Doctoral Dissertation

Bacterial microbiomes associated with rock-dwelling lichens

(Summary)

Author: Zichen HE
Supervisor: Takeshi NAGANUMA

Program of Food and AgriLife Science
Graduate School of Integrated Sciences for Life
Hiroshima University

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学 位 論 文 の 要 約

論文題目　Bacterial microbiomes associated with rock-dwelling lichens
(岩石着生性地衣類に共在する細菌相に関する研究)

広島大学大学院統合生命科学研究科
食品生命科学プログラム
学生番号　Ｄ２０６３１２
氏　　名　何　子琛 (HE Zichen)

Lichens are mutually symbiotic systems consisting of fungal and algal symbionts. While diverse lichen-forming fungal species are known, limited species of algae form lichens. Plasticity in the combination of fungal and algal species with different eco-physiological properties may contribute to the worldwide distribution of lichens, even in extreme habitats. Lichens have been studied systematically for more than 200 years; however, plasticity in fungal–algal/cyanobacterial symbiotic combinations are still unclear. In addition, the association between non-cyanobacterial bacteria and lichens has attracted attention in recent years. The types, diversity, and functions of lichen-associated bacteria have been studied using both culture-based and culture-independent methods. In this study, Rock tripe lichens (*Umbilicariaceae*) were collected from Continental and Maritime Antarctic biological, Arctic and Subarctic Areas in North America and Northern Europe, Alpine Areas in Eastern Alps and Equatorial Africa and Japanese regions; Non-*Umbilicaria* lichens were collected from Guiana and South Africa regions; in order to compare their bacterial floras and potential metabolism. Bulk DNA extracted from the

lichen samples was used to amplify the 18S rRNA gene and the V3-V4 region of the 16S rRNA gene, whose amplicons were Sanger- and MiSeq-sequenced, respectively. The data of each sample were analyzed by regions, and the results of *Umbilicaria* and Non-*Umbilicaria* lichens were comparing respectively. Finally, the results of the overall rock-dwelling lichens were integrated and analyzed.

This doctoral dissertation consists of seven chapters: Chapter 1. Introduction: Chronicle of Research into Lichen-associated Bacteria; Chapter 2. Microbiomic Analysis of Bacteria Associated with Rock Tripe Lichens in Continental and Maritime Antarctic Regions; Chapter 3. Bacterial Microbiomes Associated with Rock Tripe Lichens from Arctic/Subarctic Areas in North America and Northern Europe; Chapter 4. Microbiome Analysis of Bacteria Associated with Rock Tripe Lichens from Alpine Areas in Eastern Alps and Equatorial Africa; Chpater 5. Microbiomes associated with rock tripe lichens dwelling on montane rocks and cliffs in western Japan; Chpater 6. Microbiomes Associated with Epilithic Non-Umbilicaria Lichens from the Venezuelan Guiana Shield and the South African Highveld Plateau; Chpater 7. General discussion of approximate global rock-dwelling lichens.

Chapter 1. Introduction: Chronicle of Research into Lichen-associated Bacteria reviewed the research history of lichens, including the researches in the past more than 200 years and lichen-associated bacteria and provides insights into the current status of research in this field. As to bacterial associates of lichens, traditional culture-based methods reveal their physiological and biochemical features in vitro but allow only inferences on their roles in vivo in lichens. Culture-independent molecular approaches provide taxonomic and phylogenetic identifications of bacterial associates but only in silico speculations on their functions in lichens. Considering the variety and practicality

of secondary metabolites produced by lichen-forming fungi and bacteria, lichen-associated bacteria would represent huge treasure houses for human benefit. However, research on lichen-associated bacteria is still limited.

Chapter 2. Microbiomic Analysis of Bacteria Associated with Rock Tripe Lichens in Continental and Maritime Antarctic Regions collected rock tripe lichens from two distinct Antarctic biological regions, the continental region near the Japanese Antarctic station (Syowa Station) and the maritime Antarctic South Orkney Islands (Signy Island), in order to compare their bacterial floras and potential metabolism. Bulk DNA extracted from the lichen samples was used to amplify the 18S rRNA gene and the V3-V4 region of the 16S rRNA gene, whose amplicons were Sanger- and MiSeq-sequenced, respectively. The fungal and algal partners represented members of the ascomycete genus *Umbilicaria* and the green algal genus *Trebouxia*, based on 18S rRNA gene sequences. The V3-V4 sequences were grouped into operational taxonomic units (OTUs), which were assigned to eight bacterial phyla, *Acidobacteriota, Actinomyceota, Armatimonadota, Bacteroidota, Cyanobacteria, Deinococcota, Pseudomonadota* and the candidate phylum *Saccharibacteria* (also known as TM7), commonly present in all samples. The OTU floras of the two biological regions were clearly dis-tinct, with regional biomarker genera, such as *Mucilaginibacter* and *Gluconacetobacter*, respectively. The OTU-based metabolism analysis predicted higher membrane transport activities in the maritime Antarctic OTUs, probably influenced by the sampling area's warmer maritime climatic setting.

Chapter 3. Bacterial Microbiomes Associated with Rock Tripe Lichens from Arctic/Subarctic Areas in North America and Northern Europe collected rock tripe lichens from Arctic and Subarctic biological regions, in order to compare their bacterial floras

and potential metabolism. Bulk DNA extracted from the lichen samples was used to amplify the 18S rRNA gene and the V3-V4 region of the 16S rRNA gene, whose amplicons were Sanger- and MiSeq-sequenced, respectively. The fungal and algal partners represented members of the ascomycete genus *Umbilicaria* and the green algal genus *Trebouxia*, based on 18S rRNA gene sequences. The V3-V4 sequences were grouped into operational taxonomic units (OTUs), which were assigned to ten bacterial phyla, *Acidobacteriota*, *Actinomycota* (formerly *Actinobacteria*), *Armatimonadota*, *Bacteroidota (formerly Bacteroidetes)*, *Chloroflexota*, *Cyanobacteria*, *Gemmatimonadota*, *Planctomycetota*, *Pseudomonadota* (formerly *Proteobacteria*) and *Verrucomicrobiota*, commonly present in all samples. The OTU floras of the two biological regions were clearly distinct, with regional biomarker genera, such as *Mucilaginibacter* and *Gluconacetobacter*, respectively. Even though the lichen samples with various lichen species, the bacterial OTUs were clustered on the dendrogram between different sampling regions Arctic and Subarctic. This chapter think that the geographical and/or bioclimatic environment, rather than the different lichen-forming fungus species, is what affects the bacterial microbiomes linked to the *Umbilicaria* species investigated here, And the climate change of sampling sites may actually have similarities.

Chapter 4. Microbiome Analysis of Bacteria Associated with Rock Tripe Lichens from Alpine Areas in Eastern Alps and Equatorial Africa collected rock tripe lichens from two alpine fellfields of Mt. Brennkogel in Eastern Alps (Austria) and Mt. Stanley of the Rwenzori mountains in equatorial Africa (Uganda), to compare their bacterial compositions, diversities, possible biomarkers and potential metabolisms. Bulk genomic DNA was extracted from the lichen samples, which were used to amplify the 18S rRNA

gene by Sanger sequencing and the V3-V4 region of the 16S rRNA gene by Illumina Miseq sequencing. Based on 18S rRNA gene sequences, the symbiotic components of fungal and algal partners were represented by the genus *Umbilicaria* belonging to *Ascomycota* and the green algal linegae *Trebouxia* belonging to *Chlorophyta*, respectively. The reads of V3-V4 region were classified into operational taxonomic units (OTUs) according to a set value of similarity, which were assigned to a total of 26 bacterial phyla, were all found in both areas. Eight of the 26 phyla, i.e., *Acidobacteriota, Actinomycota* (formerly *Actinobacteria*), *Armatimonadota*, *Bacteroidota*, *Chloroflexota*, *Deinococcota*, *Planctomycetota*, and *Pseudomonadota* (formerly *Proteobacteria*), were present in all samples with read abundances of >1% of the total read number. The bacterial compositions of the Austrian and Ugandan lichens were distinct, with the OTU frequency-based regional biomarker phyla, *Pseudomonadota* and *Armatimonadota*, respectively. The OTU-based potential metabolism analysis predicted similar relative abundances of metabolic pathways in the two regions, probably influenced by similar alpine climate, although the distance between the two areas is calculated as about 5430 km.

Chpater 5. Microbiomes associated with rock tripe lichens dwelling on montane rocks and cliffs in western Japan collected rock tripe lichens from Japan, in order to compare their bacterial floras and potential metabolism. Bulk DNA extracted from the lichen samples was used to amplify the 18S rRNA gene and the V3-V4 region of the 16S rRNA gene, whose amplicons were Sanger- and MiSeq-sequenced, respectively. The fungal and algal partners represented members of the ascomycete genus *Umbilicaria* including two species *U. esculenta* and *U. muehlenbergii*, and the green algal genus *Trebouxia*, based on 18S rRNA gene sequences. The V3-V4 sequences were grouped into operational

taxonomic units (OTUs), which were assigned to ten bacterial phyla, *Acidobacteriota*, *Actinomycota* (previously *Actinobacteria*), *Bacterroidota* (formerly *Bacteroidetes*), *Chloroflexota*, *Cyanobacteria*, *Gemmatimonadota*, *Planctomycetota*, *Pseudomonadota* (before *Proteobacteria*), and *Saccharibacteria*_TM7. Although it is grouped according to two different species, in fact, the difference of bacterial communities in the clothing samples in the sampling area has nothing to do with species. That is, the associated bacterial communities are almost no-grouping, and the results were not show variated lichen species-specific grouping.

Chpater 6. Microbiomes Associated with Epilithic Non-Umbilicaria Lichens from the Venezuelan Guiana Shield and the South African Highveld Plateau collected epilithic non-*Umbilicaria* lichens from the Venezuelan Chiuri tepui and the South African Highveld Plateau, in order to compare their bacterial floras and potential metabolism. Bulk DNA extracted from the lichen samples was used to amplify the 18S rRNA gene and the V3-V4 region of the 16S rRNA gene, whose amplicons were Sanger- and MiSeq-sequenced, respectively. The fungal and algal partners represented members of the common lichens, including fruticose and foliose, and the green algal genus *Trebouxia*, based on 18S rRNA gene sequences. The V3-V4 sequences were grouped into operational taxonomic units (OTUs), which were assigned to twelve bacterial phyla, *Acidobacteriota*, *Actinomycota* (formerly *Actinobacteria*), *Armatimonadota*, *Bacteroidota (formerly Bacteroidetes)*, *Chloroflexota*, *Cyanobacteria*, *Deinococcota*, *Gemmatimonadota*, *Planctomycetota*, *Pseudomonadota* (formerly *Proteobacteria*), *Saccharibacteria*_TM7 and *Verrucomicrobiota*. Although lichens collected in these two regions are different, the microbial communities in these two regions have obvious regional characteristics especially at Class, Genus and Species ranks. On the other hand, in the same sampling

region, lichen samples of the same species or genus tended to cluster. This discovery might provide evidence for the influence of local environment and lichen species on lichen associated bacterial community, and the influence of local environment is greater than lichen species.

Chpater 7. General discussion of approximate global rock-dwelling lichens contained simple discussion of fungal and algal partners a total of 82 lichen samples; and mainly focused on and analyzed the V3-V4 MiSeq data of a total of 82 lichen samples in the above chapters to seek global overall information of rock-dwelling lichens associated bacterial microbiome. The analyses of bacterial microbiomes communities determined the detailed biogeographic information in different regions by bioinformatics, especially the Syowa Station in Antarctica showed a clear boundary with other regions regardless of lichen types. Roles or functions of different bacterial microbiomes communities were predicted according to biomarkers, however, no matter how the bacterial microbiome of lichens is, the overall structure of their metabolic pathway prediction results will not change significantly. In addition, cosmopolitan and potential cosmopolitans were found and introduced.