

Review Article

A Brief Overview on Microbial Communities Associated with Lichens

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Abstract

The investigation of microorganisms from so far unexplored ecological niches has become an exciting subject in recent years. Lichens are ecologically highly adapted and also unique because the physiological integration of organisms provides conditions for persistence under extreme environmental conditions. Lichens are generally considered as symbiosis between fungi and green algae or cyanobacteria. These partnerships give rise to light-exposed and long-living joint structures, and the unique organization of lichens provides still unexplored environments for microbial communities. Recent studies showed that lichens harbour high abundances and diversity of bacteria but their exact location, ecology and biotechnological potential are still unexplored. The present paper summarizes briefly and gives an overview about the most important topics related to the study of the microbial community inhabiting the lichen thalli.

1. Introduction

Lichens are important components of biodiversity on earth. Their species richness may be equal to or greater than that of vascular plants at given sites [1, 2]. Lichens are mutualistic symbiotic organisms and consist of two unrelated components: a fungus (the mycobiont) and one or more algae or cyanobacteria (the photobionts). Lichens are the dominant organisms of ca. 8% terrestrial ecosystems [3] and are typically found in environments subject to extremes of temperature, desiccation and nutrient status. Lichens fill an important niche in areas, where extreme climatic conditions lead to an absence or seasonal inaccessibility of higher plants. According to these environmental conditions and aspects, lichens can be considered as an interesting reservoir for diverse group of microorganisms.

Although the symbiotic components of lichens have been extensively described little is known about the diversity of the microbial community inhabiting these complex systems. Detailed scientific studies on bacteria colonizing lichens have been not so far well understood, and based on the idea that lichen represents a unique ecosystem due to its physiological and morphological characteristics; it is expected that such complex system would contain novel strains with unknown metabolic capability [4, 5].

The extent of microbial diversity in nature is still largely unknown, suggesting that there might be much more useful information yet to be identified from microorganisms in natural habitat. Significant diversity in genotype appears to exist in among and within lichens species and molecular methods are taking place to analyze this pool of diverse biosynthetic gene. Molecular approaches provide an invaluable assessment of biotechnological potential of unexplored groups of microorganisms associated to lichens [6], especially regarding bacterial species.

The presence of nonphotosynthetic bacteria in lichens has been already described since some time ago, however these early narratives were all only based on non molecular approaches, mainly of them were found only by cultivation-dependent approaches. The use of molecular fingerprints and DNA sequences has only recently been used to characterize culturable bacteria in lichens and no study has so far focused on the localization of microbial population in the lichen thalli [7, 8].

2. Lichens

Lichen is a symbiotic associative life form among a wide range of fungi, photosynthetic algae, or cyanobacteria, or possibly all of three together [9]. They are considered one of the best known groups of symbiotic organisms [10]. The lichens symbiosis has been evolved probably around 400 to 600 million years ago and they can be considered as ecosystems where the interaction of partners results in specific behaviour and life forms [11, 12]. Typically, these filamentous and unicellular organisms are involved in association to form undifferentiated plant-like structure referred to as thallus. The lichen is an ecologically obligate, stable mutualism between a fungal partner and unicellular or filamentous algae or cyanobacteria cell [12]. The thallus shows an interesting variety of morphological structures, colours and size, and present themselves completely different from either symbiotic partner grown in non-symbiotic culture, this differentiation is formed through complex interactions between mycobiont and photobiont cells growing together to form a body unlike either of the two symbionts grown alone [13].

In general, three major life forms of lichens thalli are recognized: crustose (crust-like biofilm), foliose (leaf-like) and fruticose (branched tree-like). Gelatinous thallus is also described, although it is restricted only to some cyanobacterial lichens. Even without roots, lichens can efficiently extract nutrients from recalcitrant surfaces and very often grow in habitats with extreme light, dryness, or temperature, which are less favourable or not suitable to higher plants. The symbiosis increases the tolerance to undesirable conditions than in its partner alone [12, 14, 15].

According to Joneson S and Lutzoni F [16], the sequence of events leading to a stratified thallus can be summarized in four basic stages: (i) pre-contact, which is prior to physical contact of symbionts but close enough that extracellular interactions are possible; (ii) early-contact, where fungal cells contact algal cells forming an envelope through an increase in short lateral branches; (iii) incorporation of growing fungal and algal units into an undifferentiated mass; (iv) and differentiation of layered tissues, however the molecular mechanisms involved in such event are still unknown.

Galun M. Lichenization [17] studied the development of thallus observed that if fungi and phototrophs do not interact to form any of the initial stages of lichens development, they are considered non-compatible, indeed mycobionts and photobionts are considered compatible when they enter into stage two of lichen development as shown by the envelopment of the alga through increased lateral branching. The formation of lichens associations represents one of the most successful lifestyles among the fungi; approximately 20% of the 65,000 described species are involved in this association. Almost all of the 13,500 lichen-forming species are ascomycetes; only ~ 50 belong to the basidiomycetes group [18].

As described by many authors, fungi are important for the symbiotic process and the main selective fungi advantage in becoming symbiotic in lichens is the supply of carbohydrates, as its source of energy produced by photosynthesis in the photobiont. Lutzoni *et al* [19] along of their studies found that about one-fifth of all known extant fungal species form obligate symbiotic associations with green algae, cyanobacteria or with both photobionts, as a way to meet their requirements for carbohydrates. This feature requires the photobiont to release the carbohydrate without fatal detriment to itself [20]. There are several groups of organisms responsible to produce carbohydrates (polyol or glucose); these include the major groups of the algae represented not only by green algae (Chlorophyceae), but also red algae and the brown algae. All these groups capable of multicellular organisation have physiological characteristics in common which allow them to transfer carbohydrates between cells of their tissues to the fungi [21]. At present, an estimated 100 species in 40 genera are reported to form lichen symbiosis. They are placed in at least five phylogenetically classes: Cyanophyceae, Xanthophyceae, Phaeophyceae, Chlorophyceae and Trebouxiophyceae [18].

An estimated 10% of the lichen-forming fungi form associations with cyanobionts, which provide fixed nitrogen along with photosynthetically fixed carbon [22]. In cyanobionts lichens, the fungus absorbs glucose from the cyanobacterial cells, through the movement of hexose produced by photosynthesis from the vegetative cells to the heterocysts; in non-heterocystous cyanobacteria the mechanisms of nitrogen fixation is still not yet clearly understood and most of the cyanobionts appear to be heterocystous [23].

The reproduction is carried either with fungal spores that have to find a suitable photobiont or by vegetative propagules including both partners; usually lichens grow very slow in a rate of mm up to cm per year [24], in nature it is possible to find exemplars with more than one meter heights growing over rocks and native vegetation.

The lichen community can be found in different substrates and environments, usually in places where no other organisms would be able to survive, they are able to colonize inhospitable niches, with extreme variations on temperature and humidity, and hence they are considered as pioneers in colonization of new spaces [25].

3. Microorganisms associated to lichens

Microorganisms in natural environment face conditions that are different from those experienced during assays carried in laboratory, especially growth at variable temperatures (low and high), long periods of frozen and dryness state, soil with high water content causing sometimes anaerobic conditions, low pH, and high content of organic matter on soil. These conditions may result in selectivity and evolution of different taxonomic groups of microorganisms to adapt to specific niches [26]. It is well known, that isolated microorganisms do not reflect the true composition and structure of natural environments, and actually the analyze of small subunits of rDNA from natural ecosystems has become a powerful tool to characterize microbial communities directly in situ, because it avoids limitation of culturability and provide precise information regarding phylogenetic fingerprints [26].

The ecological assessment of microbes from unexplored environmental habitats has become an interesting subject in earlier years. Niches as diverse as soil, water and air are being studied by various techniques, ranging from culture techniques to metagenomic approaches [4]. According to these new molecular assessments, several new information about microbial communities are now being clarified and this is a promising perspective for the investigation of further, biologically rich habitats. Among these new highlights, diverse groups of cryptogams including lower plants, fungi and lichens could be particularly suitable as hosts for bacterial communities. Lichens are considered as pioneers of terrestrial habitats colonization, they are found from arctic to tropical regions in a large diversity of environments developing among others on stones, arid soils, or as epiphytes on plants [27]. Although the symbiotic components of lichens have been extensively described, little is known about the diversity of the microbial community inhabiting these complex systems. Petri *et al* [28] described the presence of several microbial biofilms in the interface of saxicolous lichens; in this report they observed the existence of micro habitats constituted by a very rich diversity of microorganisms. Similar evidences were also found on the Antarctic cold desert, the place has been colonized by a considerable number of lichen community, and provide one of the most extensively locus for studying the association formed by endolithic microorganisms. The molecular examination carried by [29] revealed the presence of vast microbial diversity inhabiting this ecosystem, where it is possible to find distinct phylotypes of bacteria belonging mainly to the Actinomycetes group.

Cyanobacteria can be found in association with lichens, and they are responsible to provide nitrogenated compounds via nitrogen fixation [30]; approximately 150 species in 58 genera form association with lichens. Rai [31] proved that almost all nitrogen fixed by cyanobacteria is transferred to the fungi. Cyanobionts occurs in two type of lichen association: the first one called bipartite, where a continuous layer of photosynthetic and nitrogen-fixing cyanobionts is established, and the second one there is the integration of a second eukaryotic photobionts form with the nitrogen-fixing cyanobiont [32]. Usually, modifications on morphology and life cycles occur on cyanobacteria after the aggregation with lichens, including increase of 10% to 35% in heterocysts and enhancing the fixation rates compared with non-associated cells. However, relatively little information is known regarding the nitrogen cycling in lichens [33].

Of all fungal symbiotic relationships, however, the lichen association is a rather particular case: it contributes to a substantial primary evolutionary radiation of ascomycetous fungi [19]. Species that form lichens associations are equal or outnumber those that form parasitic associations (20%) or mycorrhizal associations (8%), and they are exceeded only by saprophytic decomposers (50%). Almost 98% of all lichen-forming species belong to the ascomycetes group; only 1% is quoted as basidiomycetes. Although some authors have been also reported Actinomycota, Mastigomycota and Myomycota, these groups are no longer considered as true fungi [18]. On the other hand, fungus-associated bacteria have now been described for several functional groups of fungi and both positive and negative effects on fungal performance have been reported [34].

The study on bacterial diversity in unexplored niches provides potential benefits by storing new gene pool as well as by finding new bacterial taxa. The understanding bacterial diversity also gives new insights into the biological mechanisms of adaptation and tolerance to inhospitable environments [35]. Bacteria have the capacity to adhere to natural or artificial surfaces, and they are able to form sessile multicellular communities very stable and resistant to almost any kind of adverse conditions, these complex microbial structural interactions include diverse species with various functions responsible to give stability to the microbial community [36].

4. Lichens-association microorganisms and their biotechnological potential

Lichens and their natural products have a long tradition of being used for decorations, brewing and distilling, perfume and dying industry, food, and natural remedies. A great number of species have proved to be source of important metabolites and with modern technology, the potential of discovering and utilizing these products has increased every day [12]. Lichenologists have studied lichen chemistry for the past hundred years and have found over 800 compounds considered as secondary metabolites, especially polyketides-derived aromatic compounds [37, 38].

The chemical substances produced by lichens can be grouped according to their location on the thallus, these products may be considered intra- or extracellular. The thallus has a complex structure, and some metabolites are synthesized by both the fungal and/or the photobiont partners [39]. The intracellular compounds (carbohydrates, carotenoids, vitamins, aminoacids and proteins) are connected to cell wall and protoplast; those compounds are formed not only in lichens, but also in fungi, algae and high plants [39, 40]. Extracellular products usually are so called secondary metabolites and are mainly found at the medulla or cortex, but very rare in both layers [40]. Although, these compounds are found in fungi and high plants, the biggest part is considered exclusive of lichens; the concentration of metabolites comprehend 0.1 to 10% of lichen dry biomass, in some cases even higher [39].

Although many natural and cultured lichens have been screened for their biological activities and several novel compounds have been isolated and identified, lichens have been essentially ignored by the modern industry. There are two contributing reasons for this: its slow growth in nature and they are difficult to propagate and resynthesize in culture [41, 42].

It is generally believed that most of secondary metabolites in lichens are provide by the mycobionts, this is evident due to the fact fungal compounds are well known in medicine. It is possible, that the photobionts also contribute to the repertoire of lichens metabolites [41].

A significant part of all lichens are colonized by cyanobacteria, and research suggests that the prokaryotic partner contributes significantly to the biosynthetically diversity of lichens. Marine and freshwater cyanobacteria produce a wide range of peptides and other bioactive compounds and are rich source of mixed peptides-polyketides. Among these metabolites, many can be considered with biotechnologically promising bioactivity; anticancer, antifungal and antiviral are also reported. Several reports has described many microcystin compounds as the most commonly isolated bioactive compound produced by the Nostoc strains, one of the most common genus in terrestrial cyanobacteria symbiosis [43].

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The investigation of bacteria in association with symbiotic organisms has attracted considerable interest because of its great unexplored biotechnological potential for different industrial processes and in sustainable agriculture. Wang [44] reported the rich source of bioactive secondary metabolites with antiviral, antitumor and antimicrobial activity from bacteria isolated from sponge; they are hosts of a large community of microorganisms, such as bacteria and fungi, and some of them are host-specifics.

Among these groups, the lichens have attracted special attention in concern to the complex structure presented by the interaction of different microorganisms in symbiosis and the huge

amount of compounds produced as primary and secondary metabolites [8].

Detailed scientific studies on bacteria colonizing lichens have been not so far well understood, and based on the idea that lichen represents a unique ecosystem due to its physiological and morphological characteristics; it is expected that such complex system would contain novel strains with unknown metabolic capability.

5. Metaproteomics and lichens

During the begging of the geological earth formation, microorganisms were responsible for the primary roles in providing the environmental conditions that we can find actually. They are the major drives of the biogeochemical and nutrients cycles, as well as degraders of natural and anthropogenic wastes on the planet [45]. Many of these important reactions are catalysed by microbial enzymes that are made up by individual proteins and are regarded as environmental catalysts.

According to the functions described above, it makes sense to study the vast range of microbial proteins in different ecosystems and unexplored niches to understand better to role of these enzymes in complex systems [46]. Actually, there is an increasing interest to understand microbial community composition and functions directly from their respective environments, and molecular analysis of environmental samples has greatly improved our knowledge about microbial diversity. Metagenomic approaches obtained directly from environmental samples provide large amount of data concerning genetic diversity and metabolic potential of microorganisms within selected environments [47]. Indeed, it is necessary to improve knowledge of microbial diversity with functional details of these microbial ecosystems.

In former time, the application of postgenomic techniques has been limited mainly to study pure culture in laboratory, and regarding studies on microbial communities only cultivation-independent experiments were done. However, these studies do not provide enough information about gene and protein expression in complex mixtures as found in the natural environment, guiding to a distorted understanding of microbial ecology.

Various strategies were used to study the relationship between the ecosystem functioning and the structure of microbial communities. Major goals of these efforts are to attribute key functions to specific community members and, in view of the ecosystem stability, to reveal cooperation between community members and functional redundancies [48]. Besides measuring enzyme activities, respiration rates, metabolites concentration and nucleic acids are often used as markers for microbial identity. However, these structural data are specific only for certain group of microorganisms and there are no specific markers for all known bacteria [49].

Although the identification of structural and functional gene can be used as a good indicator for the presence of microorganisms and their metabolic potential in the environment, it is not possible to understand completely the entire role of those organisms in situ. In comparison to molecular approaches, the use of proteins are a promising alternative since they reflect the actual functionality with respect to metabolic reactions, and give direct information about microbial activity more than functional genes. In other aspect, the use of proteins can also reveal the identity of microorganisms living in mixture systems via database analysis using homology with other described species [49, 46].

Proteomics is one of the fastest developing research areas and contributes substantially to our understanding of organisms at the cellular level. It is considered a new approach that enables the direct observation of proteins expressed by mixed microbial assemblages [50]. Recently, the large-scale characterization of the entire protein complement of environmental microbiota (often referred to as metaproteomics) has been proven useful to investigate

the most abundant proteins in environmental samples [51-53]. Environmental proteomics, due to its opportunity to study many protein functions and responses simultaneously, offers excellent possibilities to improve our understanding of microbial community architecture and composition, and ecosystem functioning. Metaproteomics can be used to study protein expression from complex systems and provide direct evidence of metabolic and physiological activities. Recently, proteomic approaches have been used to quantify and detect proteins from organisms in natural habitats and the proteomic approach is now more feasible than metagenomics sequences providing opportunity to identify proteins from microorganisms in complex mixtures [51]. Until now, several authors presented metaproteome data from different environmental systems, such as soil particles, activate sludge, biofilms, and seawater, even about stress response of mixed cultures. However, the use of metaproteomic approaches to elucidate the microbial diversity in symbiotic living being is still completely unexplored, especially in lichen research area [54].

6. Geographic and ecological signals from bacterial communities associated to lichens

Most of the biogeography studies focused on the macroorganisms as plants or animals, whereas it was still little known about the distribution of microbial species and their communities. The understanding of microbial distribution in temporal and spatial dimensions, i.e. microbial biogeography, is still in its infancy [55], but received a rapid increasing interest in the recent past. The study of biogeography offers insight in those mechanisms, which generate and maintain diversity among organisms in general, such speciation, extinction or dispersal [56].

Microbial biogeography studies have explored a wide range of habitats, taking in consideration soil bacteria communities [57], bacterial assemblages in lakes and streams [58], or extremophilous microorganisms in soda lakes [59]. Genetic methodological approaches based on fingerprint profiles and large scale sequencing make easier nowadays to discover the dominating unculturable fraction of microbial diversity. The statement of [60] that "everything is everywhere but the environment selects", meaning that species can be found where their environmental requirements are met, has been leading almost all the biogeography studies. However, not only ecological parameters determine the distribution of microorganisms. When bacteria form intimate association with macroorganisms, as many bacteria-animal and bacteria-plant symbioses, the distribution of the first is often affected by the geographic distribution of their hosts [61]. A high degree of host specificity was found for bacteria associated with two Sphagnum species independent of the geographical region [62], whereas the extent of gene deletions, duplications, and acquisitions in the genome of facultative symbiotic soil bacteria of the genus Frankia (Actinomycetes) was correlated to the biogeography distribution of the associated plants in the family Casuarinaceae [63]. However, at smaller scales the microbial biodiversity may remain hidden to us because many species occur at a density below the limit of our detection [64].

Long-living organisms may represent ecological stable niches, which can host still undiscovered microbial communities. Textbook examples for longevity are lichens. These organisms represent an ecologically obligate and stable mutualism between a fungus, the mycobiont (the exhabitant partner) and a population of extracellularly located green algae or cyanobacteria, the photobionts [65]. Recently molecular methods confirmed the conspicuous presence of microorganisms in lichen thalli of diverse growth forms [4, 7, 8]. The complex morphologies of the lichen thalli, from crustose to foliose and shrubby, offer more or less exposed surfaces, where microorganisms can form highly structured biofilm-like assemblages on the fungal surface and can reach considerable abundances [8]. More than 17.000 lichen species are described [66]. Many lichen

species are highly cosmopolitan, show a wide range of ecological tolerance, and are widespread on different substrata [67]. Alternatively there are lichen species, which have an extremely wide geographic distribution but are restricted to particular ecological conditions. In this context one of the major limiting factor affecting the distribution of some lichen species is their sensitivity to air pollution [68].

Most of the lichens, especially the large shrubby and leaf-like forms are easily recognized in the nature. Data about the geographic distribution of lichens has therefore accumulated over the past 200 years. In fact, lichens are the sole group for which a vast knowledge on biogeography already exists. Many lichens have an extremely wide geographic distribution, and the same species can be found on several continents under similar ecological conditions. There has been a lot of debate how the wide distribution patterns emerged. In the past the long range dispersal hypothesis was much favoured, but recent evidence shows that the pattern could in many cases also be explained by historic changes and the separation of previously coherent distribution ranges. In recent times anthropogenic fragmentation of distribution ranges became a major threat to lichens, especially when these are particularly sensitive to other parameters such as air pollution

Lichens are well known to be reliable indicators of air quality and have been used as biomonitors in several studies [69, 70]. One of the best-known lichen genera, which is particularly sensitive to air pollution and represents excellent indicators of forest ecosystems with long ecological continuity and with a large number of rare species is *Lobaria* [71]. In particular *Lobaria pulmonaria* is often used as a flagship species for conservation activities. *Lobaria pulmonaria* is widely distributed in temperate and circumboreal regions of the Northern Hemisphere, and tropical mountain systems, and in South Africa. While species is still abundant in North America it suffered a decline in many parts of its European range. It is now considered endangered in many parts of Central Europe, and further industrial regions.

7. Conclusion

The structure of natural communities of terrestrial habitats is often highly complex and therefore difficult to characterize. It has become possible by the use of modern molecular biology tools and analysis to evaluate, at the community level, a wide number of environmental samples and describe the microbial community structure in complex interaction, such as lichen symbiosis. Despite the acknowledged importance of soil and root associated microorganisms, little is known about the interaction of bacteria associated with lichens. Although many scientific studies have shown that the lichen thallus is considered a reservoir of many bacterial species still more studies should be conducted to understand much better the interaction and function of these bacteria in lichens and the traditional concept of lichens has to be expanded to consider multiple bacterial partners.

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