

Lichenized Bacteria: A Pioneer Source of Biomedical and Environmental Protection

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ABSTRACT

The review highlights the synthesis of secondary metabolites by symbiotic bacteria, emphasizing the importance of these compounds in various stages of symbiotic interactions. Recognizing the roles of natural products, such as complex polyketides and nonribosomal peptides, extends from partner recognition and invasion to establishing bacterial populations, providing nutrition, and contributing to chemical defense. Notably, the study focuses on unculturable symbionts, with cultivation-independent techniques playing a significant role in advancing research in this field. The notion is that bacterial symbionts, particularly in marine invertebrates like sponges, are likely the actual producers of numerous drug candidates found in these organisms. Sponges, recognized as a significant source of biologically active natural products, are often thought to harbor compounds originating from bacteria. Biofouling, the unwanted buildup of microorganisms on submerged structures and living marine organisms, presents economic challenges, including increased fuel consumption and metal corrosion. Diverse studies, spanning morphology, biochemistry, and molecular characteristics, highlight the complexity of biofouling. To address these issues and mitigate economic losses, there is a pressing need to identify effective natural antifouling agents capable of controlling the abundant biofouling bacteria in marine environments.

Keywords: Antifouling, Symbionts, Lichen, Parmeloid

INTRODUCTION

Numerous natural compounds that have been identified in marine invertebrates are thought to be produced by symbiotic bacteria. The resemblance of several chemicals from sponges, tunicates, or bryozoans to complex polyketides and non-ribosomal peptides, two metabolites normally only known from microbes, is particularly indicative of a bacterial lich origin (Faulkner, 2000; Schwarzer & Marahiel, 2001; Piel, 2009). A well-established technique for locating secondary metabolites in organisms such as plant tissue, plant cells, cyanobacteria, and dinoflagellates is immunolocalization (Gillor et al., 2000). The *ped* and *onn* systems' striking resemblances and intricacy point to the possibility that they share an ancestor gene cluster. It is highly implausible because sponges and beetles might have directly shared a symbiont or genetic material; this cluster should have just originally belonged to a free-living bacterium. The development and processes of natural product symbiosis are still unknown. The intimate link between the insect pathogenic bacterium *P. aeruginosa* and the pederin-producing beetle symbiont shows that the pederin symbiosis developed from an infection of a *Paederus* spp. progenitor. There are various possibilities for sponges. These and several other marine invertebrate species feed by filtering out food microorganisms. Some of these microbes could be lysis-resistant, allowing them to recolonize the tissue of each new generation of sponge. Due to their

meagre antibacterial action, it is unclear whether the onnamides give the producer a clear advantage over other participants in the bacterial consortium (Piel et al., 2004). Zchori Fein & Perlman (2004) stated that the Two bug species were found to harbour a second lineage of Bacteroidetes symbionts that were unrelated to *Cardinium*, according to the primers employed. The Bacteroidetes group, which was involved in many reproductive changes in their arthropod hosts, recently described *Candidatus cardinium*. It included parthenogenesis, feminization, and cytoplasmic incompatibility processes. There are several bacterial symbionts in insects and other animals. By extending the heritable genetic variety already existent in one species, many of them are mostly transmitted vertically along with the genes of their hosts (Ferrari & Vavre, 2011). In an early examination of their diversity, the following taxonomic groupings of bacteria were identified in lichen-associated microorganisms: Acetobacteraceae, Acidobacteriaceae, Alphaproteobacteria, Brucellaceae, Gammaproteobacteria, Methylobacterium, Proteobacteria, Rhizobiales, and Rhodospirillales. One clear general trend is the variation in the results for the one cyanolichen, *Peltigera phyllidiosa*. With almost every pair of primers used in the initial "RHAPSA-D" screening experiment, different bacteria were discovered in association with the cyanolichen *P. phyllidiosa* than with any of the other lichens examined (each of which has a green-algal photobiont instead of *Nostoc*). Research from different studies revealed numerous bacterial cells on the lower cortices of lichens, including clusters of cells encased in what appeared to be polysaccharides (Hodkinson and Lutzoni, 2009). Lichens are pioneers in the colonization of diverse environments and can develop on a diversity of substrates, such as soil, and stones, or live as epiphytic organisms on plants, among others.

Research methodology of lichen

Bacteriobionts

The rock outcrops from which the foliose lichens were collected contained four species: *Parmelia sulcata*, *Rhizoplaca chrysoleuca*, *Umbilicaria americana* and *Umbilicaria phaea*. All these species are known to contain bacteriobionts, making them invaluable for research into the relationship between bacteria and lichen symbiosis. This collection provides a unique opportunity to study how different members of this group interact with their environment to survive. Recent research has suggested that lichen-associated microbes are an integral component of lichen thalli, and thus the traditional view of this symbiotic relationship should be expanded to include bacteria. A bar-coded pyrosequencing technique was utilised to explore these findings further to survey bacterial communities associated with four species of lichens from multiple locations on rock outcrops. The study found that each species had distinct bacterial communities dominated by Alphaproteobacteria; numerous phylotypes closely related to sequences isolated from other known nitrogen fixers such as *Azospirillum*, *Bradyrhizobium*, and *Frankia* were also found in all four specimens surveyed. Furthermore, it was observed that a particular Rhizobiales lineage affiliated with the lichens sampled had a relatively abundant presence across all samples analyzed, suggesting its importance in sustaining the microbial diversity found within these organism's environments. Currently, the research methodology involved in lichen-associated bacteria is divided into culture-based and culture-independent methods. Culture-based methods are used for the isolation of bacteria from lichens using various physical and chemical experimental methods to study the structure, function, products, and metabolites of bacteria. Culture-independent methods including molecular methods, such as DNA extraction, polymerase chain reaction (PCR), gel electrophoresis, and denaturing gradient gel

electrophoresis (DGGE). First-generation DNA sequencing (Sanger method) and shotgun sequencing have been used to identify the isolated bacteria and perform whole-genome sequencing. This finding is particularly noteworthy given its potential implications for understanding how certain microorganisms interact within their respective ecosystems while providing insight into possible mechanisms for nitrogen fixation processes occurring therein as well. Overall, this study has provided significant evidence towards expanding our knowledge base surrounding the intricate relationships between bacteria and their host organisms, namely those involving symbiosis between two or more dissimilar organisms living together under mutually beneficial conditions, thereby presenting novel opportunities for future inquiry into such complex ecological systems at both local and global scales alike.

Various methodologies were employed in a study conducted to isolate and identify the bacterio-bionts found from the lichenized fungal species. Firstly, samples of individual lichen thalli of each species were collected from different habitats with a sterile knife and placed into individual sterile plastic collection bags. The four different lichen species collected had their thalli within a few centimeters of one another and always within 1-2 cm² area for easy identification in the lab. After transportation, back to the lab on ice all samples were immediately processed for further analysis.

To identify diversity based on present soil bacterial communities we used surface sterilization techniques followed by isolation procedures likely integral components such as those found in internal surfaces of thallus like external surface borne microbes so that our sample could be free from contamination during DNA extraction process.

Materials used for production of lichenized bacterial metabolites

The availability of producing symbionts might have a considerable influence on the development of long-term and fermentation-

based sources of invertebrate-derived medicinal prospects, practically all of which are now far away in meaningful amounts (Schmidt et al., 2000). Numerous studies have attempted to identify the producers using techniques such as cell separation, *in situ* hybridization, immune localization, and culture. The etymological root of the components, however, continues to be a mystery because none of these procedures has, to date, shown definitive results (Gillor et al., 2000). The *Pseudomonas sp.* were allowed into the system's fragmentation into three different genomic areas, the high density of transposase pseudogenes that surround the two early identified sections is consistent in the Sea Sponges (Kellner & Dettner, 1996; Piel et al., 2004). The *Pseudomonas sp.* were allowed into the system's fragmentation into three different genomic areas, the high density of transposase pseudogenes that surround the two early identified sections is consistent in the Sea Sponges (Kellner & Dettner, 1996; Piel et al., 2004). There are 99 insect species and numerous mite species are permitted in this screening, along with the estimated incidence rate of *Cardinium* and the boundaries of exploration in its host ranges. The symbiotic bacteria *Cardinium*, which was discovered in four parasitic wasp species and one armoured scale insect, was tested in 85 insects. One predatory mite from the 14 mite species investigated was confirmed to have the symbiotic bacteria (Zchori Fein & Perlman, 2004).

Similarity of the symbiont bacteria with the host

Blattabacterium species found from the *Bacteroidetes*, which was closely linked to the *Cardinium*, which was discovered to cluster hosts, that were also closely related, indicating host specialisation and horizontal transmission among such hosts (Zchori Fein & Perlman, 2004).

Advantages of the Bacterio-bionts cultivated from the lichens

Antifouling compounds from the microbes inhibit the settlement of fouling invertebrate larvae and algal spores are considered as the potential anti-biofilm agents correlates the biofilm formation and the biofouling process of fouling organisms. Development of the biofilm on a substrate attracts other fouling organisms like barnacles and algae through the surface modification and release of chemicals. The exploitation of the highly

effective and non toxic potential antifouling compounds. These compounds are introduced based on their microbial origins, and then categorized into the following different structural groups: fatty acids, butenolides, terpenoids, steroids, phenols, phenyl ethers, polyketides, alkaloids, flavonoids, amines, nucleosides, and peptides. (Adnan et al., 2023) (Figure 2).

Figure 1. Pictorial representation showing the importance of the Lichen Associated Bacteria in the Antifouling

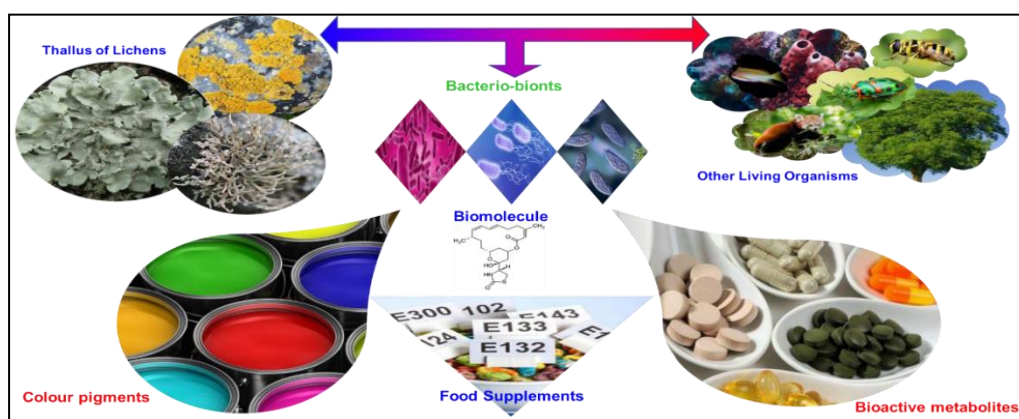
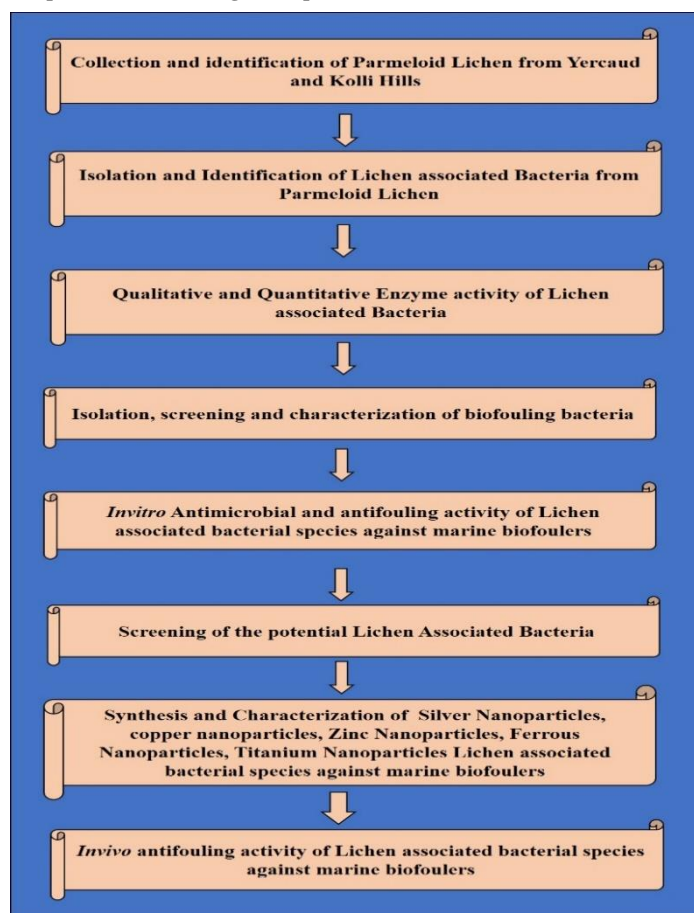


Figure 2. Beneficial impacts of the Bacterio-bionts driven from various sources for industrial and mechanical applications

Benefits of the Symbiont bacteria

drug candidates identified in marine invertebrates have long been thought to be the actual products of bacterial symbionts. It has been commonly assumed that sponges, the most significant marine source of physiologically active natural chemicals, include substances of bacterial origin. However, because of a widespread inability to culture the presumed producers, this symbiotic theory remained unverified (Piel et al., 2004). The 16S ribosomal DNA of the cardinium was amplified in part using primers (rDNA). Arthropods were examined for the presence of Wolbachia, a more well-known reproductive manipulator. A phylogenetic study of all known Cardinium 16S rDNA sequences reveals that horizontal transmission commonly occurs when closely related symbionts are carried by distantly related arthropods. This second symbiotic lineage has a tight relationship with other arthropod symbionts, including Blattabacterium, the principal cockroach symbiont, and ladybird beetle symbionts that kill males, indicating the existence of a varied assemblage of Bacteroidetes bacteria linked with arthropods that are probably making a big biological impact on their hosts (Zchori Fein & Perlman, 2004). A research finding was exploring with the three degenerate primers such as 895F, 902R, and 904R. They were created to accommodate the remaining eubacterial heterogeneity following the determination of the generally conserved sites across the eubacteria and the pinpointing of areas shared only by chloroplasts and cyanobacteria for exclusion. A particular primer (1185mR) was created to target a variety of bacteria while excluding 16S rRNA gene sequences that are exclusive to plastids. This primer was altered to solely target Rhizobiales bacteria that are linked with lichens (1185aR). There are two additional primers namely, 1381R and 1381bR to eliminate only sequences originating from *Asterochloris* sp., the main algal partner in squamulose typed *Cladonia* sp. thalli, while targeting a larger range of 16S sequences. Primers were given names

based on the location of the 3' end on the 16S rRNA molecule from *E. coli*. The approximate proportion of bacteria with sequences matching each of the primers was calculated using RDP-II Probe Match (Hodkinson and Lutzoni, 2009).

Problems faced during the research in the symbiont's isolation

Finding biosynthetic genes is technically difficult because of the complexity of sponge metagenomes (Gillor et al. 2000; Piel et al., 2004). These passengers have a variety of repercussions on the host's phenotypes: besides the cost imposed on the host for maintaining the symbiont population, they can provide fitness advantages to the host or manipulate the host's reproduction. An invention said that the insects of symbioses are ideal model systems for communal genetics. Primarily, the bacterial symbionts were directly or indirectly affecting based on their interactions with other species within a community. Examples include their involvement in modifying the use of host plants by phytophagous insects, in providing resistance to natural enemies, but also in reducing the global genetic diversity or gene flow between populations within some species. Secondary, an emerging picture in insect symbioses is that many species are simultaneously infested with more than one symbiont, which permits studying the factors that shape bacterial communities; for example, horizontal transmission, interactions between host genotype, symbiont genotype and the environment and interactions among symbionts. The insects' symbiotic complements are dynamic communities that affect and are affected by the communities in which they are embedded.

Endophytic bacterial strains found from various hosts

B. subtilis was found from the *Negombata magnifica* Keller (Toxic finger-sponge), it was tested by the Iatruinculin B for toxicity analysis (Gillor et al. 2000). Piel et al., (2004) were experimented the *P. aeruginosa*,

obtained from the *Theonella swinhoei* Gray was tested against antitumor polyketide synthesis effect. *Candidatus cardinium* (Blattabacterium sp.,) strain was isolated from the American pathogenic insect *Cockroaches*, and Ladybird beetles were experimented with the Bacterial symbiont distributed in arthropods (Zchori-Fein & Perlman, 2004).

Application of lichen symbionts

Another promising approach is the use of nanotechnology to develop novel coatings that prevent biofouling. Overall, the use of lichen-associated symbionts and nanotechnology holds great potential for developing effective and sustainable solutions to mitigate biofouling. (Subbaiyan et al., 2023). Lichen symbiotic associations, an underexplored research area, showed promising effects in the production of antimicrobial and antifouling compounds (Rubavathi et al., 2022a). These lichen-associated bacteria engage in various functional activities and have multiple bacterial counterparts. To assess the impact of these nanoparticles such as silver nanoparticles, copper nanoparticles, zinc nanoparticles synthesized from lichen-associated bacteria on biofouling activity in marine transportation. (Rubavathi et al., 2022b,2022c Rubavathi et al., 2023, 2024).

CONCLUSION

The methodology enabled to accurately compare microbial communities present across the sampled sites without any bias or interference due to environmental factors or contaminants present in them. It was successful in isolating bacteria associated with specific types of fungi while also providing insight into how these organisms interact with one another at an ecological level amongst other things. Such information is invaluable when it comes understanding biodiversity patterns among microorganisms which has implications towards conservation efforts as well as biotechnology applications related to food production etcetera.

Competing interests' policy

The authors declare that they have no competing interest.

Declaration by Authors

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