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Invited Mini Review

Lichens as natural sources of biotechnologically relevant Bacteria

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Abstract

The search for microorganisms from novel sources and in particular microbial symbioses represents a promising approach in biotechnology. In this context lichens have increasingly become subject of research in microbial biotechnology, particularly after the recognition that a diverse community of bacteria other than cyanobacteria are additional partners to the traditionally recognized algae-fungus mutualism. Here we review recent studies using culture-dependent as well as culture-independent approaches showing that lichens can harbor diverse bacterial families known for the production of compounds of biotechnological interest and that several microorganisms isolated from lichens, in particular *Actinobacteria* and *Cyanobacteria*, can produce a number of bioactive compounds, many with biotechnological potential.

Keywords: lichens - bacteria - *Actinobacteria* - *Cyanobacteria*- biotechnology.

Introduction

Recently, the World Health Organization (WHO) produced a global map of antimicrobial resistance and issued a warning that a 'post-antibiotic' world could soon become a reality and has already started (Woolhouse and Farrar 2014). Therefore, the quest for new drugs from microbial sources is currently at a crossroads, where on one hand it is widely thought that the discovery of novel compounds has passed its "golden era" with many pharmaceutical companies abandoning or downscaling their microbial natural products research, while on the other hand, full genomic sequencing and metagenomic research shows that a large number of "silent" or novel biosynthetic pathways exist (for reviews see Brady et al. 2009 ; Ochi and Hosaka 2013), promising to lead to novel bioactive compounds produced via heterologous expression (e.g. Chang and Brady 2013). The continued search for microorganisms from novel sources represents an effective strategy forward towards drug discovery. In this context microbial symbioses are of particular interest, since increasing evidence supports the hypothesis that microbial bioactive compounds, such as those used as antibiotics, might be involved in microbe-microbe and microbe-host communication (Yim et al. 2007; Yoon and Nodwell 2014). An example supporting this hypothesis, is the fact that many interesting novel bioactive compounds of microbial origin have been discovered in marine sponges, known to harbor complex symbiotic microbial communities (see Fuerst 2014 for a review).

Lichens are a well-recognized, self-supporting, mutualistic symbiosis between a dominant fungal partner (mycobiont) that provides shelter for one or several photosynthetic green algae and/or cyanobacteria (photobionts) forming a unique symbiotic structure, the lichen thallus, (De Bary 1879; Frank 1876; Schendener 1860 reviewed in Honegger 2000). Lichen-forming fungi have evolved for at least 400 million years (Taylor et al. 1995). The lichen thallus is well-adapted to a wide variety of specific ecological niches, often including extreme environmental conditions (e.g. extreme temperatures, periodic desiccation, high levels of UV radiation). At least some of the mechanisms to cope with these extreme conditions are of a chemical nature (e.g. UV screens, cryoprotectants, osmolites) providing leads and inspiration for biotechnological development. It also appears that allelopathic compounds by the holobiont could play a role in the persistence of lichens. Despite estimated individual ages of decades to centuries, there are no reports of destructive bacterial infections in lichens. The capacity of produce a broad range of interesting chemical compounds help explain the fact, that lichens have been historically also used as sources of pigments, perfumes, human medicines etc. (Rikkinen 1995).

A number of chemical structures have consistently been associated with lichens and due to their presence in axenic culture or other non-lichenizing fungi, it is generally accepted that they are produced by the mycobiont. Those include repeats of aromatic acids such as orsellinic acid (depsides, depsidones and usnic acids) that are believed to be synthesized by non-reducing fungal polyketide synthases (Huneck and Yoshimura 1996; Robinson 1999), that have been directly recovered from lichens (i.e. Grube and Blaha 2003; Muggia and Grube 2010 ; Schmitt et al. 2008). The diversity of these major compounds have been subject of recent reviews and catalogues (Boustie and Grube 2005; Elix 2014; Molnár and Farkas 2010; Shrestha and St Clair 2013; Shukla et al. 2010; Stocker-Woergoetter 2008) and therefore we will focus here on those produced by other microorganisms, in particular *Bacteria*, although we cannot rule out that these bacteria can be also involved in the biosynthesis or conversion of major compounds found in lichens.

Culturable bacteria of biotechnological interest

While the presence of microorganisms other than the mycobiont and the photobiont in the lichen holobiont has long been known by isolation techniques (Uphof 1925; reviewed in Cardinale et al. 2006), systematic attempts to study these organisms have only recently been carried out. One early study (Gonzalez et al. 2005) published 10 years ago clearly demonstrated that lichens are sources of bioactive microorganisms. That study focused on *Actinobacteria* by employing a targeted isolation strategy using nalidixic acid (an inhibitor of Gram-negative bacteria) as well as heat-treatment prior to cultivation. A standard medium (Yeast Malt Extract) was used for isolation from 25 terrestrial lichen samples from Hawaii, Alaska and the Reunion Islands. The lichens species were not identified and the samples were just classified as saxicolous (i.e. rock associated; 6 from Alaska, 5 from Hawaii) or arboricolous (i.e tree-associated; 5 from Hawaii and 9 from the Reunion Islands). A large number (337) of strains were obtained, and identified based on DNA fingerprinting and fatty acid analysis. Among these strains, 110 belonged to the family *Streptomycetaceae*, well-known to produce bioactive compounds. Other actinobacterial families also know for the production of bioactive compounds, some belonging to the "rare *Actinomycetes*" sensu Subramani and Aalbersberg (2013), such as *Micromonosporaceae* (142 strains), *Pseudonocardiaceae* (30 strains) and *Thermomonosporaceae* (7 strains) were also isolated. These strains were screened for genetic potential for biosynthesis (PCR targeting genes

involved in polyketide, small peptides, and isoprenoid biosynthesis), as well as antimicrobial activity (against *Escherichia coli, Staphylococcus aureus* and *Candida albicans*). A high percentage of strains (over 60%) potentially contained at least one biosynthetic cluster and about 30% of the strains had antimicrobial activity against at least one of the target microorganisms.

Since the pioneering study by Gonzalez and colleagues (2005), many other studies have focused on lichen-associated bacteria via cultivation. A number of these studies have targeted individual isolates or species, especially for taxonomic purposes or for full genomic sequencing (Table 1). A particular interest was given to biotechnological potential of strains, since in several cases isolation selected for *Actinobacteria* by nalidixic acid addition (9 out of 23 strains). Finally in some cases [*Actinoplanes* sp. ATCC55532 (Singh et al. 1997), *Streptomyces uncialis* (Davies et al. 2005), *Streptomyces* sp. (Motohashi et al. 2010), *S. cyaneofuscatus* (Brana et al. 2015) and *Streptomyces* sp. (Lavallee 2011)] the focus was primarily on the discovery of novel bioactive molecules, with the first strain described in a patent (Singh et al. 1997). These strains will be discussed in a later section. It is remarkable that in several of these studies there is only a poor description or a complete lack of identification of the source lichens.

A different class of culture-based studies performed a more comprehensive isolation of lichen associated bacteria, basing the isolation on criteria other than bioactivity and, except for our recent study (Parrot et al. 2015), did not target *Actinobacteria* (Table 2). The results of these studies offer a less biased view of the cultivable bacterial diversity associated with lichens, and it is clear that certain bacterial phyla and classes, in particular *Alpha-* and *Gammaproteobacteria, Firmicutes* and *Actinobacteria* are frequently isolated from lichens (Cardinale et al. 2006; Grube et al. 2009; Selbmann et al. 2010). In addition *Bacteroidetes* seem to be isolated from marine or littoral lichens (Parrot et al. 2015; Sigurbjornsdottir et al. 2014). Among all isolates, many belong to bacterial families known to contain strains know to produce bioactive compounds, enzymes of biotechnological interest such as lipases proteases and antagonistic molecules (Table 2). In a few cases this was experimentally tested (Grube et al. 2009; Lee et al. 2014), while in others studies this potential was inferred by the presence of genes involved in the biosynthesis of polyketides or 16S rRNA similarity to known bioactive strains (Parrot et al. 2015). In the latter study the authors focused on the isolation of *Actinobacteria* from marine and littoral lichens, and showed that these lichens were sources of bacteria of biotechnological interest, and that the use of Marine Agar, a medium used to the

isolation of a broad diversity of marine bacteria, yielded many strains that were not recovered with the commonly used Actinomycete Isolation Agar medium, and this did not appear to be an effect of salinity (Parrot et al. 2015). Among these stains some appeared to represent novel species or even genera. Finally that study compared the 16S rRNA genes to those in the NCBI Genbank from bacteria associated with lichens studied using culture independent methods and as expected none of the strains appeared to be prevalent in the studied lichens.

To obtain a larger representation of lichen associated strains, including strains for which only 16S rRNA sequences exist, we searched all 16S rRNA gene sequences present in the NCBI Genbank *nt* database with the query "*lichen AND bacteria [orgn] NOT Cyanobacteria [orgn] NOT uncultured"* and recovered the taxonomy information associated with these sequences (associated or not to publications), and obtained somewhat similar results, which again might be a reflection of the targeting of *Actinobacteria* in isolation efforts even though this analysis did not include the study of Gonzalez and colleagues and Parrot and colleagues that have focused on *Actinobacteria* (Gonzalez et al. 2005; Parrot et al. 2015). Among 511 bacterial strains retrieved, nearly half of isolates (246) belong to *Actinobacteria,* 185 to *Alphaproteobacteria*, 85 to *Firmicutes* and 59 to the *Gammaproteobacteria*. Focusing on bacterial families of biotechnological interest, about 30% (150 in 511) belong to the families *Bacillaceae* (40), *Paenibacillaceae* (37), *Burkholderiaceae* (24), *Pseudomonadaceae* (19), *Streptomycetaceae* (18), *Micromonosporaceae* (5), *Nocardioidaceae* (5) and *Pseudonocardiaceae* (2) known for the production of bioactive molecules.

Finally, a number of studies have attempted to cultivate cyanobacteria (mostly the main photobionts) from lichens (Table 3). In addition to the search of bioactive compounds of pharmacological the main interest a few of these studies was the production of toxins or other metabolites by these cyanobacteria strains, and in particular hepatoxins relevant to public health (see Dittmann et al. 2013 for a review). Extensive genetic and chemical screening of lichens of the lichen order *Peltigerales*, known of for their association with the cyanobacterial genus *Nostoc* have clearly shown that a non-negligible (about 5 % of specimens) contain microcystins or nodularins (Kaasalainen et al. 2013) most likely produced by symbiotic cyanobacteria. In addition to the original interest for public health, the use of microcystin congeners has also recently been proposed for cancer therapy (Niedermeyer et al. 2014), demonstrating the interest in these lichen associated cyanobacteria.

Summarizing, existing information from culture-based studies clearly show that lichens can be a source of bacterial strains of biotechnological interest such as *Bacillus, Paenibacillus, Pseudomonas, Burkholderia* and *Nostoc*. Regarding *Actinobacteria,* interesting strains in this phylum were isolated even without selective approaches. Studies using selective approaches yielded a higher diversity of *Actinobacteria* and in particular of non-*Streptomyces* genera, including possible new species. It also appears that cyanobacterial symbionts can be a promising source of bioactive molecules of biotechnological interest.

Strains known to produce bioactive compounds

Despite the large number of bacterial strains of relevance to drug discovery isolated from lichens, the presence of structurally-identified bioactive molecules have only been reported for a few of these strains (*Streptomyces uncialis* and *Actinoplanes* sp. ATCC55532, *Streptomyces* sp. RI104-LiC106, *Streptomyces* sp. RI104-LiB101, *Streptomyces* sp. L-91-3 *Nostoc* sp., *Nostoc* sp. ATCC 53789 and *Nostoc* sp. IO-102-I Tables 1 and 3). In addition, identified molecular structures have also been reported for strain *S. cyaneofuscatus* T178 reported as being phenotypically and metabolically indistinguishable from *S. cyaneofuscatus* M-27. The relative small number of reported molecules is partly due to the fact that interest in lichen-associated bacteria is relatively recent but also likely due to the fact that publications tend to focus on novel chemical structures, and thus presence of known compounds, even in novel organisms, is seldom reported. In the following paragraphs we describe several lichen-associated bacteria for which bioactive compounds have been identified. The molecular structures of selected compounds are presented in Figure 1.

Actinoplanes sp. ATCC55532 was isolated from a non-identified arboricolous lichen from Spain has been reported to produce actinoplanic acids A and B (Singh et al. 1997) . This patent also claims that actinoplanic acid B **(1)** inhibits farnesyl-protein transferase (FPTase) and the farnesylation of the oncogene protein Ras, *in vitro* at (IC_{50} at 50 nM) and thus have a potential for the treatment of colorectal carcinoma, exocrine pancreatic carcinoma and myeloid leukemias.

Streptomyces uncialis, isolated by Davies and co-workers from a *Cladonia uncialis,* has been the been well characterized and reported to produce the enediyne antibiotic uncialamycin **(2)** highly active against *Escherichia coli*, *Staphylococcus aureus* as well as

Burkholderia cepacia (Davies et al. 2005). The same strain produces the alkaloids Cladoniamides A-G with the latter **(3)** showing significant *in vitro* toxicity against MCF-7 breast cancer cells at 10 µg/mL (Williams et al. 2008). A second lichen-derived *Streptomyces* strain isolated from lichens by the Davies' group (L-91-3) was reported to produce several inhibitors of Catepsin K (a protease identified as a drug target for osteoporosis therapy Yasuda et al. 2005). These molecules included the oligopeptide Antipain and two analogues described as vince-2 and lichostatinal (Lavallee 2011).

Strains RI104-LiC106 and RI104-LiB101 reported as belonging to a new *Streptomyces* species have been shown to produce JBIR-88 **(4),** a 1,1-dichlorocyclopropane-containing angucycline and JBIR-89 **(5),** a butenolide. JBIR-88 was cytotoxic to HeLa and ACC-MESO-1 cells IC50 36 and 52 mM, respectively. It also showed antibacterial activity against *Micrococcus luteus*, but not against *Candida albicans* and *E. coli* (Motohashi et al. 2010).

S. cyaneofuscatus M-27 was isolated from the algae *Fucus spiralis* and reported to be metabolically indistinguishable from *S. cyaneofuscatus* T-178 isolated from an unidentified lichen. This strain was reported to produce a number of anthracycline family antitumor antibiotics daunorubicin, cosmomycin B **(6)** and galtamycin B and the antifungal macrolactam maltophilin **(7)**. Daunorubicin is already used as a drug for cancer chemotherapy, cosmomycin B shows in vitro cytotoxicity against the KB human epidermoid carcinoma cell line (Ando et al. 1985) and galtamycin B shows in vitro cytotoxicity against gastric adenocarcinoma (HMO2), breast carcinoma (MCF7), and hepatocellular carcinoma (HepG) cell lines at (10 µg/mL Antal et al. 2005). Maltophilin has been shown to inhibit the growth of a number of fungi (Jakobi et al. 1996). It has to be noted however that all molecules reported to be produced by *S. cyaneofuscatus* M-27 were only identified based on their HPLC retention time and UV-vis spectra (Brana et al. 2015).

Nostoc strain. ATCC 53789 was isolated from lichens and was shown to produce cryptophycin 1 **(8)**, which was originally patented for its antifungal properties (Moore and Patterson 1997). Further analysis of other molecules in this class resulted in the discovery of it biosynthetic pathway, and of a strategy for chemoenzymatic synthesis of analogous molecules (Magarvey et al. 2006), Furthermore, a synthetic analog (cryptophycin 52) achieved Phase II clinical trials for lung cancer therapy (Edelman et al. 2003) at which stage development was stopped. More recently, the use of cryptophycin analogs in antibody-drug conjugates has also been proposed (Verma et al. 2012).

An unnamed *Nostoc* sp. strain isolated from *Peltigera canina* was shown to produce the chlorine containing nostoclides I and II, which have moderate toxicity (10 µg/ml) against cell lines Neuro-Pa CCL 131 and KB CCL17. *Nostoc* sp. strain IO-102-I isolated from a *Pannaria pezizoides* from Finland, appears in turn to be the only lichen-derived isolate shown to produce a number of microcystins (Oksanen et al. 2004), despite the report that many lichen specimens contain this class of molecules.

Finally, a recent study has reported that a *Nostoc* sp. strain N6 isolated from *Peltigera membranaceae* produces nosperin **(9)** a pederin like-molecule, produced by a predominantly trans-AT polyketide synthase biosynthetic pathway (Kampa et al. 2013). In this remarkable study Kampa and colleagues predicted - using shotgun metagenomic sequencing and sequence-based biosynthetic analysis - that a *Nostoc* strain was likely to produce a pederin-like molecule. Based in this information the authors were able to perform targeted isolation of the cyanobacterium that was followed by mass cultivation and isotopic incorporation to finally determine the structure of this molecule present in very low abundance, linking it to its most likely biosynthetic pathway (Kampa et al. 2013).

In summary, although interest in lichen-associated biotechnologically relevant bacteria is relatively recent, a number of bioactive and structurally identified molecules were produced by *Actinomycetes* and *Cyanobacteria* isolated from lichens, with some novel structures of high potential and some already in drug development.

The yet untapped potential seen through cultivation independent approaches

For the past 10 years, lichen-associated bacteria have been subject of an increasing number of cultivation-independent molecular surveys. These studies employed a number of techniques including fingerprinting of rRNA genes, fluorescence *in situ* hybridization and sequencing from mixed microbial populations (e.g., Cardinale et al. 2006, 2008, Grube et al. 2009). The cultivation-independent approaches used DGGE, SSCP, 16S rRNA gene cloning and Sanger sequencing, or next generation sequencing (454 pyrosequencing, Illumina sequencing) either of PCR amplified 16S rRNA genes or of shotgun fragments (Table 4). These studies clearly show a difference in abundance and diversity of culture-dependent versus -independent fractions of the lichen associated bacterial communities and demonstrated that the bacterial communities of most lichens studied so far are dominated by bacteria

belonging to the *Alphaproteobacteria* class of the *Proteobacteria*, with other taxa such as the *Beta-*, *Gamma-* and *Deltaproteobacteria* and the phyla *Bacteroidetes, Actinobacteria, Firmicutes* and *Verrucomicrobia* frequently present (Grube et al. 2015). The general relationships between microbiomes, lichen morphology, photobiont and substrates have recently been summarized by Aschenbrenner and colleagues, indicate that most lichens do have *Alphaproteobacteria* as predominant symbionts, although at a finer taxonomic resolution some patterns emerge such as the importance of *Acidobacteria* and *Acetobacteraceae (Rhodospirillales*) in acid rock and soil lichens, and higher actinobacterial percentages in intertidal lichens (Aschenbrenner et al. 2014).

Even though lichens are hosts of phyla and classes known to contain species with potential of producing bioactive compounds, such as the *Actinobacteria* and *Firmicutes*, an analysis of diversity at a finer resolution indicates that although present, bacterial families of biotechnological interest (apart from the Cyanobacteria) are relatively minor members of the community, with the *Burkholderiaceae* and *Paenibacillaceae* representing at a maximum one percent of the community, while the other families are about 10 to 100 times less abundant (Table 4). That does not decrease the potential for biodiscovery based on cultivation approaches, as it has been clearly pointed out in the previous sections. However, lower relative abundances have to be taken in account when evaluating the role of these organisms and their bioactive molecules to the lichen symbiosis (i.e. their spatial localization and aggregation becomes important), and during the design of culture independent strategies to uncover the biotechnological potential of these microorganisms (i.e. via metagenomics).

One interesting point emerging from cultivation independent studies, is the presence *Myxococcales* in relative abundances of the same range or sometimes higher than those of *Actinobacteria* (Table 4). This group of microorganisms is particularly interesting with large genomes and potential to the production of bioactive compounds (Dworkin 2001). *Myxobacteria* have a particular life style (Dworkin 2001) which is also conducive to the use of targeted isolation (e.g. Zhang et al. 2003), but to date a single lichen-associated *Myxobacteria* [ATCC 25944 (M 155)] has been described (McCurdy 1971). This is interesting, since in the original description of the *Myxobacteriaceae* Thaxter indicates that a number of species including *Corallococcus* (*Myxococcus*) *coralloides* and *Melittangium* (*Chondromyces*) *lichenicolus* were associated with lichens (Thaxter 1892), and myxobacteria have been reported as associated to decaying lichens (Reichenbach 1999) .

Regarding the application of metagenomic techniques in biotechnological development of lichen-associated bacteria, the nosperin example discussed above (Kampa et al. 2013) clearly shows that the direct shotgun sequencing and assembly and analysis of cyanobacterial genomes can lead to the discovery of potentially new novel interesting molecules and biosynthetic pathways, as well as guide targeted cultivation of microorganisms of interest. Other recent studies have applied shotgun sequencing targeting lichen associated bacteria (Grube et al. 2015; Sigurbjornsdottir et al. 2015), but the complexity of community did not allow the assembly of contigs long enough to permit analyses of biosynthetic pathways. A focus on lichens with chlorophyte symbionts, with a possible physical separation of bacteria (or bacterial DNA) would increase the chance of assembling larger genomic fragments and allow a better access to biosynthetic pathways, in particular those possibly present in the genomes of dominant *Alphaproteobacteria* symbionts (with the caveat that these organisms are not the most prolific in terms of the biosynthesis of bioactive molecules). Other techniques such as the construction and massive screening of fosmid libraries (e.g. Freeman et al. 2012) or single cell genomics (e.g. Wilson et al. 2014) likely represent better alternatives to access the genomes of rarer bacteria from lichens. However, the fact that many of the bacteria with biosynthetic potential present in lichens belong to groups for which targeted cultivation methods do exist, seem to indicate that for the near future, cultivation based-methods might be in fact a more promising approach.

In a first metaproteomic study, proteomics using one-dimensional gel electrophoresis combined with LC-MS/MS and normalized spectral counting was applied to characterize the active proteins of the *Lobaria pulmonaria* symbiosis (Schneider et al. 2011). Interestingly, not only the fungal partner was characterized by a rich secondary metabolism, also the bacterial partners showed a high activity supporting the hypothesis of biotechnologically relevant bacteria in symbioses.

Further biotechnological potential of lichen-associated bacteria

Irrespective of their capacity to produce small metabolites, lichen-associated bacteria have a wider biotechnological potential. According to a pioneering study by Gasser et al. (2011), one third of the detected lichen-associated bacteria have the potential to produce PHA biopolymers, yet, the strains isolated also showed a remarkable high antagonistic potential against plant pathogens. Up to 100% were antagonistic especially against leave pathogens,

such as *Alternaria alternata*. Similarly, antagonistic properties were also detected by Kim et al. (2013, 2014). Cernava et al. (2015) studied the antagonistic potential of a lichen-associated bacterial community of the lung lichen (*Lobaria pulmonaria*) with an integrative approach combining isolate screening, multi-omics techniques and high resolution mass spectrometry. The highly diverse microbiome contained an abundant antagonistic community dominated by *Stenotrophomonas, Pseudomonas* and *Burkholderia.* In these examples, the precise principle of antagonisms, and whether this also comprises interesting novel compounds, still needs to be established. The examples for antagonisms will be continued with future studies, especially after Zachow et al. (2013) proposed a promising screening strategy using plant rhizospheres as bait for lichen-associated bacteria. These studies further support lichens as important reservoirs of bacteria that are biotechnologically attractive.

Considering the high estimate of 18500 described lichens, much work apparently lays ahead of us to exploit their biotechnological potential. A more focused search for the most promising habitats and species may be guided by ecological knowledge. This possibly includes habitats where microbial regulation and competition becomes a major factor due to certain abiotic parameters, such as temperature and water availability. Thus expected hot spots for further research will include warmer zones such as tropical rain forests, or those, which are more frequently exposed to water inundation, including water streams or intertidal habitats.

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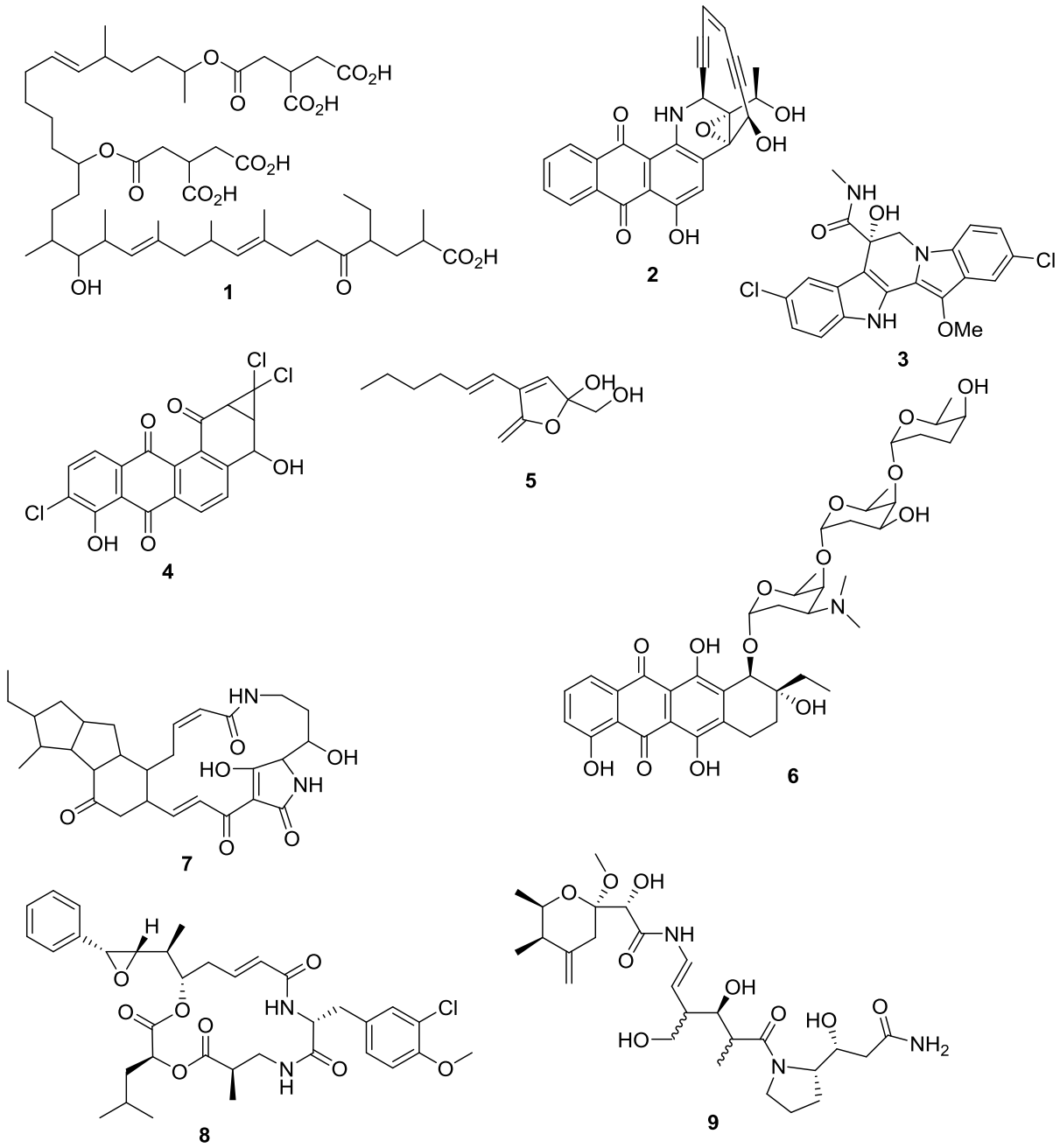

Figure 1. Structures of some molecules produced by lichen associated bacteria **(1)** actinoplanic acid B, **(2)** uncialamycin, **(3)** cladoniamide G **(4)** JBIR-88 **(5)** JBIR-89 **(6)** cosmomycin B **(7)** maltophilin **(8)** cryptophycin 1 **(9)** nosperin

Table 1. Bacterial strains isolated from lichens that 1) are officially described and/or 2) have a fully sequenced genome and/or 3) produced molecules that have been identified

Strain	Phylum or Class - Famlly	Location, Lichen	Isolation Medium	Nalidixic Acid	Reference
Corallococcus sp. ATCC 25944	*Deltaproteobacteria* - *Myxococcaceae*	n.r.	n.r.	no	McCurdy et al, 1971
Actinoplanes sp. ATCC 55532	*Actinobacteria* - *Actinoplanacee*	Spain, Madrid Unidentified	ISP3	no	Singh et al 1997
Streptomyces uncialis "ex Julian Davis et al."	*Actinobacteria* - *Streptomycetaceae*	Canada, British Columbia Surface of *Cladonia uncialis*	n.r.	no	Davies et al. 2005
Herminiimonas saxobsidens NS11	*Betaproteobacteria* - *Oxalobacteraceae*	Turkey, Mugla Lichen-Rock interface	Potassium Oxalate	no	Lang et al. 2007
Nocardioides exalbidus RC825	*Actinobacteria* - *Nocardioidaceae*	Japan, Izu Oshima Island unidentified	IAM-A1	no	Li et al 2007
Schumannella luteola KHIA	*Actinobacteria* - *Microbacteriaceae*	Japan, Tokyo unidentified	Modified Dettmer	no	An et al 2008
Leifsonia lichenia 2Sb	*Actinobacteria* - *Microbacteriacee*	Japan, Tokyo unidentified	Modified Dettmer	no	An et al 2009
Mucilaginibacter lappiensis ANJLI2	*Bacteroidetes*- *Sphingobacteriaceae*	Finland, Lapland Decaying Lichen	Lichenin	no	Mänistö et al 2010
Streptomyces sp. RI104-LiC106	*Actinobacteria* - *Streptomycetaceae*	Japan, Rishiri Island unidentified	HV agar	yes	Motohashi et al 2010

Streptomyces sp. RI104-LiB101	*Actinobacteria - Streptomycetaceae*	Japan, Rishiri Island unidentified	HV agar	yes	Motohashi et al 2010
Streptomyces sp. L-91-3	*Actinobacteria - Streptomycetaceae*	Canada, British Columbia Unidentified	n.r.	nr	Lavallée 2011
Frondihabitans cladoniiphilus CafT13	*Actinobacteria - Microbacteriaceae*	Austrian alps	TYE	no	Cardinale et al. 2011
Actinomycetospora iriomotensis IR73-Li102	*Actinobacteria - Pseudonocardiaceae*	Japan, Iriomote Island crustose unidentified	humic acid vitamin agar	yes	Yamamura et al. 2011a
Actinomycetospora rishiriensis RI109-Li102	*Actinobacteria - Pseudonocardiaceae*	Japan, Rishiri Island unidentified	humic acid vitamin agar	yes	Yamamura et al. 2011b
Sphingomonas sp PAMC26605	*Alphaproteobacteria - Sphingomonadales*	Arctic Norway (Svalbard) *Ochrolechia* sp.	n.r.	no	Shin et al 2012
Sphingomonas sp PAMC26621	*Alphaproteobacteria - Sphingomonadales*	Arctic Norway (Svalbard) *Cetaria* sp.	n.r.	no	Lee et al 2012a
Sphingomonas sp PAMC26617	*Alphaproteobacteria - Sphingomonadales*	Arctic Norway (Svalbard) *Umbilicaria* sp.	n.r.	no	Lee et al 2012c
Sphingobacterium cladoniae No.6	*Bacteroidetes- Sphingobacteriaceae*	Korea, Geogeum Island Cladonia sp	LB	no	Lee et al 2013
Luteimicrobium album RI148-Li105	*Actinobacteria - Promicromonosporaceae*	Japan Rishiri Island foliaceous unidentified	humic acid vitamin	yes	Hamada et al 2013

			agar		
Streptomyces cyaneofuscatus T178	*Actinobacteria - Streptomycetaceae*	Spain, Muniellos Unidentified	TSA	yes	Braña et al 2015
Streptomyces cyaneofuscatus T35	*Actinobacteria - Streptomycetaceae*	Spain, La Vecilla Unidentified	TSA	yes	Braña et al 2015
Streptomyces cyaneofuscatus T140	*Actinobacteria - Streptomycetaceae*	Spain, Cuntis Unidentified	TSA	yes	Braña et al 2015
Streptomyces cyaneofuscatus T163	*Actinobacteria - Streptomycetaceae*	Portugal, Guimarães Unidentified	TSA	yes	Braña et al 2015

Table 2. A list of studies that surveyed the diversity of microbial isolates associated with lichens

Lichen species	Lichen origin	target of isolation	Medium	Potentially interesting families, activities or others caracteristics of biotechnological interest	Reference
Not identified	Sitka and Kodiak, Alaska Hawaii and Reunion Islands	Biotechnology	soil extract agar, humic acid agar and glycerol asparagine agar	*Streptomycetaceae, Micromonosporaceae, Pseudonocardiaceae, Thermomonosporaceae.* Antimicrobial activity, Genes involved in biosynthesis of secondary metabolites.	Gonzalez et al. 2005
Cladonia digitata, C. rangiferina, C coniocracea, C. pyxidata, C. coccifera, Pseudoevernia furfuracea, Hypogminia physodes, Rocella phycopsis, R. fuciformis**	Styria, Austria and Normandy, France*	Untargeted, Diazotrophs	TYE, Sugar-rich N free	*Bacillaceae, Paenibacillaceae, Burkholderiaceae, Micromonosporaceae, Streptomycetaceae, Streptosporangiaceae*	Cardinale et al. 2006
Canoparmelia caroliniana, Canoparmelia crozalsiana, Canoparmelia texana, Parmotrema sancti-angeli Parmotrema tinctorum	Rain Forest, Brazil	Diazotrophs	N-free NFb	*Pseudomonadaceae*	Liba et al. 2006

Cladonia arbuscula, Lecanora polytropa, Umbilicaria cylindrica	wind-swept heath above the tree line Austria	Untargeted	R2A	*Bacillaceae, Paenibacillaceae Burkholderiaceae*. Chitinolytic, glucanolytic, and proteolytic activity, hormone production (indole-3-acetic acid). Antagonistic activity	Grube et al. 2009
Lecanora fuscobrunnea, Umbilicaria decussata, Usnea antarctica, Xanthoria elegans	Antarctic	Psychrophiles	TYE	*Pseudomonadaceae, Bacillaceae*	Selbman et al 2010
Cladonia sp. Cladonia rangiferina	Spagnun Bog, Tundra; Russia	Acidobacteria	Lichen extract, M3	None, as only *Acidobacteria* isolated described	Pankratov 2012
Ochrolechia sp	Arctic	Antimicrobial Antioxidants	MY/R2A	*Burkholderiaceae, Sphingomonadaceae*. Antimicrobial, Antioxidant, Prod. Carotenoids	Kim et al. 2014
Usnea sp., Cladonia borealis, Psoroma sp., Stereocaulon sp., Umbilicaria sp., Cetraria sp., Ochrolechia sp.	Antarctic and Arctic	Biotechnology	R2A, ISP4, MY	*Streptomycetaceae, Paenibacillaceae, Burkholderiaceae, Pseudomonadaceae*; Extracellular proteases, Lipases	Lee et al. 2014
Lecanora helicopis, Verrucaria ceuthocarpa, Hydropunctaria maura, Caloplaca verruculifera	Northern Iceland seashore Crag,	Untargeted	Marine Agar	*Streptomycetaceae, Bacillaceae*	Sigurbjörnsdòti r et al. 2014

Lobaria pulmonaria	Styria, Austria	Biotechnology	R2A, SCA, ISP2	*Bacillaceae, Paenibacillaceae, Burkholderiaceae, Pseudomonadaceae, Nocardioidaceae, Xanhtomonadaceae* Antagonisitic activity, Volatile Organic Compound production, Spermidine production, hydrogen cyanide production	Cernava et al. 2015a Cernava et al. 2015b
Lichina confinis, Lichina pygmaea, Rocella fuciformis, Collema auriformis	Marine, France; Riparian, Austria	Littoral Lichens; Actinobacteria	Marine Agar, AIA,ISP2 with Nalidixic acid	*Bacillaceae, Pseudomonadaceae, Nocardioidaceae, Promicromonosporaceae, Pseudonocardiaceae, Streptomycetaceae*. Pks I and Pks II genes.	Parrot et al. 2015

Table 3. Cyanobacteria strains isolated from lichens with characterized secondary metabolites

Strain	Lichen species	Lichen origin	Metabolites Described	Reference
Nostoc sp. ATCC 53789	Not described	Lichen, Arron Island, Scotland	Cryptophycin 1	Hisrsch et al 1990
Nostoc sp.	*Peltigera canina*	Not reported	Nostoclide I and II	Yang et al 1993
Nostoc sp. ATCC 53789	Not described	Lichen, Arron Island, Scotland	Nostocyclopeptide A1 and A2	Golakoti et al 2001
Nostoc sp. IO-102-I	*Pannaria pezizoides*	Rock Southern Finland	[ADMAdda5]microcystin-LR [DMAdda5]microcystin-LR [D-Asp3,ADMAdda5]microcystin-LR [ADMAdda5]-XR type variants	Oksanen et al. 2004
Nostoc sp N6	*Peltigera membranacea*	Iceland	Nosperin	Kampa et al 2013

Table 4. Cultivation independent studies broadly targeting the diversity of lichen associated bacteria

Lichen species	Lichen origin	Techniques applied	Phyla/Class described, average and range of percentage of Total¶, Prokaryotes, Bacteria§	Potentially interesting families*, activities or others caracteristics of biotechnological interest	Reference
Cladonia digitata, C. rangiferina, C. coniocracea, C. pyxidata, C. coccifera, Pseudevernia furfuracea, Hypogymnia physodes, Roccella phycopsis, R. fuciformis**	Styria, Austria and Normandy, France*	ITS fingerprinting, Sequencing of bands	*Gammaproteobacteria* *Actinobacteria* *Betaproteobacteria*	*Bacillaceae*	Cardinale et al. 2006
Cladonia arbuscula	Styria, Austria	FISH, SSCP	*Alphaproteobacteria* 75% *Actinobacteria* 6% *Betaproteobacteria* 4%	Not described	Cardinale et al. 2008
Cladonia cristatella, C. cryptochlorophaea, C. cf. *sobolescens, C. peziziformis, C. subtenuis, Flavoparmelia caperata, Parmotrema perforatum, Peltigera phyllidiosa, Lasallia pensylvanica, Umbilicaria*	Virginia and N. Carolina, USA	Direct Sanger sequencing of PCR products using universal primers (RHAPSA-D)	*Alphaproteobacteria* *Acidobacteria* *Gammaproteobacteria*	None	Hodkinson and Lutzoni 2009

mammulata					
Cladonia arbuscula, Lecanora polytropa, Umbilicaria cylindrica	Styria, Austria	FISH, SSCP	*Alphaproteobacteria* 45-75% *Actinobacteria* 6% *Betaproteobacteria* 4%	*Burkholderiaceae*[a], *Pseudomonadaceae*[a]	Grube et al 2009
Xanthoparmelia plittii, X. somloënsis	Massachusetts, USA	Pyro-sequencing of 16S rRNAs	*Alphaproteobacteria* (30-60% §) *Acidobacteria* (~17% §) *Bacteoidetes* + *Gammaproteobacteria* + *Deltaproteobacteria* + *Fibrobacteres* (<2% §)	Not described	Mushegian et al 2011

Parmelia sulcata, Rhizoplaca chrysoleuca, Umbilicaria americana, Umbilicaria phaea	Colorado, USA	Pyro-sequencing of 16S rRNAs	*Alphaproteobacteria*[b] (47%; 30-85%) *Acidobacteria*[b] (14%; 4-25%) *Gammaproteobacteria*[b] (8%; 0-52%) *Firmicutes*[b] (7%; 1-13%) *Verrucomicrobia*[b] (6%; 0-24%) *Planctomycetes*[b] (5%; 1-10%) *Actinobacteria*[b] (4%; 0-10%) *Betaproteobacteria*[b] (1%; 0-4%) *Bacteroidetes*[b] (3%; 0-8%) *Deltaproteobacteria*[b] (1% 0-8%)	*Paenibacillaceae*[b] (1.6%) *Micromonosporaceae*[b] (0.31%) *Kineosporiaceae*[b] (0.25%) *Burkholderiaceae*[b] (0.18%) *Myxococcales*[b] (0.16%) *Pseudonocardiaceae*[b] (0.09%) *Pseudomonadaceae*[b] (0.03%) *Actinomycetaceae*[b] (0.01%)	Bates et al 2011
Hydropunctaria maura, Ophioparma ventosa, Pertusaria corallina, Rhizocarpon geographicum	SW Norway	DGGE, Clone libraries	*Alphaproteobacteria*[c] (30% §) *Betaproteobacteria*[c] (21% §) *Actinobacteria*[c] (~16% §) *Acidobacteria*[c] (~10% §) *Cyanobacteria*[c] (8% §) *Firmicutes*[c] (7% §) *Chloroflexi*[c] (2% §) *Bacteroidetes*[c] (1% §)	*Burkholderiaceae*[c] *Pseudomonadaceae*[c] *Nocardioidaceae*[c]	Bjelland et al 2011
Lobaria pulmonaria	Styria, Austria	FISH, SSCP	*Alphaproteobacteria* (47.3-93.9%)	*Burkholderiaceae*[d]	Cardinale et al 2012a
Cetraria islandica, Lobaria pulmonaria, Lecanora polytropa, Cladonia arbuscula, Umbilicaria cylindrica,	Styria, Austria	FISH, SSCP	*Alphaproteobacteria* 15.5-80% *Betaproteobacteria*[e] 0-55% *Gammaproteobacteria*[e] 0-28% *Actinobacteria*[e] 0-6%	Not described	Cardinale et al 2012b

Cladonia coccifera					
Solorina crocea	Styria, Austria	Pyro-sequencing of 16S rRNAs†	*Acidobacteria* (42.4-66.4%) *Proteobacteria* (11-30%) *Planctomycetes* (7.2-25.2%) *Actinobacteria* (1.3-5.2%)	*Myxococcales*	Grube et al 2012
21 samples of *Cladonia* sp., *Flavocetraria* sp., *Ophioparma*sp., *Umbilicaria* sp., *Usnea* sp., *Dictyonema* sp., *Leptogium* sp., *Peltigera* sp., *Sticta* sp.	Cerro de la Muerte, Costa Rica Alaska, USA North Carolina, USA	Pyro-sequencing of 16S rRNAs†	*Alphaproteobacteria*[f] (60%; 5-93%§) *Acidobacteria*[f] (25%; 1-92%§) *Betaproteobacteria*[f] (5.0%; 0-19%§) *Gammaproteobacteria*[f] (3.3%; 0-9%§) *Actinobacteria*[f] (1.7%; 0-13%§) *Verrucomicrobia*[f] (1.5%; 0-15%§) *Planctomycetes*[f] (0.6%; 0-22%§) *Bacteoidetes*[f] (1.2%; 0-6%§) *Deltaproteobacteria*[f] (0.9% 0-6%§) *Firmicutes*[f] (0.3; 0-2%§)	*Burkholderiaceae*[f] (0.74%) *Myxococcales*[f] (0.70%) *Pseudomonadaceae*[f] (0.43%) *Actinomycetaceae*[f] (0.04%) *Nocardioidaceae*[f] (0.02%) *Pseudonocardiaceae*[f], (0.007%) *Micromonosporaceae*[f] (0.007%) *Kineoporiaceae*[f] (0.005%)	Hodkinson et al 2012
Arthrorhaphis citrinella, Baeomyces placophyllus, B. rufus, Icmadophila ericetorum, Psora decipiens Trapeliopsis granulosa	Styria, Austria	FISH	*Alphaproteobacteria* *Acidobacteria*	Not described	Muggia et al 2013

Rhizocarpon spp	South Tyrol, Italy	DGGE, 20 sequenced bands	*Alphaproteobacteria* 11 bands *Acidobacteria* 3 bands *Bacteroidetes*, *Firmicutes*, *Cyanobacteria* and *Actinobacteria* 1 band.	Not described	Esposito et al 2013
Lobaria pulmonaria	Styria, Austria	Pyro-sequencing of rRNAs† FISH	*Alphaproteobacteria*[g] (35%) *Bacteroidetes*[g] (14%) *Verrucomicrobia*[g] (8%) *Deltaproteobacteria*[g] (7.5%) *Actinobacteria*[g] (12%) *Betaproteobacteria*[g] (2.7%) *Gammaproteobacteria*[g] (2.4%) *Acidobacteria*[g] (2.3%) *Planctomycetes*[g] (1.2%)	*Myxococcales*[g] (6.9%) *Pseudonocardiaceae*[g] (3.6%) *Micromonosporaceae*[g] (1.3%) *Kineoporiaceae*[g] (0.9 %) *Pseudomonadaceae*[g] (0.8%) *Nocardioidaceae*[g] (0.8%) *Burkholderiaceae*[g] (0.1%) *Streptomycetaceae*[g] (0.1%)	Aschenbrenner et al 2014
Peltigera membranaceae	Iceland	Shotgun pyro-sequencing followed by rRNA analysis	*Alphaproteobacteria* (40%) *Bacteroidetes* (14%) *Actinobacteria* (11%) *Betaproteobacteria* (9%) *Verrucomicrobia* (8%) *Gammaproteobacteria* (5%) *Deltaproteobacteria* (3%)	*Nocardiaceae*[h] *Myxococcales*[h] *Burkholderiales*[i]	Sigurbjörnsdòttir et al 2015

a. Estimated from PCR amplification with primers targeting these clades; b.Estimated from OTU table graciously provided by S. Bates; c Estimated from figure 3B in the reference; d. Estimated from PCR amplification with primers targeting this clade; e.Estimated from Figure 2A in the reference; f Estimated from the OTU table associated with the publication; g. Estimated from OTU table associated to the publication; h. Used as reference genomes i. based on blastx analysis of contigs

* Order in the case of *Myxococcales* †*Cyanobacteria* excluded