

Chasing *Legionella* spp. in Antarctic human-made and natural environments

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Legionella spp., potential pathogens of severe pneumonia, are ubiquitous bacteria that inhabit in freshwater environments. Man-made water facilities are also common reservoirs which can be a source of infectious outbreaks. Generally, described *Legionella* species are known to be mesophilic bacteria (ideal range of growth conditions, 25°C to 45°C) and research focusing on their biology in the low-temperature environment is still lacking. In polar regions, little is known about the occurrence and distribution of this bacteria, but from our previous investigations, *Legionella* DNA was continuously detected by PCR from Japanese Syowa station in Antarctica (69°S, 39°E). Our next aim was to elucidate whether these DNAs were brought in from outside of Antarctica by human activities or were inflow from the local environment and determine the presence of pathogenic species that may be a potential risk for expeditioners. We investigated the occurrence and diversity of *Legionella* spp. in both artificial and natural environments in Antarctica using *Legionella*-specific 16S rRNA gene-based amplicon sequencing and also attempted isolating *Legionella* spp. from Antarctic environments.

For *Legionella*-specific 16S rRNA gene-based amplicon sequencing analysis, we collected environmental samples from glacier lakes in East Antarctica and water facilities of Syowa Station. 1 L of water from each site was filtered through 0.22 µm filter unit and biofilm was collected by rubbing with sterile swab. A total of 36 samples (Lake water samples, 20 samples; Syowa station water and biofilm samples, 16) were analyzed. Lake water samples confirmed the presence of diverse *Legionella* in the lake environments that were distinctly different from the amplicon sequence variants (ASVs) detected in the station. The majority of the *Legionella* ASVs inhabiting Antarctic lake habitats were phylogenetically distinct from known *Legionella* species, whereas the ASVs detected in the human-made station tended to contain ASVs highly similar to well-described mesophilic species with human pathogenicity. Our data suggest that unexpected *Legionella* diversity exists in remote Antarctic cold environments and that environmental differences (e.g., temperature) in and around the station affect the community structure.

For culture experiment, we attempted to isolate *Legionella* spp. inhabiting Antarctic cold environments by the enrichment method with minor modification and finally obtained a first psychrotolerant *Legionella* strain (growth range 4°C–25°C) designated as TUM19329 from the sediment of Lake Naga-ike in the Skarvsnes ice-free area, East Antarctica (0°C–10°C (7); 69°29'S, 39°35'E). Whole genome sequencing was performed for this strain using MinION (Oxford Nanopore Technologies, Oxford, United Kingdom) and MiSeq (Illumina, San Diego, CA, USA). The final assembly resulted in one large closed chromosome with a total length of 3,750,805-bp. which contained 3,538 proteins and G+C content of 39.1%. To assess similarity, 16S rRNA gene sequence were blast searched against NCBI database and average nucleotide identity (ANI) were calculated against genomes of *Legionella* spp. downloaded from RefSeq database. The phylogenetic tree showed *Legionella fallonii* as a close relative of this strain (Figure 1). However, strain TUM19329 showed relatively

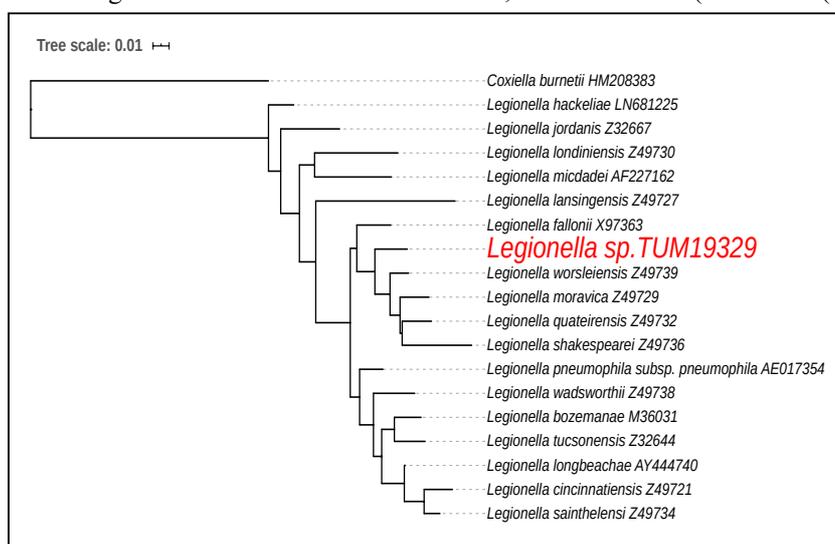


Figure 1. Maximum likelihood tree based on 16S rRNA of the genus *Legionella*.

low 16S rRNA gene sequence similarity (<97.5%) and the ANI value (<79%) for all known *Legionella* species. Considering the threshold for taxonomic assignment, our data suggest this strain is a candidate of novel species of the genus *Legionella*. Whereas, the strain shared high 16S rRNA gene sequence similarity (99.9%) with the environmental sequence (AB630760) recovered from a benthic moss colony (moss pillar) of another freshwater lake in East Antarctica. These results suggest the possibility that cold-adapted closely related members of strain TUM19329 are present in other cold freshwaters.

From these results, we revealed that unexpected diverse unknown *Legionella* spp. are present even in Antarctica. Our findings suggest that *Legionella* spp. encompasses a greater variety of cold-adapted species in a low-temperature environment than are currently known.

References

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