fig. 9.3). A short summary is provided below.

Nutrient supply: High number of contigs were found to represent Ton and Tol transport systems, some of which involving in iron uptake. Phosphate metabolism was represented with a relatively small amount of contigs, including proteins involved in solubilization of phosphates.

Pathogen defense: Some contigs were corresponding to the multidrug resistance efflux pumps, as well as various antibiotic (luoroquinolone, vancomycin, methicillin, penicillin and cephalosporine) resistance. Relatively small amount of contigs represent the production of secondary metabolites which are well-known for their biological activity.

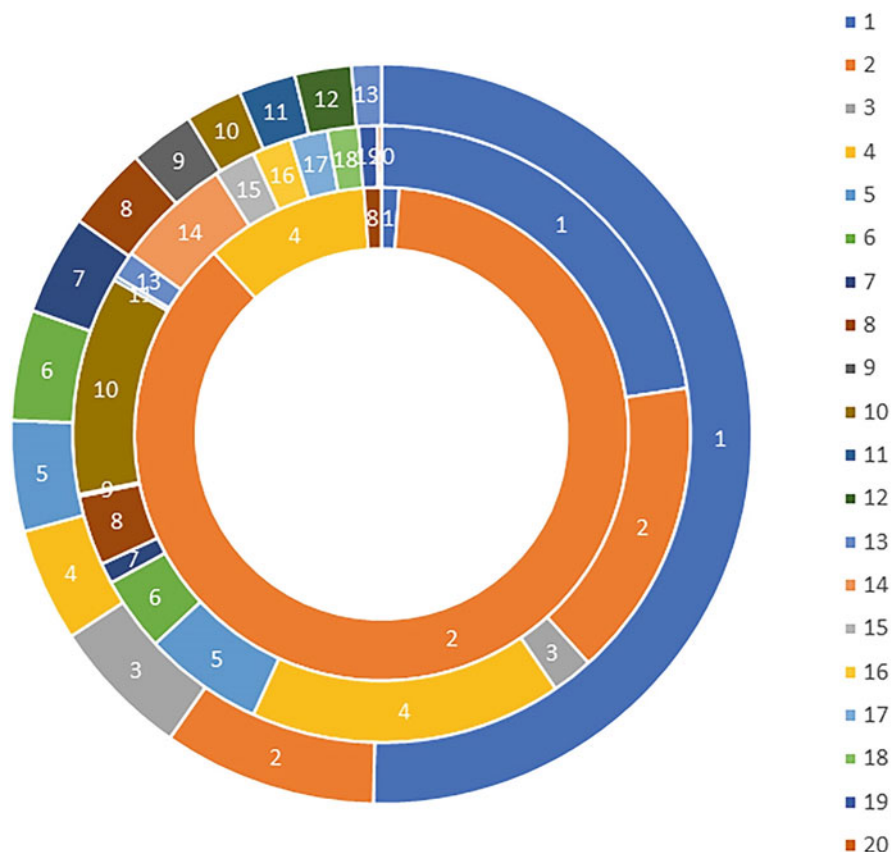


Fig. 9.2 Comparative description of orthologous group (COG/KOG) functions in bacteria (outer circle), fungus (middle circle), and algae (inner circle) associated to the lichen thalli. Compiled from data presented in Schneider et al. (2011). Note: 1—Posttranslational modification, protein turnover, chaperones; 2—Energy production and conversion; 3—Lipid metabolism; 4—Carbohydrate transport and metabolism; 5—Amino acid transport and metabolism; 6—General function prediction only; 7—Inorganic ion transport and metabolism; 8—Signal transduction mechanisms; 9—DNA replication, recombination, and repair; 10—Translation, ribosomal structure, and biogenesis; 11—Nucleotide transport and metabolism; 12—Transcription; 13—Secondary metabolites biosynthesis, transport, and catabolism; 14—Cytoskeleton; 15—Coenzyme metabolism; 16—Cell wall/membrane/envelope biogenesis; 17—Cell cycle control, cell division, chromosome partitioning; 18—Intracellular trafficking, secretion, and vesicular transport; 19—RNA processing and modification; 20—Chromatin structure and dynamics

Resistance against abiotic stress factors: Relatively high amount of contigs corresponds to the metal resistance. Moreover, about the same number of oxidative-stress protectants were observed.

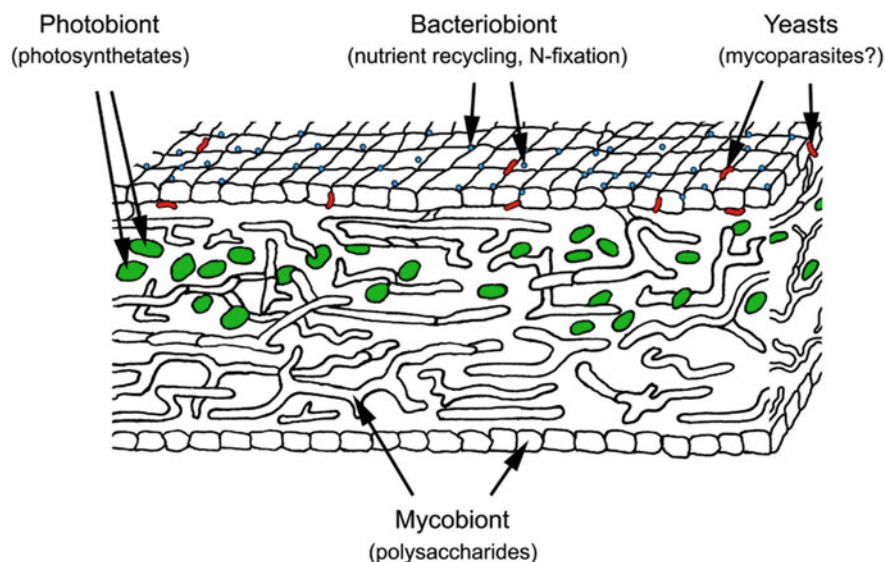


Fig. 9.3 Reconstruction of the lichen thallus based on data from Grube et al. (Grube and Berg 2009; Grube et al. 2015) and Spribille et al. (2016)

Photosynthesis support by vitamin B₁₂ and hormone production. Numerous contigs were corresponding to biosynthesis of tetrapyrrole, coenzyme-B₁₂, thiamine, and biotin. Small amount of auxin biosynthesis corresponding contigs were found.

Detoxication of metabolites and lytic activity: High amount of indicated contigs were found to participate in xenobiotics metabolism and biodegradation. Some contigs corresponding to chitin and *N*-acetylglucosamine utilization were also detected, which can be responsible to degradation of older sections of the thallus for obtaining nutrients for the growing parts.

In contrast to bacteria, the role of basidiomycete yeasts in lichen symbiosis still remains unclear. Despite Spribille et al. (2016) shown the presence of yeast is correlated with amount of lichen secondary metabolites, Mark et al. (2020) confirmed the absence of any effect of medullary chemotype on the distribution of yeast community. The author supposed low probability of metabolites production directly by the yeasts.

It is also known that some Cyphobasidiomycetes cause gall formation and therefore can be associated with mycoparasites (Millanes et al. 2016) similar to different filamentous basidiomycete fungi (Diederich et al. 2018; Tuovinen et al. 2019). However, most of host lichen specimens lacked galls (Spribille et al. 2016; Cernava et al. (2015); Mark et al. 2020). More studies are required to make clear the interaction between basidiomycete yeasts, mycobiont, photobiont, and microbial consortia in lichens.

Table 9.3 Some secondary metabolites produced by lichen endobiont bacteria (Calcott et al. 2018)

Lichen endobiont	Metabolite	Activity
<i>Streptomyces uncialis</i>	Uncialamycin	Antibiotic, antitumor
<i>Micromonospora chersina</i>	Dynemicin	Antitumor, antibiotic
<i>Streptomyces</i> sp. L-4-4	Aminocoumarin, coumabiocins A–F, novobiocin	Antibiotic
<i>Streptomyces</i> sp. L-9-10	2'- <i>O</i> -demethylherbicidin F (1), 9'-deoxy-8',8'-dihydroxyherbicidin B, 9'-deoxy-8'-oxoherbicidin B, 8'-epimer of herbicidin B, 9-(β -D-arabinofuranosyl) hypoxanthine (Ara-H)	Herbicidal agents, antibiotics
<i>Streptomyces</i> sp. isolated from <i>Stereocaulon</i> sp.	Lichostatalin	Inhibitor of the cysteine protease cathepsin K (CatK)
Unculturable <i>Streptomyces</i> sp.	1,1-Dichlorocyclopropane-containing angucycline	Antitumor, antibiotic
<i>Streptomyces cyaneofuscantus</i>	Aneodimycin, cyaneomycin, usnic acid	Antioxidant, antitumor, antibacterial, UV protectant
<i>Nocardia ignorata</i>	Brominated diketopiperazines	Antioxidant, anti-inflammatory

9.4 Biotechnological Potential/Relevance

Lichens have a huge role in traditional and evidence-based medicine practice. Recent discoveries of lichen-inhabiting bacteria and their biotechnological relevance can solve many issues connected with the lichen biotechnology. Obtaining biologically active substances from slow-growing lichen thalli is a difficult and time-consuming task, which can be solved with use of bacteria.

A short list of secondary metabolites produced by lichen endobionts and their application are presented in Table 9.3.

Definitely, this is not a really vast amount of characterized secondary metabolites from lichen endobionts. The main problem is the uncultivability of most bacteria, which makes the discovery of novel metabolites challenging task. In order to address this issue, novel methods such as transcriptomics may become handful.

9.5 Conclusions

The recent discoveries of diverse metacommunities of lichen thalli forced to redefine lichens as complex and dynamic ecosystems (Hawksworth and Grube 2020; Smith et al. 2020) and catalyzed a call to reconceptualize the symbiotic concept of lichen (Spribille et al. 2016; Hawksworth and Grube 2020). The traditional approach of

lichens as bicomponent mutualistic association are outdated, and “ecosystem-like” concept is gaining increasing recognition.

However, physiological processes operating within the symbiosis as well as symbiont interactions remain to be a significant gap in our understanding the functioning of the association. Future studies are highly expected to uncover the diversity of the lichen-associated microbial communities and reveal their biotechnological potential.

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