Assembly of Bacterial Genomes from the Metagenomes of Three Lichen Species

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ABSTRACT  Bacteria have recently emerged as important constituents of lichen holobionts. Here, 29 bacterial metagenome-assembled genome (MAG) sequences were reconstructed from lichen metagenomes and taxonomically classified in four phyla. These results provide a pivotal resource for further exploration of the ecological roles played by bacterial symbionts in lichen holobionts.

Lichenized fungi are known as symbiotic associations of a mycobiont (fungus) and a photobiont (green alga and/or cyanobacterium). Recently, evidence was found for the presence of bacterial communities that play important roles in this symbiotic system (1–4). Surprisingly little is known about the genomes of dominant but so far uncultured bacteria in these miniature ecosystems. Here, we present bacterial metagenome-assembled genomes (MAGs) that were reconstructed from three lichen metagenome samples, i.e., Lobaria pulmonaria (L.) Hoffm., Cladonia furcata (Huds.) Schrad., and Peltigera polydactylon (Neck.) Hoffm. (3, 5). The selected lichens represent variants of symbiotic associations of the mycobiont with one or two types of photobionts. The lung lichen L. pulmonaria includes a green alga (Dictyochloropsis reticulata) and a cyanobacterium (Nostoc sp.) as photobionts (6), and P. polydactylon includes a cyanobacterium (Nostoc sp.) (7). In contrast, the genus Cladonia includes only a green alga (Asterochloris sp.) (8).

Lichen samples were obtained from three locations in Austria (3, 5). Metagenomic DNA was extracted using the MO BIO PowerSoil DNA isolation kit. The metagenomic DNA was sequenced by GATC Biotech (Konstanz, Germany) after libraries were prepared with the Illumina TruSeq DNA library kit. The Illumina HiSeq 2000 (L. pulmonaria) and HiSeq 2500 (C. furcata and P. polydactylon) instruments were used for paired-end 100- or 150-bp sequencing, resulting in >35 million reads per metagenome. Community-level assessments of bacterial functioning using these metagenome data sets were reported elsewhere (3, 5).

Default parameters were used for all software unless otherwise noted. Illumina adaptor removal and initial filtering of low-quality reads (Phred scores of <20) were performed using Trimmomatic v0.39 and VSEARCH v2.14.2 (9, 10). Metagenome data sets were then de novo assembled using metaSPAdes v3.14.0 (11). Totals of 103,819, 135,511, and 68,049 contigs with a length of >1 kb were generated from the Cladonia, Lobaria, and Peltigera metagenome data sets, respectively. The generated contigs were binned using MaxBin2 v2.2.7, MetaBAT2 v2.12.1, and CONCOCT v1.1.0 (12–14) and were further dereplicated and aggregated into MAGs using DAS Tool v1.1.1 (15). The completeness and the percentage of contaminations in the MAGs were estimated using CheckM v1.0.13 (16). The quality of the MAGs was classified according to the Minimum Information about a Metagenome-Assembled Genome (MIMAG) standards (17). The Bin Annotation Tool v4.6 was used to obtain the taxonomic classification for each MAG (18).

Twenty-nine MAGs with contamination of <10% were recovered. Among them, 7...
17, and 5 MAGs originated from the Cladonia, Lobaria, and Peltigera metagenomes, respectively. The MAGs were assigned to Proteobacteria (20 MAGs), Acidobacteria (3 MAGs), Bacteroidetes (3 MAGs), and Verrucomicrobia (1 MAG) (Table 1). One MAG each was classified in the candidate phylum “Candidatus Parcubacteria” and the superphylum Terrabacteria. We recovered 8 high-quality, 18 medium-quality, and 3 low-quality draft MAGs. The estimated completeness of the MAGs ranged from 26.9 to 98.9%, and genome sizes ranged from 492,776 to 5,800,883 bp. To the best of our knowledge, our MAGs provide an extended basis for further exploration of the symbiotic function of lichen-associated bacteria that will be conducted in follow-up studies.

**Data availability.** This shotgun metagenome project with three lichen metagenomes has been deposited in the European Nucleotide Archive (ENA) database under the study number PRJEB38505 and accession numbers ERR4179389 to ERR4179391 for the data sets. The MAG sequences are accessible under the accession numbers provided in Table 1.

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**REFERENCES**


