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Comprehensive structural and functional diversity of bacterial communities in lichens : A review

Aliffa Yusti Sadila[1], Ummi Zubaidah[1], Annisa Nur Fatimah[1], Diaz Fathania Adani[1] and Almando Geraldi[1,2]*

[1] Department of Biology, Faculty of Science and Technology, Universitas Airlangga, Surabaya, Indonesia
[2] Research Center for Bio-molecule Engineering, Universitas Airlangga, Surabaya, Indonesia

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ABSTRACT

The structural form of lichens mostly occur as moss-like substances.Theygrow on barks, branches, rocks, exposed soil surfaces, rain forests, and temperate woodland. Lichens are holobionts with more than one participant in the association. They are usually comprised of a filamentous fungal partner called mycobiont and an algal partner called photobiont. Lichen as a mutualistic association between a fungal and an algal partner, is now, a traditional concept. Recent and advanced molecular level studies revealed the fact, that lichens harbor diverse microbial communities such as heterotrophic bacteria. Bacterial communities in lichen vary as they are also influenced by abiotic environment that builds up their habitat. The metabolic activities of bacteria in lichen may provide benefits both to the bacteria itself and the lichen where it lives. This review pivots diversity of bacterial communities, the influence of abiotic environments on bacterial communities, and functional roles of bacterial communities in lichens.

Key words : *Lichen, Bacteria, Plant growth promoting bacteria*

Introduction

Lichens are ecologically diverse and many, often pioneering species, can grow with exceptionally low supply of nutrients. That survival ability of lichen is related to mutualistic association with many microorganisms. Lichens are usually comprised of a filamentous fungal partner called mycobiont and an algal partner called photobiont which can be either an eukaryotic chlorobiont (green algae) or a prokaryotic cyanobiont (cyanobacteria) and a very few are from other classes of algae. About 85% of lichens have chlorobionts, 10% have cyanobionts and less than 5% have other photobionts as their photosynthetic partner(Zachariah and Varghese, 2018). It became a traditional concept knowing lichen as mutualistic association between fungal and algal partner. Because recent and advanced molecular level studies have revealed the fact, that lichens harbor diverse microbial communities including heterotrophic bacteria. Several strains of bacteria on lichen which have been confirmed are *Azotobacter, Bacillus, Beijerinckia, Clostridium,* and *Pseudomonas* (Zachariah and Varghese, 2018). The objectives of this review are to identify the diversity of bacterial communities in lichen which live in different habitats, to elaborate how surrounding environment give influences on bacterial- associated lichen, and to show the comprehensive roles of bacteria on life and survival of lichen will be explained too.

Bacterial Communities in Lichen Species

Lichen is a form of moss-like substance that happen to exist through symbiosis of fungi, algae, and bacterias. They build up the shapes, textures, sizes, and colors of lichens. Lots of aspects implicate bacterial communities in lichen even as simple as reproduction.Bacterial communities at the centers of diferent lichens resemble each other, while edges house more random assemblages (Mushegian *et al.*, 2011). Lichens often reproduce asexually, and within asexual propagules, the lichen packages its bacteria (Aschenbrenner *et al.*, 2014). Samples were taken from a gravestone in North Cemetery, Petersham, Massachusetts. The location was surrounded by forest before the land was used for agriculture. Agricultural lands were then abandoned, and forests grew up around the stone walls whereas became novel habitat for lichens. Mushegian *et al.*, (2011) studied microbial communities taken from the lichens genus *Xanthoparmelia* ; *X. plittii* & *X. somloënsis*. *X. plittii* contains stictic acid in its internal layer, while *X. somloënsis* contains salazinic acid, but they have similar leaf-like foliose morphologies. All samples were overwhelmingly dominated by *Proteobacteria* and *Acidobacteria*. The *Proteobacteria* were dominated by *Alphaproteobacteria* whereas almost all of the sequences within the *Alphaproteobacteria* belonged to the orders *Sphingomonadales*, *Rhodospirillales*, and *Rhizobiales* (Mushegian *et al.*, 2011). Bates *et al.*, (2011) used *Parmelia sulcata, Rhizoplaca chrysoleuca, Umbilicaria americana*, and *Umbilicaria phaea* were collected from granite rock outcrops at a site in northern Colorado as their samples. The studies uses sterilization and isolation of community DNA, and its is found that there are some taxa were across their lichen samples, namely, *Actinobacteria, Firmicutes*, and *Gammaproteobacteria*, which all the species were also dominated by *Alphaproteobacteria*. From study conducted by Sierra *et al.*, (2020), which used seven genera, namely *Cora, Hypotrachyna, Usnea, Cladonia, Peltigera, Stereocaulon* and *Stictasaid* as collected samples from which Paramoecosystems within two national parks in Colombia. The study uses DNA from its samples and they isolated samples to identify microbial community profiles by 16S rRNA sequencing. The result shows that *Proteobacteria* and *Cyanobacteria* were the most dominated bacteria in their samples too. The other study conducted by Grube *et al.*, (2009) analyzed the structure and composition of associated bacterial communities of the lichen species *Cladonia arbuscula, Lecanora polytropa* and *Umbilicaria cylindrica* by a polyphasic approach, using combined microscopic fluorescence in situ hybridization (FISH) and confocal laser-scanning microscopy (CLSM) and molecular techniques (microbial fingerprints by PCR-single-strand conformation polymorphism analysis (SSCP) using different primer systems, shows a result of a prevalence of *Alphaproteobacteria* (45–75%) in these communities, which other groups of bacteria were present at lower abundances.

Previous studies suggest that the centers of lichens house both greater numbers of bacterial species and more-consistent communities. In contrast, edges are species-poor and the bacterial communities of different edges are more variable. The thicker center of a lichen has more biomass per unit area and thus more available habitat (Mushegian *et al.*, 2011). It was proven by Mushegian's study (2011) using *Xanthoparmelia* lichens, the center of the thallus is the moldest part of the individual and appears to grow as a combination of original and regenerating tissues; the edges of the lichen are recent growth. Center modules have been exposedto ambient, colonizing bacteria for a longer period of time than edge modules. There may also be chemical and physical differencesbetween the different locations; centers may be more likely to fragment, and data from, e.g., *Cetraria islandica* suggest that nitrogen concentrations are significantlylower in older fragments (Parinkina *et al.*, 2000). In the *Xanthoparmelia* lichens Mushegian (2011) has sampled, the centers housed dense numbers of column shaped reproductive structures termed isidia, while the edgeslacked isidia and were therefore flat.

Although it is known about groups of bacteria that are symbiotic with lichen, their roles are still difficult to be understood than the role of algae and fungi, which are also known to have symbiosis with lichen. A study conducted by Grube *et al.,* (2015), explained the functional concept of bacterial symbiosis with lichen *Lobaria pulmonaria*. After conducting the analysis, it was found that the taxonomic structure of the bacteria formed the symbiosis with *L. pulmonaria*. The most dominant phylas are *Proteobacteria*, *Cyanobacteria*, and *Acidobacteria*. In *Proteobacteria*, *Alphaproteobacteria*is the most abundant taxon, of which the most frequently occurring orders are *Rhizobiales* and *Spingomandales*. Within the *Rhizobiales*, *Methylobacteriaceae* and

Bradyrhizobiaceae are prominent, while *Rhizobiaceae*, *Beijerinckiaceae*, *Xanthobacteriaceae*, and *Phylobacteriaceae* were found in small quantities. In addition, using Complementary In Situ Fluorescence Hybridization (FISH) visualization with *Alphaproteobacteria* and *Betaproteobacteria* specific probes, together with nonspecific eubacteria probes can illustrate the distribution of bacteria by metagenome analysis. *Alphaproteobacteria* have a dominant number and are widespread on both the upper and lower surfaces of the lichen thallus, whereas *Betaproteobacteria* are less abundant and locally limited. Together with nonspecific eubacterial probes, bacterial distribution by metagenome analysis can be illustrated.

Another study conducted by Elacher*et al.*, (2015) discussed more specifically the symbiosis of *Rhizobiales* bacteria on lichen *L. pulmonaria*. The most abundant phylum is *Alphaproteobacteria* with the *Rhizobiales* being the most dominant order. The identified families in the *Rhizobiales* are *Methylobacteriaceae*, *Bradyrhizobiaceae*, and *Rhizobiaceae*. The only identified genus is *Methylobacterium*. *Methylobacteriaceae* and *Methylobacteriumradiotolerans* were the most frequently found species. Meanwhile, species that are less frequently identified are*M. nodulans*, *M. populi*,and members of the *M. extorquens* group. Other identified families of *Rhizobiales* are *Bradyrhizobiaceae* which are more diverse with four distinctive genera, namely: *Bradyrhizobium*, *Rhodopseudomonas*, *Nitrobacter* and *Oligotropha*. The most abundant species in *Bradyrhizobiaceae* was identified as *Rhodopseudomonas palustris*. The fewer families of the order *Rhizobiales* are included in the genera *Beijerinckiaceae*, *Xanthobacteraceae*, *Phyllobacteriaceae*, and *Brucellaceae*.

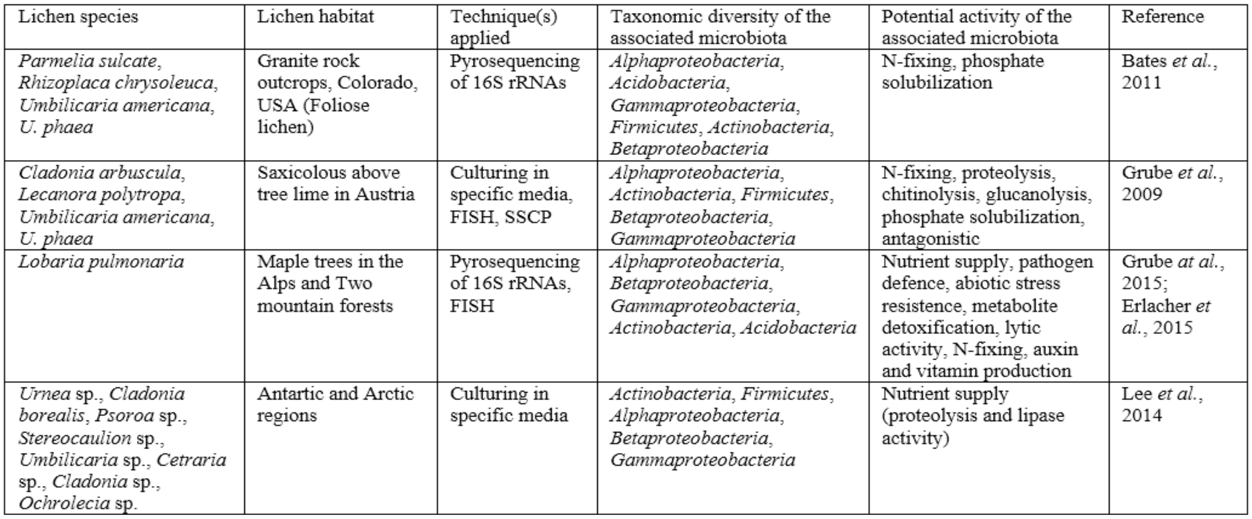
Roles of Bacterial Communities in Lichen

In traditional concept, it is commonly known that lichens are generally defined only as bipartite or tripartite mycobiont-photobiont symbiosis. However, in recent study the presence of other microorganisms in the lichen thalli has been conducted. Begin with Johannes Uphof's early report on "purple bacteria" in lichens, suggesting that non-cyanobacterial prokaryotes were present in the lichen. The following series of published papers are focusing to reveal structural and functional diversity of bacterial communities in lichens including their ability to produce secondary metabolites with biological activities (Sigurbjörnsdóttir, 2016). The nonphotoautotrophic bacteria of the lichen microbiome areincreasingly regarded as significant players in the ecologyof lichens. Because many lichens are able to grow onextremely nutrient-poor substrates, it has been suggestedthat these bacteria provide some lichen thalli with a substantial source of crucial nutrients (Hodkinson, 2012)(See Table 1 for more overview).

Bacteria associate with lichen as nitrogen-fixing agent.

Recent studies have revealed association between bacterial communities and lichen by microbial fingerprint technique(Bates *et al.*, 2011; Grube *et al.*,

Table 1. List of bacterial communities associated with lichen and their functional roles (Sigurbjörnsdóttir, 2016)



Lichen species	Lichen habitat	Technique(s) applied	Taxonomic diversity of the associated microbiota	Potential activity of the associated microbiota	Reference
Parmelia sulcate, *Rhizoplaca chrysoleuca*, *Umbilicaria americana*, *U. phaea*	Granite rock outcrops, Colorado, USA (Foliose lichen)	Pyrosequencing of 16S rRNAs	*Alphaproteobacteria*, *Acidobacteria*, *Gammaproteobacteria*, *Firmicutes*, *Actinobacteria*, *Betaproteobacteria*	N-fixing, phosphate solubilization	Bates *et al.*, 2011
Cladonia arbuscula, *Lecanora polytropa*, *Umbilicaria americana*, *U. phaea*	Saxicolous above tree lime in Austria	Culturing in specific media, FISH, SSCP	*Alphaproteobacteria*, *Actinobacteria*, *Firmicutes*, *Betaproteobacteria*, *Gammaproteobacteria*	N-fixing, proteolysis, chitinolysis, glucanolysis, phosphate solubilization, antagonistic	Grube *et al.*, 2009
Lobaria pulmonaria	Maple trees in the Alps and Two mountain forests	Pyrosequencing of 16S rRNAs, FISH	*Alphaproteobacteria*, *Betaproteobacteria*, *Gammaproteobacteria*, *Actinobacteria*, *Acidobacteria*	Nutrient supply, pathogen defence, abiotic stress resistence, metabolite detoxification, lytic activity, N-fixing, auxin and vitamin production	Grube *at al.*, 2015; Erlacher *et al.*, 2015
Urnea sp., *Cladonia borealis*, *Psoroa* sp., *Stereocaulion* sp., *Umbilicaria* sp., *Cetraria* sp., *Cladonia* sp., *Ochrolecia* sp.	Antartic and Arctic regions	Culturing in specific media	*Actinobacteria*, *Firmicutes*, *Alphaproteobacteria*, *Betaproteobacteria*, *Gammaproteobacteria*	Nutrient supply (proteolysis and lipase activity)	Lee *et al.*, 2014

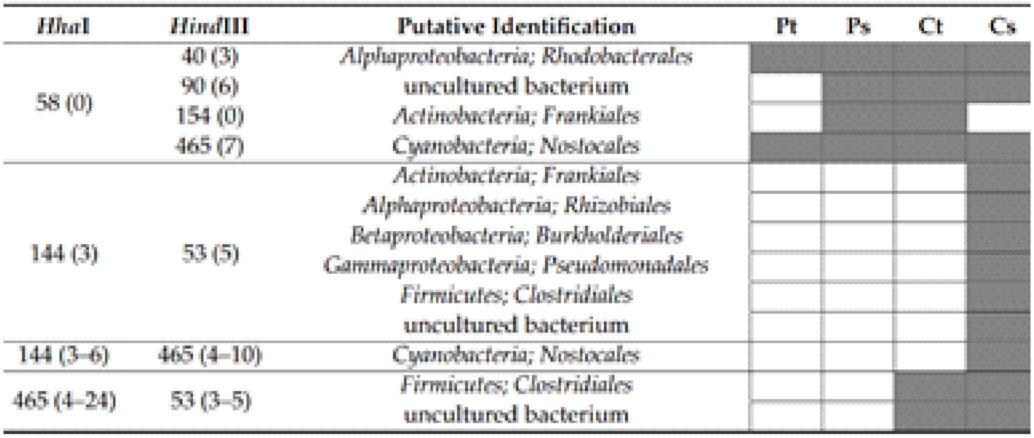
2009). Regarding that, many researchersintensely looked for the roles of bacterial communities in lichen. Nitrogen (N) is one of crucial macronutrients which all organisms have to obtain as nitrogen plays an important part in building up protein, protoplasm, chlorophyll, and nucleic acids (Banati and Supryanto, 2014). Because the majority of lichens have green algal photobionts that are not capable of fixing atmospheric N_2 like their cyanobacterial counterparts and N acquisition is thought to occur exclusively via aerial deposition of inorganic N forms(Banati and Supryanto, 2014), they need an agent to fulfill their nitrogen needs. The number of Nitrogen is abundant in nature, however, it is not always in an available form for assimilation. It must thus be fixed to usable form and the transformation is mediated by nitrogenase enzymes (Mushegian *et al.*, 2011). Bates *et al.*, (2011) reported that bacteria associated with lichen are very closely related to several other known N_2-fixing taxa, such as *Frankia* (*Actinobacteria*), *Beijerinckia*, *Bradyrhizobium*, and *Azospirillum* (*Alphaproteobacteria*), and others with less certain affiliations were found close to or within genera known to contain N_2 fixers (e.g., *Acinetobacter*, *Burkholderia*, *Gluconobacter*, and *Rhodospirillum*). By culturing bacteria-associated lichen on a semisolid Brown's N-free medium, Grube *et al.*, (2009) confirmed Diazotrophic bacterias (*Gammaproteobacteria* and *Firmicutes*) are able to live in culture media and those strains potentially complement the nitrogen budget in lichens with eukaryotic algae (Grube *et al.*, 2009).

In the comparative study between bipartite chlorolichens of the genus *Cladonia* and bipartite cyanolichens of the genus *Peltigera* in N_2-acquisition, chlorolichens have a higher diversity of nitrogen-fixing bacteria than cyanolichens (Almendras *et al.*, 2018). Bipartite cyanolichenreserve nitrogen needs due to the cyanobacteria capability in fixing nitrogen. While in chlorolichen, the green algae are not able to fix nitrogen and only contributes with products of photosynthesis. Therefore, bacteria associated with bipartite chlorolichen thalli could supply nitrogen needs. Using Terminal restriction fragment length polymorphism (TRFLP) technique to profile nitrogen-fixing bacteria communities in both *Cladonia* and *Peltigera*, Almendras *et al.*, (2018) confirmed that the diazotrophs in *Cladonia* is more diverse than in *Peltigera*. Examining Table 2, *Cladonia* which is chlorolichen become the host of several bacterial groups like *Alphaproteobacteria, Actinobacteria, Firmicutes, Cyanobacteria,* andin addition to *Betaproteobacteria and Gammaproteobacteria.* While in Peltigera which is cyanolichen, *Betaproteobacteria* and *Gammaproteobacteria* are absence (Table 2). Hodkinson and Lutzoni (2009) also presented preliminary evidence that the structure of bacterialcommunities associated with cyanobacterial lichens (cyanolichens) differ from those foundwith chlorolichens.

Lichen-associated bacteria play a role on phosphorus acquisition

As same as nitrogen, phosphorus (P) is also another essential macronutrient for plants and lichens and is

Table 2. Tentative identification of the nifH terminal restriction fragments (TRFs) by an in silico analysis of the profiles obtained from thalli and substrates of Peltigera and Cladonia lichens (Almendras *et al.*, 2018)



*Hha*I	*Hind*III	Putative Identification	Pt	Ps	Ct	Cs
58 (0)	40 (3)	*Alphaproteobacteria; Rhodobacterales*	■	■	■	■
	90 (6)	uncultured bacterium		■	■	■
	154 (0)	*Actinobacteria; Frankiales*		■	■	
	465 (7)	*Cyanobacteria; Nostocales*	■	■	■	■
144 (3)	53 (5)	*Actinobacteria; Frankiales*				■
		Alphaproteobacteria; Rhizobiales				■
		Betaproteobacteria; Burkholderiales				■
		Gammaproteobacteria; Pseudomonadales				■
		Firmicutes; Clostridiales				■
		uncultured bacterium				■
144 (3–6)	465 (4–10)	*Cyanobacteria; Nostocales*				■
465 (4–24)	53 (3–5)	*Firmicutes; Clostridiales*			■	■
		uncultured bacterium			■	■

Pt: Peltigerathallus; Ps: Peltigerasubstrate; Ct: Cladoniathallus; Cs: Cladoniasubstrate. The presence/absence of a TRF in each type of sample is indicated by a dark/empty square.

involved in all major metabolic pathways. Phosphate solubilizing bacteria (PSB) have the ability to release P and make it available to lichens[15]. Related publication, Bates *et al.*, (2011) confirmed that lichen-associated bacteria recovered in their survey may also play a role in the acquisition of phosphorus. Members of the *Acetobacteraceae* which were dominant in their samples, and a number of strains from this family are known to solubilize phosphate. Once again, dominant phylotypes that is coded as PT01560 was closely related to *Gluconobacteroxydans*, an organism known to have genes coding for histidine acid phosphatase (HAP), an enzyme involved in the mineralization of phytate (a highly abundant organic phosphorus compound). Moreover, Grube *et al.*, (2009) also reported about one-tenth of the culturable colonies retrieved from the lichen species (N = 261), 23% of the strains showed phosphate-solubilizing activity (Table 1). Regarding that finding, phosphate-solubilizing bacteria may significantly enhance the mobilization of nutrients in lichen (Grube *et al.*, 2009).

Bacterial communities function as pathogen defence, stress amelioration, and UV protection

Lichens represent one of most diversified and oldest symbiotic lifestyles of fungi. Lichens are commonly found in extreme habitat with poor-nutrient substrate. One reason for the ecological success and survive on oxidative stress of this fungal-algal partnership is related with bacterial communities association. In lichen species *Lobaria pulmonaria*, by using pyrosequencing of 16S rRNAs technique to trace targeted bacterial gene, Grube *et al.*, (2015) has found 891 indicated genes conferring resistance to metals (copper, cobalt-zinc-cadmium, silver, mercury and arsenic). Besides the evidence for adapting in metals, the microbiome provided a first evidence for the involvement in the detoxification of inorganic substances (e.g., As, Cu, Zn), the detailed mechanisms remaining unknown (Cernava, 2015). Additionally, a culture collection of bacterial isolates obtained from three lichen species was screened for the arsM gene. Detected carriers of arsM were later identified as members of the genera *Leifsonia, Micrococcus, Pedobacter, Staphylococcus,* and *Streptomyces*. Grube *et al.*, (2015) also discovered several genes involved in dormancy and sporulation, so that this to be an important aspect of bacteria adapted to the poikilohydric life on lichens. Beside that bacteria associated with *L. pulmonaria* has certain genes involved in the biosynthesis of stress-reducing metabolites. Regarding pathogen defence, the presence of genes for the metabolism of typical bacterial antibiotics in *L. pulmonaria* suggests potential competition among bacterial strains on the lichen surfaces or a defence against other strains entering the microbial surface community (Grube *et al.*, 2015).

Most lichens are extremely tolerant to desiccation, low temperature surviving for months to years in a state of cryptobiosis, and UV radiation, because many of their diverse secondary metabolites act as UV filters (Grube *et al.*, 2009). Lee *et al.*, (2014) isolated several samples of lichen species (*Usnea* sp., *Cladonia borealis, Psoroma* sp., *Stereocaulon* sp., *Cladonia borealis, Umbilicaria* sp., *Cetraria* sp., *Cladonia* sp., and *Ochrolechia* sp.) from Arctic and Antarctica pole which are noted as high UV exposure region. Bacterial taxa of *Frondihabitans, Hymenobacter,* and *Burkholderia* were recovered across lichen samples from both the Arctic and Antarctica, and most of them produced extracellular lipases. Members of this genus form pink to red-pigmented colonies and these pigments mayconfer resistance to UV by absorbing maximum in the UV region (Fuji *et al.*, 2010; Peeters *et al.*, 2011; Singh and Gabani, 2011). These bacteria act as an UV shield (Lee *et al.*, 2014).

Lichens are sources for plant growth promoting bacteria which potentially used as biofertilizer

Several studies have shown that lichen-symbiotic bacteria form a community with specific patterns. However, a study conducted by Cardinale *et al.*, (2008) concluded that the structure of the bacterial community in lichen was not correlated with the host species and provided evidence that some lichen-symbiotic bacteria may be opportunistic, or as extensions of the immediate surrounding soil environment, rather than symbiotic partners.

Related with nitrogen-fixing bacteria which lives inside lichen, it is not surprising that lichen can potentially be used as a biofertilizer. Biofertilizers are often referred to fertilizer containing living or latent microorganisms that activate biological process for making fertilizers or forming an unavailable elemental being available to plants. Some metagenomic studies done by Annette *et al.*, (2013) proved that *Nostoc* existed as a symbiont of the lichen *Peltigeramembranacea* and showed its expression in natural thalli. *Nostoc*, one of the most widespread genera of nitrogenfixing filamentous cyanobacteria, is able to form macroscopic or microscopic colonies (Potts, 2000). According to Esch (2014), *Nostoc*'s N fixing ability has been investigated in a number of studies that have indicated its potential to contribute N to soils. Esch has clearly demonstrated *Nostoc*'s potential as a biofertilizer as it was proven by good hydration and good function as a supplement for corn plants in Kandy Korn by *Nostoc*'s ability. The results of the hydration studies demonstrated the ability of *Nostoc* to hydrate rapidly when exposed to water and to continuously take on water over time.The bioassays concluded with promising results for the use of *Nostoc*as noted by the significant increases in height, and leaf number from both of the studies demonstrating that *Nostoc* can function as a N supplement.

Another study leads by Shridhar (2012), *Azospirillum* species belong to thefacultative endophytic diazotrophs groups which also associated with lichenis considered as the starting point of mostongoing BNF (Biological Nitrogen Fixation) programs withnon-legume plants worldwide. This bacteria is directly benefits plants improvingshoot and root development and increasing the rate ofwater and mineral uptake by roots. Beside *Azospirillum*, *Azotobacter* also become promising candidate as biofertilizer. *Azotobacter* species are efficient in fixation of highest amount of nitrogen (29.21 μgNmL^{-1} day^{-1}). Moreover, *Azotobacter* plays different beneficial roles by producing different types of vitamins, amino acids, plant growth hormones (Chennappa *et al.*, 2017).

Conclusion

Undoubtedly in recent study lichens are not enough to be known as fungal-alga mutualistic association but more than that bacterial communities also exist inside lichens. In this study review, several strains of bacteria live inside certain lichen species. The most dominant bacteria phylas are *Proteobacteria*, *Cyanobacteria*, and *Acidobacteria.* In their associated life, lichenous bacteria is influenced by surrounding environment (e.g. temperature, UV light, and sunexposure). Being an important symbiont, bacterial communities have several roles in lichen. Using microbial fingerprinting technique, bacteria associated with lichen very closely related to several other known N_2-fixing taxas. Beside fixing N_2, lichenous bacteria also play a role in phosphorous acquisition, pathogen defense, stress amelioration, and UV protection. Due to the presence of nitrogen fixing bacteria, lichen is potentially used as biofertilizer.

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