Examination of cultivation conditions for novel bacteria in an Antarctic Lake

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Antarctica has many lakes with varying salinity, area, and depth; thus, it has the most remarkable diversity of lakes on Earth (Laybourn-Parry & Pearce, 2016; Pickard, et al. 1986). These lakes have been considered to have low biodiversity due to their low primary productivity, which is attributed to long periods of ice-covering, low temperatures, and oligotrophic conditions. However, recently, it has been revealed that diverse prokaryotes are present in those lakes (Vincent, et al. 2008; Kurosawa, et al. 2010; Laybourn-Parry & Pearce, 2016; Chaya, et al. 2019). The community structural analyses of prokaryotes and eukaryotes in several lakes in the ice-free area near the Showa station, Antarctica, were conducted using Next-generation sequencing. As a result, a lake was discovered where sequences were presumed related to the superphylum *Parcubacteria,* which accounted for 4.6% of the total. The superphylum *Parcubacteria* is one of the Candidate Phyla Radiation, and there have been few reports of its successful cultivation for this group. They are considered parasitic or symbiotic organisms because of their small cell and genome size and lack of synthetic pathways for amino acids and nucleotides (Brown et al., 2015; Castelle et al., 2018; Vigneron et al., 2020). If the superphylum *Parcubacteria* can be cultivated, physiological, biochemical, and ecological analysis will be possible. These would provide new knowledge into the evolution of the entire bacteria and their symbiotic mechanisms with hosts. Therefore, this study aimed to identify cultivatable conditions for the novel bacteria belonging to the superphylum *Parcubacteria* detected from Antarctic Lake.

Enrichment cultures were carried out using lake water containing bottom sediment collected from a lake of the coastal ice-free area in the Lützow-Holm Bay as a source of microorganisms. The basic medium was prepared according to the pH and salinity of the lake in which the sample was taken. To that, yeast extract, Ferric citrate, sodium pyruvate, and glucose were added singly or in combination to achieve a final concentration of 0% or 0.1% to make a total of 16 media types. The cultures were incubated at -5℃, 3℃, and 10℃ under 8,000 lux for 24 hours of light exposure. The headspace of the culture tubes was filled with nitrogen gas or air. A total of 96 different culture conditions were used for Enrichment cultivation. Microorganisms were collected from enrichment cultures where microbial growth was confirmed and DNA extracted. Prokaryotic genes were amplified by PCR using the extracted DNA as templated DNA. Then, 16S rRNA gene sequences in enrichment cultures were acquired by Next-generation sequencing. After that, bacterial community structure was revealed using analysis pipeline QIIME2, and the homology of the 16S rRNA gene sequences to that of the closest related species was examined using the Basic Local Alignment Search Tool of the National Center for Biotechnology Information.

Microbial growth was confirmed in 30 out of 96 conditions by 102 days after the start of the cultivation, and bacterial base sequences in these enrichment cultures were classified into 62 ASVs (Amplicon sequence variant). Of these, 2 ASVs were presumed novel bacteria belonging to the superphylum *Parcubacteria* based on the homology with the closest related species (Table 1). These 2 ASVs were detected in the enrichment culture under nitrogen added to the air at 3℃ after yeast extract, ferric citrate, and sodium pyruvate to the basic medium, accounting for 11% and 5% of the total, respectively. Therefore, it revealed that the superphylum *Parcubacteria* and their hosts detected in the Antarctic lake could grow in the presence of the additive mentioned above when cultivated around 3℃, with nitrogen added to the air in the gas phase. Furthermore, besides the superphylum *Parcubacteria*, 10 ASVs were identified to be derived from novel bacteria. In the future, we will attempt to isolate these novel bacteria, including the superphylum *Parcubacteria,* using the limiting dilution method.

Table 1. The sequences and classification derived from novel bacteria detected in enrichment cultures.

Classification	The closest relatives or sequences	Homology (%)
Superphylum *Parcubacteria*	*"Ca.* Kaiserbacteria"	81.5
	"Ca. Kaiserbacteria"	81.9
Class *Alphaproteobacteria*	*"Ca.* Pelagibacter giovannonii"	100
Order *Longimicrobiales*	*Longimicrobium terrae*	83.8
Family *Pirellulaceae*	*"Ca.* Laterigemmans baculatus"	99.1
Family *Phyllobacteriaceae*	*Mesorhizobium wenxiniae*	86.5
	Psychrobacter proteolyticus	91.2
Genus *Pseudomaribius*	*Pseudomaribius aestuariivivens*	96.3
Genus *Psychrobacter*	*Psychrobacter namhaensis*	96.8
Genus *Maribacter*	*Maribacter aquivivus*	97.5
Genus *Neolewinella*	*Neolewinella aurantiaca*	97.9
Genus *Rubrobacter*	*Rubrobacter* sp. CBF L56	97.9

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