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A Study on Prokaryotic Community Diversity and Metabolic Functions of Surface-sediments in the West Antarctica

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A Study on Prokaryotic Community Diversity and Metabolic Functions of Surface-sediments in the West Antarctica

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Abstract

The Antarctic environments are closely linked to the atmosphere, hydrosphere, lithosphere, cryosphere and biosphere systems. These environments are extremely important parts of the global physical and biological system as the region is experiencing dramatic changes such as glacier melting. Also, Antarctic benthic environments are harsh conditions for survival to prokaryotes due to high pressures, low temperature and low nutrient availability depending on the areas.

Prokaryotes predominate in terms of biomass and perform a significant role in the biogeochemical flux of organic matters and nutrients in the marine ecosystem. Prokaryotes have undergone changes for the growing and survival under harsh environments and thus show enormous genetic diversity for exploration. Thus, understanding the community structures and metabolic functions of these organisms in the Antarctica are may provide insights into expected response of the microbial communities to the Antarctic environment which are rapidly changing and distribute excellent source for biotechnological applications. However, the prokaryotic community diversity and their ecological functions are still poorly understood in the west Antarctica. This study investigated how do benthic prokaryotic communities differ by overlying seawater properties according to areas and how do physical and chemical characteristics of surface sediments relate with benthic prokaryotic diversity and their functions in the west Antarctica. In this study, to investigate the benthic prokaryotic diversity and their metabolic functions to better understand the ecosystem in the West Antarctica sediments, 16S rDNA pyrosequencing and shotgun sequencing were applied.

Pyrosequencing of V5–V8 region of bacterial and archaeal 16S rRNA genes

revealed that prokaryotic community distribution exhibited obvious geographical differences. While Phylum *Crenarchaeota* were found to be more dominant in the Amundsen Sea, *Alpha*- and *Gammaproteobacteria* were relatively more abundant in the Ross Sea. Moreover, mainly Ammonia-oxidizing archaea (AOA) *Nitrososphaeria*-type *Crenarchaeota* was less abundant in the polynya sites than non-polynya sites in the Amundsen Sea and have shown positive correlation with temperature and negatively correlated with TOC. On the other hand, *Proteobacteria* have shown negatively correlated with temperature, but positively correlated with TN and TOC in the Ross Sea. The data suggests that the environmental conditions influence to prokaryotic diversity and community structures. The slightly high temperature, low TOC and lower $\delta^{13}C$ allow that AOA *Nitrososphaeria*-type *Crenarchaeota* flourish in the Amundsen Sea and lower temperature and more labile nitrogen and carbon allow predominance of *Proteobacteria* in the Ross Sea.

The analyses of three shotgun sequencing data of pelagic sediment revealed that there were similar taxonomic groups in three stations but prokaryotic community structure have differed between the stations. Although *Proteobacteria* were the most abundant phylum in all three stations, it was identified that *Gammaproteobacteria* were the most abundant class in the station R6, while *Alphaproteobacteria* were the most abundant class in the station R7 and R8. Archaea were less abundant in all stations and contributed substantially less to the benthic prokaryotic communities, *in situ*. The phylum *Thaumarchaeota* was less abundant in the station R6 than in the station R7 and R8. An ammonia oxidizing *Thaumarchaeota* are lives in the oligotrophic marine environment, thus, more refractory nitrogen and carbon (e.g., higher $\delta^{15}N$ and lower $\delta^{13}C$) conditions allow these organisms are more abundant in the station R7 and R8. Therefore, there are differences in community structure and

functional potential of benthic prokaryotes between inner continental shelf and outer of continental shelf. The difference benthic prokaryotic distribution was strongly affected by distinct environmental conditions, and organic matter utilization is an important factor to shape benthic prokaryotic community in pelagic sediments of the Ross Sea.

An identification and characterization of culturable bacteria were performed in surface deep-sea sediments in the west Antarctica. 73 bacteria strains were isolated and identified from 4 sediment samples, of which two strains $M2^T$ and R106 in the genus *Shewanella* were subjected to analyses of physiological, chemotaxonomical, phylogenetic and genomic characteristics. Two novel species in the genus *Shewanella* were facultatively anaerobic, and had the lowest optimal temperature for growth. Also, a new species had two sets of the *feoA–feoB* operon involved in ferrous iron transport. Hence, these results may indicate that the strain $M2^T$ adapt to the environment in which the strain was isolated using different strategies among their closest phylogenetic relatives *Shewanella* species. Pyrosequencing and metagenome analyses also confirmed that *Shewanella* species were dorminant in surface sediment of the study areas. Based on the data obtained by polyphasic approach, this study provide insight that *Shewanella* species plays a important roles in iron cycling in the study areas.

Over all, this study suggested that prokaryote might play a important roles in benthic environment in the west Antarctica. And these finding will increase our knowledge about the ecological functions of the benthic prokaryotes in the west Antarctica as well as help preddict their ability for adaptation against environmrnts changing.

Keyword: Western Antarctica, marine sediment, benthic prokaryotic community, 454 pyrosequencing, shotgun sequencing, metagenomics

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CHAPTER 1

General Introduction

Prokaryotes (bacteria and archaea) have evolved to be able to inhabit in a wide range of habitats on the earth from the aquatic and terrestrial ecosystem to even extreme environments, and the number of prokaryotes is estimated to be over 4–6 × 10^{30} of prokaryotic cells (Whiteman *et al*., 1998). Marine prokaryotic communities also exist in diverse environments extending from sea surface microlayer to deep sea. Marine surface sediment layers typically support high numbers of microbial cells (1.7 × 10^{28}; Whiteman *et al*., 1998; Madsen, 2008) and prokaryotes in subseafloor sediments may make up approximately 13% of the global prokaryotic biomass (Parkes et al., 1994; Whitman et al., 1998; Bar-On et al., 2018). Prokaryotic communities are critical to the functioning of marine ecosystems and the dominant players in carbon cycling and other biogeochemical cycles such as nitrogen, phosphorus, iron and sulphur (Parkes *et al*., 2000; Azam and Malfatti, 2007; Falkowski *et al*., 2008). Hence, the investigation structure of prokaryotic communities and their distribution is a key to understanding the ecological and biogeochemical functions in deep-sea ecosystems (Smith *et al*., 2009; Kimes *et al*., 2013).

The Antarctic environment is closely connected to the atmosphere, hydrosphere, lithosphere, cryosphere and biosphere systems. Thus, changes in this environmental system have affected global climate, oceanographic process and sea

level change. The consequences of global warming have been especially observed over most of the Antarctic Peninsula and West Antarctica (Meredith and King, 2005; Reid *et al*., 2009; Steig *et al*., 2009) and a rapid melting of sea ice has been noted (e.g., Amundsen Sea and Bellingshausen Sea; Pritchard *et al*., 2012; Shepherd *et al*., 2012; Rignot *et al*., 2013). In addition, the Antarctic Seas are also critical to global elements cycles, as they are regions of extensive air-sea heat and gas exchange as well as high biological productivity associated with returning of nutrients to surface waters by global deepwater formation (Sarmiento *et al*., 2004).

The Antarctic Ice Sheet presently covers an area of 14×10^6 km^2 and contains about 27×10^6 km^3 of ice (Fretwell *et al*., 2013). It accounts for about 90% of the Earth's total ice volume and, if the entire Antarctic Ice Sheet melted, sea level would rise by about 60 meters (Fretwell *et al*., 2013). An ice shelf is a floating extension of the ice sheet. Ice shelves gain ice in several ways: (1) surface accumulation processes include snow fall, (2) flow of ice from the continent and (3) the freezing of marine ice to their undersides. Ice shelves lose mass through processes of ablation: (1) melting from underneath the ice shelf (from relatively warm ocean currents), (2) melting above (from warm air temperatures) and (3) from calving icebergs. Especially, the glaciers and ice shelves of the Western Antarctic Ice Sheet (WAIS) are rapidly losing mass (Pritchard *et al*., 2012; Shepherd *et al*., 2012; Rignot *et al*., 2013).

A large extent of the west Antarctic continental shelves is covered by ice shelves or by WAIS. And the west Antarctic continental shelves show the unique morphologic features including great depth, irregular topography and land-ward slopping profile. The most part of this unusual topography is due to glacial erosion

and isostatic depression also contribute to ~ 25% of this unique characteristic (Anderson, 1999). The isostatic depression is responded to the great weight of the continental ice sheet (Anderson, 1999) and cause a crustal forebulge, thus, influences the inner shelf. Glaciers spread out over the coast and erode the bedrock or other sediment, and therefore glacial erosion also affects the inner continental shelf.

The vertical structure of water currents and the features of the water masses are largely determined by distribution of physic-chemical parameters and by vertical mixing. Antarctica's continental shelf plays a significant role in the changes in major water masses. The water cycle on the Ross Ice Shelf differs from the Amundsen Ice Shelf with respect to formation of bottom water (Jenkins *et al*., 2016). In the Ross ice shelf, dense Shelf Water, formed by brine injection beneath growing sea ice, influence melting of the sub-ice cavity (Jenkins *et al*., 2016). While the Amundsen ice shelf revealed that the intrusion of the warm Circumpolar Deep Water (CDW) on the inner shelf helps to rapidly melt the ice shelves and glaciers (Jenkins *et al*., 2016).

The Ross Sea continental shelf is a biologically productive system (Smith *et al*., 2012), forming dense shelf waters (DSW; Jenkins *et al*., 2016). The Ross Sea Polynya is the largest polynya on Earth, and is continuously formed due to the continuous blowing away sea ice by strong katabatic winds from the land over the ocean (Arrigo and van Dijken, 2004). Due to the largest Polynya formation, the Ross Sea is one of the most spatially productive marginal sea in the Antarctic Seas, which also means that Polynya is one of the most productive Antarctic shelf systems (Arrigo *et al*., 1998; Smith *et al*., 2012). With high phytoplankton biomass mainly dominated by diatoms or haptophytes (Mangoni *et al*., 2017), the organic carbon exported from these biomasses in the water column sink and reach the bottom

(Bercovici *et al*., 2017). The Amundsen Sea polynya has occurred through when the body with the higher temperature transports sensible heat to the body with the lower temperature, mainly caused by warmer CDW to rise to the surface and provides the source of heat needed to melt the glaciers. The rapid melting of glaciers and ice shelf in this region may alter ecosystem change by flux of energy flow. The Amundsen Sea polynyas are also reported as the most productive polynyas with high phytoplankton biomass dominated by *Phaeocystis antarctica* in the Antarctic shelf (Arrigo *et al*., 2012; Yang *et al*., 2016). In these polynyas, flux of organic matter from the water column affect to benthic sediment (Kennedy et al., 2002; Baldi et al., 2010; Gillies et al., 2012).

Based on the above context, the continental shelf sediments in the West Antarctica are considered to be dynamically affected by hydrographic and biological processes, possibly affecting the structures and functions of benthic prokaryotic communities. Therefore, it is important to understand the dynamics of prokaryotic community for understanding benthic environment in these areas which are rapidly changing ecosystem.

About 80% of the Earth's biosphere is mostly extreme environments such as high pressure and low temperature, and in marine environments, more than 90% of the environment has temperatures below 5°C (Margesin and Schinner, 1994). Microorganisms play a crucial role in maintaining stability of the ecosystem, and they have undergone changes for the growing and survival under harsh conditions and thus show huge genetic and metabolic diversity for exploration. Therefore, understanding the community structures and metabolic functions of these cold adapted prokaryotes in the West Antarctica with physiochemical differences may

provide insights into expected response of the microbial communities to the Antarctic environment which are rapidly changing and may provide excellent source of cold-active enzymes and biocatalysts for biotechnological applications (Mirete *et al*., 2016).

Prokaryotes predominate in terms of biomass and perform a significant role in the biogeochemical flux of organic matters and nutrients in the marine ecosystem (Azam and Malfatti, 2007; Falkowski *et al*., 2008). Investigation of structure and diversity of prokaryotic communities is an important first step to understand the ecological and biogeochemical functioning of prokaryotes (Kimes *et al*., 2013). However, most of the prokaryotic biodiversity is still unexplored. Because it is difficult to reproduce marine environment in laboratory, it has been estimated that more than 99% of the bacteria have not been cultured yet (Lewis, 2009). Therefore, it is clear that there are remained most of the marine prokaryotes due to uncultured. To overcome these limitations associated with cultivation-dependent techniques for investigation prokaryotic community structures, molecular techniques based on 16S rRNA gene have been widely applied for sequencing and phylogenetic studies to assess the prokaryotic community compositions (Weisburg *et al*., 1991; Pereira *et al*., 2010). There are diverse methodological approaches to assess prokaryotic diversity based on 16S rRNA gene have been developed such as polymerase chain reaction (PCR), denaturing gradient gel electrophoresis (DGGE), Terminal restriction fragment length polymorphism (T-RFLP), Fluorescent *in situ* hybridization (FISH), cloning. The traditional molecular techniques have the advantage of being able to identify dominant groups of microbial community that has not been cultured, but minor groups can't detect. Thus, high-throughput sequencing techniques (i.e.,

pyrosequencing) are used and enable rapidly characterize a deeper level (major & rare group) of prokaryotic diversity and understand of the prokaryotic communities in a sample. With the advent of next-generation sequencing (NGS), there were the enormous progress in the investigation of prokaryotic community compositions, but the community structures cannot directly allow to obtain the functional potential of the prokaryotes in nature. Hence, in order to overcome the limitation of NGS for elucidating the *in situ* metabolic activity and ecological functions of prokaryotes, large-scale shotgun metagenome sequencing approaches have performed recently (Quince *et al*., 2017). These metagenomic techniques can provide powerful advantages such as metabolism potential and putative ecological functions of the prokaryotes, and be used to profile an accurate numerous prokaryotic communities and community-wide comparisons in natural samples (Quince *et al*., 2017).

In the Ross Sea, sediments have been sampled for the purpose of identifying bacterial communities and a few studies of the bacterial diversity have been investigated by cultivation-dependent approaches or cultivation-independent approaches (Table 1.1). A few studies of bacterial diversity have been investigated with relating of available source of energy on organic matter or nutrient in sediments (Table 1.1). *Proteobacteria*, *Bacteroidetes* and *Actinobacteria* were relatively dominant bacterial community (Baldi *et al*., 2010; Carr *et al*., 2013; Lee *et al*., 2014; Learman *et al*., 2016) and archaeal phyla *Thaumarchaeota* were predominant in sediments (Learman *et al*., 2016). However, their composition has known to be related to quality and quantity of organic matter or nutrient and thus, to affect the carbon and nitrogen cycling in this environment (Fabiano *et al*., 1998; Baldi *et al*., 2010; Carr *et al*., 2013; Lee *et al*., 2014; Learman *et al*., 2016). The studies in

Amundsen Sea have only just started recently, when Jacobs *et al*. (1996) made the first investigation of oceanographic measurements on the continental shelf (Jenkins *et al*., 2016). A limited analysis of prokaryotic diversity in the Amundsen Sea sediment has been performed (Learman *et al*., 2016), and showed prokaryotic communities in sediments could be distinguished along the quantity and quality of organic matter.

However, despite the importance of prokaryotic community studies in sediments, a few studies using traditional molecular approaches and high-throughput sequencing techniques have been conducted in the West Antarctic sediment to date. It means that prokaryotic community structures and diversity in the West Antarctic sediment needs more studies using pyrosequencing and shotgun metagenome sequencing. These techniques would help us to understand the relationship between prokaryotic diversity and environmental condition, and to elucidate the in situ metabolic activity of prokaryotes in the West Antarctic ecosystems.

Table 1.1. Summary of previous studies on prokaryotic diversity in the Ross Sea and the Amundsen Sea.

Location	Main interests	Method	Reference
Ross Sea	Enzymatic activity and bacterial distribution in surface sediments	Bacterial counting and enzymatic activity	Fabiano *et al*. (1998)
	Bacterial diversity of surface sediment	T-RFLP and cloning of 16S rRNA gene	Baldi *et al*. (2010)
	Bacterial diversity along the depth	Pyrosequencing of 16S rRNA gene	Carr *et al*. (2013)
	Diversity and physiological characteristics of bacteria from surface sediment	Cultivation and enzyme activity	Lee *et al*. (2014)
	Prokaryotic diversity of surface sediment	Illumina MiSeq sequencing of 16S rRNA gene	Learman *et al*. (2016)
	Diversity and physiological characteristics of culturable bacteria	Cultivation and enzyme activity	Lo Giudice *et al*. (2006)
	Bacterial diversity of surface sediment	Illumina MiSeq sequencing of 16S rRNA gene	Li *et al*. (2019)
	Bacterial diversity of surface sediment	Illumina MiSeq sequencing of 16S rRNA gene	Li *et al*. (2020)
Amundsen Sea	Prokaryotic diversity of surface sediment	Illumina MiSeq sequencing of 16S rRNA gene	Learman *et al*. (2016)
	Prokaryotic diversity of surface sediment	Pyrosequencing of 16S rRNA gene	Cho *et al*. (2020)

Considering importance of understanding the diversity and ecological functions of prokaryotic communities, patterns of prokaryotic diversity and distribution are still scarcely understood in sediments from the West Antarctica mostly due to little investigation. Thus, the overall objective of this study was to investigate the benthic prokaryotic diversity and their metabolic functions to better understand the ecosystem in the West Antarctica sediments (Fig. 1.1 and Fig. 1.2). In Chapter 2, prokaryotic community structures using 16S rDNA pyrosequencing was used to investigate of relationship between prokaryotic community structures and environmental parameters in surface sediment at edges of west Antarctic ice shelves. In Chapter 3, to gain insights into metabolic functions and taxonomic structures of benthic prokaryotic communities, metagenomic analysis using shotgun DNA metagenome sequencing was performed on the surface sediments in the Ross Sea. In Chapter 4, identification and characterization of culturable bacteria, which are abundant and play an important role in iron cycling in the study areas, was investigated in surface sediments using physiological, chemotaxonomical, phylogenetic and genomic analyses. Through these studies, understanding of the compositions and ecological functions of sediment prokaryotic communities would be expanded in the West Antarctica.

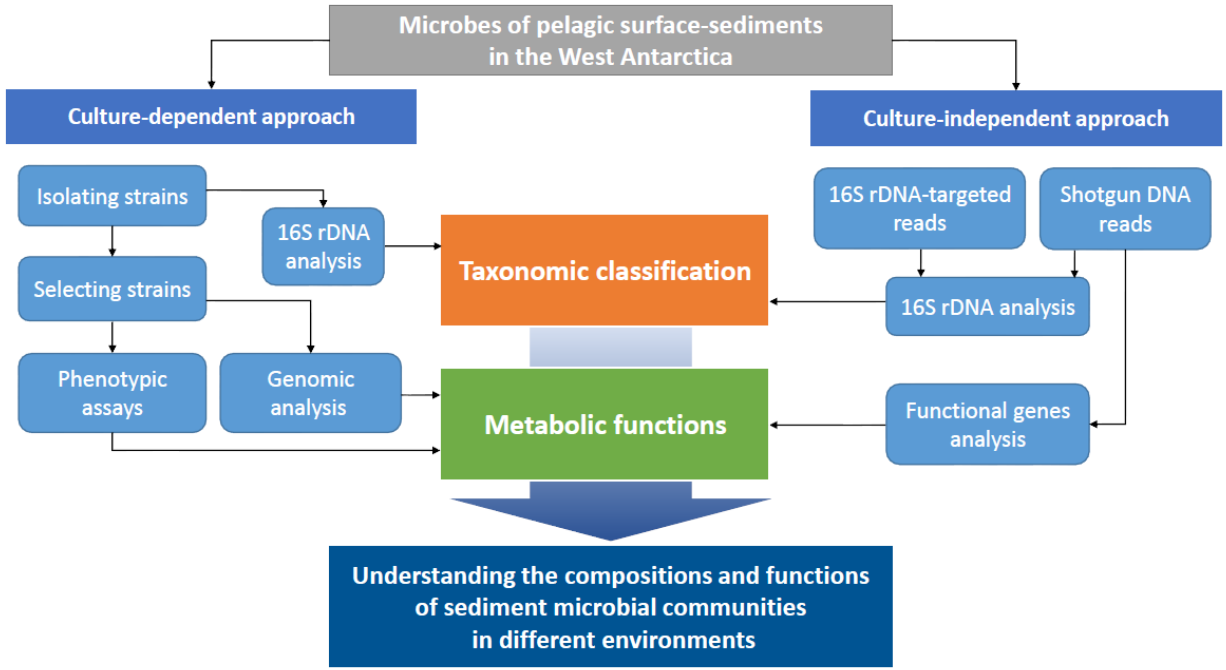


Figure 1.1. Schemes and purposes of this study

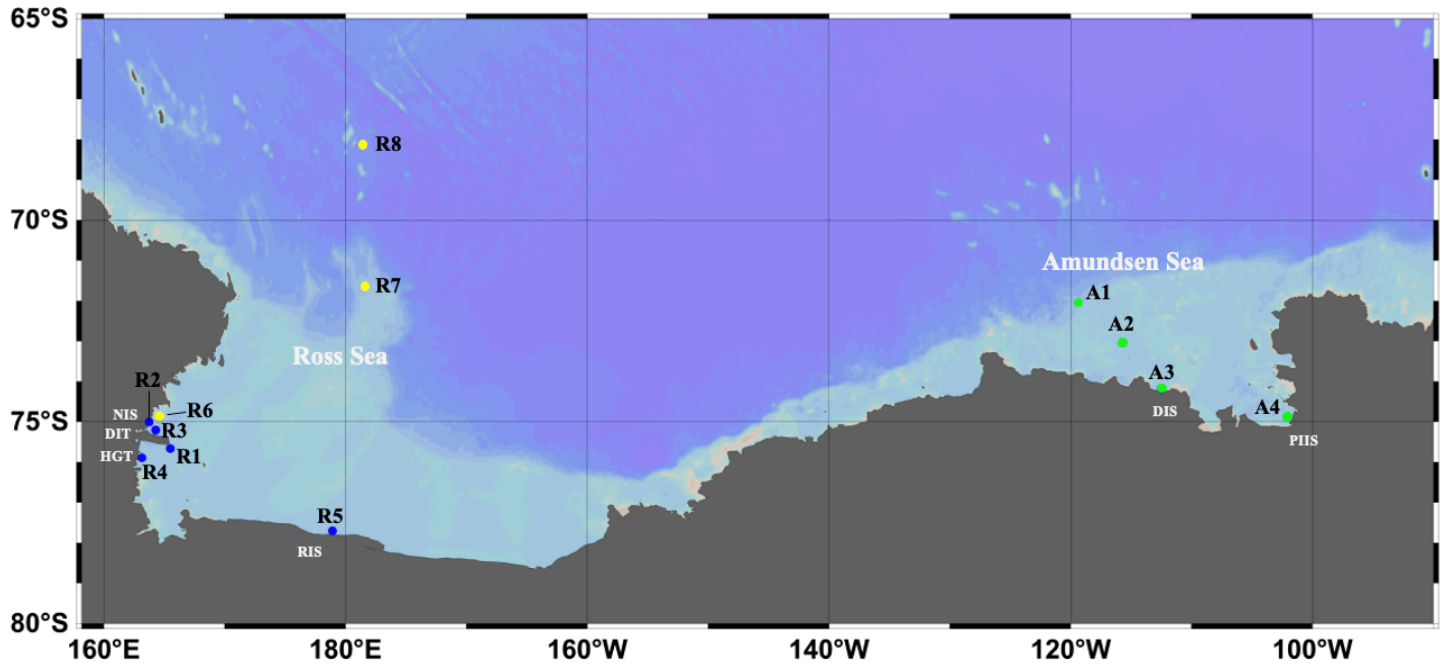


Figure 1.2. Map of 12 sampling stations in this study. Station R1–R5 and A1–A4 for study in chapter 2, station R6– R8 for study in chapter 3, and R1 and A1–A3 for study in chapter 4.NIS: Nansen Ice Shelf, DIT: Drygalski Ice Tongue, HGT: Harbord Glacier Tongue, RIS: Ross Sea Ice Shelf, DIS: Dotson Ice Shelf, PIIS: Pine Island Ice Shelf.

CHAPTER 2

A wide variety of prokaryotic community structures in surface sediments at edges of west Antarctic ice shelves

2.1 Introduction

Marine prokaryotic communities exist in diverse environments extending from sea surface microlayer to deep sea. Prokaryotic communities are critical to the food web dynamics as a major component of the microbial food web and the dominant players in biogeochemical cycles of organic matters and nutrients (Parkes *et al*., 2000; Azam and Malfatti, 2007; Falkowski *et al*., 2008). Especially benthic prokaryotic communities inhabiting subseafloor sediments may make up approximately 13% of the global prokaryotic biomass (Bar-On *et al*., 2018). Hence, the identification of structure and distribution of prokaryotic communities is a key to understanding the ecological and biogeochemical functioning in deep-sea sediment ecosystems (Smith *et al*., 2009; Kimes *et al*., 2013).

The continental shelf sediments in the west Antarctica are considered to be dynamically affected by geological, biological and hydrographic factors. The west Antarctic continental shelf is great depth, irregular topography and land-ward slopping profile (Anderson, 1999). The glacial erosion contributed the most part of this unusual topography, and would influence the sedimentation near the grounding-line by production of glacial till. The highest primary production in the Antarctic Seas is generally associated with coastal polynyas (Arrigo and van Dijken, 2003; Arrigo *et al*., 2007) and the continental shelf (Smith and Gordon, 1997; Arrigo and van Dijken, 2004). In these polynyas, the organic matter exported from the phytoplankton blooms in the water column affects to benthic sediment (Kennedy *et al*., 2002; Baldi *et al*., 2010; Gillies *et al*., 2012). However, bottom water masses are different between the Amundsen Sea (Circumpolar Deep Water, CDW; the warmer

and less saline waters) and the Ross Sea (Shelf Water, the cold and saline waters; Jenkins *et al*., 2016). The rapidly melting of the ice shelves and glaciers is related to the intrusion of the warm CDW onto the continental shelf in the Amundsen Sea and related to Shelf Water, formed by brine injection beneath growing sea ice in the Ross Sea (Walker *et al*., 2007; Pritchard *et al*., 2012; Jenkins *et al*., 2016). The combined effects of these factors possibly affect to variations of benthic prokaryotic community in the inner continental shelf.

In Antarctica, previous studies of benthic prokaryotic diversity have been investigated by cultivation-independent approaches such as cloning and high-throughput sequencing techniques (Bowman *et al*., 2003; Bowman and McCuaig, 2003; Powell *et al*., 2003 Baldi *et al*., 2010; Carr *et al*., 2013; Lee *et al*., 2014; Ruff *et al*., 2014; Learman *et al*., 2016; Franco *et al*., 2017; Li *et al*., 2019; Cho *et al*., 2020; Li *et al*., 2020). The previous studies were mainly investigated in coast of Antarctic Peninsula (Bowman *et al*., 2003; Bowman and McCuaig, 2003; Franco *et al*., 2017), in Polar Front (Lee *et al*., 2014; Ruff *et al*., 2014). Learman *et al*. (2016) focused on the surface sediments in outlying areas of the mid continental shelf and Carr *et al*. (2013) studied long core sediment by the depth of at one station in the Ross Ice Shelf. In recent studies, Li *et al*. (2019) and Cho *et al*. (2020) studied microbial diversity in sediments in the Ross Sea and the Amundsen Sea, respectively, and Li *et al*. (2020) studied microbial diversity in the 3 regions at the Ross Sea, Prydz Bay and Antarctic Peninsula.

Therefore, there are a few studies on the relationship between benthic prokaryotic communities and environmental data at edges of ice shelves and inner areas of the continental shelf in the west Antarctica.

The ultimate goal of this study is to understand how environmental factors affect benthic prokaryotic communities at edges of ice shelves in which dynamic sedimentation processes occur. Specific questions addressed in present study include: How do benthic prokaryotic communities differ at the edges of remote ice shelves in the Ross Sea and Amundsen Sea? Which environmental factors are important in shaping the benthic prokaryotic community in each ice shelf? To address these questions, surface sediment samples were collected from the west Antarctic continental shelf during three Araon expeditions. The investigation of environmental variables and the high-throughput sequencing (i.e., 16S rRNA gene-based pyrosequencing) for identifying prokaryotic community structures were performed on surface sediments in the Amundsen Sea and Ross Sea. This study is the first study that explores benthic prokaryotic communities at edges of ice shelves in inner continental shelf across a wide area in west Antarctica and would contribute to expand understanding of benthic prokaryotic communities with different physical and chemical characteristics of surface sediments in west Antarctica.

2.2 Materials and Methods

2.2.1 Sediment sample collection and environmental parameters

Sediment samples were collected at 9 stations from Amundsen Sea (4 stations) and Ross Sea (5 stations) in Antarctica during three research cruises on the IBRV Araon (Fig. 2.1). The sediment samples from the Ross Sea were collected by colleagues. The samples were obtained from water depth between 669 m and 1224 m using a box corer equipped with weights of ca. 350 kg that are attached to the top of rectangular steel tube (30 × 10 × 60 cm). Surface sediments (0–2 cm depth) were sub-sampled by ethanol-steriled steel spatula and placed into sterile 50 ml conical tube for analysis of prokaryotic abundance, DNA extraction and environmental parameters. All the samples were frozen immediately at -80 °C on board the ship and transferred to a land-based laboratory and stored at -80 °C until further analysis. Duplicate subsamples were collected from sediments of the Amundsen Sea. The remaining procedure was the same as that of the Ross Sea sample. Temperature and salinity data in the water column were obtained at the same stations of box core sampling by a conductivity-temperature-depth (CTD) system (SBE 911; Sea-Bird Electronics). In the Ross Sea, total carbon (TC) and total nitrogen (TN) contents were determined with an Organic Elemental Analyzer (FLASH, 2000 NC Analyzer), and the total inorganic carbon (TIC) concentration was determined using the UIC CO_2 coulometer (Model CM5240) at the Korea Polar Research Institute. The TOC concentration was estimated the difference between the TC and TIC (Kim *et al*., 2018). In the Amundsen Sea, TIC was removed from the sediments, and then TOC and TN were measured by using an Organic Elemental Analyzer. Stable isotope (^{13}C

and ^{15}N) was analyzed using Isotope Ratio Mass Spectrometry (IRMS) at the Beta Analytic Corporation, USA.

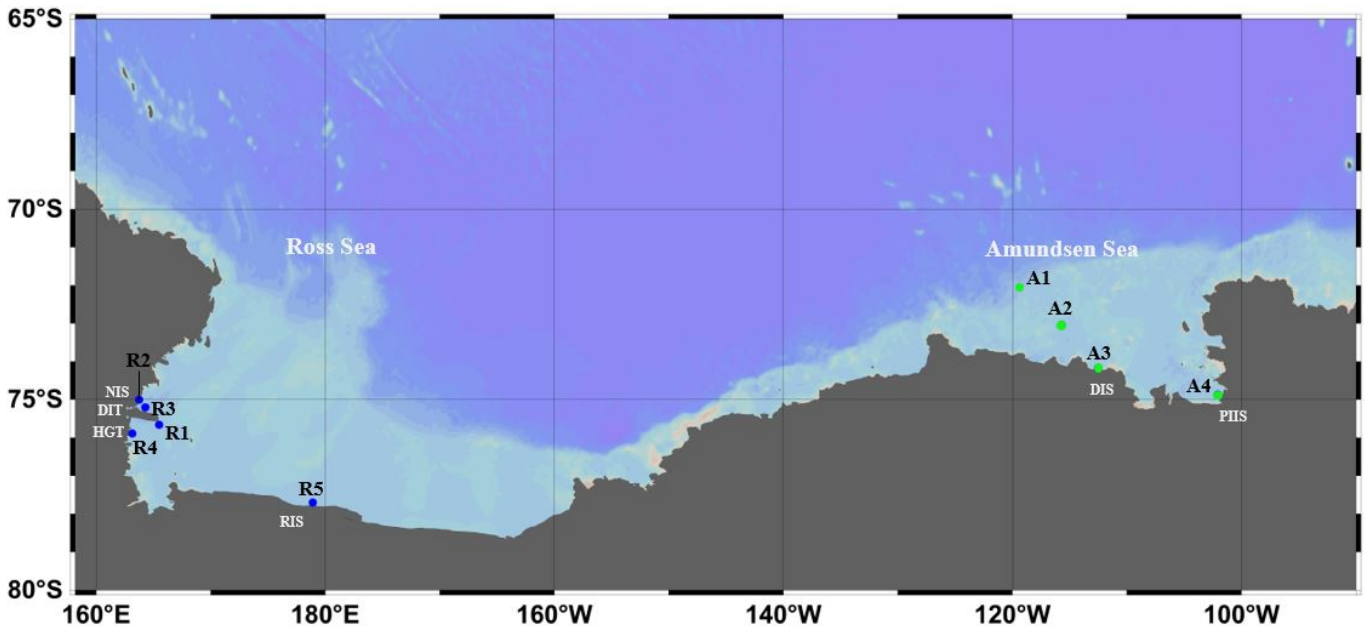


Figure 2.1. Map of 9 sampling sites, A1-A4 (green circle) in the Amundsen Sea and R1-R5 (blue circle) in the Ross Sea. NIS: Nansen Ice Shelf, DIT: Drygalski Ice Tongue, HGT: Harbord Glacier Tongue, RIS: Ross Sea Ice Shelf, DIS: Dotson Ice Shelf

2.2.2 Direct counts of prokaryotic abundance (PA)

Prokaryotic abundance (PA) was measured using SYBR gold staining method based on Breuker *et al*. (2013) and with some modifications. SYBR gold staining method was used instead of SYBR green I in this study (Weinbauer *et al*. 1998; Shibata *et al*. 2006). Ca. 1 g of each sediment sample was 10-fold diluted with 0.2 μm filter-sterilized neutral formalin (final conc. of 2%) in 3% sea-salt water. The samples were vortexed for 10 s to homogenize, and 2 ml of the slurry was transferred in a new 50 ml tube and added 5 ml of 0.2 μM hydrochloric acid after filter-sterilize to dissolve carbonates. Samples were placed on rotator for 5 min and centrifuged for 20 min at 16,000 × g, and then 7 ml of supernatant was carefully removed. Five ml tris-ethylenediaminetetraacetic acid (TE) buffer was added to the pellet and centrifuged. This step was repeated twice for washing. And the pellet was suspended with 25 ml TE buffer followed by an ultrasonic treatment for 20 s (Weinbauer *et al*., 1998). Seven hundred μl of samples was stained on Anodisc™ filters (0.2 μm) with SYBR Gold as described Shibata *et al*. (2006). Cells were counted using a epifluorescence microscope (BX51 Olympus). A minimum of 500 cells were counted for each sample.

2.2.3 DNA extraction and PCR amplification of the 16S rRNA genes

The sediment samples were homogenized with sterilized pipette tip and freeze-dried (Miller *et al*. 1999). Genomic DNAs were extracted from 0.2–0.9 g of freeze-dried samples using FastDNATM SPIN Kit for soil (MP Biomedicals®), according to the manufacturer's instruction with bead beating for 2 min. Extracted DNAs were stored at -20 °C until further analysis. Genomic DNA was extracted in duplicate for each sample and negative control was performed for checking contamination. Concentration of extracted DNA was measured with Quant-iT™ PicoGreen® dsDNA Assay Kit (Life Technologies) and a Multilabel Plate Readers (2103 EnVision™ PerkinElmer).

The V5–V8 region of bacterial and archaeal 16S rRNA genes were amplified by polymerase chain reaction (PCR) using the universal primers set Uni787F (5′-ATTAGATACCCNGGTAG-3′) with barcodes and linkers and Uni1391R (5′-ACGGGCGGTGWGTRC-3′) (Jorgensen *et al*., 2012). PCR was performed in a 50 µl PCR mixtures containing 5 µl of the DNA extract, 0.4 µM of each primer, 1 × Takara EX Taq Buffer (Mg2+ plus), 0.2 mM dNTPs, 1.25 U/µl Takara EX Tag DNA polymerase, 0.05% BSA (Bovine serum albumin) and 2.5% DMSO (dimethyl sulfoxide). PCR amplifications was conducted under the following conditions: 94 °C for 15 min; 30 cycles of denaturing (94 °C for 45 s), annealing (53 °C for 45 s) and extension (72 °C for 1 min) followed by the final extension at 72 °C for 7 min. PCR amplicons were identified using agarose gel electrophoresis and were purified using LaboPassTM PCR purification kit (CosmoGenetech).

2.2.4 Pyrosequencing data and statistical analyses

All amplicons with different sample-specific barcodes (Table S2.1) were pooled in equal amount and pyrosequencing was performed in 1/2 region of PicoTiterPlate device on the 454 GS FLX Titanium according to manufacturer's instructions at the Macrogen Corporation, Korea.

Recently, Edgal (2018) identified that optimal clustering threshold of operational taxonomic units (OTUs) were ~99% for full-length sequences to species. New amplicon sequence variants (ASVs) methods combined the benefits for subsequent analysis of closed-reference and de novo OTUs have been developed (Callahan *et al*., 2017) for surveying microbial communities. The sequence reads from pyrosequencing were trimmed and denoised using the AmpliconNoise program (version 1.29; Quince *et al*., 2009). With the QIIME 2 software package (version 0.21.4; Bolyen *et al*., 2018) using ASVs methods, the amplicon reads assigned to unknown and chloroplast were removed, and the remaining amplicon reads were normalized with the lowest reads number 1,596 in all samples for the subsequent analyses. Functional abundances were predicted using MetaCyc pathway abundances and coverages with PICRUSt2.0 (Phylogenetic Investigation of Communities by Reconstruction of Unobserved States; Douglas *et al*., 2020) software.

The SILVA reference database (version 138; Quast *et al*., 2013) was used to assign taxonomy of OTUs. And data in percent or a ratio were transformed using a square root for t-test and correlation analysis. Analysis of similarities (ANOSIM) based on the Bray-Curtis similarity distances of square root transformed ASVs

abundance were used to test differences in the prokaryotic communities by seas from PRIMER (version 6; Clarke and Gorley, 2006). Alpha diversity was evaluated using QIIME 2 software package (version 0.21.4). The beta-diversity between samples was analyzed using Bray-Curtis similarity using non-metric multidimensional scaling (NMDS) with mothur (version 1.48.0; Schloss *et al*., 2009). To determine which environmental variables, affect alpha-diversity, Spearman's rank correlation analysis was investigated in R (version 4.0.5; R Core Team, 2020)). Correlations between taxa and functional abundances were statistically analyzed via Spearman's rank correlation in R (version 4.0.5).

The Sequence Tag-based Analysis of Microbial Population dynamics (STAMP; Parks *et al*., 2014) and Linear discriminant analysis effect size (LEfSe) analyses were conducted to compare the prokaryotic compositions and between two groups.

2.3 Results

2.3.1 Environmental Characteristics and Total prokaryotic abundance

Principal component analysis (PCA) of environmental variables confirmed that environmental variables in the Amundsen Sea stations differed from variables in the Ross Sea on the first PCA axis (Fig. 2.2).

Bottom water temperature in the Amundsen Sea (0.3–1.1 °C) was significantly higher than that in the Ross Sea (-1.9 °C, $p < 0.05$, t-test; Table 2.1). Bottom water salinity in the Ross Seas (34.78–34.80) was higher than that in the Amundsen Sea (34.52–34.71, $p < 0.05$, t-test; Table 2.1).

The TN and TOC concentration and the C/N ratio among samples was not different in the Amundsen Sea and the Ross Sea (Table 2.1; $p > 0.05$, t-test, respectively). The station A1 (Sea-ice zone) and A3 (DIS) showed the lowest and the highest TN and TOC concentration, respectively, in the Amundsen Sea and the station R2 (NIS) and R1 (DIT, Tip) were the lowest and highest TN and TOC concentration in the Ross Sea, respectively (Table 2.1). Station R5 (RIS) presented the lowest C/N ratio (7.2) and station R2 (NIS) was the highest C/N ratio (8.7). The TN concentration was correlated with TOC concentration (Fig. 2.3a; $r = 0.91$, $p < 0.05$). The δ^{13}C-values measured in the samples ranged from -25.9 to -22.7‰ and the δ^{15}N-values range from 2.8 to 5.1‰ (Table 2.1). The station R2 (NIS) was the highest δ^{13}C–values (-22.7‰), while the station A3 (DIS) was the highest δ^{15}N–values (5.1‰) among all stations (Table 2.1). The δ^{15}N-values were negatively correlated with salinity (Figure 2.3b; $r = 0.62$, $p = 0.02$).

Prokaryotic abundance of wet sediment samples ranged from 1.3–8.6 × 10^8 cells

g^{-1} (R1 (DIP, tip)-R5 (RIS)) to 1.3–2.2 × 10^8 cells g^{-1} (A1 (Sea-ice zone)–A4 (PIIS); Table 2.1). There were no significant differences in the prokaryotic abundance ($p > 0.05$, t-test) among the Amundsen Sea and the Ross Sea stations.

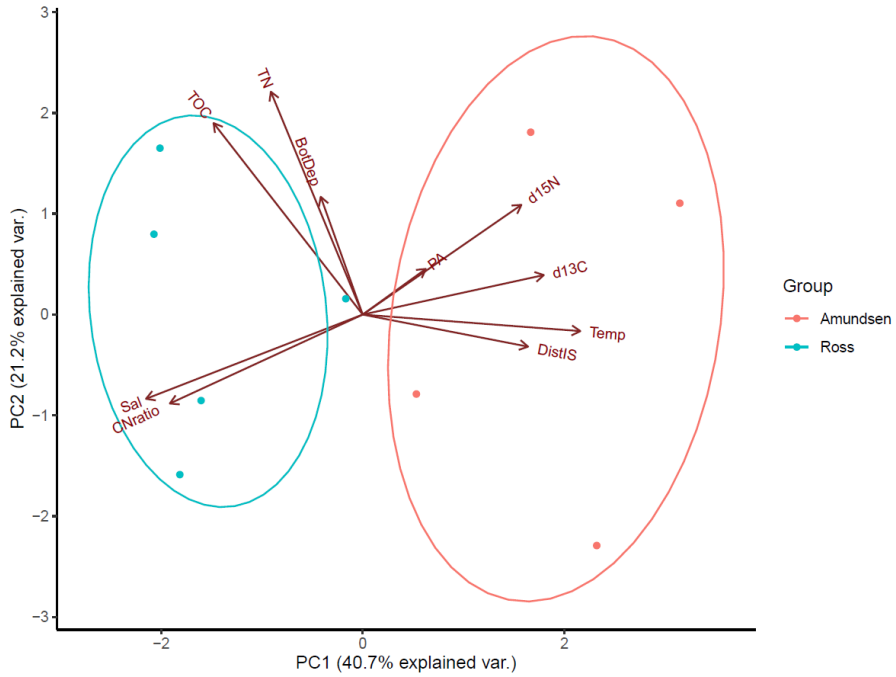


Figure 2.2. Principal component analysis (PCA) of environmental variables. Stations from the Amundsen Sea are indian red and Ross Sea are turquoise. Each line represents the direction and strength of environmental gradient (BotDep, Bottom Depth; Temp, Temperature; DistIS, Distance from Ice shelf; Sal, Salinity; TN, Total Nitrogen; TOC, Total Organic Carbon; CNratio, Carbon to Nitrogen ratio; d15N, δ15N-values; -d13C, converted δ13C-values to positive values) that statistically correlate with the ordination.

Table 2.1. Description of sampling stations, bacterial abundances and environmental parameters in sediment samples obtained in the Amundsen and Ross Seas. Environmental parameters of the Amundsen Sea samples were analyzed in duplicate and the value means average of duplicate measure value ± standard deviation.

Study area	Sample	Date of sampling	Latitude	Longitude	Bottom Depth (m)	Temperature (℃)	Salinity (PSU)	PA (x 10^8 cells/ g of wet sediment) (n=2)	TN (%)	TOC (%)	C/N Ratio	$\delta^{15}N$ (‰)	$\delta^{13}C$ (‰)
Amundsen Sea	A1	16-Jan-2016	72°05.03'S	118°53.05'W	745	0.78	34.71	1.46 ± 0.04	0.03 ± 0.01	0.18 ± 0.06	6.61 ± 0.31	3.40 ± 0.42	-24.40 ± 0.20
	A2	18-Jan-2016	73°02.40'S	115°43.50'W	710	0.31	34.52	2.19 ± 0.13	0.09 ± 0.02	0.56 ± 0.13	6.24 ± 0.10	4.70 ± 0.00	-25.00 ± 0.20
	A3	20-Jan-2016	74°10.29'S	112°31.79'W	1034	0.57	34.57	1.33 ± 0.57	0.10 ± 0.00	0.62 ± 0.01	6.51 ± 0.23	5.05 ± 0.21	-24.70 ± 0.10
	A4	03-Feb-2016	74°52.03'S	102°04.80'W	945	1.07	34.67	1.67 ± 0.15	0.06 ± 0.00	0.51 ± 0.01	8.42 ± 0.31	3.20 ± 0.00	-24.60 ± 0.20
Ross Sea	R1	12-Jan-2015	75°39.57'S	165°28.98'E	848	-1.89*	34.79*	1.81 ± 0.51	0.14	1.07	7.56	3.20	-23.30
	R2	13-Jan-2015	75°00.10'S	163°43.50'E	669	-1.89*	34.79*	1.33 ± 0.74	0.06	0.49	8.73	3.80	-22.70
	R3	08-Dec-2015	75°12.28'S	164°16.58'E	1224	-1.88	34.80	1.41 ± 0.38	0.10	0.81	7.82	3.20	-22.80
	R4	13-Dec-2015	75°53.65'S	163°07.58'E	742	-1.90	34.79	1.51 ± 0.46	0.08	0.63	7.67	2.80	-23.00
	R5	12-Dec-2015	77°42.27'S	178°54.89'E	709	-1.90	34.78	8.63 ± 3.34	0.10	0.70	7.18	3.80	-25.90

*CTD data from adjacent station of each sampling station

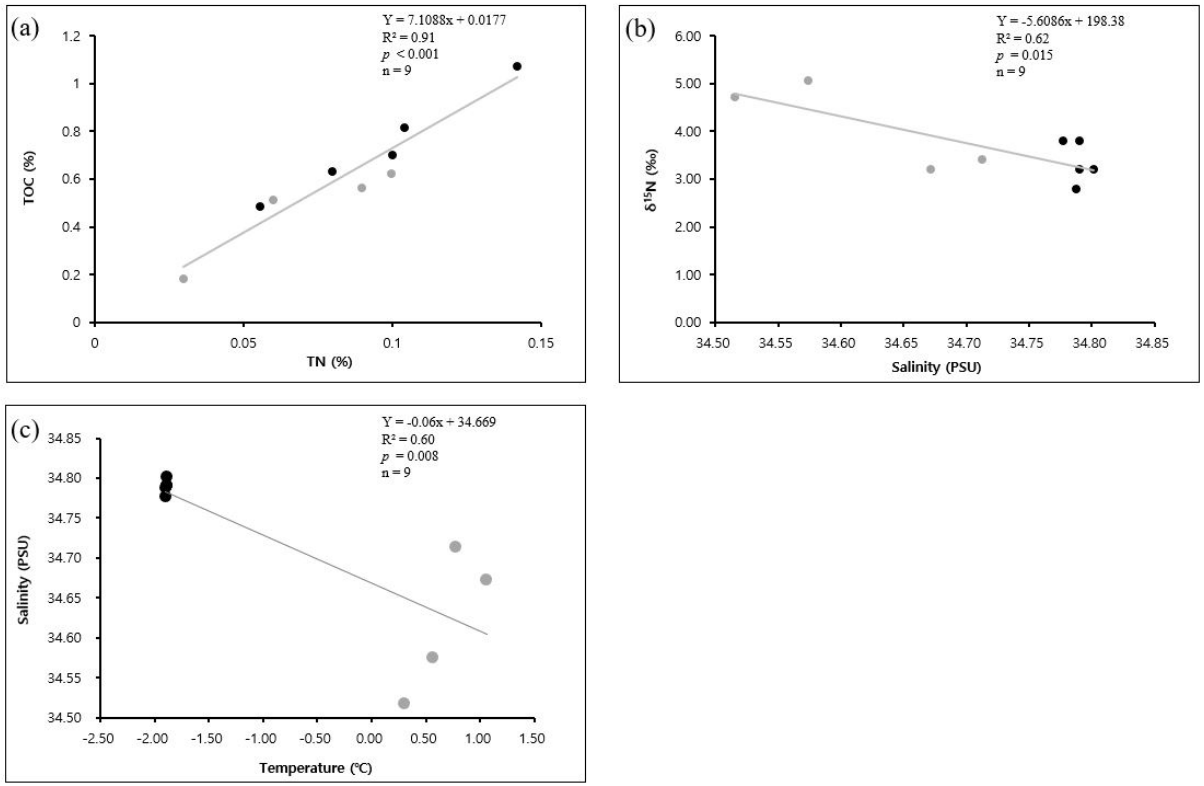


Figure 2.3. Relationship between (a) TN (%) and TOC (%) (b) Salinity (PSU) and δ15N (‰) (c) Temperature (℃) and Salinity (PSU) in the Amundsen Sea (grey) and Ross Sea (black) samples.

2.3.2 Prokaryotic community composition

A total of 81,142 quality-controlled reads (1,596–6,366 reads per sample) were obtained from 13 sediment samples at 9 study stations. All subsequent analyses relied on libraries normalized with the lowest reads number 1,596.

The number of observed features of each sample ranged from 204 (station A1 (Sea-ice zone)) to 656 (station R1 (DIT, Tip); Table 2.2). The number of observed features detected in samples of the Ross Sea (average 513 ± 94) was higher than Amundsen Sea (average 393 ± 95; Wilcoxon test, $p < 0.05$; Table 2.2 and Figure 2.10). Bacterial sequences were clustered into 9282 ASVs and classified into 23 formally described bacterial phyla and 23 candidate phyla. When analyzing the shared ASVs between the Amundsen Sea and Ross Sea, there were common 2670 ASVs (32.7%) and unique 1564 ASVs (19.1%) in the Amundsen Sea and unique ASVs (48.25%) in the Ross Sea (Fig. 2.4a). And there was common 1515 ASVs (21.6%) between the inner continental shelf in the Amundsen Sea (A3 (DIS) and A4 (PIIS)) and those in the Ross Sea (all stations), while unique 821 ASVs (11.4%) in the Amundsen Sea and unique 4705 ASVs (67.0%) in the Ross Sea (Fig. 2.4b). The analysis results of shared ASVs among all stations showed that there were the least common ASVs between station A2 (Amundsen polynya center) and R1 (DIP, tip) (26 ASVs) and there was the most common ASVs between station R3 (DIT) and R5 (RIS) (93 ASVs; Fig. 2.5).

The relative abundance analysis showed that there were variations in prokaryotic community in sample replicates (Fig. 2.6). Ten phyla accounted for 83.9–95.8% of bacterial groups (Fig. 2.6 and Fig. S2.1). Major bacterial groups in

all samples, *Planctomycetes* (5.2–58%), *Proteobacteria* (3.9–28.7%), *Actinobacteriota* (0.63–15.98%), Candidate division *Dadabacteria* (1.9–12.3%) and Acidobacteriota (1.6–8.7%) were predominant (Table S2.2). *Chloroflexi* (0.9–10.2%), *Bacteroidota* (0.1–6.3%), *Desulfobacterota* (0.0–12.3%), Candidate division NB1-j (0.2–3.3%), Candidate division *Verrucomicrobiota* (0.2–4.1%) and Candidate division *Dependentiae* (0.1–4.6%) were also dominant bacterial groups (Table S2.2). The rest 33 phyla were rarely detected (< 1% in all samples). At the class level, *Planctomycetes* (4.3–52.8%), *Gammaproteobacteria* (2.8–18.7%), *Alphaproteobacteria* (0.6–13.4%), *Dadabacteriia* (1.9–12.3%) were the most abundant (Table S2.2). *Planctomycetes* was predominated by *Pirellulales* (3.9–52.7%, mainly *Pirellulaceae*) and *Planctomycetales* (0.2–7.8%, mostly *Gimesiaceae*). *Alphaproteobacteria* was mainly comprized of *Rhizobiales* (0.0–6.0%, mainly *Methyloligellaceae*) and *Kiloniellales* (0.1–4.8%, mostly *Kiloniellaceae*). Most *Dadabacteria* were classified to *Dadabacteriales* (0.1–9.8%, mainly *Dadabacteriales*) and *Actinomarinales* were belonged to *Acidimicrobiia* (0.1–12.8%, mainly *Actinomarinales*_uncultured; Table S2.2).

Archaeal sequences were clustered into 74 ASVs and classified into 3 formally described archaeal phyla and 1 candidate phyla. In all samples, *Crenarchaeota* was mainly most predominant, and the relative abundance analysis showed that *Crenarchaeota* accounted for more than 10%, except for sample R1-1 (8.3%), R1-2 (2.4%), R2-2 (9.3%) and R4-2 (6.0%; Fig. 2.6 and Table S2.2). *Crenarchaeota* was predominated by class *Nitrososphaeria* (2.4–74.3%, mainly *Nitrosopumilales* (0.8–74.1%); Table S2.2).

Relative abundance of *Planctomycetota* was positively correlated with

Acidobacteriota (R = 0.73, $p < 0.001$; Fig. 2.7 and Table 2.3). *Alphaproteobacteria* was also positively correlated with *Gammaproteobacteria* (R = 0.87, $p < 0.001$), *Desulfobacterota* (R = 0.80, $p < 0.001$), *Verrucomicrobiota* (R = 0.80, $p < 0.001$), *Dependentiae* (R = 0.80, $p < 0.001$), Candidate division *NB1-j* (R = 0.78, $p < 0.001$), *Actinobacteriota* (R = 0.72, $p < 0.001$) and *Myxococcota* (R = 0.72, $p < 0.001$). There was positively correlation between *Gammaproteobacteria and Desulfobacterota* (R = 0.80, $p < 0.001$), Candidate division NB1-j (R = 0.77, $p < 0.001$), *Bacteroidota* (R = 0.75, $p < 0.001$) and Myxococcota (R = 0.72, $p < 0.001$; Fig. 2.7 and Table 2.3). *Actinobacteriota* and *Desulfobacterota* were also positively correlated with Candidate division NB1-j (0.2–3.3%), Candidate division *Verrucomicrobiota* and *Myxococcota* (Fig. 2.7 and Table 2.3). On the other hand, *Crenarchaeota* was negatively correlated with Candidate division Verrucomicrobiota (R = -0.81, $p < 0.001$), *Alphaproteobacteria* (R = -0.76, $p < 0.001$), *Acidobacteriota* (R = -0.71, $p < 0.001$) and *Planctomycetota* (R = -0.69, $p < 0.001$; Fig. 2.7 and Table 2.3).

Table 2.2. Value of alpha-diversity estimates. Data were normalized by randomly subsampling to 1596 reads.

Samples	observed features	Chao1	Ace	Shannon	Simpson
A1 (Sea-ice zone)-1	448	1054	1191	6.7	0.96
A1 (Sea-ice zone)-2	323	849	889	5.5	0.93
A1 (Sea-ice zone)-3	246	676	719	4.5	0.87
A1 (Sea-ice zone)-4	204	497	514	3.9	0.80
A2 (Amundsen polynya center)-1	556	1501	1627	7.5	0.97
A2 (Amundsen polynya center)-2	400	862	1004	6.0	0.90
A2 (Amundsen polynya center)-3	505	1588	1606	7.1	0.96
A2 (Amundsen polynya center)-4	470	1278	1444	6.8	0.95
A3 (DIS)-1	436	1083	1112	6.5	0.95
A3 (DIS)-2	406	995	1163	6.1	0.93
A3 (DIS)-3	339	912	1072	5.6	0.92
A3 (DIS)-4	396	1030	1179	6.2	0.94
A4 (PIIS)-1	472	880	962	7.7	0.99
A4 (PIIS)-2	399	866	1009	6.5	0.94
A4 (PIIS)-3	403	1071	1194	6.0	0.91
A4 (PIIS)-4	282	644	720	5.1	0.88
R1 (DIT, Tip)-1	656	1522	1808	8.4	0.99
R1 (DIT, Tip)-2	610	1480	1633	8.3	0.99
R2 (NIS)-1	425	1076	1123	7.0	0.97
R2 (NIS)-2	498	1201	1340	7.5	0.98
R3 (DIT)-1	393	952	1022	6.3	0.94
R3 (DIT)-2	400	1028	1112	6.0	0.93
R4 (HGT)-1	605	1403	1486	7.9	0.99
R4 (HGT)-2	574	1252	1494	7.9	0.99
R5 (RIS)-1	486	1136	1289	6.7	0.93
R5 (RIS)-2	478	1237	1249	6.6	0.92

Table 2.3. Spearman's correlation analysis between taxa. Red color indicate positive correlations (R > 0.4), blue indicate negative correlations (R < -0.4) and white indicate not having correlations.

Taxa	*Crenarchaeota*	*Planctomycetota*	*α-proteobacteria*	*γ-proteobacteria*	*Bacteroidota*	*Actinobacteriota*	*Desulfobacterota*	*Acidobacteriota*	*NB1-j*	*Verrucomicrobiota*	*Dependentiae*	*Chloroflexi*	*Dadabacteria*	*Patescibacteria*	*Myxococcota*	*Marinimicrobia_(SAR406 clade)*
Crenarchaeota		R = -0.69 p < 0.001	**R = -0.76** **p < 0.001**	R = -0.63 p < 0.001	R = -0.62 p < 0.001	R = -0.49 p = 0.012	R = -0.67 p < 0.001	**R = -0.72** **p < 0.001**	R = -0.56 p = 0.003	**R = -0.81** **p < 0.001**	R = -0.6 p = 0.001	R = 0.32 p = 0.11	R = 0.25 p = 0.21	R = -0.55 p = 0.004	R = -0.54 p = 0.004	R = -0.39 p = 0.046
Planctomycetota			R = 0.26 p = 0.20	R = 0.14 p = 0.50	R = 0.37 p = 0.06	R = -0.02 p = 0.93	R = 0.33 p = 0.10	**R = 0.73** **p < 0.001**	R = 0.12 p = 0.56	R = 0.41 p = 0.04	R = 0.16 p = 0.43	R = -0.35 p = 0.08	R = -0.25 p = 0.22	R = 0.66 p < 0.001	R = 0.09 p = 0.68	R = 0.37 p = 0.06
α-proteobacteria				**R = 0.87** **p < 0.001**	R = 0.59 p = 0.001	**R = 0.72** **p < 0.001**	**R = 0.8** **p < 0.001**	R = 0.47 p = 0.015	**R = 0.78** **p < 0.001**	**R = 0.8** **p < 0.001**	**R = 0.8** **p < 0.001**	R = -0.39 p = 0.047	R = -0.33 p = 0.09	R = 0.39 p = 0.05	**R = 0.72** **p < 0.001**	R = 0.29 p = 0.15
γ-proteobacteria					**R = 0.75** **p < 0.001**	R = 0.62 p < 0.001	**R = 0.8** **p < 0.001**	R = 0.29 p = 0.15	**R = 0.77** **p < 0.001**	R = 0.69 p < 0.001	R = 0.65 p < 0.001	R = -0.41 p = 0.037	R = -0.36 p = 0.07	R = 0.1 p = 0.64	**R = 0.72** **p < 0.001**	R = 0.27 p = 0.19
Bacteroidota						R = 0.31 p = 0.12	**R = 0.72** **p < 0.001**	R = 0.23 p = 0.26	R = 0.56 p = 0.003	R = 0.58 p = 0.002	R = 0.35 p = 0.08	R = -0.45 p = 0.02	R = -0.33 p = 0.09	R = 0.11 p = 0.61	R = 0.49 p = 0.011	R = 0.44 p = 0.025
Actinobacteriota							R = 0.58 p = 0.002	R = 0.29 p = 0.15	**R = 0.7** **p < 0.001**	**R = 0.77** **p < 0.001**	R = 0.62 p < 0.001	R = -0.12 p = 0.54	R = -0.36 p = 0.07	R = -0.06 p = 0.76	**R = 0.72** **p < 0.001**	R = 0.08 p = 0.70
Desulfobacterota								R = 0.37 p = 0.06	**R = 0.83** **p < 0.001**	**R = 0.76** **p < 0.001**	R = 0.65 p < 0.001	R = -0.55 p = 0.003	R = -0.67 p < 0.001	R = 0.23 p = 0.26	**R = 0.73** **p < 0.001**	R = 0.51 p = 0.008
Acidobacteriota									R = 0.32 p = 0.11	R = 0.61 p = 0.001	R = 0.45 p = 0.02	R = -0.18 p = 0.39	R = -0.14 p = 0.49	R = 0.62 p < 0.001	R = 0.43 p = 0.029	R = 0.19 p = 0.36
NB1-j										**R = 0.74** **p < 0.001**	R = 0.67 p < 0.001	R = -0.54 p = 0.004	R = -0.58 p = 0.002	R = 0.08 p = 0.70	R = 0.69 p < 0.001	R = 0.55 p = 0.003
Verrucomicrobiota											**R = 0.7** **p < 0.001**	R = -0.3 p = 0.14	R = -0.44 p = 0.024	R = 0.32 p = 0.11	**R = 0.72** **p < 0.001**	R = 0.29 p = 0.15
Dependentiae												R = -0.3 p = 0.13	R = -0.17 p = 0.41	R = 0.46 p = 0.019	R = 0.53 p = 0.005	R = 0.22 p = 0.27
Chloroflexi													R = 0.69 p < 0.001	R = -0.25 p = 0.22	R = -0.18 p = 0.38	R = -0.53 p = 0.005
Dadabacteria														R = 0.03 p = 0.89	R = -0.46 p = 0.019	R = -0.37 p = 0.15
Patescibacteria																R = 0.28 p = 0.16
Myxococcota																R = 0.22 p = 0.29

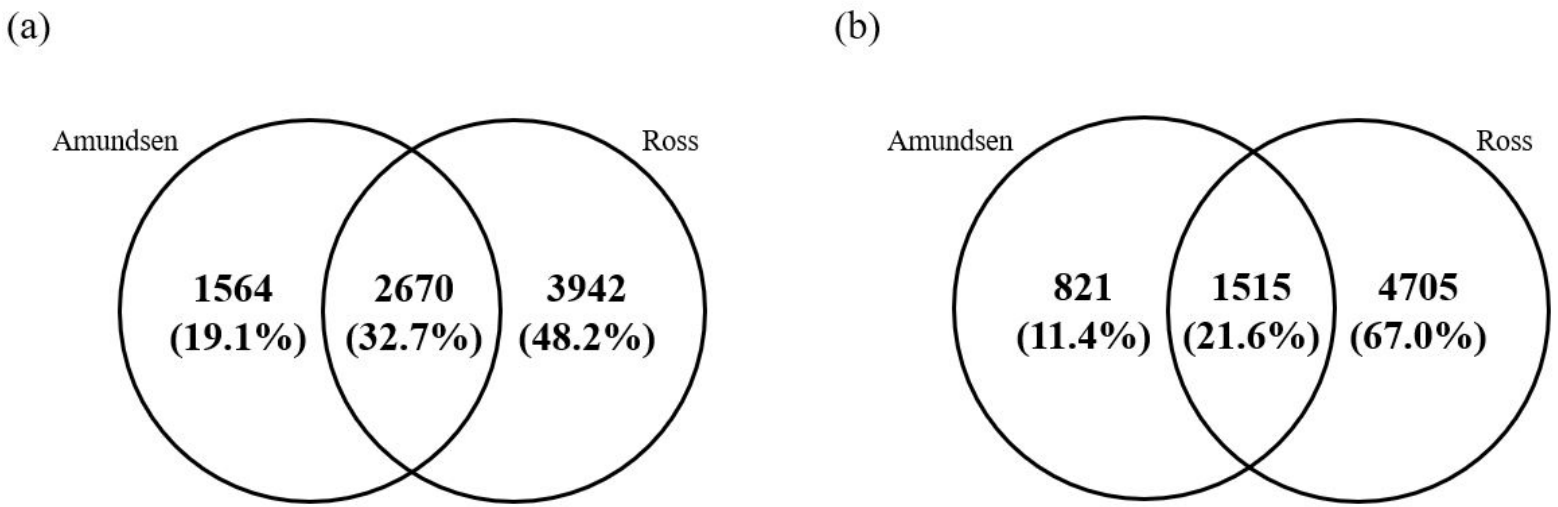


Figure 2.4. Venn diagram showing the unique and common ASVs between the Amundsen Sea and Ross Sea. (a) the unique and common ASVs between all stations of the Amundsen Sea (A1-A4) and Ross Sea (R1-R5) (b) the unique and common ASVs between inner continental shelf stations of the Amundsen Sea (A3 and A4) and Ross Sea (R2-R5).

	A1	A2	A3	A4	R1	R2	R3	R4	R5
A1	550	57	53	58	39	62	39	53	53
A2	57	399	55	59	26	52	34	43	51
A3	53	55	393	48	27	47	34	47	32
A4	58	59	48	495	34	45	42	56	45
R1	39	26	27	34	590	31	42	80	58
R2	62	52	47	45	31	470	41	73	39
R3	39	34	34	42	42	41	731	62	93
R4	53	43	47	56	80	73	62	1067	76
R5	53	51	32	45	58	39	93	76	884

Figure 2.5. Shared ASVs between the samples. The numbers correspond to the shared ASVs. The redder color, the more ASVs are shared and the bluer color, the loss ASVs are shared.

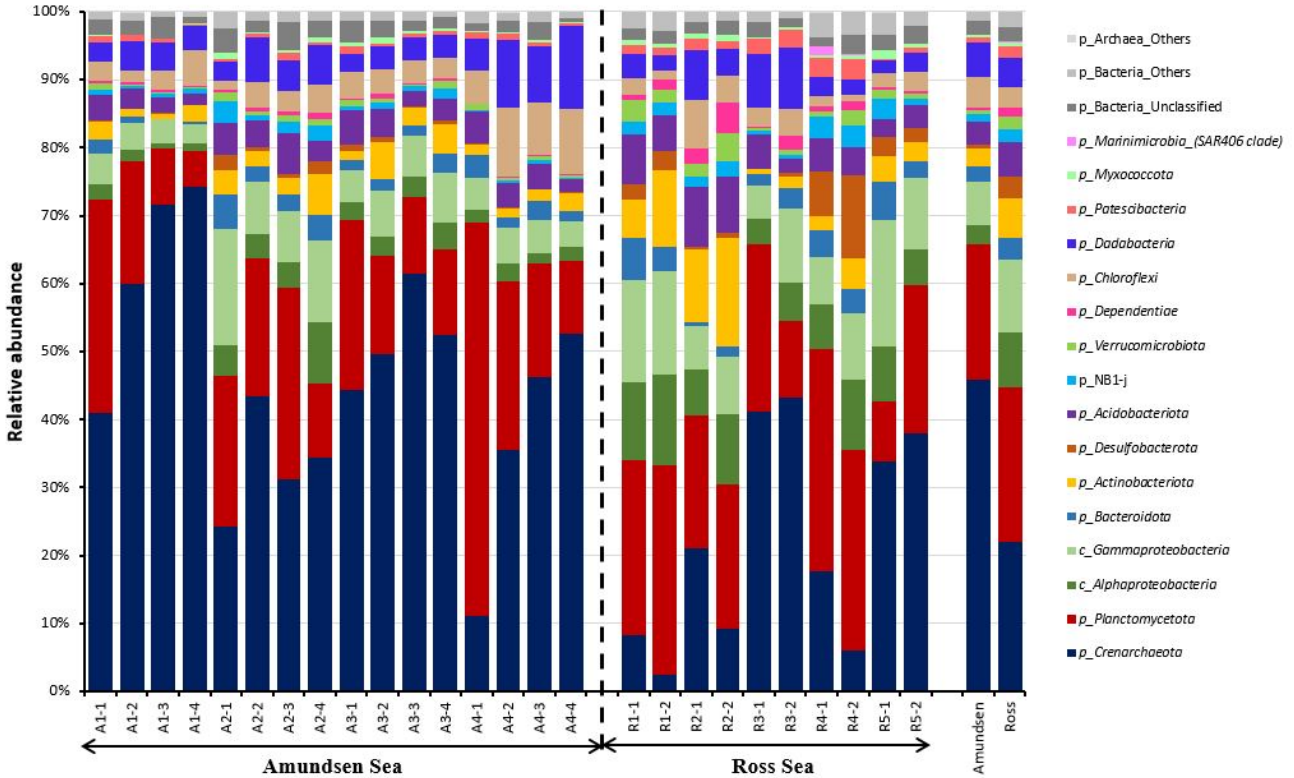


Figure 2.6. Taxonomic composition of bacterial and archaeal phyla that appeared at ≥ 1% relative abundance at any of the samples. Each sample was analyzed in duplicate or quadruplicate and relative abundance of all replicate in each sample is shown. The description of sample names is the same as in Table 2.1.

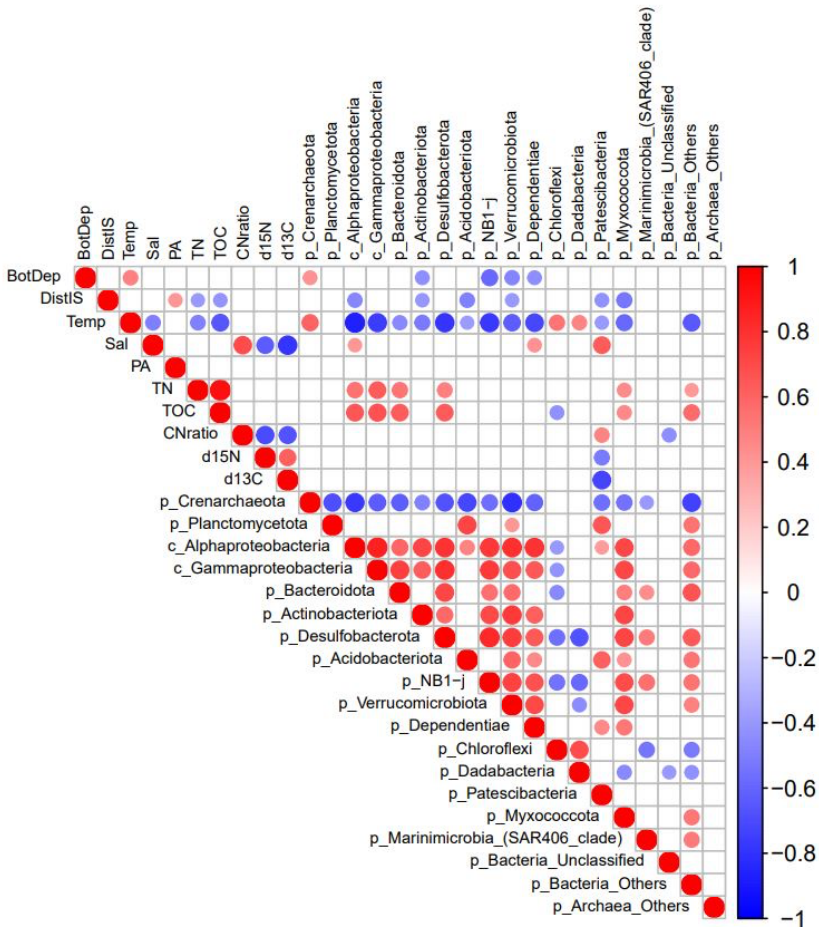


Figure 2.7. Spearman's correlation analysis between environmental variables and taxa of all replicates. Only significant correlations ($p \leq 0.05$) are indicated. Red color indicates positive correlations and blue color indicates negative correlations.

2.3.3 Spatial variation of prokaryotic community composition

The ANOSIM analysis indicated that the prokaryotic community in the Amundsen Sea was different from those in the Ross Sea ($R = 0.51$, $p = 0.001$). While archaea were found to be more dominant in the Amundsen Sea, bacteria were relatively more abundant in the Ross Sea. Phylum *Crenarchaeota* within the class *Nitrososphaeria* in the Amundsen Sea (11.1–74.3%) were more abundant than in the Ross Sea (2.4–43.2%; Fig. 2.8 and Fig. S2.2). In the Ross Sea stations, *Proteobacteria* (8.7–28.7%) within the class *Alphaproteobacteria* (3.8–13.3%) and *Gammaproteobacteria* (4.9–18.7%) were more abundant than in the Amundsen Sea (0.6–9.0% and 3.6–12.0%, respectively; Fig. 2.8 and Fig. S2.2). There was also significant difference phylum *Dependentiae*, Candidate division NB1-j, *Patescibacteria* and Candidate division Verrucomicrobiota between in the Amundsen Sea and in the Ross Sea (Fig. 2.8 and Fig. S2.2). At the class level, *Babeliae*, *Chlamydiae*, *Desulfobulbia*, NB1-j, *Polyangia*, *Sa*ccharimonadia and *Thermoleophilia* in the Ross Sea stations were more abundant than in the Amundsen Sea (Fig. S2.2). While the relative abundance of the class JG30-KF-CM66 within *Chloroflexi* (0.9–7.1%) in the Amundsen Sea (0.0–1.1%) were more abundant than in the Ross Sea (0.5–3.1%; Fig. S2.2).

Linear discriminant analysis (LDA) effect size using LEfSe indicated that there were significantly ASVs abundant discrimination between Amundsen Sea and Ross Sea. Eighteen clades were identified to have higher bacterial ASVs abundance in the Amundsen Sea: they were mainly *Planctomycetota*, including *Planctomycetes* (mostly *Rubinisphaeraceae*, *Pirellulaceae*), *Burkholderiales*

(*Gammaproteobacteria*), *Vicinamibacteria* (*Acidobacteriota*) and *Flavobacteriales* (*Bacteroidota*; Fig. S2.3). However, 24 ASV clades were more abundant in the Ross Sea than in the Amundsen Sea, in where Candidate division NB1-j was mostly different ASVs (Fig. S2.3). In the Ross Sea, there were also more abundant *Planctomycetota* including *Phycisphaerae* (mainly *Phycisphaeraceae*) and *Planctomycetes* (mainly *Pirellulaceae*). *Alphaproteobacteria* (mainly *Puniceispirillales*), *Chloroflexi* (mainly JG30-KF-CM66), *Dependentiae* (mainly *Babeliales*), *Bacteroidota* (mainly *Crocinitomicaceae*), and *Desulfobacterota* (mainly *Bradymonadales*; Fig. S2.3).

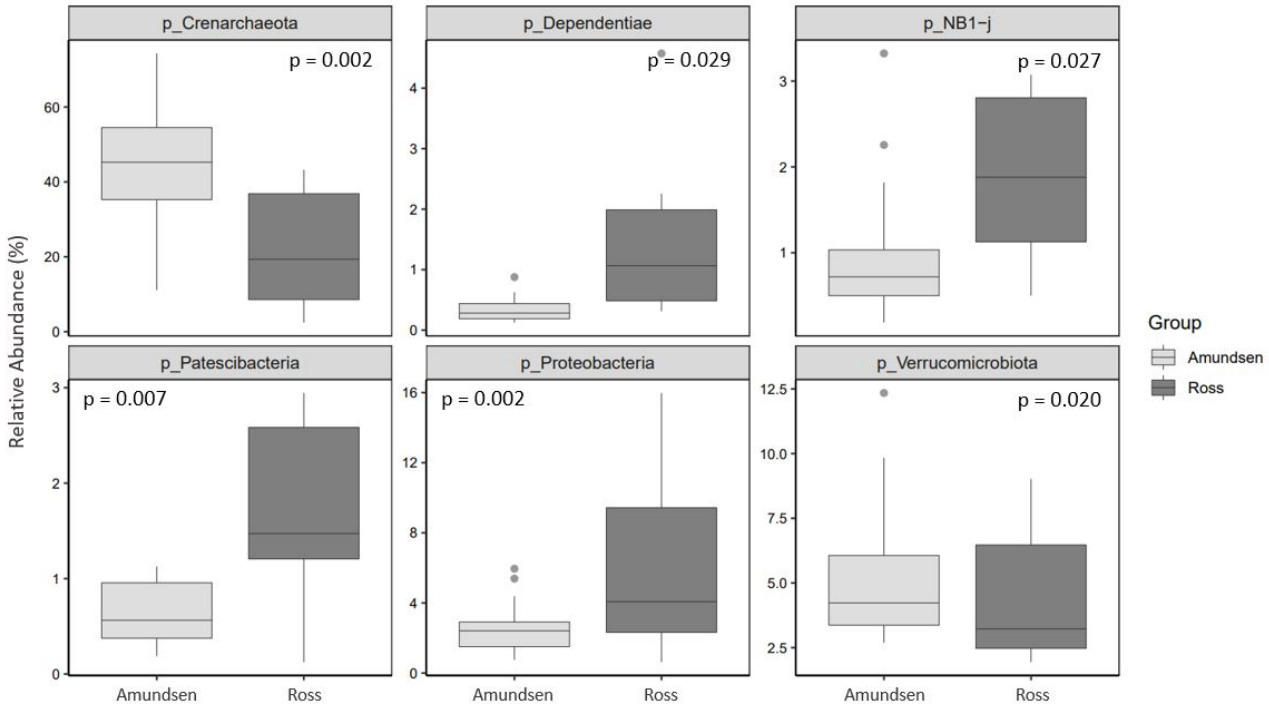


Figure 2.8. Boxplots of significant difference phylum between in the Amundsen Sea and in the Ross Sea.

2.3.4 Alpha- and Beta-diversity

Variations were observed in prokaryotic diversity among the different stations. Overall, species richness (i.e., ACE, $p < 0.05$) and evenness (i.e., Shannon, $p < 0.05$ and Simpson, $p < 0.05$) were higher in the Ross Sea compared to the Amundsen Sea (Table 2.2 and Figure 2.9). Estimates of Chao1 were also turned out to be significant difference between the Amundsen Sea and Ross Sea (Table 2.2 and Figure 2.9; $p < 0.05$). The index of Chao1 varied from 497 (sample A1 (Sea-ice zone)-4) to 1588 (sample A2 (Amundsen polynya center)-3) and that of ACE varied from 514 (sample A1 (Sea-ice zone)-4) to 1808 (sample R1 (DIT, Tip)-1; Table 2). Shannon diversity index varied from 3.9 (sample A1 (Sea-ice zone)-4) to 8.4 (sample R1 (DIT, Tip)-1) and Simpson diversity index varied from 0.80 (sample A1 (Sea-ice zone)-4) to 0.99 (sample R1 (DIT, Tip)-1). Station R1 (DIT, Tip) and R3 (DIT) in the Ross Seas, showed the highest species richness and presented the lowest diversity. Rarefaction curves for all samples indicated that number of sequence reads would reveal more observed features and increase diversity (Fig. 2.10).

To determine of beta-diversity, all replicate samples were analyzed using NMDS (Fig. 2.11). Prokaryotic community was distinguished according to the coordinate NMDS1. It was showed that distribution of the prokaryotic community had a difference between the Amundsen Sea samples and the Ross Sea ones.

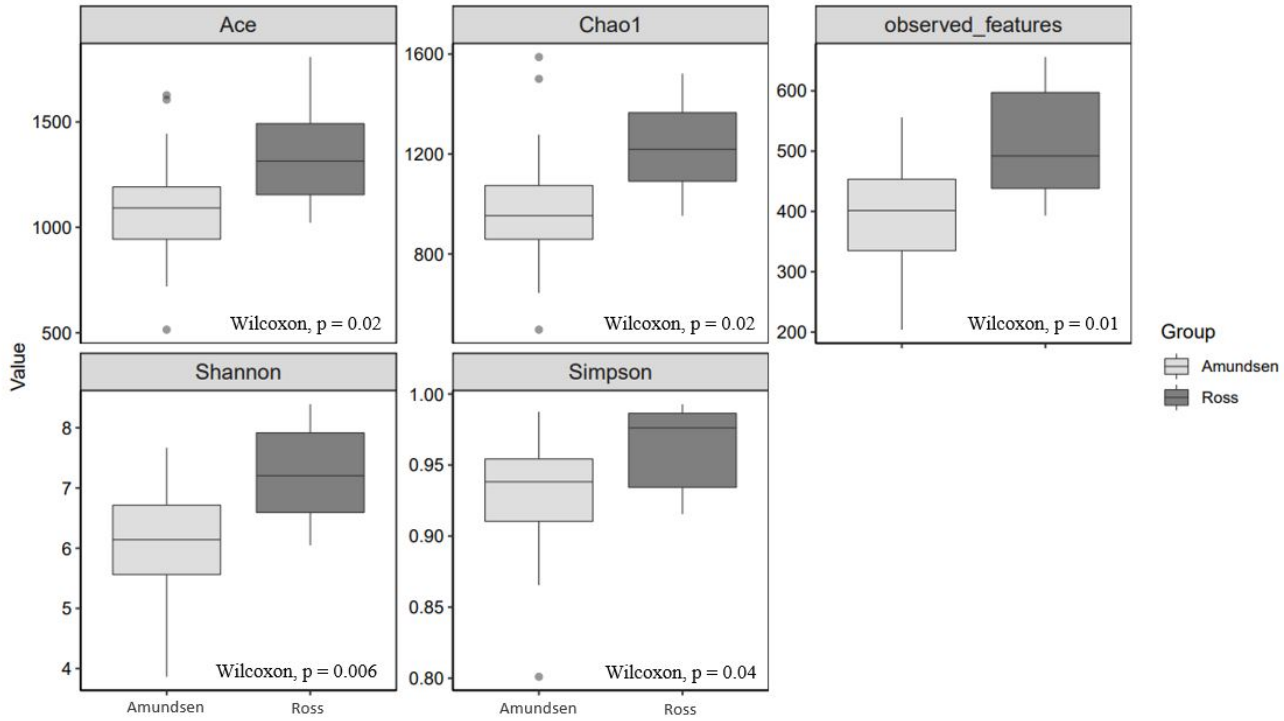


Figure 2.9. Boxplots of alpha diversity between in the Amundsen Sea and in the Ross Sea.

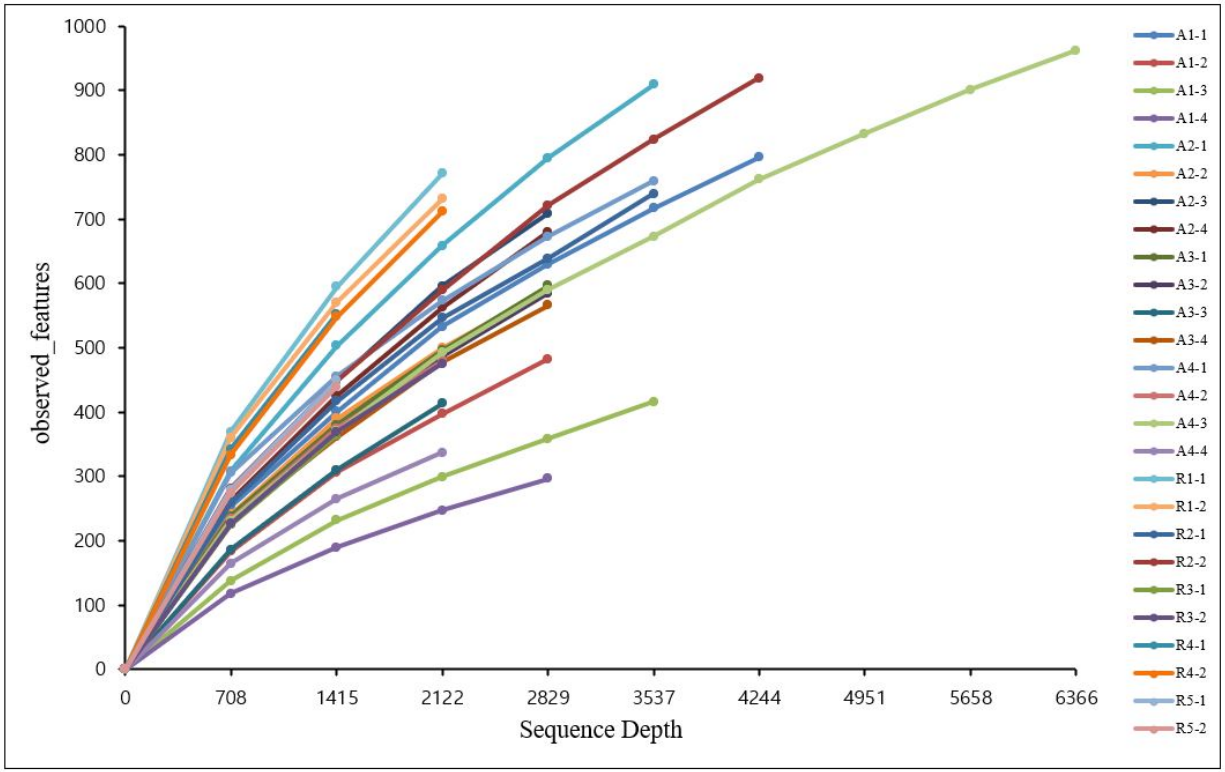


Figure 2.10. Rarefaction curve of all replicates.

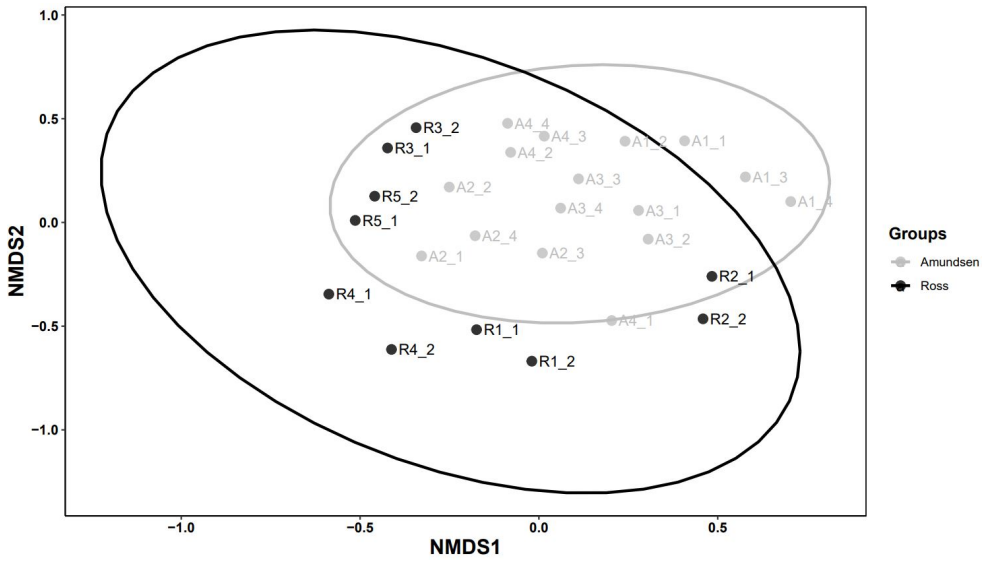


Figure 2.11. Non-metric multidimensional scaling (NMDS) using Bray-Curtis similarity among all samples.

2.3.5 Prokaryotic differentiation and their relationship with environmental properties

Relative abundance of *Crenarchaeota* were positively correlated with Temperature (R = 0.60, p = 0.001; Fig 2.7). *Alpha*- (R = -0.87, p < 0.001) and *Gamma-Proteobacteria* (R = -0.76, p < 0.001) were negatively correlated with Temperature, and positively correlated with TN and TOC (R > 0.50, p < 0.001). And predominant bacteria phylum *Planctomycetota* showed no correlation with environmental factors. Temperature was negatively correlated with *Actinobacteriota* (R = -0.51, p < 0.05) and was positively correlated with *Dadabacteria* (R = 0.47, p = 0.02; Fig 2.7).

The relationship between environmental factors and alpha diversity was measured by Spearman's rank correlation (Table 2.4). Prokaryotic richness (Chao1 and Ace) and evenness (Shannon and Simpson) were both negatively correlated with temperature. TN and TOC showed positive correlation with richness (Chao1 and Ace), moreover, TOC indicated positive correlation with evenness (Shannon).

Table 2.4. Spearman's correlation analysis between environmental factors and alpha-diversity.

Factor	Observed_features	Chao1	Ace	Shannon	Simpson
Bottom Depth (m)	-0.43*	-0.4*	-0.36	-0.37	-0.16
Temperature (℃)	-0.65**	-0.67**	-0.65**	-0.59*	-0.4*
Salinity (PSU)	0.18	0.1	0.12	0.26	0.27
TN (%)	0.34	0.43*	0.44*	0.28	0.2
TOC (%)	0.48*	0.51*	0.52*	0.42*	0.3
C/N Ratio	0.03	-0.12	-0.1	0.17	0.21
$\delta^{15}N$ (‰)	-0.15	0.03	-0.02	-0.23	-0.24
$-\delta^{13}C$ (‰)	-0.01	-0.06	0.04	-0.17	-0.35

*, $p < 0.05$; **, $p < 0.001$

2.3.6 Predicted function differentiation

Prokaryotic functions between the Amundsen Sea and the Ross Sea were predicted by PICRUSt2.0 at MetaCyc level 2. Overall, 407 MetaCyc pathways were predicted and the results showed that all samples had a high number of sequences assigned to biosynthesis such as nucleoside and nucleotide biosynthesis; amino acid biosynthesis; cofactor, prosthetic group, electron carrier, and vitamin biosynthesis; fatty acid and lipid biosynthesis; and carbohydrate biosynthesis (Table S2.3). All samples also depicted functional categories such as C1 compound utilization and assimilation, inorganic nutrient metabolism, nucleoside and nucleotide degradation, fermentation, TCA cycle, respiration, pentose phosphate pathways, glycolysis (Table S2.3).

There were statistically significant differences ($p < 0.05$ by t-test) between two groups (Fig. 2.12). The mean relative abundance of TCA cycle, nucleoside and nucleotide biosynthesis, aromatic compound biosynthesis pathways were higher in the Amundsen Sea, while fermentation, inorganic nutrient metabolism, nucleotide and nucleotide degradation, C1 compound utilization and assimilation, and amine and polyamine biosynthesis pathways were strengthened in the Ross Sea.

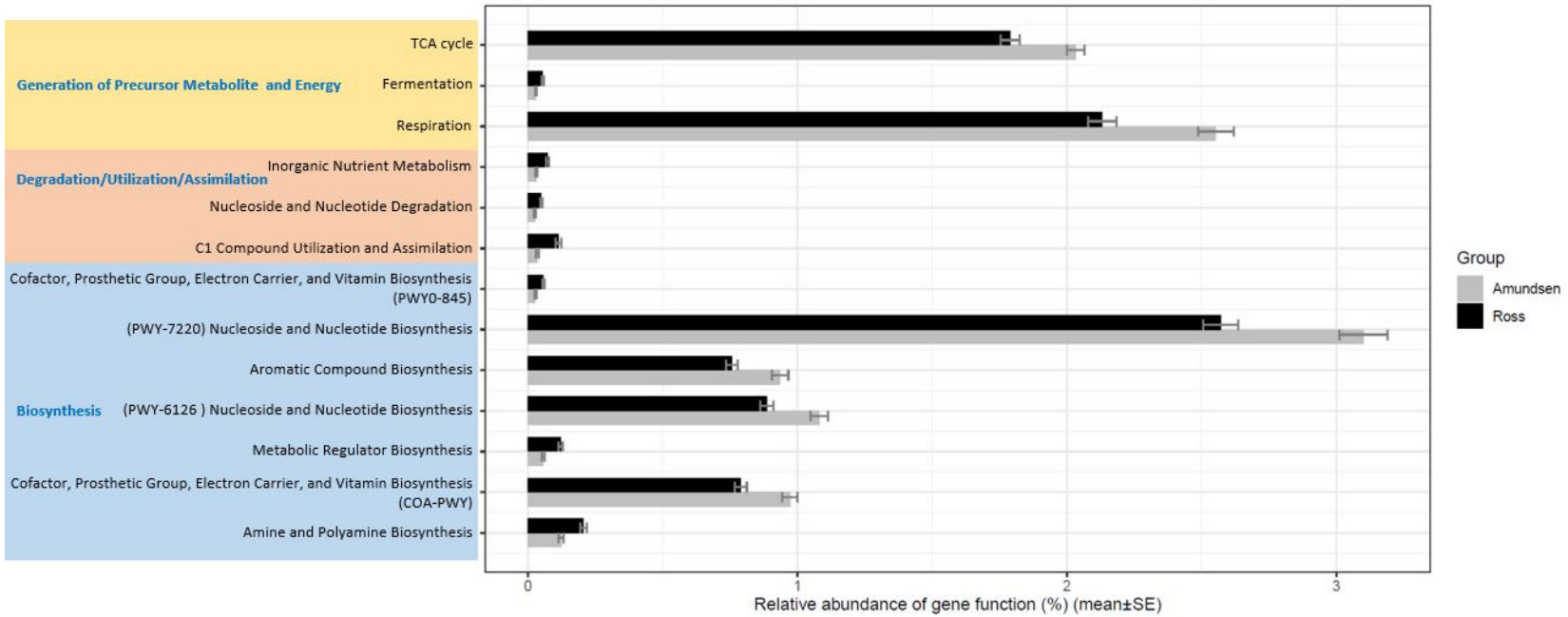


Figure 2.12. Significant differences in the relative abundance of PICRUSt2.0-generated pathways at level 2 between the Amundsen Sea and the Ross Sea ($p < 0.05$).

2.4 Discussion

To our best knowledge, this is the first comprehensive comparison of prokaryotic communities in surface sediments at edges of various ice shelves in west Antarctica (Amundsen Sea and Ross Sea). Overall, it was identified that there were similar taxonomic groups in all stations but prokaryotic community structure and alpha-diversity values have differed between the stations in the Amundsen Sea (more abundant *Crenarchaeota*) and those in the Ross Sea (more abundant *Proteobacteria*), indicating relationships with the distinct physical (e.g., temperature and salinity) and chemical environmental data of the surface sediments (e.g., TN and TOC concentration). Moreover, the water mass in the study stations, influenced by the warmer and less saline waters in the Amundsen Sea and the cold and saline waters in the Ross Sea, can influence the benthic community structure. Phytoplankton blooms can also affect to the transport of organic matter to the benthic sediments. It was proved that phytoplankton-derived organic matter contributes benthic prokaryote communities and increased energy sources (organic matter) support the high prokaryotic abundance (Carr *et al*., 2013; Ruff *et al*., 2014). The $\delta^{13}C$–values measured in the surface sediments ranged from -25.9 to -22.7‰ (average -24.1‰) and the $\delta^{15}N$–values range from 2.8 to 5.1‰ (average 3.7‰). The data in this study does suggest that phytoplankton may are contributors of organic matter source to benthic sediments (Meyers, 1994; Xiao *et al*., 2010). The C/N ratio generally remained around 7.9 except station A1–A3 (average 6.5), which is higher than the Redfield ratio (6.625) and falls within the range of marine source (5–10; Meyers, 1994). Thus, these results indicate that marine organic matter from phytoplankton or

melting sea ice has been degraded due to higher N-consumption.

In the classification of archaea, there are some problems in the SILVA database because the proposed *Thaumarchaeota* phylum is still classified as *Crenarchaeota* (Kan *et al*., 2011). Dharmesh *et al*. (2012) compared three different databases (SILVA, RDP, and EzTaxon-e) for identification of archaea classification using their own fasta file. The archaeal sequences was assigned to be *Crenarchaeota* (95.8%) in SILVA, but in EzTaxon-e, most of the sequences classified to *Thaumarchaeota* (96.4%), and no sequences were belonged to *Crenarchaeota* (Dharmesh *et al*., 2012).

Crenarchaeota (reclassified *Thaumarchaeota*) have been reported an extremely common archaeon living in Antarctic environments (Murray *et al*., 1998; Gillan and Danis, 2007; Hernandez *et al*., 2015). *Crenarchaeota* abundance was relatively higher in this study when compared with the Learman *et al*. (2016) which were investigated in mid continental shelf. These observations are consistent with data from Liu *et al*. (2018) which confirmed that *Crenarchaeota* population became abundant at inshore than at mid-shelf stations in the South Atlantic. In this study, the abundance of *Crenarchaeota* was also relatively higher than that of Cho *et al.*, (2020), but the same tendency showed that *Crenarchaeota* was less abundant in the polynya sites (A2) than non-polynya sites (A1, A3 and A4, $p < 0.05$ by u-test). *Crenarchaeota* are also ubiquitous with the oligotrophic marine environment and lives by oxidizing ammonia to nitrite (Leininger *et al*., 2006: Pester *et al*., 2011; Hatzenpichler, 2012). The most abundant archaeal phylum *Crenarchaeota* was mainly Ammonia-oxidizing archaea (AOA) *Nitrososphaeria*-type in the Amundsen Sea. AOA are were only known to be in the *Crenarchaeota* (Beman *et al*., 2010), but Parks *et al*. (2020)

suggests that AOA is reclassified into the class *Nitrososphaeria* within the *Crenarchaeota* phylum. The relative abundance of *Crenarchaeota* have shown positive correlation with temperature ($p = 0.001$) and negatively correlated with TOC ($p = 0.05$). The environmental conditions such as slightly low TOC and more refractory carbon (e.g., lower $\delta^{13}C$) allow that these organisms flourish in the Amundsen Sea. These result also can be explained as a consequence that *Crenarchaeota* are more abundant with increased sediment age (e.g., higher $\delta^{15}N$ and lower $\delta^{13}C$; Mateos-Rivera *et al*., 2016). Furthermore, AOA activity was known to be increased with temperature, for freshwater, terrestrial, and cultivated AOA (Lehtovirta-Morley, 2018). Thus, the results suggest that *Crenarchaeota* are dominant prokaryotic group at non-polynya sites in the Amundsen Sea where input of C_{org} from the water mass is less and temperature is higher.

Proteobacteria was reported in several marine, benthic environments (Sogin *et al*., 2006; Danovaro *et al*., 2010; Williams *et al*., 2010; Zinger *et al*., 2011) and Antarctic sediments (Bowman *et al*., 2003; Bowman and McCuaig, 2003; Ruff *et al*., 2014; Carr *et al*., 2013; Learman *et al*., 2016; Li et al., 2019; Li et al., 2020), which is also indicated by this study. Many members of that phylum which include metabolically diverse groups such as chemolithotrophs, heterotrophs and phototrophs, can be well adapted to ecosystems with limited access to organic matter and nutrient (Hodkinson, Coulson and Webb 2003; Zumsteg *et al*.2012) by changing environment (Mateos-Rivera *et al*., 2016). *Proteobacteria* was the predominant bacterial phylum in all stations, and more abundant in the Ross Sea. At the class level, a noticeable feature is that *Alpha* and *Gammaproteobacteria* abundance was relatively higher in in the Ross Sea than in the Amundsen Sea. These observations

are inconsistent with previous studies known to be dominated by *Gamma* and *Deltaproteobacteria* in the Ross Sea sediments (Bowman and McCuaig, 2003; Baldi *et al*., 2010; Learman *et al*., 2016), but consistent with data from Li *et al*. (2016) which confirmed that *Alpha* and *Gammaproteobacteria* were abundant at inshore in the Ross Sea.

The relative abundance of *Alpha* and *Gammaproteobacteria* have shown negatively correlated with temperature ($p < 0.001$), but positively correlated with TN ($p < 0.05$) and TOC ($p < 0.001$). The environmental conditions such as lower temperature and more labile nitrogen and carbon (e.g., lower $\delta^{15}N$ and higher $\delta^{13}C$) allow predominance of *Proteobacteria* in the Ross Sea (Mateos-Rivera *et al*., 2016; Liu *et al*., 2018).

Planctomycetota were dominant phylum at all stations, and its relative abundance were higher than those of other studies on Antarctic sediments (Baldi *et al*., 2010; Carr *et al*., 2013; Learman *et al*., 2016). A particular group of *Planctomycetota* are autotrophic bacteria using anaerobic ammonium oxidation (Anammox) reaction which is supplied by various respiratory and/or dissimilatory pathways such as aerobic respiration, sulfate reduction and these reactions play a key role in the global nitrogen cycle by releasing fixed nitrogen back to the atmosphere as N_2 (Devol, 2003; Engström *et al*., 2009).

The dominant phylum *Bacteroidota*, mainly the order *Flavobacteriales*, are very diverse and are widespread in marine environment, and they degrade complex carbon including phytoplankton cells and phytoplankton-derieved particles (Kirchman, 2002; Bowman and Nichols, 2005; Gómez-Pereira *et al*., 2012; Teeling *et al*., 2012). Phylum *Actinobacteriota* and *Acidobacteriota* were also dominant in

study stations, and may play important roles in the decomposition of organic matters (Jensen *et al*., 2005; Kielak *et al*., 2016). *Actinobacteriota* have ability to break down organic compounds in the carbon cycle (Duran *et al*., 2015) and some *Acidobacteriota* are able to fix nitrogen and correlated with sulfur cycling in deep-sea sediments (Flieder *et al*., 2021). *Dadabacteria* (formerly SBR1093) belong to candidate phylum that have been found in numerous environments but have not been extensively evaluated for their potential contributions to biogeochemical circulation. *Dadabacteria* are thought to have the potential capability to degrade dissolved organic matter in microorganisms, specifically peptidoglycan and phospholipids (Graham and Tully, 2021).

In the PICRUSt2.0 analysis, bacteria were more similar in functional aspects as predicted by the metabolic MetaCyc pathways in contrast to having different community compositions and abundance in the Amundsen Sea and Ross Sea. The proportion of gene families related with ecological function were highest for nucleoside and nucleotide biosynthesis, followed by amino acid biosynthesis and carbohydrate biosynthesis. The organic compounds, nucleosides and nucleotides, are not only important components of all living organisms, but are also involved in several basic biological processes. “The amino acid synthesis pathway constitutes a significant portion of bacterial metabolic activity during growth in minimal medium, and amino acids are not only protein precursors, but also precursors to many other important compounds such as nucleotides (Reitzer, 2009).” Thus, these results represent that the sediment bacterial community have a similar role such as the significant ecological function in carbon and nitrogen cycling in the harsh Antarctic marine environment.

In this study, analyses revealed that there are differences in benthic prokaryote diversity and community structure between the Amundsen Sea and Ross Sea. The benthic prokaryotic distribution appeared to be strongly dependent on the quality and quantity of sediment organic matter which are influenced by water mass, sub-ice shelf circulations, primary production and glacier melting at edges of ice shelves in the west Antarctica.

CHAPTER 3

Metagenomic analysis of surface sediment in the Ross Sea (Antarctica)

3.1 Introduction

Prokaryotes exist almost in every environment including an extreme environment such as low temperature and high pressures, and play an important role in the biogeochemical cycling of carbon, nitrogen, sulfur and various metals (Azam and Malfatti, 2007; Falkowski *et al*., 2008). Deep-sea marine sediment occupied more than two-thirds of the Earth's surface, and this environment is considered to be an extreme environment. In the subseafloor sediment environments, the prokaryotes are continuously exposed to harsh conditions in low temperature, high pressure and low organic matter availability (Kennedy *et al*., 2008), thus, they have undergone various adaptation for the growing and survival under extreme environment and are expected to have enormous genetic diversity.

The physicochemical parameters of the oceans are mainly influenced by the characteristics of ocean current circulation and water mass. Water mass mainly affecting the Ross shelf is dense Shelf Water (SW) with low temperature and high salinity, while Circumpolar Deep Water (CDW) being characterized by higher temperature and lower salinity and Antarctic Bottom Water (AABW) with intermediate temperature between SW and CDW affect to continental slope and deep basin of the Ross Sea, respectively (Jenkins *et al*., 2016). The Ross Sea continental shelf is one of the most spatially productive marginal sea in the Southern Ocean due to the development of the Ross Sea polynya with high phytoplankton blooms (Arrigo *et al*., 1998; Smith *et al*., 2012). Hence, it is known that a relatively large amount of organic matter sinks and reaches the bottom sediment n the Ross Sea (Bercovici *et al*., 2017).

Based on these feature, organic matters of the subseafloor sediment in the Ross Sea are considered to be dynamically affected by hydrographic and biological processes, inevitably affecting the structures and metabolic functions of benthic prokaryotic communities. The benthic prokaryotic structure, dynamics and function are important in understanding the roles of the prokaryote in terms of ecological and biogeochemical functioning. However, most of identification and characterization of prokaryotes is still unexplored because of limitations associated with cultivation. To overcome these limitations for investigating of the prokaryotic community structures, metagenomic sequencing approaches have been applied to rapidly characterize and understand of the prokaryotic communities *in situ*. The metagenomic sequencing also contribute to directly obtain the functional potential of the prokaryotes in nature. Therefore, these metagenomic approaches can provide substantial insights into the profiles of an accurate numerous prokaryotic communities and understanding of metabolism potential and putative ecological functions of the prokaryotes.

In this study, to gain insights into metabolic functions and taxonomic structures of benthic prokaryotic communities, metagenomic analyses using shotgun sequencing as well as investigations of environmental variables were performed on the surface sediments in the Ross Sea. The present study is the first metagenomic study to assess the functional potential of the benthic prokaryotic community and to identify the active community members in the Ross Sea.

3.2 Materials and Methods

3.2.1 Sediment sample collection and environmental parameters

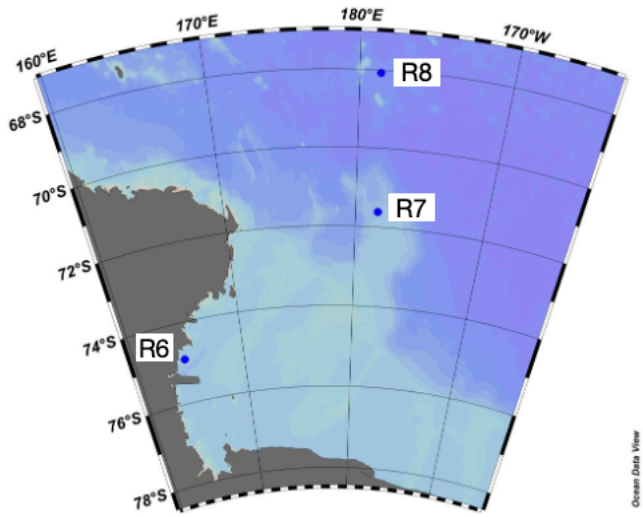
Sediment samples were collected in 2015 during two research cruises on the *IBRV* Araon at the 3 stations, all of which are located within the Ross Sea, Antarctica (Fig. 3.1 and Table 3.1). The samples were obtained using a box corer at stations with different water depths 543 m (R6, inner continental shelf), 1083 m (R7, continental slope) and 3050 m (R8, deep basin). Surface sediments (0–2 cm depth) were sub-sampled and all the samples were frozen immediately at -80 °C on board the ship and later transferred to a land-based laboratory and stored at -80 °C until further analysis. And the environmental data (bottom temperature, bottom salinity, PA, TN, TOC, C/N ratio, $\delta^{15}N$, $\delta^{13}C$) were analyzed in the same way as those analyzed in Chapter 2.

3.2.2 Pyrosequencing, metagenomic library preparation and shotgun sequencing

For both the 16S rRNA gene pyrosequencing and shotgun sequencing, metagenomic DNAs were extracted in duplicate or quadruplicate for each freeze-dried sediment sample (0.2–0.9 g) using FastDNA™ SPIN Kit for soil (MP Biomedicals®), according to the manufacturer's instruction. Negative control was also performed for checking contamination.

Amplification of extracted DNA were performed in the same way as those

(a)



(b)

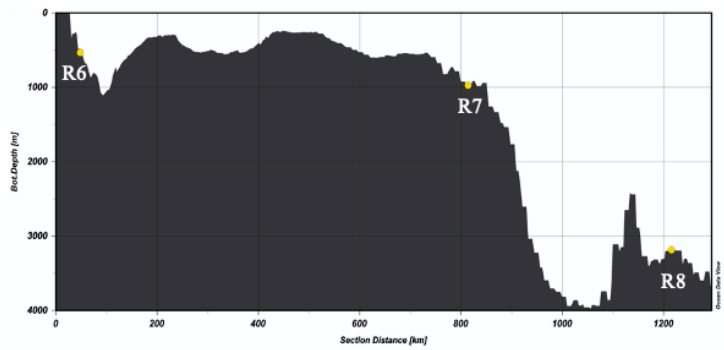


Figure. 3.1. (a) Map of 3 sampling sites in the Ross Sea and (b) bathymetric map.

Table 3.1. Description of sampling stations, prokaryotic abundances (PA) and environmental parameters in sediment samples obtained in the Ross Sea. The value of PA means averages of duplicate measure value ± standard deviation. IS:Inner Continental Shelf, CS: Continental Slope, DB: Deep Basin.

Sample names	Date of sampling	Latitude	Longitude	Bottom Depth (m)	Bottom Temperature (℃)	Bottom Salinity	PA (x 10^8 cells/g of wet sediment, n=2)	TN (%)	TOC (%)	C/N Ratio	$\delta^{15}N$ (‰)	$\delta^{13}C$ (‰)
R6 (IS)	13-Jan-2015	74°54.83'S	164°31.19'E	543	-1.89*	34.78*	1.64 ± 0.55	0.03	0.14	4.51	0.6	-21.0
R7 (CS)	20-Jan-2015	71°36.99'S	178°17.45'W	1083	0.99*	34.72*	1.76 ± 0.03	0.04	0.67	18.22	2.5	-24.3
R8 (DB)	21-Dec-2015	68°04.08'S	178°37.89'W	3050	0.39*	34.68*	1.26 ± 0.12	0.07	0.43	5.95	5.1	-25.1

*CTD data from adjacent station of each sampling station

analyzed in Chapter 2. All amplicons with different sample-specific barcodes were pooled in equal amount and pyrosequencing was performed in 1/2 region of PicoTiterPlate device on the 454 GS FLX Titanium at the Macrogen Corporation, Korea.

To obtain sufficient DNA (a minimum of 200 ng DNA) for shotgun sequencing, quadruplicate DNA samples for each sediment sample were pooled after DNA extractions. Metagenomic shotgun sequencing libraries were prepared with Illumina's TruSeq Library kit. Each library was sequenced in a single lane using the Illumina HiSeq 2 × 250 bp paired-end technology according to manufacturer's instructions at ChunLab Inc., Korea.

3.2.3 Metagenomic DNA analyses

To analyze and annotate the metagenomic data, the raw fastq reads of samples were uploaded to the Metagenome Rapid Annotation using Subsystem Technology (MG-RAST) server (Glass *et al*., 2010; version 4.0.3). Quality control and annotation pipeline of the samples was carried out the following steps: Adapter sequences were trimmed by a bit-masked k-difference matching algorithm (Jiang *et al*., 2014). Dereplication, artificially duplicated reads filtering, quality filtering (Cox *et al*., 2010), and length filtering (50 bp) were performed. Based on the BLAST-Like Alignment Tool (BLAT; Kent, 2002), metagenomic sequences were compared to those of gene and protein-coding databases. Taxonomic identification was generated using contigLCA algorithm of the M5NR database (Meyer *et al*., 2008) for comparison with multiple metagenomes. And functional identification was done against COGs (Clusters of Orthologous Groups), KOs (KEGG Orthology), NOGs,

and Subsystems with e-value cut-off of $e < 1 \times 10^{-5}$.

3.2.4 Pyrosequencing data analyses

A new amplicon sequence variants (ASVs) methods (Callahan *et al.*, 2017) were used to identify prokaryotic communities. With the QIIME 2 software package (version 0.21.4; Bolyen *et al*., 2018), the sequence reads from pyrosequencing were trimmed, denoised, removed of unknown and chloroplast, and normalized with the lowest reads number 1,596 in all samples for classification. The SILVA reference database (version 138; Quast *et al*., 2013) was used to assign taxonomy of OTUs.

4.3 Results

4.3.1 Environmental Characteristics

Bottom water temperature in the station R6 (IS; -1.9 °C) was significantly lower than that in the station R7 (CS) and R8 (DB) (1.0 °C and 0.4 °C, respectively; Table 3.1 and Fig. 3.2). Bottom water salinity in study stations ranged from 34.68 to 34.78, and station R6 (IS) was the highest bottom water salinity (34.78; Table 3.1 and Fig. 3.2). Prokaryotic abundance of wet sediment samples ranged from 1.3–1.8 $\times 10^8$ cells g^{-1} (Table 3.1 and Fig. 3.2). The station R6 (IS) was the lowest TN (0.03%) and TOC concentration (0.14%; Table 3.1 and Fig. 3.2) among the study stations. The highest TN and TOC concentration was shown in the station R8 (DB; 0.07%) and in the station R7 (CS; 0.67%), respectively (Table 3.1 and Fig. 3.2). The station R6 (IS) presented the lowest C/N ratio (4.5) and station R7 (CS) was the highest C/N ratio (18.2). The δ^{15}N-values ranged from 0.6 to 5.1‰ and the δ^{13}C-values range from -25.1 to -21.0‰ (Table 3.1 and Fig. 3.2). The station R6 (IS) was the highest δ^{13}C–values (-21.0‰), while the lowest δ^{15}N–values (0.6‰) among all stations (Table 3.1 and Fig. 3.2). But, the station R8 (DB) was the lowest δ^{13}C–values (-25.1‰), while the highest δ^{15}N–values (5.1‰) among all stations (Table 3.1 and Fig. 3.2). Principal component analysis (PCA) of environmental variables confirmed that environmental conditions between the study stations were distinguished. Especially, environmental variables in the station R6 (IS) significantly differed from variables in other stations on the first PCA axis (Fig. 3.3).

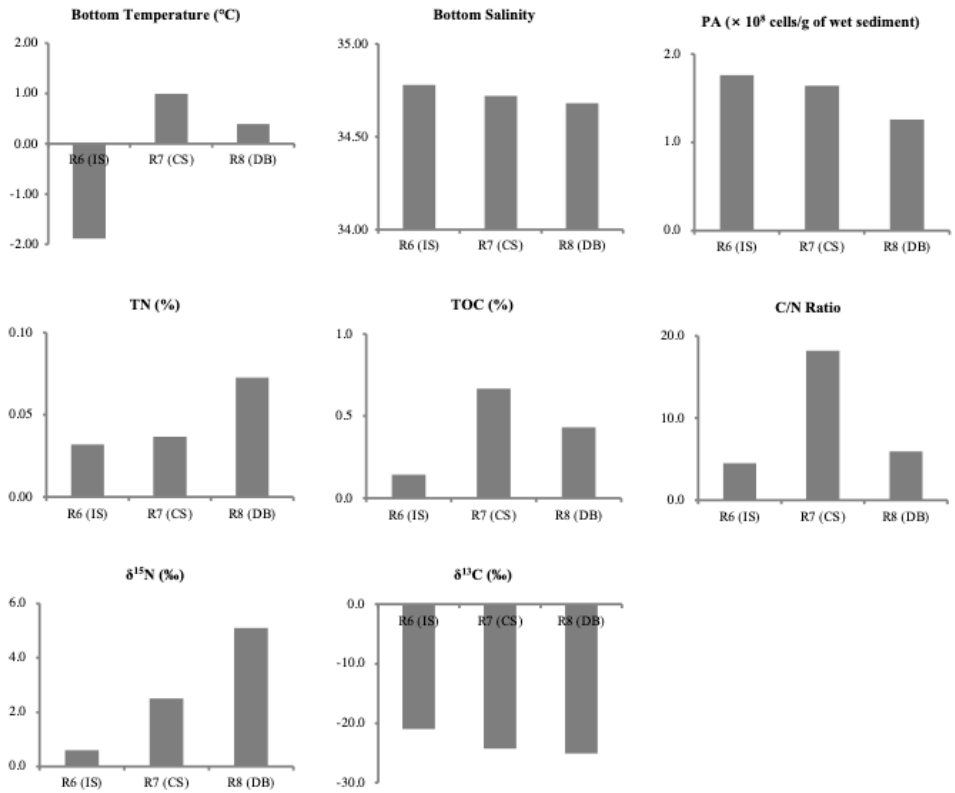


Figure 3.2. Graph of environmental data in the study stations. IS:Inner Continental Shelf, CS: Continental Slope, DB: Deep Basin, PA; Prokaryotic Abundance, TN: Total Nitrogen, TOC: Total Organic Carbon, CNratio: Carbon to Nitrogen ratio, $\delta^{15}N$: $\delta^{15}N$–values, $\delta^{13}C$: $\delta^{13}C$–values.

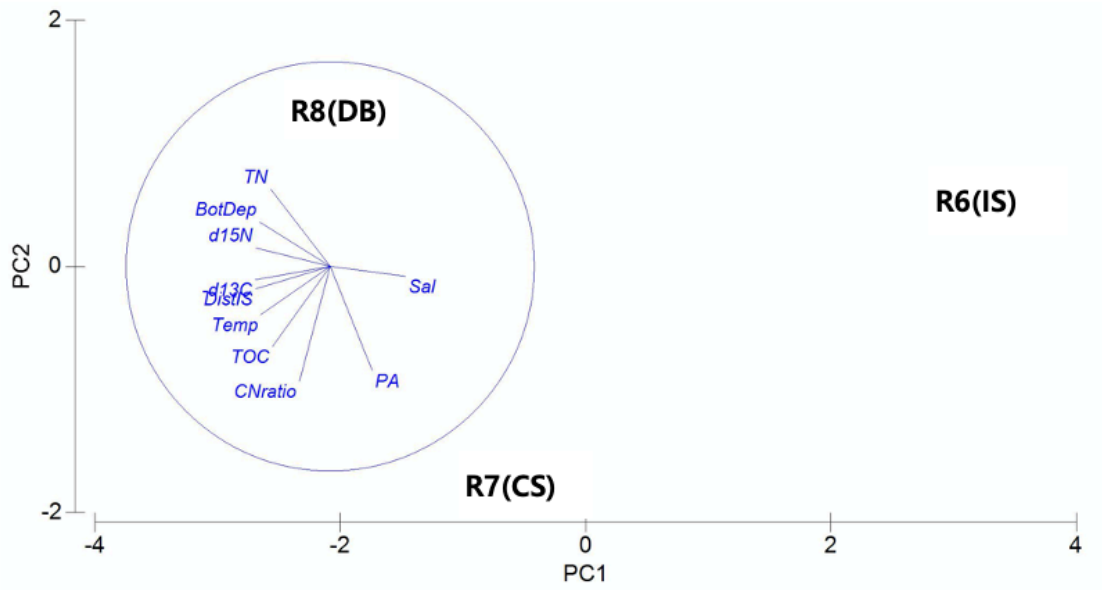


Figure 3.3. Principal component analysis (PCA) of environmental variables in the study stations. Each line represents the direction and strength of environmental gradient (BotDep, Bottom Depth; DisIS, Distanc from Ice Shelf; Temp, Temperature; Sal, Salinity; PA, Prokaryotic Abundance; TN, Total Nitrogen; TOC, Total Organic Carbon; CNratio, Carbon to Nitrogen ratio; $\delta^{15}N$, $\delta^{15}N$–values; $-\delta^{13}C$, converted $\delta^{13}C$–values to positive values). IS:Inner Continental Shelf (R6 station), CS: Continental Slope (R7 station), DB: Deep Basin (R8 station).

3.3.2 Metagenomic sequencing statistics

The sequencing from the three dataset resulted in a total of 61,610,704,318 bases, of which 165,066,909 reads were obtained with an average length from 360 ± 70 bp to 386 ± 69 bp (Table 3.2). A total of 17.2 Gbp–22.3 Gbp and 47,972,017–59,994,749 reads per station were obtained, and the station R6 (IS) presented the highest bases and reads (Table 3.2). Among all sequences, 43,560,526 reads (72.6%, R6 (IS)), 35,757,201 reads (74.5%, R7 (CS)) and 33,125,268 reads (58.0%, R8 (DB)) passed the quality control (QC), respectively (Table 3.2). After quality control pipeline, in the station R6 (IS), 41,147,880 (94.5%) reads and 299,061 (0.7%) reads were represented to predicted protein and rRNA features, respectively (Table 3.2). In the station R7 (CS), 35,613,745 (99.6%) reads and 96,414 (0.3%) reads were represented to predicted protein and rRNA features, respectively (Table 3.2). And in the station R8 (DB), 32,054,490 (96.8%) reads and 63,092 (0.2%) reads were represented to predicted protein and rRNA features, respectively (Table 3.2). Of these putative protein- and rRNA-related reads, 47.6 ± 5.2% and 0.5 ± 0.5% matched known protein and rRNA sequences, respectively. And 51.9 ± 4.8% reads were predicted proteins with unknown function (hypothetical proteins). The average GC content was 56 ± 12% for the sequences that passed quality control (Table 3.2).

Table 3.2. Statistical analysis of the annotation results for all metagenomic samples used from MG-RAST.

	R6 (IS)	**R7 (CS)**	**R8 (DB)**
Upload: bp Count	22,306,856,999 bp	17,275,263,941 bp	22,028,583,378 bp
Upload: Sequences Count	59,994,749	47,972,017	57,100,143
Upload: Mean Sequence Length	372 ± 71 bp	360 ± 70 bp	386 ± 69 bp
Upload: Mean GC percent	50 ± 11 %	59 ± 10 %	58 ± 10 %
Artificial Duplicate Reads: Sequence Count	15,949,737	11,795,708	23,494,658
Post QC: bp Count	16,270,895,405 bp	12,877,780,115 bp	12,880,903,477 bp
Post QC: Sequences Count	43,560,526	35,757,201	33,125,268
Post QC: Mean Sequence Length	374 ± 72 bp	360 ± 72 bp	389 ± 70 bp
Post QC: Mean GC percent	50 ± 11 %	59 ± 10 %	59 ± 9 %
Processed: Predicted Protein Features	41,147,880	35,613,745	32,054,490
Processed: Predicted rRNA Features	299,061	96,414	63,092
Alignment: Identified Protein Features	14,806,040	15,266,758	14,154,185
Alignment: Identified rRNA Features	24,328	13,312	10,980

3.3.3 Metagenomic prokaryotic community composition

All three stations had sequences that were identified as the Bacteria, Archaea, Eukaryota and Viruses at the domain level (Fig. 3.4a). However, 0.3–0.8% of the sequences were not assigned to any organism. Bacteria were the most abundant domain in all three sediment samples, ranging from 85.4 to 92.6% of the total, while the archaea (3.1–5.9%) contributed substantially less to the benthic prokaryotic communities (Fig. 3.4a and Table 3.3). Differences among the three stations were observed at the domain eukaryota. The abundance of eukaryotes in the station R6 (IS, 10.2%) was higher than those of other stations (0.9–1.4%; Fig. 3.4a and Table 3.3). Forty-one eukaryotic phyla from the Animalia, Fungi, Plantae, and Protista kingdoms were classified. The Animalia phyla *Arthropoda* (0.1–0.5%), *Chordata* (0.2–1.3%) and *Nematoda* (0.02–0.1%) increased in abundance as the study station approached to inner continental shelf (e.g., station R6, IS). And the abundant of viruses was relatively low (0.6–0.7%).

The sequences were classified into 28 bacterial phyla, 5 archaeal phyla and 42 eukaryotic phyla, in total of 75 phyla were recovered from all three stations. The most abundant phylum in all three stations was *Proteobacteria*, which accounted for 55.9 to 64.7% of the sequences, followed by *Planctomycetes* (6.1–8.4%), *Bacteroidetes* (3.3–8.6%), and *Actinobacteria* (3.4–5.5%; Fig. 3.4b and Table 3.3). *Planctomycetes* was the second most frequent bacterial phyla in the station R7 (CS) and R8 (DB; 6.1% and 8.4%, respectively), while the second abundant bacteria in the station R6 (IS) was *Bacteroidetes* (Fig. 3.5a and Table 3.3). *Verrucomicrobia* (1.9–2.3%, exception the station R8 (DB; 0%)), *Firmicutes* (1.8–2.4%),

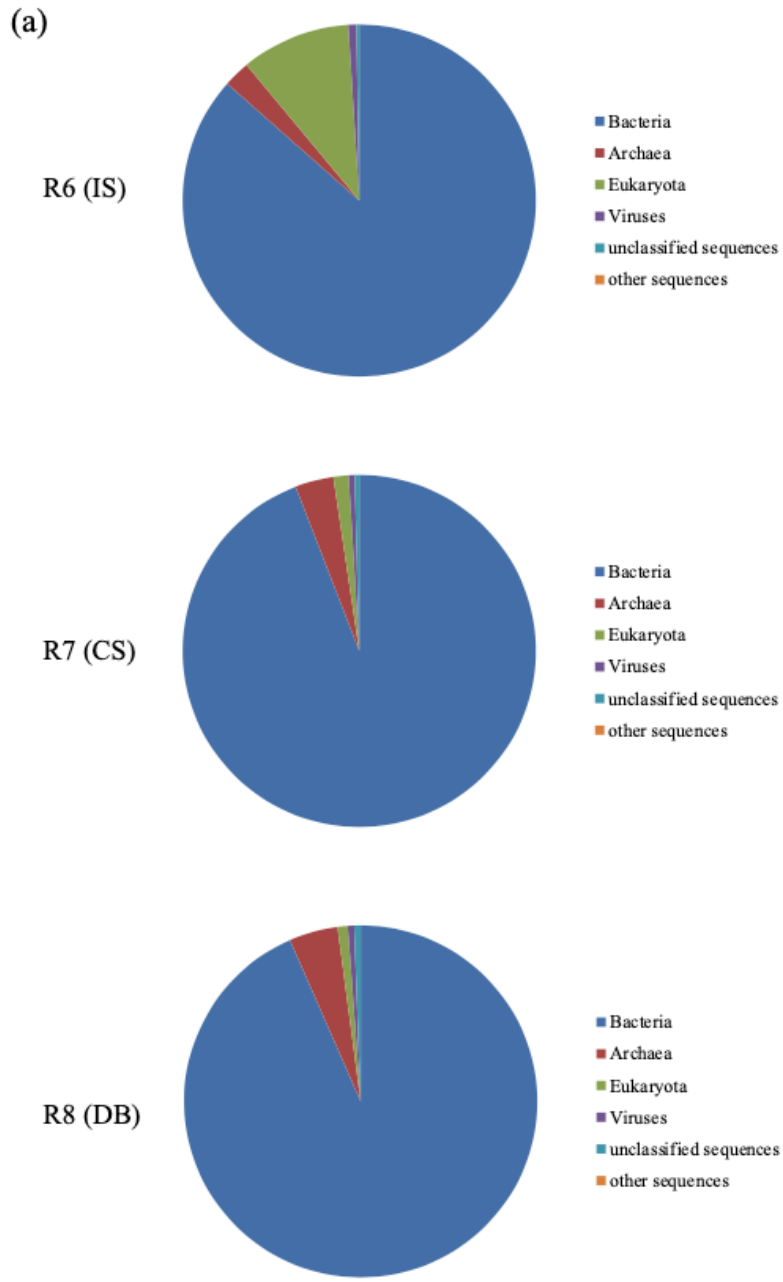


Figure 3.4. Taxonomic distribution of metagenomes (a) at domain level and (b) at phylum level between three stations. Organisms that appeared at ≥0.1% were showed in chart.

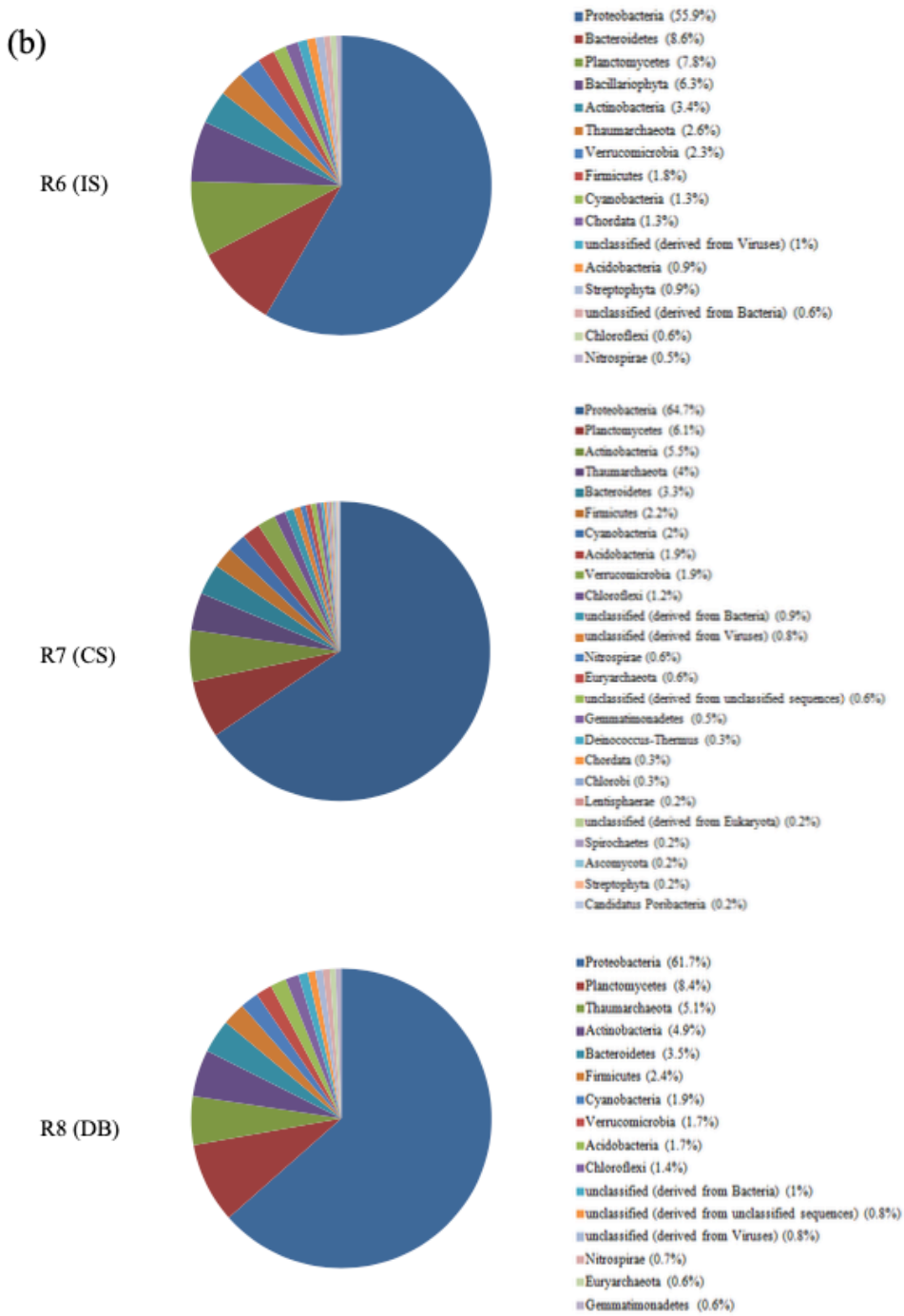


Figure 3.4. (Continued) Taxonomic distribution of metagenomes (a) at domain level and (b) at phylum level between three stations. Organisms that appeared at ≥0.1% were showed in chart.

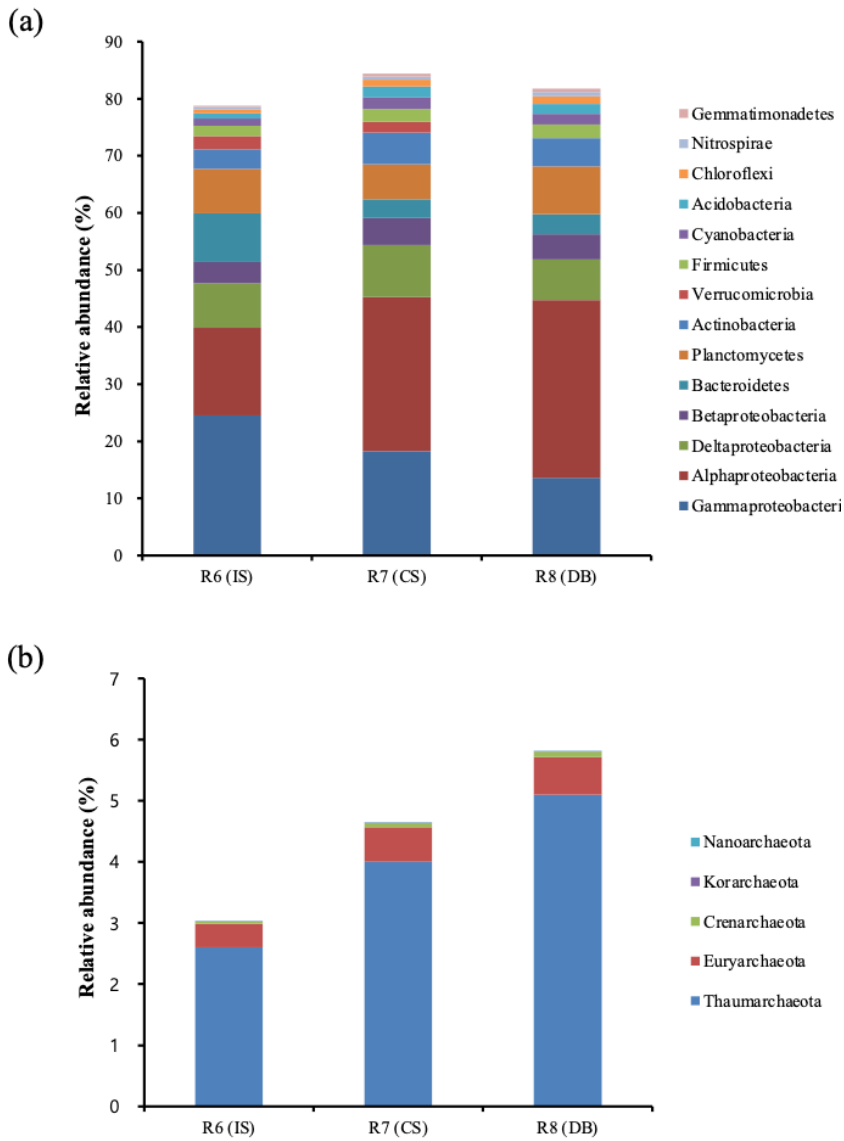


Figure 3.5. Taxonomic distribution of metagenomes (a) bacterial phyla and (b) archaeal phyla in three stations.

Table 3.3. Composition of organisms detected in the metagenome data. Organisms that appeared at ≥0.5% in any of the stations are shown.

	R6 (IS)		R7 (CS)		R8 (DB)	
	No. of sequences	% of metagenome	No. of sequences	% of metagenome	No. of sequences	% of metagenome
Bacteria	**10030323**	**85.4**	**9960689**	**92.6**	**9647701**	**89.9**
Proteobacteria	6558464	55.9	6963309	64.7	6621377	61.7
Bacteroidetes	1005534	8.6	354652	3.3	377472	3.5
Planctomycetes	911195	7.8	660129	6.1	902985	8.4
Actinobacteria	404419	3.4	590281	5.5	527325	4.9
Verrucomicrobia	274000	2.3	206697	1.9	0	0.0
Firmicutes	207245	1.8	240293	2.2	256770	2.4
Cyanobacteria	154994	1.3	212633	2.0	205981	1.9
Acidobacteria	104823	0.9	208968	1.9	179246	1.7
Chloroflexi	72414	0.6	128620	1.2	147716	1.4
Nitrospirae	60389	0.5	61912	0.6	76509	0.7
Gemmatimonadetes	21345	<0.5	54580	0.5	63904	0.6
Other bacteria	255501	2.2	278615	2.6	288416	2.7
Archaea	**361670**	**3.1**	**505976**	**4.7**	**631673**	**5.9**
Thaumarchaeota	305747	2.6	430198	4.0	547133	5.1
Euryarchaeota	44785	<0.5	60703	0.6	66044	0.6
Other archaea	5114	0.0	8305	<0.5	10464	<0.5
Eukaryota	**1196225**	**10.2**	**146906**	**1.4**	**101094**	**0.9**
Others	113041	1.0	82480	0.8	85177	0.8
unclassified sequences	37821	<0.5	60042	0.6	84956	0.8

Cyanobacteria (1.3–2.0%), *Acidobacteria* (0.9–1.9%) and *Chloroflexi* (0.6–1.4%) were also abundant (>1%) in the all stations (Fig. 3.5a and Table 3.3). At the class level, *Gammaproteobacteria* (13.6–24.5%) and *Alphaproteobacteria* (15.3–31.1%) were more abundant than *Deltaproteobacteria* (7.1–9.2%) and *Betaproteobacteria* (3.7–4.7%) across all stations (Fig. 3.5a). Differences among the three stations were observed when examined at the class level, *Gammaproteobacteria* (24.5%) was the most abundant class in the station R6 (IS), while *Alphaproteobacteria* was the most abundant class in the station R7 (CS) and R8 (DB, 27.0% and 31.1%. respectively; Fig. 3.5a). Five archaeal phyla (*Crenarchaeota*, *Euryarchaeota*, *Korarchaeota*, *Nanoarchaeota* and *Thaumarchaeota*) were recovered from all stations (Fig. 3.5b). Within the archaeal domain, all three stations had similar distributions. *Thaumarchaeota* (2.6–5.1%) was the most predominant archaeal phylum, and *Euryarchaeota* (0.4–0.6%) was the second abundant phylum across all stations (Fig. 3.5b).

3.3.4 Comparison of prokaryotic community structure determined via the amplicon and shotgun-metagenomic approaches

Six sediment samples at 3 study stations were analyzed using an amplicon of the 16S rRNA gene (e.g., pyrosequencing). Bacterial sequences were classified into 47 bacterial phyla. *Acidobacteria*, *Actinobacteria*, *Bacteroidetes*, *Proteobacteria*, and *Planctomycetes* were dominant bacterial groups in all three stations (Fig. 3.6). The most abundant phylum in all stations was *Planctomycetes*, which accounted for 18.3 to 36.6% of the sequences, followed by *Proteobacteria* (6.0–28.6%), *Actinobacteria* (5.1%–14.3%) and *Bacteroidetes* (0.7–10.2%; Fig. 3.6). Especially, *Bacteroidetes* was more abundant in the station R6 (IS, 10.2%) than those of other stations (0.7–1.7%), while *Actinobacteria* (14.3%) and *Chloroflexi* (5.5%) were more abundant in the station R7 (CS) and R8 (DB) compared with other stations, respectively (Fig. 3.6). *Alphaproteobacteria* (2.3–14.9%) and *Gammaproteobacteria* (3.6–13.7%) were more abundant than *other Proteobacteria* (< 1%) across all stations (Fig. 3.6). Archaea were relatively lower abundant than bacteria in the station R6 (IS, 3.0%) and R7 (CS, 17.0%) but were the most abundant prokaryote in the station R8 (DB, 47.8%; Fig. 3.6). The whole abundance of archaea was belonged to *Thaumarchaeota* which was classified as Crenarchaeota in chapter 2.

Prokaryotic community structures were directly compared with the 16S rRNA gene data obtained from both the amplicon and shotgun sequencing. The result comparison showed that *Proteobacteria* and *Planctomycetes* were the most abundant bacterial phylum when shotgun sequences and amplicon sequences were analyzed,

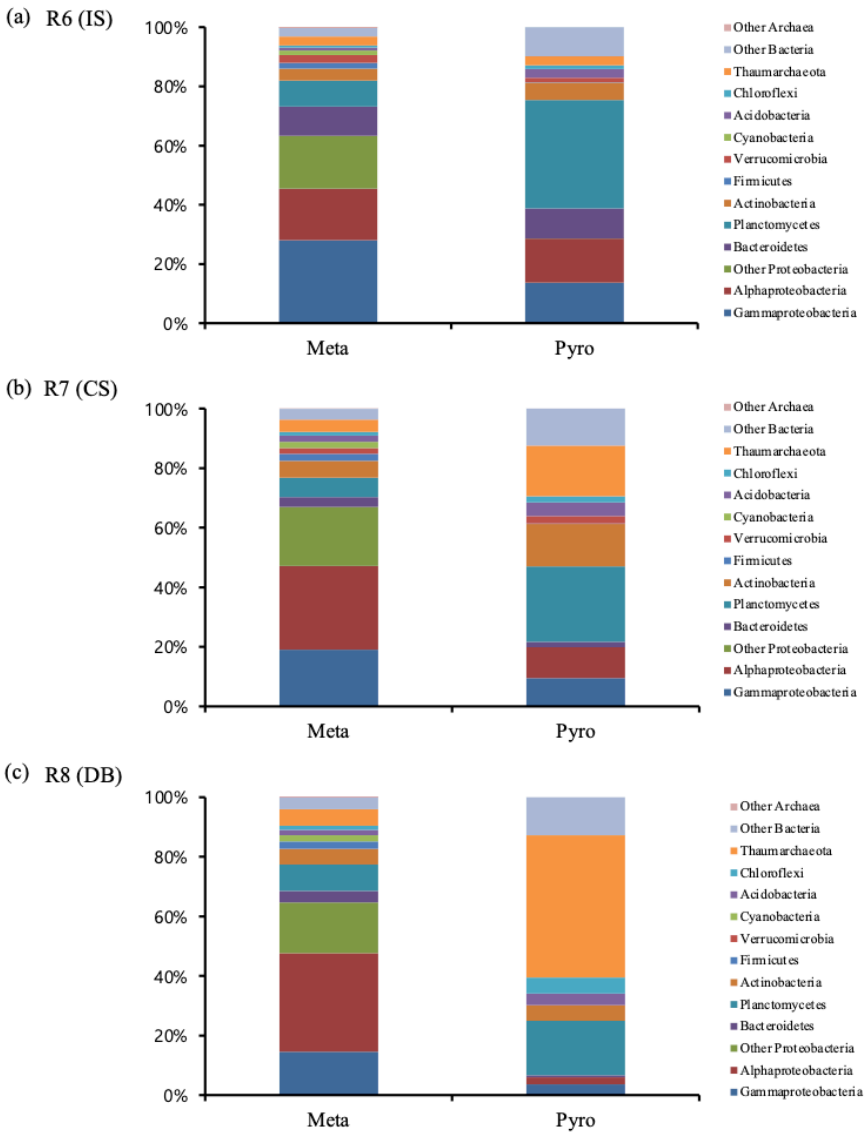


Figure 3.6. Comparisons of the relative abundances (percentage of reads) of prokaryotes in (a) station R6 (IS), (b) station R7 (CS) and (c) station R8 (DB), determined using 16S rRNA gene data obtained using the shotgun metagenomic approach (lane 1, Meta) versus the 16S rRNA gene data obtained using the amplicon sequencing approach (lane 2, Pyro).

respectively (Fig. 3.6).

Proteobacteria accounted for 63.3 to 66.9% of the metagenomic sequences, while occupied 6.0 to 28.6% of amplicon sequences (Fig. 3.6). *Planctomycetes* was the most dominant bacterial group of amplicon sequences (18.3–36.6%), while accounted for 6.4 to 8.9% of the metagenomic sequences (Fig. 3.6). There was a difference in the abundance of each *Proteobacteria* class, but the community structure, in which *Alphaproteobacteria* and *Gammaproteobacteria* were more abundant than other *Proteobacteria* class, showed the similar distribution. When compared with the amplicon sequences, *Betaproteobacteria* was abundant in the shotgun sequences (4.2–4.9%; Fig. 3.6). And *Cyanobacteria* was a distinguished phylum between shotgun sequences (1.5–2.1%) and amplicon sequences (0.0%; Fig. 3.6). Within *Thaumarchaeota* phylum, there was also a distinct difference between two approaches that corresponded to 3.0–47.8% in amplicon sequences and 3.0–5.1% in shotgun sequences (Fig. 3.6).

3.3.5 Functional gene profiles of the metagenome

All three stations revealed a similar functional distribution for subsystems and COGs (Fig. 3.7 and Fig. 3.8). Genes coding for clustering-based subsystems (14.2–14.4%), carbohydrates (11.6–11.8%), amino acids and derivatives (10.7–11.4%) and protein metabolism (6.52–7.3%) represented the four most abundant categories for subsystems (Fig. 3.7). The functional genes for sulfur metabolism (1.3–1.6%), nitrogen metabolism (0.8–1.1%), phosphorus metabolism (0.6–0.8%) and iron acquisition and metabolism (0.6–0.7%) were detected (Fig. 3.7). The most abundant functional groups were related to metabolism (42.1–43.4%), followed by cellular processes and signaling (20.6–21.3%), poorly characterized categories (18.1–19.9%), and information storage and processing (16.5–17.4%) for COGs (Fig. 3.8).

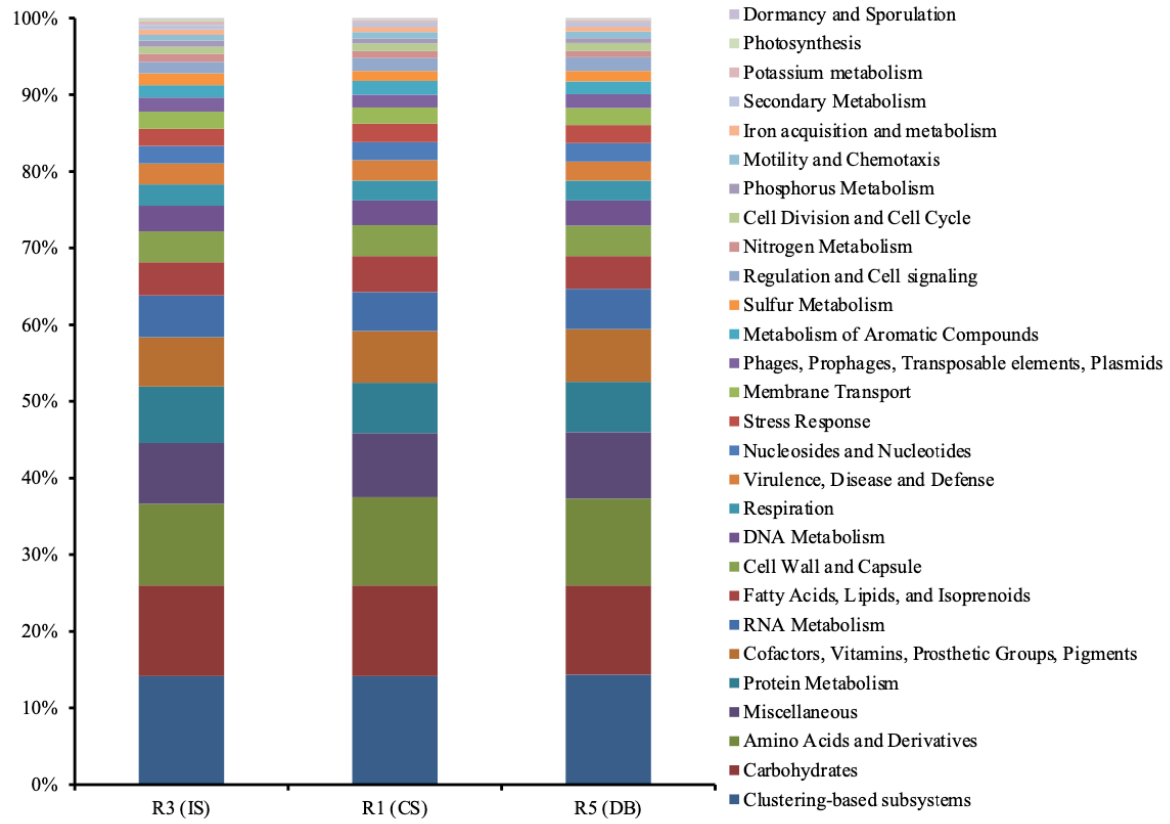


Figure 3.7. Functions annotated against subsystems in each station.

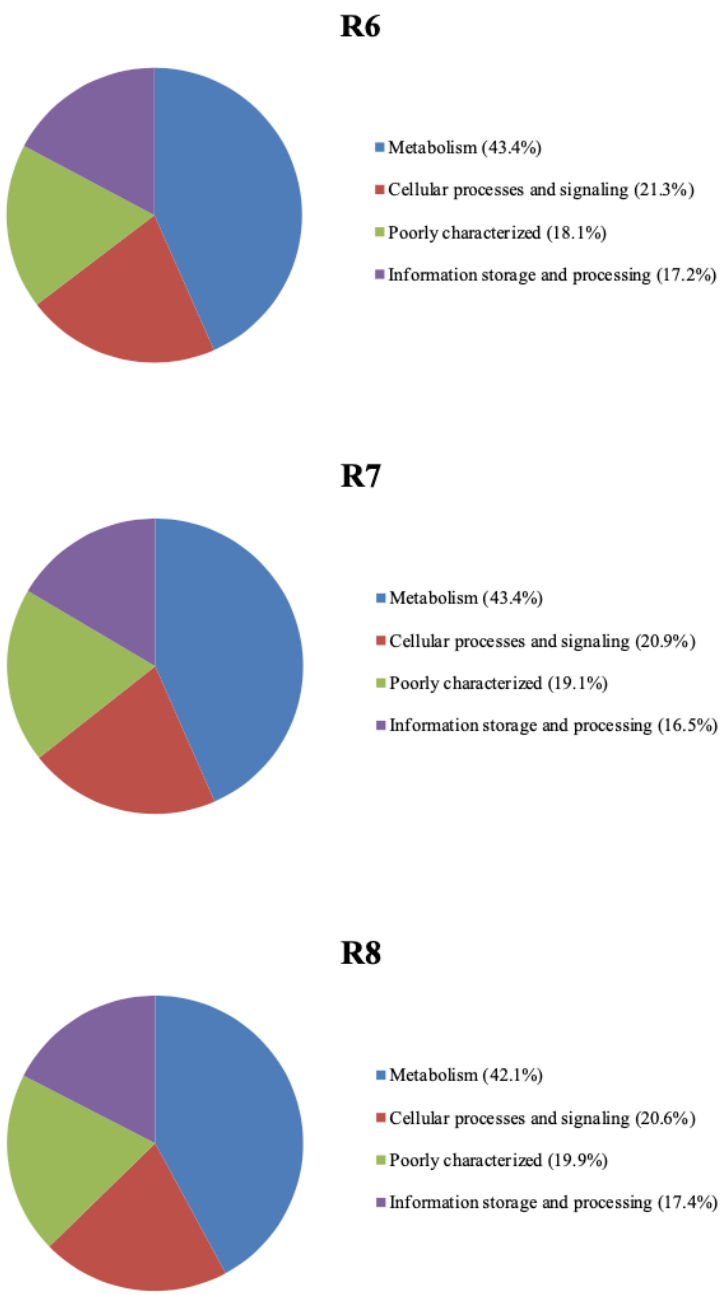


Figure 3.8. Distribution of functional categories for Clusters of Orthologous Groups (COGs).

4.4 Discussion

To our best knowledge, this is the first study to compare of prokaryotic communities and functional gene contents in surface sediments at the continental margin and ocean basin in the Ross Sea, simultaneously. The benthic community composition studies were conducted with limited technique using T-RFLP or amplicon sequencing and limited areas of continental shelf in the Ross Sea (Baldi *et al*., 2010; Carr *et al*., 2013; Learman *et al*., 2016). And functional potential of the benthic prokaryotes was also investigated in limited areas such as fresh pond and soil in Antarctica (Varin *et al*., 2012; Koo *et al*., 2018). The present study, however, has combined a metagenomic approach with pyrosequencing analyses of prokaryotic 16S rDNA to assess the functional potential of the benthic prokaryotic community and to identify the active community members in the Ross Sea.

In this study, we present three new metagenomic data sets from surface sediment to examine the prokaryotic community structure and functional contents using the shotgun sequencing. The analyses of three metagenomic data of pelagic sediment revealed that there were similar taxonomic groups in three stations but prokaryotic community structure have differed between the stations. Although *Proteobacteria* were the most abundant phylum in all three stations, it was identified that *Gammaproteobacteria* were the most abundant class in the station R6 (IS), while *Alphaproteobacteria* were the most abundant class in the station R7 (CS) and R8. *Proteobacteria* are abundant in Antarctic sediments (Ruff *et al*., 2014; Carr *et al*., 2013; Learman *et al*., 2016) and exhibits in several marine environment (Danovaro *et al*., 2010; Williams *et al*., 2010; Zinger *et al*., 2011), and has a metabolic diversity

with playing a significant role in nutrient cycles (Kersters *et al*., 2006). This characteristic of this phylum can allow to be well adapted to diverse environment with different conditions of organic matter and nutrient (Zumsteg *et al*., 2012). A majority of the *Gammaproteobacteria* are chemoorganotrophs in nutrient-rich environments (Gao *et al*., 2009), and the family *Alteromonadaceae* of *Gammaproteobacteria* requires sodium to grow (López-Pérez and Rodriguez-Valera, 2014). Thus, environmental conditions such as more labile nitrogen and carbon (e.g., lower $\delta^{15}N$ and higher $\delta^{13}C$) and hypersaline enable to predominate of *Gammaproteobacteria* in the station R6 (IS; Mateos-Rivera *et al*., 2016; Liu *et al*., 2018), which are influenced by water mass, primary production and glacier melting in inner cintinental shelf. And the genus *Pseudomonas* and *Shewanella* of *Gammaproteobacteria* were abundant in the station R6 (IS). *Pseudomonas*, denitrification bacteria (Chen *et al*., 2003), would be potentially expected to participate in nitrogen cycles and iron-reducing bacteria *Shewanella* (Hwang *et al*., 2019) is also involved in iron cycles in the station R6 (IS). *Alphaproteobacteria* are oligotrophs which can survive and thrive in environments with low nutrients (Falkow *et al*., 2006). The more refractory nitrogen and carbon (e.g., higher $\delta^{15}N$ and lower $\delta^{13}C$) conditions allow these organisms are more abundant in the station R7 (CS) and R8 (DB). The order *Rhizobiales* of *Alphaproteobacteria* was abundant in the station R7 (CS) and R8 (DB), thus, this nitrogen-fixing bacteria (Tsoy *et al*., 2016) are potentially participated in nitrogen cycles in these stations. *Rhodobacterales*, marine hydrocarbon-degrading bacteria (Kim and Kwon, 2010), were also dominant order of *Alphaproteobacteria* in all stations, and this result is consistent with high abundance of carbohydrates function genes in metagenomes. The order

Desulfuromonadales, *Desulfobacterales* and *Desulfovibrionales* of *Deltaproteobacteria* which are bacterial sulfate reducers, were also abundant groups in all stations, especially the station R7 (CS), and whose metabolic activity would be contribute to the sulfur metabolism in these stations.

Planctomycetes were the second or the third most abundant phylum at all stations. The phylum *Planctomycetes* is mainly aerobic and neutrophilic, but the anaerobic ammonium oxidation (anammox) species are also present. Their diversified metabolism allows them to survive in a wide variety of ecosystems such as hypersaline environments (Schneider *et al*., 2013) and glacial waters (Liu *et al*., 2006; Zeng *et al*., 2013) and Antarctic soils and waters (Newsham *et al*., 2010; Piquet *et al*., 2010). Thus, despite the differences in the quality and quantity of sediment organic matter in the study areas, *Planctomycetes* could be thrive in all stations. In the station R6 (IS), *Bacteroidetes* was dominant phylum, and they degrade complex carbon including phytoplankton cells and phytoplankton-derieved particles (Gómez-Pereira *et al*., 2012; Teeling *et al*., 2012). This metabolism of this phylum can allow to be well adapted to the environment which are influenced by organic matter source exported from phytoplankton bloom in inner continental shelf. *Actinobacteria* was also one of the dominant phyla in all stations, and may play important roles in the decomposition of organic matters (Kielak *et al*., 2016). And this phylum is more abundant in the station R7 (CS) than in the station R6 (IS) and R8 (DB), which may be adapt to high TOC concentration in benthic sediment.

Archaea were less abundant in all stations and contributed substantially less to the benthic prokaryotic communities, *in situ*. The phylum *Thaumarchaeota* was less abundant in the station R6 (IS) than in the station R7 (CS) and R8 (DB). An

ammonia oxidizing *Thaumarchaeota* are lives in the oligotrophic marine environment (Pester *et al*., 2011; Hatzenpichler, 2012), thus, more refractory nitrogen and carbon (e.g., higher $\delta^{15}N$ and lower $\delta^{13}C$) conditions allow these organisms are more abundant in the station R7 (CS) and R8 (DB).

One important result of the sequence approach comparison was the detection and classification of *Cyanobacteria* sequences. *Cyanobacteria* are photoautotrophic organisms in a wide range of habitats and some *Cyanobacteria* contribute to nitrogen-fixing of atmospheric nitrogen. Thus, this phylum also potentially participated in nitrogen cycles in study stations. The community differences between amplicon sequences and shotgun sequences are introduced by bias of both approaches (Steven et al., 2012). In this study, amplicon sequencing only targets the V5–V8 region, whereas metagenomic sequence can span the entire length of the gene. Because different regions of the 16S rRNA gene is used to assign taxonomic of prokaryotes, the two approaches may not naturally give identical results.

In this study, metagenomic analyses revealed that there are differences in community structure and functional potential of benthic prokaryotes between inner continental shelf and outlying of continental shelf. The difference benthic prokaryotic distribution was strongly affected by distinct environmental conditions, and organic matter utilization is an important factor to shape benthic prokaryotic community in pelagic sediments of the Ross Sea.

CHAPTER 4

A new psychrophilic iron-reducing bacterium, *Shewanella psychromarinicola*, isolated from pelagic sediment of the Ross Sea (Antarctica)

Contents of this article are modified from the online published paper "*Shewanella psychromarinicola* sp. nov., a psychrophilic bacterium isolated from pelagic sediment of the Ross Sea (Antarctica), and reclassification of *Shewanella arctica* Kim *et al*. 2012 as a later heterotypic synonym of *Shewanella frigidimarina* Bowman *et al*. 1997"

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4.1 Introduction

The genus *Shewanella* was proposed with *S. putrefaciens*, *S. benthica* and *S. hanedai* based on 5S rRNA sequence analysis (MacDonell and Colwell, 1985). An intensive study on phylogenetic analyses of 16S rRNA gene sequences proposed that this genus represents the type genus of the family *Shewanellaceae* with 18 *Shewanella* species in *Gammaproteobacteria* (Ivanova *et al*., 2004). At the time of writing, the genus *Shewanella* comprises 68 species with validly published names (Parte, 2018; Yun *et al*., 2018). Members of the genus *Shewanella* are facultative anaerobic, Gram-stain-negative, motile and straight or curved rods, and have been isolated from a wide range of environments including clinical samples, dairy products, marine or freshwater sediments, aquatic samples, marine host organisms and cryospheric samples (Bowman, 2015).

A novel lineage of *S. frigidimarina* within the genus *Shewanella* was reported for psychrotrophic iron-reducing strains isolated from diverse Antarctic habitats including sea ice, sea ice algae, cyanobacterial mats, saline lake water, glacier mud and sediment (Bowman *et al*., 1997; Bozal *et al*., 2002). Later, type strains of 3 *Shewanella* spp. including *S. livingstonensis*, *S. vesiculosa* and *S. arctica* were isolated from coastal water and sediment samples in polar regions (Bozal *et al*., 2002; Bozal *et al*., 2009; Kim *et al*., 2012) and formed a robust clade with *S. frigidimarina* on the basis of 16S rRNA gene sequences (hereafter, the SF clade). In the present study, we isolated two novel bacterial strains affiliated with the SF clade, and performed a polyphasic taxonomic analysis to determine the taxonomic position of the strains. Furthermore, phylogenetic relationships of the recognized *Shewanella*

species in the SF clade were revised, for the first time, by a genomic taxonomy analysis using complete or nearly complete whole genome sequences of our 2 strains along with the type strains belonging to the SF clade.

In this chapter, two novel strains belonging to the genus *Shewanella* were described, which were designated M2^T (= KCCM 43257^T = JCM 32090^T) and R106 (= KCCM 43258 = JCM 32089) isolated from pelagic surface-sediment of the Ross Sea, Antarctica. Based on the data obtained by polyphasic approach, these strains were proposed to belong to a novel species in the genus *Shewanella*.

4.2 Materials and Methods

4.2.1 Study area and sampling

Four sediment samples were obtained using a box corer from water depth between 710 m and 1034 m in the Ross Sea and Amundsen Sea in the Antarctica during the Araon expedition (Table S4.1). A surface sediment was taken by a sterile stainless-steel spatula and stored in a 50 ml conical tube at 4 °C until further analysis. For isolating bacteria, the surface sediment sample was diluted approximately 100-fold with autoclaved 3% (w/v) sea salts (Sigma) solution. An aliquot (100 μl) of the diluted sample was spread on marine agar (MA; Difco) and saline Reasoner's 2A agar (R2A; Difco) plates containing 3% (w/v) sea-salts. These plates were incubated at 4, 15 and 20 °C under aerobic conditions for 1 week. To calculate colony-forming units (CFU), colonies on agar plate were observed and counted after 1 week in culture. Strains $M2^T$ and R106 were picked from MA incubated at 20 °C and saline R2A incubated at 15 °C, respectively. Each strain was subsequently purified four times on fresh MA at 20 °C and saline R2A at 15 °C, respectively. Both strains were able to grow well on MA at 15 °C. The two strains were maintained on MA at 15 °C and preserved in marine broth (MB; Difco) supplemented with 30% (v/v) glycerol at -80 °C.

Prokaryotic abundance (PA) was measured using SYBR gold staining method based on Breuker *et al*. (2013) and with some modifications. SYBR gold staining method was used instead of SYBR green Ⅰ in this study (Weinbauer *et al*. 1998; Shibata *et al*. 2006). Cells were counted using an epifluorescence microscope (BX51 Olympus) and a minimum of 500 cells were counted for each sample. There

were the large discrepancy between CFU and direct microscopic counts (i.e., PA), thus, the ratio of culturable bacteria in sediment samples was calculated from the percentage of cultivable cells from CFU in proportion to PA.

For a comparative study, 4 type strains of *S. vesiculosa* LMG 24424^T, *S. livingstonensis* LMG 19866^T, *S. arctica* KCTC 23109^T and *S. frigidimarina* KCCM 41815^T were obtained from the Bacteria Collection of the Laboratory for Microbiology of the Faculty of Sciences of the Ghent University (BCCM/LMG), the Korean Collection for Type Cultures (KCTC) or the Korean Culture Center of Microorganisms (KCCM). Genomic, phenotypic and chemotaxonomical characteristics of strains M2^T and R106 were compared to those of the type strains. Unless otherwise specified, characteristics of all strains were based on cultures grown aerobically on MA for 3 days except for incubation temperatures. Due to different optimal growth temperatures, strains M2^T and R106 were incubated at 15 °C, while other 4 type strains of *Shewanella* spp. at 20 °C.

4.2.2 Phylogenic analyses of marker genes

For 16S rRNA gene amplification by PCR, DNA were extracted from a single colony by the boiling method (Englen and Kelley, 2000). The crude extracts served as DNA templates for PCR, which included *Taq* DNA polymerase (TaKaRa) and primers 27F and 1492R (Lane, 1991). Direct sequencing of a purified PCR product was performed using sequencing primers (27F, 518F, 800R and 1492R) (Lane, 1991; Anzai *et al*., 1997) with an Applied Biosystems sequencer (ABI 3730XL) at Cosmo Genetech (Seoul, Korea).

The almost complete 16S rRNA gene sequence of strain M2^T (1464 bp) and R106 (1417 bp) were obtained and analyzed using BLAST searches against the GenBank and EzBioCloud databases (Altschul *et al*., 1990; Yoon *et al*., 2017). The 16S rRNA gene sequences of closely related taxa obtained from the GenBank database were aligned using the RDP aligner (Cole *et al*., 2014) based on secondary structures. Phylogenetic analyses were performed using the program MEGA (ver. 7.0; Kumar *et al*., 2016). Neighbour-joining (NJ; Saitou *et al*., 1987) trees were reconstructed using the Kimura two-parameter model (Kimura, 1980) with the uniform rates and the pairwise deletion options. Maximum-parsimony (MP; Fitch, 1971) trees were reconstructed using the Tree-Bisection-Reconnection (Swofford *et al*., 1996) heuristic search method with the number of initial trees (random addition) as 10 and complete deletion options. Maximum-likelihood (ML; Felsenstein, 1981) trees were reconstructed using the Kimura two-parameter model (Kimura, 1980) with the Nearest-Neighbour-Interchange heuristic method, the uniform rates and complete deletion options. The robustness of the phylogenetic trees reconstructed by each tree-making method was confirmed by bootstrap analyses based on 1000 replications.

A representative marker gene in the genus *Shewanella* (Venkateswaran *et al*., 1999), gyrase B subunit (*gyrB*) gene, was retrieved from genome sequences of each strain after genome annotation using the Pathosystems Resource Integration Center (PATRIC) web service (Wattam *et al*., 2017). The *gyrB* gene sequences of strains M2^T, R106, and the 4 type strains in the SF clade were aligned with those of 13 phylogenetically related *Shewanella* species from the GenBank database using Clustal W (ver. 2.1; Larkin *et al*., 2007). Phylogenetic trees of *gyrB* gene using

nucleic acids sequences were reconstructed as described above in the phylogenetic analysis of 16S rRNA gene.

4.2.3 Genome characteristics and phylogenomic analyses

To obtain genome sequences, genomic DNA of the 6 strains were extracted using a commercial kit (DNeasy Blood & Tissue kit; Qiagen). Two DNA sequencing platforms were employed for genome sequencing. For all strains except for *S. livingstonensis* LMG 19866^T, sequencing libraries were constructed with Nextera DNA Preparation Kit (Illumina; fragment size of ca. 600 bp) and sequenced with Illumina MiSeq by ChunLab Inc. (Seoul, Korea). For the two strains M2^T and *S. livingstonensis* LMG 19866^T, sequencing libraries were constructed with SMRTbell Template Prep Kit (Pacific Biosciences; fragment size of ca. 20 kb) and sequenced with PacBio RS II (Pacific Biosciences) by DNA Link Inc. (Seoul, Korea).

From the two independent sets of (sub)reads for strain M2^T, a reliable and complete genome sequence of this strain was obtained using hybrid assembly, genome-wide alignment and manual correction as previously described (Cho *et al*., 2018). For other 3 strains subjected to Illumina sequencing, *de novo* genome assembly for each strain was performed with paired-end reads (300 bp × 2) using the complete genome sequence of M2^T as a reference sequence by the SPAdes program (ver. 3.12; Bankevich *et al*., 2012). *De novo* assembly of PacBio data was performed using SMRT Analysis (ver. 2.3.0; Pacific Biosciences) by DNA Link Inc. Contamination was checked for all genomes obtained in this study on the basis of 16S (Lee *et al*., 2017) and protein-coding genes (Parks *et al*., 2015).

Genome completeness was estimated by CheckM (ver. 1.0.8; Parks *et al*., 2015). Genome size, N50 and GC content were calculated using QUAST (ver. 4.5; Gurevich *et al*., 2013). Genome coverages of Illumina and PacBio data were measured using BBMap (ver. 38.26; Bushnell *et al*., 2014) and Geneious (ver. 7.1.9) (Kearse *et al*., 2012), respectively. Overall genome relatedness index (OGRI) values including average nucleotide identity (ANI) by the BLAST-based method (Goris *et al*., 2007) and digital DNA-DNA hybridization (dDDH) by the genome-to-genome distance calculator (GGDC) (Auch *et al*., 2010) were obtained for all pairwise comparisons.

To infer more robust bacterial phylogeny of members in the genus *Shewanella*, phylogenomic analysis of concatenated 120 ubiquitous single-copy proteins was employed based on the Genome Taxonomy Database (GTDB) taxonomy (Parks *et al*., 2018). All genomes of the 24 type strains of validly named *Shewanella* species available from the NCBI Genome (Table S1) and genomes of the 6 strains in the present study were subjected to retrieving marker genes and making a multiple sequence alignment of amino acids by GTDB-Tk (https://github.com/Ecogenomics/GtdbTk). In a set of 120 bacterial marker genes (Parks *et al*., 2018), 110 genes that are present with a single copy number were selected in a dataset comprising the 30 genomes of *Shewanella* spp. (Table S2.3). For a given set of alignment, the best model for ML trees was selected using Smart Model Selection (ver. 1.8.1) (Lefort *et al*., 2017). ML phylogeny trees with the best model were reconstructed with bootstrap analyses based on 1000 replications using PhyML (ver. 3.0; Guindon *et al*., 2010).

For a comparative genomic investigation, protein-coding genes in each *Shewanella* genome available in the NCBI Genome were retrieved and subjected to homology searching by BLASTP with the e-value cut-off of $1e^{-5}$ (Zhong *et al*., 2018).

4.2.4 Morphological, physiological and biochemical characteristics

Phenotypic characteristics of strain $M2^T$ and R106 were performed in duplicate along with the type strains of *Shewanella* spp., with repeated experiments on different days.

Growth conditions. The temperature range for growth was examined by the ability of colony-formation on MA incubated at 4 and 10–50 °C (in increments of 5 °C). The pH range (pH 4.1, 4.4, 4.9, 5.5, 6.2, 6.5, 7.0, 7.2, 7.5, 8.0, 8.5, 9.0, 9.4, 9.5, 9.9 and 10.4 for strains $M2^T$, R106, *S. vesiculosa* LMG 24424^T, *S. arctica* KCTC 23109^T and *S. frigidimarina* KCCM 41815^T; pH 3.6, 3.9, 4.2, 4.7, 5.0, 5.7, 6, 6.5, 7.0, 7.5, 8.0, 8.4, 8.9, 9.0, 9.5 and 10.1 for *S. livingstonensis* LMG 19866^T) for growth was determined by assessing change in OD_{600} in pH-buffered MB (Hwang and Cho, 2008) using citric acid-phosphate buffer for pH 4.0–5.0, MES for pH 5.5–6.5, MOPS for pH 7.0–7.5, AMPD for pH 8.0–9.5 and CAPS for pH 10.0–11.0, each at a final concentration of 50 mM at 15 or 20 °C for up to 2 weeks. Salt tolerance was determined by assessing turbidity measured as OD_{600} at 15 or 20 °C using synthetic ZoBell broth (Bacto peptone, 5 g; yeast extract, 1 g; ferric citrate, 0.1 g; distilled water, 1 L) supplemented with 0, 0.5, 1 and 2–20% (at intervals of 1%, w/v) of sea salts (Sigma).

Morphology of cells. Cell morphology and size were determined using transmission

electron microscopy (EX2; JEOL).

Gram-staining. Gram-staining was performed as previously described (Smibert and Krieg, 1994).

Motility. Motility of the cells was assessed by the hanging drop method (Skerman, 1967) with cells grown in MB for 4 days at 15 or 20 °C.

Anaerobic growth. Anaerobic growth of strain M2^T and R106 were tested with a strictly aerobic bacterium (*Rhodococcus aerolatus* PAMC 27367^T) (Hwang *et al*., 2015) and facultatively anaerobic bacteria *S. vesiculosa* LMG 24424^T, *S. arctica* KCTC 23109^T and *S. frigidimarina* KCCM 41815^T, as controls in an Anaerobic jar (BBL) containing an AnaeroPak (Mitsubishi Gas Chemical) at 15 °C for 18 days.

Catalase/oxidase. Catalase and oxidase tests were performed according to the methods described by Smibert & Krieg (Smibert and Krieg, 1994) and Cappuccino & Sherman (Cappuccino and Sherman, 2002), respectively.

Hydrolysis. Hydrolysis of DNA, starch, Tween 80 was investigated as described by Hansen & Sørheim (Hansen and Sørheim, 1991). Decomposition of casein was determined as described by Smibert & Krieg (Smibert and Krieg, 1994).

H_2S production. The production of H_2S was tested triple-sugar iron agar (Difco), which was contained 3% (w/v) NaCl.

Enzyme activities and Acid productions. Other enzyme activities and acid productions were assayed using the API ZYM, API 20NE and API 50CH kits (bioMérieux) according to the manufacturer's instructions, except that the cell suspension was prepared with artificial seawater (per litre distilled water: 24 g NaCl, 10.9 g $MgCl_2.6H_2O$, 4 g Na_2SO_4, 1.5 g $CaCl_2.2H_2O$, 0.7 g KCl, 0.2 g $NaHCO_3$, 0.1 g KBr, 0.027 g H_3BO_3, 0.03 g $SrCl_2.6H_2O$, 0.003 g NaF) (Lyman and Fleming, 1940).

Sole carbon utilization. Sole carbon source utilization was tested according to Bruns *et al*. (2001) with a final concentration of 0.4% carbon source. Sole carbon source utilization was scored as negative when growth was equal to, or less than, that in the negative control with no carbon source. Growth was measured by monitoring changes in the OD_{600} for 3 weeks at 15 or 20 °C.

Iron reduction assay. Iron reduction assay was performed as described by Lovley *et al*. (1993) using a modified medium supplemented with NaCl (18 g L^{-1}) and $MgCl_2.6H_2O$ (4.24 g L^{-1}) (Coates *et al*., 1995). Sodium DL-lactate (1 mM) and ferric citrate (10 mM) served as an electron donor and an electron acceptor, respectively. Anaerobic culturing methods were employed with anaerobic pressure serum bottles capped with thick butyl-rubber stoppers (Miller and Wolin, 1974). Uninoculated controls, in the presence or the absence of the electron donor, were also incubated. After 7-12 days of incubation, iron reduction was confirmed by the presence of a black precipitate (magnetite) (Bowman et al., 1974).

4.2.5 Chemotaxonomic characteristics

Fatty acids. The fatty acid methyl esters in whole cells grown on MA for 3 days were analyzed with gas chromatography according to the instruction of the Microbial Identification System version 6.3 (MIDI) using the RTSBA 6.21 database at the KCCM (Seoul, Korea).

Isoprenoid quinines. Isoprenoid quinone composition was determined as

previously described (Minnikin *et al*., 1984) and analyzed by HPLC as described by Collins (1985) at the KCCM.

Polar lipids. Polar lipids were extracted using the procedures described by Minnikin *et al*. (1984), separated by two-dimensional TLC and identified by spraying with appropriate detection reagents (Komagata *et al*., 1987).

4.2.6 Deposit of sequences

The GenBank/EMBL/DBBJ accession numbers for the 16S rRNA gene sequence, the retrieved *gyrB* gene sequence from genome sequence and the whole genome sequence of strain M2^T are MH630172, MK133330 and CP034073, respectively, and those of strain R106 are MH630150, MK133331 and RKKB00000000, respectively. The retrieved *gyrB* gene sequence and the genome sequence of *Shewanella vesiculosa* LMG 24424^T (MK133334 and RKKD00000000), *Shewanella livingstonensis* LMG 19866^T (MK133335 and CP034015), *Shewanella arctica* KCTC 23109^T (MK133332 and RKKE00000000) and *Shewanella frigidimarina* KCCM 41815^T (MK133333 and RKKC00000000) are available in those databases.

4.3 Results and Discussion

4.3.1 Phylogenetic identification of the isolates and the ratio of cultivable bacteria

Seventy-three isolates, affiliated with *Actinobacteria*, *Bacteroidetes*, *Alphaproteobacteria* and *Gammaproteobacteria*, were obtained from four sediment samples (Table S4.1). Four of the isolates belonged to the phylum *Actinobacteria*, represented by the genera *Nesterenkonia* (1 isolates), and *Nocardioides* (3 isolates); 5 isolates belonged to the phylum *Bacteroidetes*, represented by the genera *Lacinutrix*; 5 isolates of the class *Alphaproteobacteria* belonged to the genera *Erythrobacter* (1 isolates), *Sphingorhabdus* (1 isolates), and *Sulfitobacter* (3 isolate); and 8 to the genus *Alcanivorax*, 10 to the genus *Halomonas*, 7 to the genus *Marinobacter*, 12 to the genus *Paraglaciecola*, 2 to the genus *Photobacterium*, 2 to the genus *Pseudoalteromonas*, 5 to the genus *Spongiibacter*, and 13 member of the genus *Shewanella* of *Gammaproteobacteria*. The largest groups in terms of the number of isolated species were those belonging to the genera *Shewanella* (13 isolates), *Paraglaciecola* (12 isolates), *Halomonas* (10 isolates), *Alcanivorax* (8 isolates), and *Marinobacter* (7 isolates). Isolates affiliated with the genera *Shewanella* were obtained from only one sample (R2 station). The overall 16S rRNA gene sequence similarity with the nearest type strains ranged from 98.5% to 99.9% and 13 *Shewanella* isolates had 98.8%-98.9% similarity (Table S4.2).

Prokaryotic abundance of 4 sediment samples ranged from 1.33–2.19 × 10^8 cells g^{-1} and cultivable numbers of bacteria obtained by agar plates ranged from 0.01–4.09 × 10^6 CFU g^{-1} (Table S4.2). Through these measurements, it was estimated

that average 1.1% of bacteria were cultivable cells in the study sediment but about 99% still remains to be cultivated in the study environments (Table S4.2).

4.3.2 Phylogenetic analyses of marker genes

The 16S rRNA gene sequences of strains M2^T and R106 were nearly identical (99.9%). Comparison of 16S rRNA gene sequences showed that these strains were mostly close to *S. vesiculosa* LMG 24424^T with the similarity value of 98.9%, and followed by *S. livingstonensis* LMG 19866^T, *S. arctica* KCTC 23109^T and *S. frigidimarina* KCCM 41815^T with values of 98.8–98.1%. Phylogenetic analyses of the 16S rRNA gene sequences showed that strains M2^T and R106 were affiliated with the genus *Shewanella*, specifically, with the SF clade including *S. vesiculosa* LMG 24424^T, *S. livingstonensis* LMG 19866^T, *S. arctica* KCTC 23109^T and *S. frigidimarina* KCCM 41815^T (Fig. 4.1). In spite of a high level of sequence similarities between strain M2^T and the type strains of the above species, strains M2^T and R106 formed a distinct subline in the SF clade (Fig. 4.1).

The *gyrB* gene sequence of strain M2^T was identical with that of strain R106. On the basis of *gyrB* gene sequences, the closest relative of strain M2^T was the type strain of *S. frigidimarina* with the sequence similarity of 89.4%. This value is lower than the *Shewanella* species cut-off value of 90% (Venkateswaran *et al*., 1999), indicating that strains M2^T and R106 represent a new *Shewanella* species. As shown in the 16S rRNA gene phylogeny, phylogenetic analyses of *gyrB* gene sequences strongly support the SF clade, in which strains M2^T and R106 formed a distinct subline (Fig. 4.2).

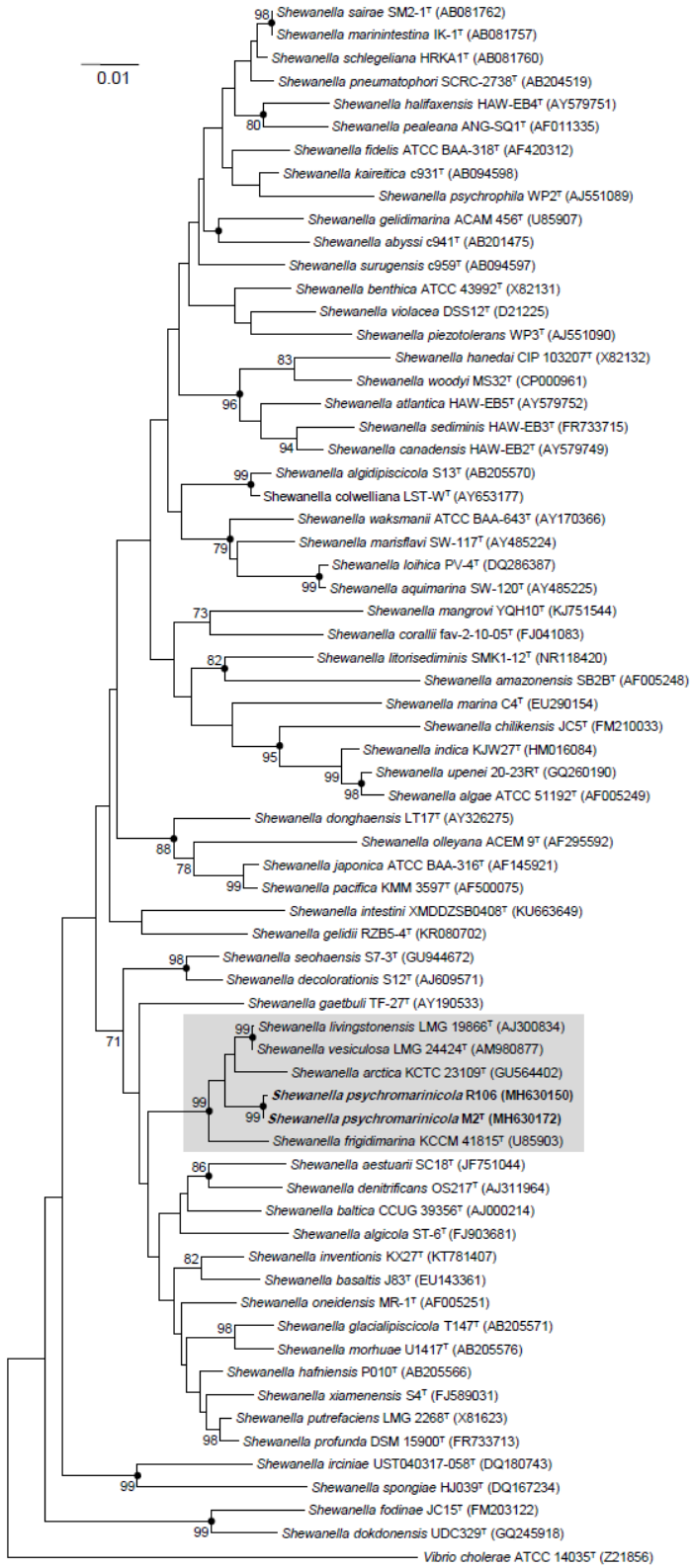

0.01
Shewanella sairae SM2-1T (AB081762)
Shewanella marinintestina IK-1T (AB081757)
Shewanella schlegeliana HRKA1T (AB081760)
Shewanella pneumatophori SCRC-2738T (AB204519)
Shewanella halifaxensis HAW-EB4T (AY579751)
Shewanella pealeana ANG-SQ1T (AF011335)
Shewanella fidelis ATCC BAA-318T (AF420312)
Shewanella kaireitica c931T (AB094598)
Shewanella psychrophila WP2T (AJ551089)
Shewanella gelidimarina ACAM 456T (U85907)
Shewanella abyssi c941T (AB201475)
Shewanella surugensis c959T (AB094597)
Shewanella benthica ATCC 43992T (X82131)
Shewanella violacea DSS12T (D21225)
Shewanella piezotolerans WP3T (AJ551090)
Shewanella hanedai CIP 103207T (X82132)
Shewanella woodyi MS32T (CP000961)
Shewanella atlantica HAW-EB5T (AY579752)
Shewanella sediminis HAW-EB3T (FR733715)
Shewanella canadensis HAW-EB2T (AY579749)
Shewanella algidipiscicola S13T (AB205570)
Shewanella colwelliana LST-WT (AY653177)
Shewanella waksmanii ATCC BAA-643T (AY170366)
Shewanella marisflavi SW-117T (AY485224)
Shewanella loihica PV-4T (DQ286387)
Shewanella aquimarina SW-120T (AY485225)
Shewanella mangrovi YQH10T (KJ751544)
Shewanella corallii fav-2-10-05T (FJ041083)
Shewanella litorisediminis SMK1-12T (NR118420)
Shewanella amazonensis SB2BT (AF005248)
Shewanella marina C4T (EU290154)
Shewanella chilikensis JC5T (FM210033)
Shewanella indica KJW27T (HM016084)
Shewanella upenei 20-23RT (GQ260190)
Shewanella algae ATCC 51192T (AF005249)
Shewanella donghaensis LT17T (AY326275)
Shewanella olleyana ACEM 9T (AF295592)
Shewanella japonica ATCC BAA-316T (AF145921)
Shewanella pacifica KMM 3597T (AF500075)
Shewanella intestini XMDDZSB0408T (KU663649)
Shewanella gelidii RZB5-4T (KR080702)
Shewanella seohaensis S7-3T (GU944672)
Shewanella decolorationis S12T (AJ609571)
Shewanella gaetbuli TF-27T (AY190533)
Shewanella livingstonensis LMG 19866T (AJ300834)
Shewanella vesiculosa LMG 24424T (AM980877)
Shewanella arctica KCTC 23109T (GU564402)
Shewanella psychromarinicola R106 (MH630150)
Shewanella psychromarinicola M2T (MH630172)
Shewanella frigidimarina KCCM 41815T (U85903)
Shewanella aestuarii SC18T (JF751044)
Shewanella denitrificans OS217T (AJ311964)
Shewanella baltica CCUG 39356T (AJ000214)
Shewanella algicola ST-6T (FJ903681)
Shewanella inventionis KX27T (KT781407)
Shewanella basaltis J83T (EU143361)
Shewanella oneidensis MR-1T (AF005251)
Shewanella glacialipiscicola T147T (AB205571)
Shewanella morhuae U1417T (AB205576)
Shewanella hafniensis P010T (AB205566)
Shewanella xiamenensis S4T (FJ589031)
Shewanella putrefaciens LMG 2268T (X81623)
Shewanella profunda DSM 15900T (FR733713)
Shewanella irciniae UST040317-058T (DQ180743)
Shewanella spongiae HJ039T (DQ167234)
Shewanella fodinae JC15T (FM203122)
Shewanella dokdonensis UDC329T (GQ245918)
Vibrio cholerae ATCC 14035T (Z21856)

Figure 4.1. Neighbour-joining tree of the 16S rRNA gene sequences for strains M2^T, R106 and the members in the genus *Shewanella* with *Vibrio cholerae* ATCC 14035^T (Z21856) as an outgroup. Only bootstrap values above 70% are shown (1000 resamplings) at the branching points. Filled circles indicate that the corresponding nodes were also obtained in both the maximum-parsimony and the maximum-likelihood trees. Bar, 0.01 nucleotide substitution per site.

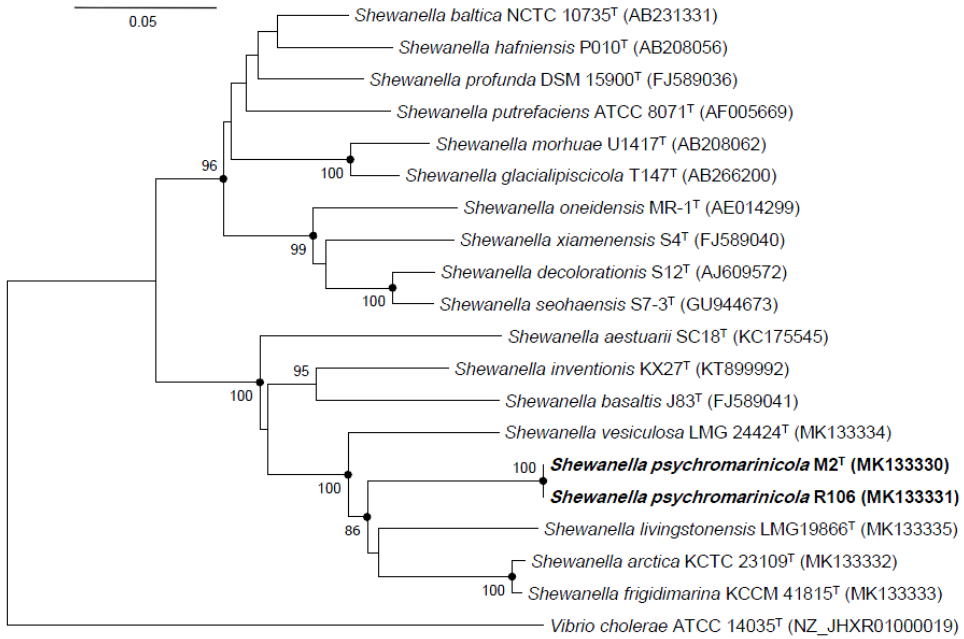


Figure 4.2. Neighbour-joining tree of the *gyrB* gene sequences for strains M2^T, R106 and selected members in the genus *Shewanella* with *Vibrio cholerae* ATCC 14035^T (NZ_JHXR01000019) as an outgroup. Only bootstrap values above 70% are shown (1000 resamplings) at the branching points. Filled circles indicate that the corresponding nodes were also obtained in both the maximum-parsimony and the maximum-likelihood trees. Bar, 0.05 nucleotide substitution per site.

4.3.3 Genome characteristics and phylogenomic analyses

Phylogenomic analysis of the concatenated 110 ubiquitous single-copy proteins revealed that strains M2^T and R106 formed a long branch separated from the type strain of *S. livingstonensis* supported by a bootstrap value of 76% in the ML tree (Fig. 4.3). Although the topologies of phylogeny trees were somewhat different depending on selected marker genes (i.e., 16S rRNA gene, *gyrB* gene or concatenated single-copy proteins), strains M2^T and R106 formed a robust clade that could be phylogenetically distinguished from the previously recognized *Shewanella* species (Fig. 4.1, 4.2 and 4.3).

The genome sizes of strains of M2^T and R106 were 5.13 and 5.09 Mbp, respectively (Table 4.1). Other statistics of the final genome assembly for each strain were given in Table 4.1, according to the minimal standards for the use of genomic data in prokaryotic taxonomy (Chun *et al*., 2018). Genome relatedness for strains M2^T, R106 and 4 type strains of *Shewanella* spp. in the SF clade was summarized in Table 4.2. The ANI value generated by pairwise genome-based relatedness between strains M2^T and R106 was 99.9%, indicative of a single genomic species. The ANI values calculated for pairwise comparisons between strain M2^T and the 4 type strains of *S. vesiculosa* LMG 24424^T, *S. livingstonensis* LMG 19866^T, *S. arctica* KCTC 23109^T and *S. frigidimarina* KCCM 41815^T were 84.3–85.5% (Table 4.2). This level is obviously below the proposed cut-off ANI values, 95–96% for the definition of a novel species (Goris *et al*., 2007, Richter and Rosselló-Móra, 2009). A high value of dDDH (98.2%) was observed between strains M2^T and R106 (Table 4.2). As such in the ANI results, dDDH values between strain M2^T and the 4 type strains of

Shewanella spp. were low as 28.0–29.7% (Table 4.2), indicating that strains M2^T and R106 are members of a novel novel species of the genus *Shewanella* (Rosselló-Mora and Amann1 2001).

Based on the compositions of genome sequences, DNA G+C contents of strains M2^T and R106 were 42.1–42.2 mol%, which are slightly higher than those of the phylogenetically nearest relatives *S. vesiculosa* LMG 24424^T, *S. livingstonensis* LMG 19866^T, *S. arctica* KCTC 23109^T and *S. frigidimarina* KCCM 41815^T (41.1–41.7 mol%; Table 4.3). The genomic DNA G+C contents of strains M2^T and R106 were within the range reported in the genus *Shewanella* (40–54 mol%; Bowman, 2015).

Comparison of whole genome sequences of *Shewanella* strains revealed that members in the SF clade have a unique configuration of the *mtr–omc* cluster (Fig. 4.4) that is involved in metal-reducing pathway (Zhong *et al*., 2018). The *mtr–omc* cluster was also recovered from draft genome sequences of other *Shewanella* members in the SF clade and displayed a highly conserved gene cluster (Fig. 4.5). Although a synteny of the *mtr–omc* cluster like the SF clade was apparently found in a distantly related *Shewanella* strain (i.e., *S. benthica* DB21MT-2; Fig. 4.4), those gene contents between the *feoA–feoB* operon and the *omcA* gene of *S. benthica* DB21MT-2 were turned out to be clearly different from those of members in the SF clade (Fig. 4.5). Two sets of the *feoA–feoB* operon involved in ferrous iron transport were detected in the complete genome sequence of strain M2^T, for the first observation in complete genome sequences of *Shewanella* strains, while others have one set (Fig. 4.4). All of 6 strains tested in the present study gave positive results of iron reduction, which might be reflected by such genomic features.

Table 4.1. Genome sequences of *Shewanella* spp. used in the phylogenomic analysis. Genome sequences of 6 strains in bold were obtained from the present study. ND, No data.

No.	Type strain	Organism Name	Strain	BioSample	Assembly	Level	Size (Mb)	Coverage	N50 (Mb)	GC%	Scaffolds
1	**Type**	***Shewanella psychromarinicola***	**M2^T**	**SAMN10397594**	**GCA_003855155.1**	**Complete**	**5.13495**	**282**	**5.13495**	**42.2**	**1**
2	**Non-type**	***Shewanella psychromarinicola***	**R106**	**SAMN10397511**	**GCA_003797165.1**	**Scaffold**	**5.09425**	**174**	**0.58765**	**42.1**	**53**
3	**Type**	***Shewanella vesiculosa***	**LMG 24424^T**	**SAMN10397593**	**GCA_003797885.1**	**Scaffold**	**4.55650**	**375**	**0.30605**	**41.7**	**45**
4	**Type**	***Shewanella livingstonensis***	**LMG 19866^T**	**SAMN10397595**	**GCA_003855395.1**	**Complete**	**4.83988**	**70**	**4.83988**	**41.1**	**1**
5	**Type**	***Shewanella arctica***	**KCTC 23109^T**	**SAMN10397596**	**GCA_003797125.1**	**Scaffold**	**4.67759**	**200**	**0.98664**	**41.4**	**19**
6	**Type**	***Shewanella frigidimarina***	**KCCM 41815^T**	**SAMN10397592**	**GCA_003797845.1**	**Scaffold**	**4.50047**	**272**	**0.21332**	**41.3**	**57**
7	Type	*Shewanella algae*	NBRC 103173^T	SAMD00046904	GCA_001598875.1	Contig	4.81725	120	0.06505	53.1	143
8	Type	*Shewanella amazonensis*	SB2B^T	SAMN02598298	GCA_000015245.1	Complete	4.30614	ND	4.30614	53.6	1
9	Type	*Shewanella baltica*	NCTC 10735^T	SAMEA4442456	GCA_900456975.1	Contig	5.30084	100	5.28747	46.3	2
10	Type	*Shewanella chilikensis*	JC5^T	SAMN08777254	GCA_003217175.1	Scaffold	4.44126	215	0.09770	52.4	90
11	Type	*Shewanella colwelliana*	ATCC 39565^T	SAMN02584968	GCA_000518705.1	Scaffold	4.57562	ND	0.17156	45.4	58
12	Type	*Shewanella decolorationis*	S12^T	SAMN02469853	GCA_000485795.1	Scaffold	4.84397	1200	0.15554	47.1	77
13	Type	*Shewanella denitrificans*	OS217^T	SAMN02598300	GCA_000013765.1	Complete	4.54591	ND	4.54591	45.1	1
14	Type	*Shewanella fidelis*	ATCC BAA-318^T	SAMN02584974	GCA_000518605.1	Scaffold	4.79869	ND	0.36025	42.8	27
15	Type	*Shewanella halifaxensis*	HAW-EB4^T	SAMN02598431	GCA_000019185.1	Complete	5.22692	ND	5.22692	44.6	1
16	Type	*Shewanella indica*	KJW27^T	SAMN07175133	GCA_002836975.1	Scaffold	4.40281	500	0.12984	52.4	55
17	Type	*Shewanella japonica*	KCTC 22435^T	SAMN06628905	GCA_002075795.1	Complete	4.97568	205	4.97568	40.8	1
18	Type	*Shewanella loihica*	PV-4^T	SAMN00623064	GCA_000016065.1	Complete	4.60259	ND	4.60259	53.7	1

Table 4.1. (Continued)

19	Type	*Shewanella mangrovi*	YQH10^T	SAMN02887322	GCA_000753795.1	Contig	4.21579	191	0.23334	48.1	49
20	Type	*Shewanella marina*	JCM 15074^T	SAMD00011086	GCA_000614975.1	Contig	4.42465	24	0.14216	40.4	75
21	Type	*Shewanella morhuae*	ATCC BAA-1205^T	SAMN05421840	GCA_900156405.1	Scaffold	4.19037	355	0.25122	44.0	32
22	Type	*Shewanella oneidensis*	MR-1^T	SAMN02604014	GCA_000146165.2	Complete	5.13142	ND	5.13142	45.9	2
23	Type	*Shewanella pealeana*	ATCC 700345^T	SAMN02598386	GCA_000018285.1	Complete	5.17458	ND	5.17458	44.7	1
24	Type	*Shewanella piezotolerans*	WP3^T	SAMN02603456	GCA_000014885.1	Complete	5.39648	ND	5.39648	43.3	1
25	Type	*Shewanella psychrophila*	WP2^T	SAMN04370084	GCA_002005305.1	Complete	6.35341	180	6.35341	44.3	1
26	Type	*Shewanella putrefaciens*	NBRC 3908^T	SAMD00046716	GCA_001591325.1	Contig	4.33612	110	0.08178	44.3	84
27	Type	*Shewanella sediminis*	HAW-EB3^T	SAMN02598412	GCA_000018025.1	Complete	5.51767	ND	5.51767	46.1	1
28	Type	*Shewanella violacea*	DSS12^T	SAMD00060963	GCA_000091325.1	Complete	4.96210	ND	4.96210	44.7	1
29	Type	*Shewanella waksmanii*	ATCC BAA-643^T	SAMN02584962	GCA_000518805.1	Scaffold	4.97148	ND	0.10159	45.3	82
30	Type	*Shewanella woodyi*	ATCC 51908^T	SAMN02598391	GCA_000019525.1	Complete	5.93540	ND	5.93540	43.7	1

Table 4.2. Pairwise comparisons of overall genome relatedness based on the average nucleotide identity (ANI) (Auch *et al*., 2010) and digital DNA-DNA hybridization (dDDH) inferred by the genome-to-genome distance (Auch *et al*., 2010). Genome sequences of strains M2^T, R106, *Shewanella vesiculosa* LMG 24424^T (SV), *Shewanella livingstonensis* LMG 19866^T (SL), *Shewanella arctica* KCTC 23109^T (SA) and *Shewanella frigidimarina* KCCM 41815^T (SF) are available in GenBank/EMBL/DBBJ databases (for accession numbers, see Table 4.1).

	ANI (%)						dDDH (%)					
	M2^T	R106	SV	SL	SA	SF	M2^T	R106	SV	SL	SA	SF
M2^T		99.9	84.3	85.0	85.4	85.5		98.2	28.0	29.4	29.7	29.7
R106			84.3	85.0	85.3	85.4			27.9	29.4	29.7	29.6
SV				85.2	84.9	85.0				29.2	28.9	29.0
SL					86.4	86.5					31.7	31.9
SA						98.0						81.7

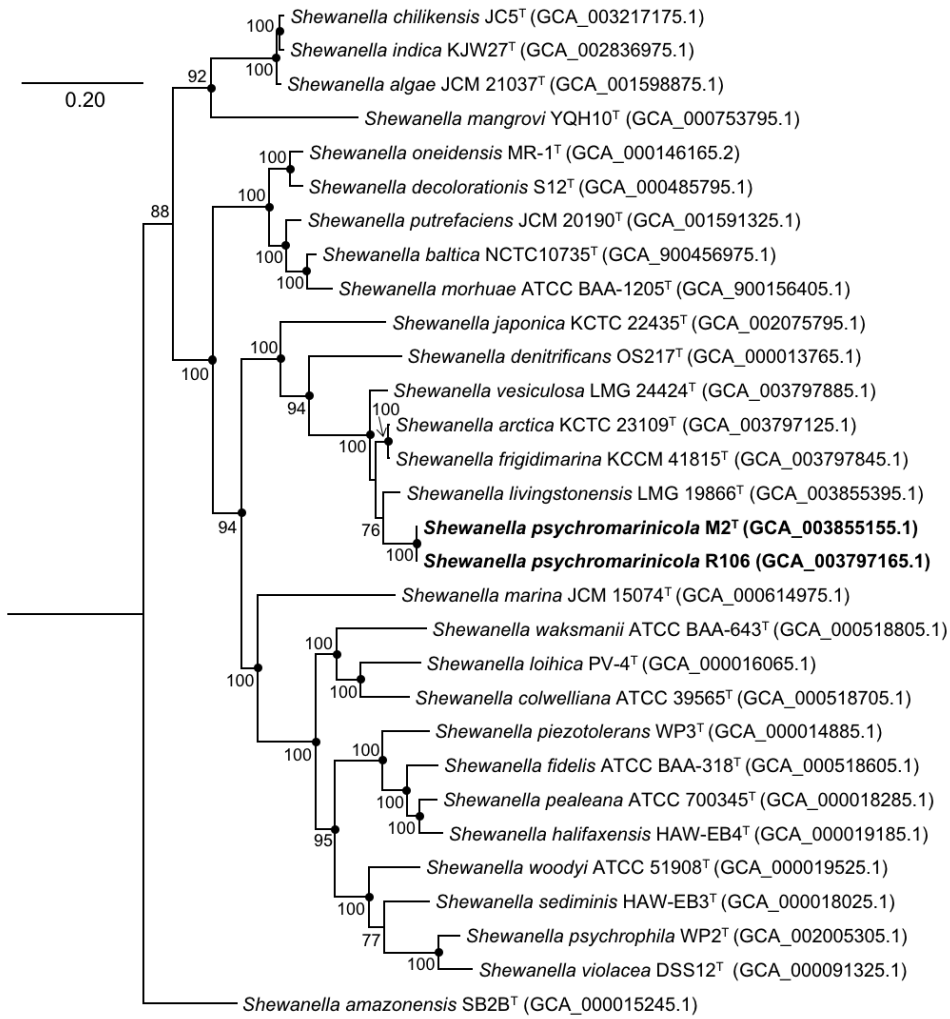


Figure 4.3. Maximum-likelihood tree of concatenated 110 ubiquitous single-copy proteins retrieved from the genome sequences of strains M2^T, R106 and selected members in the genus *Shewanella* with *Vibrio cholerae* ATCC 14035^T (GCA_000621645.1) as an outgroup. Only bootstrap values above 70% are shown (1000 resamplings) at the branching points. Filled circles indicate that the corresponding nodes were also obtained in both the neighbour-joining and the maximum-parsimony trees. Bar, 0.20 substitutions per site.

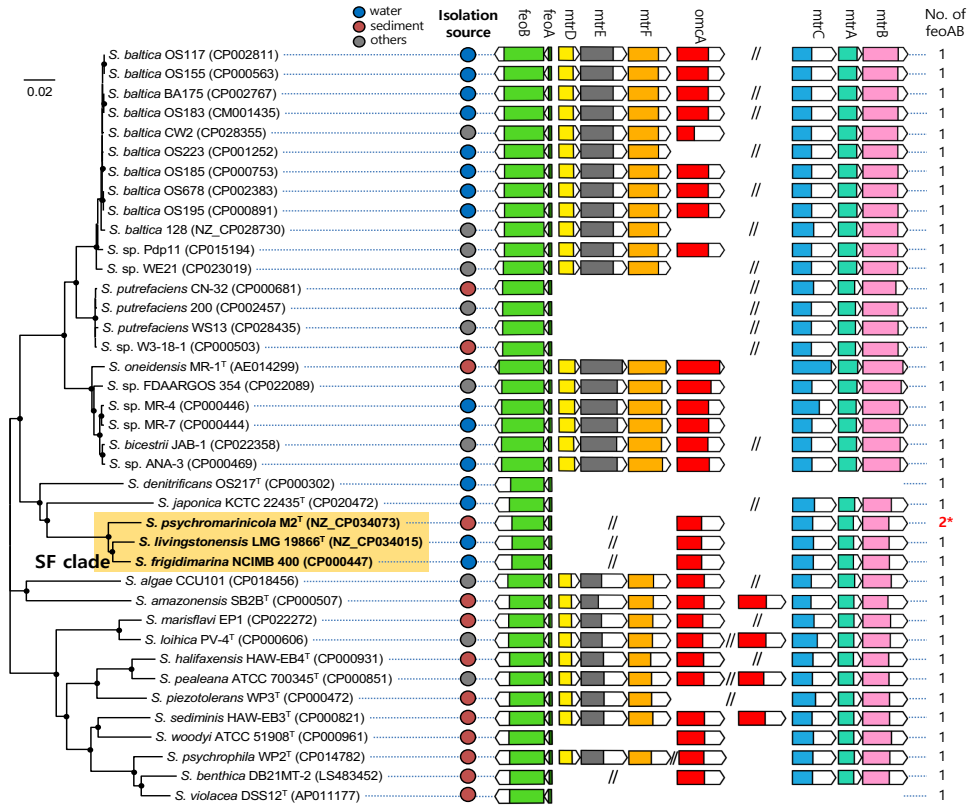
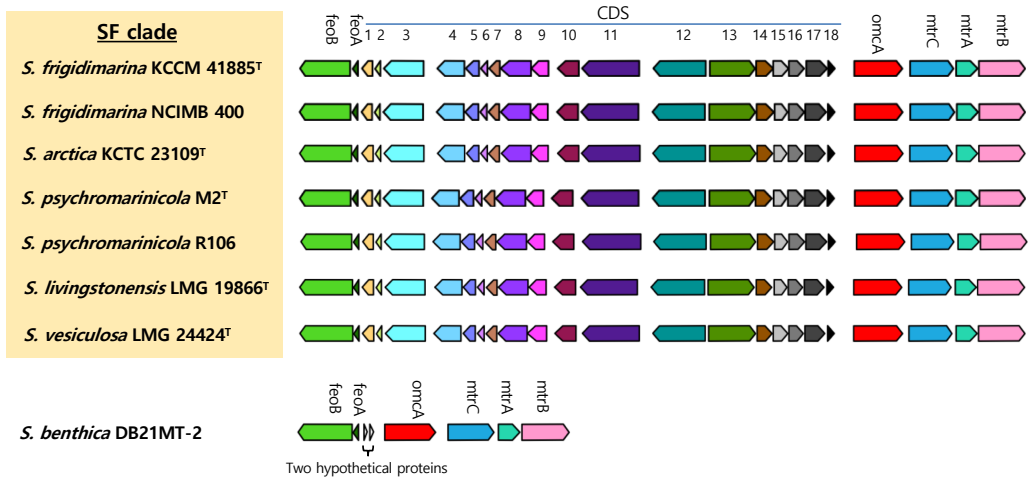


Figure 4.4. Comparison of the *mtr–omc* clusters in whole genome sequences of *Shewanella* strains that are available in the NCBI Genome. Arrows represent genes and their orientations. The length of the colored box is proportional to gene homology with those in *S. oneidensis* MR-1^{T}. The double slash indicates the presence of extra genes. The asterisk indicates the presence of additional *feoA-feoB* operon in different location. The tree on the left is a phylogenomic tree of concatenated 110 ubiquitous single-copy proteins (see Materials and Methods). Filled circles indicate that the corresponding nodes were recovered with bootstrap values above 70% in the neighbour-joining algorithm. Bar, 0.20 substitutions per site.



CDS	Product	CDS	Product
1	*N*-acetyltransferase	10	class I SAM-dependent methyltransferase
2	peptidylprolyl isomerase	11	TonB-dependent receptor
3	DUF885 domain-containing protein	12	DNA polymerase II
4	hypothetical protein	13	ATP-dependent DNA helicase DinG
5	energy transducer TonB	14	hypothetical protein
6	biopolymer transporter ExbD	15	DUF2057 domain-containing protein
7	MotA/TolQ/ExbB proton channel family protein	16	prepilin peptidase
8	MotA/TolQ/ExbB proton channel family protein	17	histone deacetylase
9	DUF3450 domain-containing protein	18	competence protein ComFB

Figure 4.5. Detailed comparison of the *mtr–omc* clusters in whole or draft genome sequences of *Shewanella* strains in the SF clade and *S. benthica* DB21MT-2. Arrows represent genes and their orientations. CDS: coding sequence.

4.3.4 Morphological, physiological and biochemical characteristics

Cells of strains M2^T and R106 were Gram-negative-stain, facultative anaerobic, iron-reducing rods (approximately 0.2–1.3 μm wide and 0.6–5.7 μm long) with motility by means of a single polar flagellum (Fig. 4.6). After 3–5 days on marine agar plates at 15 °C, colonies are orange, circular and convex, and approximately 2–3 mm in diameter. Growth occurs at 4–20 °C (optimum 10–15 °C), pH 5.5–8.5 (optimum pH 6.5) at sea salts concentration of 0.5–10.0% (w/v) (optimum 3.0–4.0% and 2.0–3.0% for strains M2^T and R106, respectively). Oxidase, catalase, iron reduction and H_2S production are positive. Casein and Tween 80 are hydrolyzed, but DNA and starch are not. According to the API ZYM test, positive for *N*-acetyl-β-glucosaminidase, acid phosphatase, alkaline phosphatase, cystine arylamidase, esterase (C4), and α-glucosidase, but negative for α-chymotrypsin, esterase lipase (C8), α-fucosidase, α-galactosidase, β-galactosidase, β-glucosidase, β-glucuronidase, leucine arylamidase, lipase (C14), Naphthol-AS-BI-phosphohydrolase, α-mannosidase, trypsin and valine arylamidase. According to the API 20NE test, positive for nitrates reduction and esculin hydrolysis, but negative for arginine dihydrolase, β-galactosidase, gelatinase, glucose fermentation, indole production and urease. According to the API 50CH test, acid is produced from *N*-acetylglucosamine, D-cellobiose, esculin ferric citrate, D-glucose, D-mannitol, D-ribose and sucrose, but not from D-adonitol, amygdalin, DL-arabinose, DL-arabitol, arbutin, dulcitol, erythritol, D-fructose, DL-fucose, glycerol, glycogen, inositol, inulin, D-lactose, D-lyxose, D-mannose, D-melezitose, D-melibiose, methyl α-D-glucopyranoside, methyl α-D-mannopyranoside, methyl β-D-xylopyranoside,

potassium gluconate, potassium 2-keto-gluconate, potassium 5-ketogluconate, D-raffinose, L-rhamnose, salicin, D-sorbitol, L-sorbose, D-tagatose, D-trehalose, D-turanose, xylitol and DL-xylose. Depending on strains, acid production is variable from D-galactose, gentiobiose, D-maltose and starch. *N*-Acetyl-D-glucosamine, L-arabinose, butyrate, D-fructose, fumarate, D-galactose, D-glucose, glycerol, L-lactate, lactose, D-lyxose, maltose, D-mannitol, melibiose, starch, D-sorbitol, sucrose (variable depending on strains), L-proline, propionate, D-trehalose and D-xylose are utilized as a sole carbon source, but cellobiose, D-malic acid and D-ribose are not. The selected morphological, physiological and biochemical characteristics of strain M2^T, R106 and closely related *Shewanella* species are summerized in Table 4.3.

Strains M2^T and R106 showed almost identical results for phenotypic characteristics except the salinity range for optimal growth, the acid production from 4 carbohydrates and the utilization of sucrose as sole carbon source (Table 4.3). Strains M2^T and R106 had the optimal temperature for growth at 10–15 °C and a maximal temperature for growth at 20 °C (Table 4.3), that can be regarded as psychrophilic bacteria (Morita, 1975). The low temperature range for optimal growth (10–15 °C) could differentiate strains M2^T and R106 from their closest phylogenetic relatives *S. vesiculosa* LMG 24424^T, *S. livingstonensis* LMG 19866^T, *S. arctica* KCTC 23109^T and *S. frigidimarina* KCCM 41815^T (20–25 °C; Table 4.3). The maximum temperature for growth of strains M2^T and R106 (i.e., 20 °C) was distinguishably lowest among the related *Shewanella* species (Table 4.3). Strains M2^T and R106 could be distinguished from those type strains of 4 *Shewanella* species in the SF clade by their inabilities to utilize cellobiose as sole carbon source

(Table 4.2). In addition, combination of certain phenotypic characteristics [e.g., salt tolerance range, gelatin hydrolysis, lipase (C14), utilization of D-mannitol and D-ribose as sole carbon source] could be used to differentiate strains $M2^T$ and R106 from those related *Shewanella* species (Table 4.3).

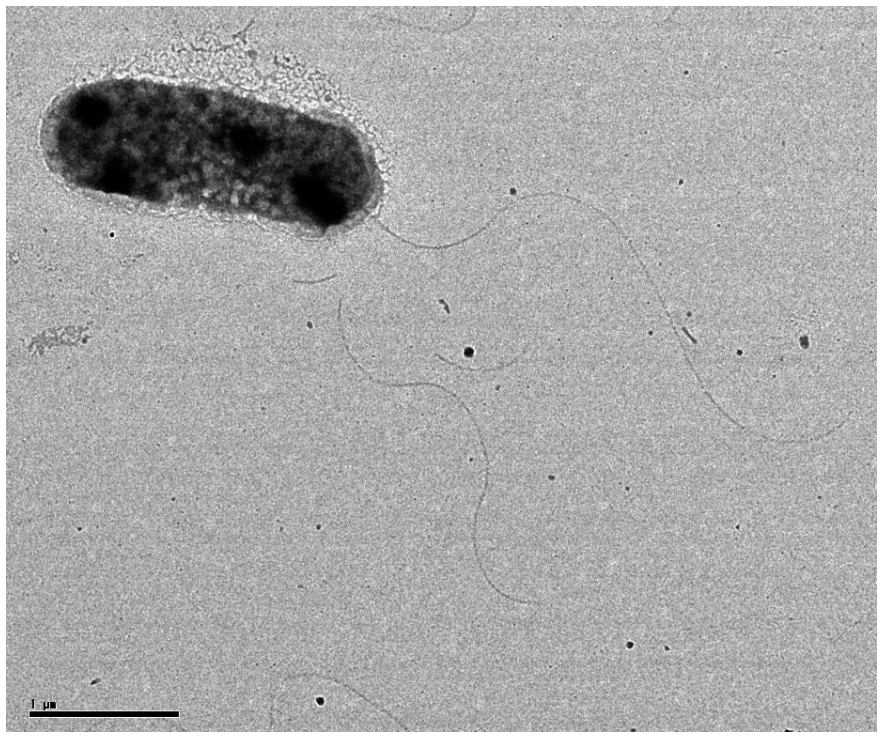


Figure 4.6. Transmission electron micrograph of negatively stained cell of strain M2^T grown on marine agar at 15 °C for 3 days. Bar, 1.0 µm.

Table 4.3. Differential characteristics of strains M2^T, R106 and related *Shewanella* species.

Taxa: 1, M2^T; 2, R106; 3, *S. vesiculosa* LMG 24424^T; 4, *S. livingstonensis* LMG 19866^T; 5, *S. arctica* KCTC 23109^T; 6, *S. frigidimarina* KCCM 41815^T; 7, *S. putrefaciens* (Venkateswaran *et al*., 1999; Brettar *et al*., 2002); 8, *S. aestuarii* SC18^T (Park and Jeon, 2013); 9, *S. basaltis* J83^T (Chang *et al*., 2008); 10, *S. inventionis* KX27^T (Wang and Sun, 2016); 11, *S. denitrificans* OS217^T (Brettar *et al*., 2002); 12, *S. japonica* KCTC 22435^T (Ivanova *et al*., 2001) Data from this study unless otherwise indicated. +, Positive; –, negative; ND, no data.

Characteristic	1	2	3	4	5	6	7*	8*	9*	10*	11*	12*
Temperature range (optimum) (°C)	4–20 (10–15)	4–20 (10–15)	4–30 (20–25)	4–25 (20–25)	4–30 (20)	4–30 (20)	4–35 (25–35)	4–37 (25–30)	4–45 (ND)	4–37 (28)	4–30 (20–25)	10–37 (20–25)
pH range (optimum)	5.5–8.5 (6.5)	5.5–8.5 (6.5)	4.9–9.4 (6.2–7.2)	5.7–9.0 (6.0–7.0)	5.5–9.4 (6.2–6.5)	5.5–9.4 (6.2–6.5)	ND (7–8)	5.0–9.0 (7.0)	5.5–9.5 (ND)	6.0–9.0 (7.0)	5.7–7.8 (ND)	6.0–9.0 (7.5)
Salt tolerance range (optimum) (%, w/v)	0.5–10.0 (3.0–4.0)	0.5–10.0 (2.0–3.0)	0–10.0 (2.0–3.0)	0.5–9.0 (1.0–2.0)	0.5–9.0 (2.0–3.0)	0.5–9.0 (2.0)	0–6 (ND)	0–5.0 (0–2.0)	5.0 (ND)	0–5.0 (2.0)	0–6.0 (1.0–3.0)	0–3.0 (1.0–3.0)
Gelatin hydrolysis	–	–	+	+	+	–	–	+	–	–	+	+
Lipase (C14)	–	–	–	–	+	+	–	–	–	–	–	+
Acid production from:												
D-Fructose	–	–	+	–	–	+	ND	–	ND	ND	ND	ND
D-Galactose	–	+	+	+	–	–	ND	–	ND	ND	ND	ND
Gentiobiose	–	+	+	–	–	–	ND	ND	ND	ND	ND	ND
Glycogen	–	–	+	–	–	–	ND	ND	ND	ND	ND	ND
Maltose	–	+	+	+	+	+	ND	ND	ND	ND	ND	ND
D-Mannitol	+	+	+	–	–	+	ND	–	ND	ND	ND	ND
Starch	–	+	+	+	–	+	ND	ND	ND	ND	ND	ND
D-Xylose	–	–	–	+	–	–	ND	–	ND	ND	ND	ND

Table 4.3. (Continued)

Utilization of sole carbon:												
Cellobiose	–	–	+	+	+	+	–	ND	ND	ND	–	+
Maltose	+	+	–	+	–	–	+	+	+	–	+	+
D-Mannitol	+	+	–	+	+	+	–	–	+	+	–	–
D-Ribose	–	–	+	+	–	–	ND	ND	ND	ND	ND	ND
Sucrose	+	–	–	–	–	+	+	–	–	ND	–	–
D-Trehalose	+	+	+	+	+	–	ND	–	ND	ND	ND	–
DNA G+C content (mol%) by genome analysis	42.2	42.1	41.7 (42)†	41.1 (41.0)†	41.4 (40)†	41.3 (40–43)†	(43–47)†	(41.3)†	(ND)†	(43)†	(46.8)†	(43–44)†

*All data were taken from the previous studies indicated.

† Data in parentheses for taxa 3–12 were from Bozal *et al*. (2009), Bozal *et al*. (2002), Kim *et al*. (2012), Bowman *et al*. (1997), Venkateswara *et al*. (1999), Park and Jeon (2013), Chang *et al*. (2008), Wang and Sun (2016), Brettar *et al*. (2002) and Ivanova *et al*. (2001), respectively.

4.3.5 Chemotaxonomic characteristics

The polar lipids of strain M2^T were phosphatidylglycerol (PG), phosphatidylethanolamine (PE), two unidentified aminophospholipids (APL1–2), an unidentified aminolipid (AL) and an unidentified phospholipid (PL1; Fig. 4.7). Both PG and PE were major polar lipids in the examined strains of *Shewanella* species (Fig. 4.7), which are commonly found in different *Shewanella* species (Bowman 2015).

Strain M2^T included ubiquinone-7 (Q-7; 31.1%), ubiquinone-8 (Q-8; 24.7%), menaquinone-7 (MK-7; 37.3%), and with smaller amounts of methylmenaquinone-7 (MMK-7; 6.9%). These quinone compositions are commonly detected in *Shewanella* species (Bozal *et al*., 2002; Morita, 1975).

The fatty acid profiles of strains M2^T and R106 were similar (Table 4.4); the major components were summed feature 3 ($C_{16:1}$ ω7*c* and/or $C_{16:1}$ ω6*c*; 19.0–26.5%), $C_{17:1}$ ω8*c* (10.5–18.1%) and $C_{16:0}$ (7.5–10.7%). These fatty acids are also found as major components in the phylogenetically close-related *Shewanella* species (Table 4.4).

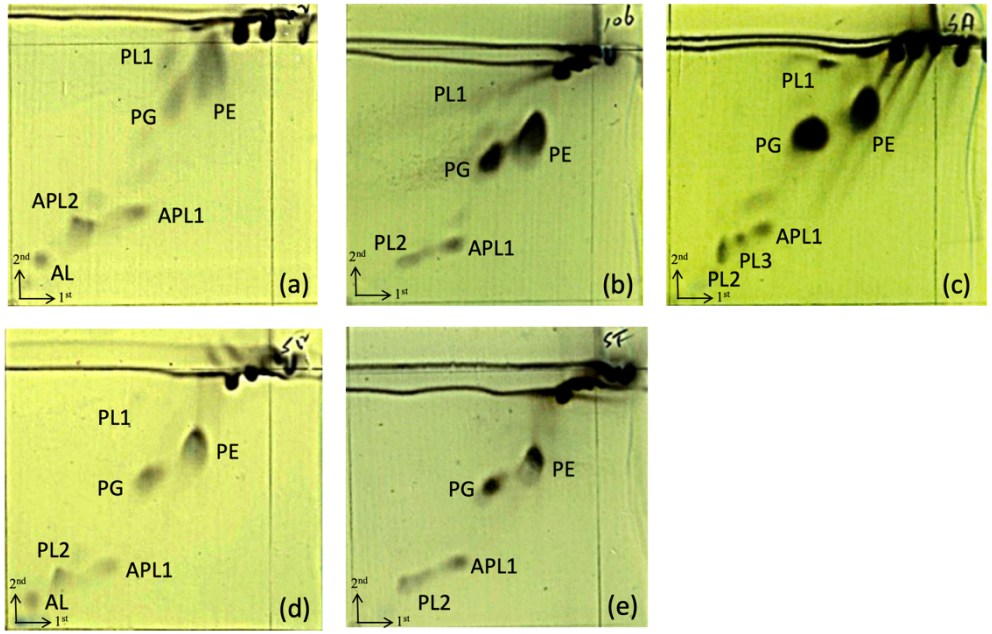


Figure 4.7. Two-dimensional TLC of the polar lipids of (a) strain M2^T, (b) strain R106, (c) *Shewanella arctica* KCTC 23109^T, (d) *Shewanella vesiculosa* LMG 24424^T and (e) *Shewanella frigidimarina* KCCM 41815^T. PG, Phosphatidylglycerol; PE, phosphatidylethanolamine; APL, unidentified aminophospholipid; PL, unidentified phospholipid; AL, unidentified aminolipid.

Table 4.4. Cellular fatty acid compositions of strains M2^T, R106 and phylogenetically close-related *Shewanella* species.

Taxa: 1, M2^T; 2, R106; 3, *S. vesiculosa* LMG 24424^T; 4, *S. livingstonensis* LMG 19866^T; 5, *S. arctica* KCTC 23109^T; 6, *S. frigidimarina* KCCM 41815^T. All data were obtained in this study; cells were grown on marine agar for 3 days at 15 °C (taxa 1 and 2) or 20 °C (taxa 3–6). Values are percentages of total fatty acids. ND, Not detected; Tr, trace (< 1%).

Fatty acids	1	2	3	4	5	6
Saturated						
$C_{12:0}$	4.2	3.1	3.5	2.8	4.9	1.2
$C_{13:0}$	1.4	2.3	1.6	9.8	3.1	Tr
$C_{14:0}$	2.3	1.4	2.3	2.3	2.1	Tr
$C_{16:0}$	10.7	7.5	9.9	2.5	8.0	2.3
$C_{17:0}$	2.1	3.9	2.1	1.4	2.8	2.1
Unsaturated						
$C_{15:1}$ ω6*c*	Tr	1.1	1.5	9.6	2.1	1.4
$C_{15:1}$ ω8*c*	Tr	Tr	Tr	4.2	1.5	Tr
$C_{17:1}$ ω6*c*	1.0	1.3	1.3	1.3	1.2	2.0
$C_{17:1}$ ω8*c*	10.5	18.1	9.6	18.7	12.9	11.4
$C_{18:1}$ ω9*c*	2.2	1.8	1.3	Tr	1.3	1.6
Branched						
iso-$C_{13:0}$	7.0	8.4	9.6	12.1	5.9	9.9
iso-$C_{14:0}$	1.5	2.0	Tr	Tr	Tr	Tr
iso-$C_{15:0}$	9.7	9.7	9.7	3.1	5.4	26.8
iso-$C_{17:0}$	Tr	Tr	Tr	Tr	Tr	4.7
iso-$C_{13:0}$ 3-OH	4.1	5.0	3.5	2.4	3.0	8.1

Table 4.4. (Continued)

Hydroxy						
$C_{11:0}$ 3-OH	Tr	1.0	Tr	2.1	1.7	Tr
$C_{12:0}$ 3-OH	2.2	1.9	1.1	1.2	2.8	Tr
Summed features[*]						
1	1.2	2.1	Tr	3.5	2.1	Tr
2	1.2	Tr	1.2	Tr	1.5	Tr
3	26.5	19.0	30.2	16.1	28.5	13.6
8	3.1	1.8	4.0	Tr	3.2	3.4

[*]Summed features are groups of two or three fatty acids that cannot be separated by GLC with the MIDI System. Summed feature 1 comprises iso-$C_{15:1}$ H and/or $C_{13:0}$ 3-OH; Summed feature 2 comprises $C_{14:0}$ 3-OH, iso-$C_{16:1}$ I and/or $C_{12:0}$ aldehyde; Summed feature 3 comprises $C_{16:1}$ ω7*c* and/or $C_{16:1}$ ω6*c*; Summed feature 8 comprises $C_{18:1}$ ω7*c* and/or $C_{18:1}$ ω6*c*.

[‡]Compositions of $C_{13:0}$ 3-OH, iso-$C_{15:1}$ H and $C_{15:1}$.

[§]Composition of $C_{16:1}$ ω7*c*.

[ǀ]Compositions of $C_{18:1}$ ω7*c*, $C_{18:1}$ ω9*t* and $C_{18:1}$ ω12*t*.

4.3.6 Reclassification of *Shewanella arctica* Kim *et al*. 2012 as a later heterotypic synonym of *Shewanella frigidimarina* Bowman *et al*. 1997

The genomic taxonomy analyses revealed that *S. frigidimarina* KCCM 41815^T and *S. arctica* KCTC 23109^T belong to a single genomic species with the ANI and dDDH values of 98.0% and 81.7%, respectively (Table 4.2), which are higher than the thresholds of current species delineation (Goris *et al*., 2007; Rosselló-Mora and Amann, 2001). The *gyrB* gene sequence similarity between both type strains is 99.2%, which is much higher than the *Shewanella* species cut-off value of 90% (Venkateswaran *et al*., 1999). In the phylogenomic tree, no sequence divergence was observed between those type strains (Fig. 4.3). In this study, the presence of MMK-7 along with MK-7, Q-7 and Q-8 was confirmed in *S. arctica* KCTC 23109^T, showing congruent quinone compositions of *S. frigidimarina* KCCM 41815^T (Table S2.4). Most phenotypic characteristics (102 out of 108) examined under an identical test condition gave the same results between *S. frigidimarina* KCCM 41815^T and *S. arctica* KCTC 23109^T (Table S2.4). Based on the polyphasic data obtained, therefore, we also propose to reclassify *S. arctica* Kim *et al*. (2012) as a later heterotypic synonym of *S. frigidimarina* Bowman *et al*. (1997).

4.3.7 Ethymology of novel species

Shewanella psychromarinicola (psy.chro.ma.ri.ni'co.la. Gr. adj. *psychros*, cold; L. adj. *marinus*, marine; L. fem. suff. -*cola* (from L. masc. or fem. n. *incola*), inhabitant; N.L. fem. n. *psychromarinicola*, a marine inhabitant that grows at low temperatures).

In conclusions, two novel strains in the genus *Shewanella* were facultatively anaerobic, and had the lowest optimal temperature for growth among their closest phylogenetic relatives *Shewanella* species. These results indicate that these novel species were well adapted to both the aerobic environment of surface sediments and the anaerobic environment of the subsurface sediments. In addition, it was confirmed that they are psychrophilic bacteria which is well adapted to cold environment. The strain M2^T was identified the only *Shewanella* strain that had two sets of the *feoA–feoB* operon involved in ferrous iron transport, while others have one set. This strain gave positive results of iron reduction, which might be reflected by such genomic features. Hence, these results may be indicating that the strain M2^T uses different strategies to adapt to the environment in which the strain was isolated. Pyrosequencing analysis revealed that *Shewanella* species were frequently detected in 4 samples out of 9 sediment samples (data were analyzed in Chapter 3). Metagenome analysis also confirmed that genus *Shewanella* were dorminant (0.6–1.1%) in surface sediment (data were analyzed in Chapter 4). The results of this study provide insight that genus *Shewanella* play an important role in iron cycling in the study areas.

CHAPTER 5

General Conclusion

The Antarctic environments are closely linked to the atmosphere, hydrosphere, lithosphere, cryosphere and biosphere systems. These environments are extremely important parts of the global physical and biological system as the region is experiencing dramatic changes such as glacier melting. Also, Antarctic benthic environments are harsh conditions for survival to prokaryotes due to high pressures, low temperature and low nutrient availability depending on the areas. Prokaryotes have undergone changes for the growing and survival under harsh environments and thus show enormous genetic diversity for exploration. Thus, understanding the community structures and metabolic functions of these organisms in the Antarctica are may provide insights into expected response of the microbial communities to the Antarctic environment which are rapidly changing and distribute excellent source for biotechnological applications. In recent years, with the advancement of molecular ecological technologies and analysis methods, biogeochemical characteristics and prokaryotic diversity have been studied. However, the prokaryotic community diversity and their ecological functions are still poorly understood in the west Antarctica. This study investigated how do benthic prokaryotic communities differ by overlying seawater properties according to areas and how do physical and chemical characteristics of surface sediments relate with benthic prokaryotic diversity and their functions in the west Antarctica.

An identification and characterization of culturable bacteria were performed in surface deep-sea sediments in the west Antarctica. 73 bacteria strains were isolated and identified from 4 sediment samples, of which two strains M2^T and R106 in the genus *Shewanella* were subjected to analyses of physiological, chemotaxonomical, phylogenetic and genomic characteristics. Two novel species in the genus *Shewanella* were facultatively anaerobic, and had the lowest optimal temperature for growth. Also, a new species had two sets of the *feoA–feoB* operon involved in ferrous iron transport. Hence, these results may indicate that the strain M2^T adapt to the environment in which the strain was isolated using different strategies among their closest phylogenetic relatives *Shewanella* species. Pyrosequencing and metagenome analyses also confirmed that *Shewanella* species were dorminant in surface sediment of the study areas. Based on the data obtained by polyphasic approach, this study provide insight that *Shewanella* species plays a important roles in iron cycling in the study areas.

The benthic prokaryotic communities and spatial distribution were investigated and were compared among the edges of ice shelves in the west Antarctica. This study demonstrates that there were similar taxonomic groups in all stations but prokaryotic community structure and alpha-diversity values have differed between the stations in the Amundsen Sea and those in the Ross Sea. In the Amundsen Sea stations, *Thaumarchaeota* mainly of genus *Nitrosopumilus* were predominant, while *Proteobacteria* were more abundant in the Ross Sea. And *Planctomycetes, Acidobacteria, Actinobacteria, Chloroflexi, Bacteroidetes* were dominant bacterial groups. It was revealed that the water mass in the study stations and organic matter exported from phytoplankton blooms affected on the benthic

prokaryotic communities. The results of this study offer the insights into the benthic prokaryotic distribution appeared to be strongly dependent on the quality and quantity of sediment organic matter which are influenced by water mass, sub-ice shelf circulations, primary production and glacier melting at edges of ice shelves in the west Antarctica.

The present study has combined a metagenomic approach with pyrosequencing analyses of prokaryotic 16S rDNA to identify the active community members and to assess the functional potential of the benthic prokaryotic community in the Ross Sea. There were similar taxonomic groups in three stations but prokaryotic community structure has differed between the stations. Bacteria were more abundant than Archaea in study areas. *Proteobacteria* were the most abundant phylum in all three stations, but the most abundant class of *Proteobacteria* was different. The class *Gammaproteobacteria* were the most abundant in inner continental shelf, while *Alphaproteobacteria* were the most abundant class in the continental slope and deep-basin. The difference benthic prokaryotic distribution was strongly affected by distinct environmental conditions, and organic matter utilization in pelagic sediments of the Ross Sea.

Over all, this study suggested that prokaryote might play a important roles in benthic environment in the west Antarctica. And these finding will increase our knowledge about the ecological functions of the benthic prokaryotes in the west Antarctica as well as help preddict their ability for adaptation against environmrnts changing.

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Supplementary Information

Table S2.1. Sample-specific barcode, linker and Uni787F* primer sequences (5'→3') used in this study.

Study area	Sample names	Barcode	Linker	Uni787F[a] primer sequence
Amundsen Sea	A2-1	AACCTACG	TC	ATTAGATACCCNGGTAG
	A2-2	AACCTAGC		
	A2-3	AACCTTCC		
	A2-4	AAGGAACC		
	A3-1	AAGCGGAT		
	A3-2	AACCGCTA		
	A3-3	AACCGGAA		
	A3-4	AACCGGTT		
	A4-1	AACGAACG		
	A4-2	AACGAAGC		
	A4-3	AACGATCC		
	A4-4	AACGATGG		
Ross Sea	R1-1	AACGCGAA		
	R1-2	AACGCGTT		
	R2-1	AACGTACC		
	R2-2	AACGTAGG		
	R3-1	AAGCAACG		
	R3-2	AAGCAAGC		
	R4-1	AAGCCGAA		
	R4-2	AAGCCGTT		
	R5-1	AAGCATCC		
	R5-2	AAGCATGG		

[a]Uni787F primers were from Jorgensen *et al*. (2012).

Table S2.2. Relative abundance of major archaeal and bacterial taxa that appeared at ≥ 1% in any of the samples. Data were normalized by randomly subsampling to 1596 reads. Each taxon was identified using the SILVA database.

Taxa	Amundsen																Ross										Average	
	A1-1	A1-2	A1-3	A1-4	A2-1	A2-2	A2-3	A2-4	A3-1	A3-2	A3-3	A3-4	A4-1	A4-2	A4-3	A4-4	R1-1	R1-2	R2-1	R2-2	R3-1	R3-2	R4-1	R4-2	R5-1	R5-2	Amundsen	Ross
p_*Crenarchaeota*	40.91	60.03	71.62	74.31	24.31	43.48	31.27	34.40	44.36	49.69	61.53	52.44	11.09	35.59	46.18	52.69	8.33	2.44	20.99	9.27	41.10	43.23	17.74	6.02	33.77	37.91	45.87	22.08
p_*Planctomycetota*	31.39	17.98	8.33	5.20	22.12	20.24	28.13	10.96	25.00	14.35	11.28	12.66	57.96	24.81	16.79	10.71	25.75	30.89	19.55	21.24	24.62	11.22	32.66	29.57	8.96	21.93	19.87	22.64
p_*Proteobacteria*	6.77	5.64	4.20	3.88	21.62	11.22	11.22	20.99	7.46	9.59	9.02	11.22	6.45	8.02	6.45	5.83	26.44	28.70	13.28	18.80	8.71	16.54	13.42	19.99	27.07	15.66	9.35	18.86
p_*Bacteroidota*	2.13	1.00	0.13	0.50	5.08	2.32	2.57	3.82	1.50	1.82	1.44	2.82	3.45	1.50	2.76	1.44	6.27	3.45	0.56	1.44	1.75	3.01	3.95	3.57	5.64	2.44	2.14	3.21
p_*Actinobacteriota*	2.57	1.07	0.75	2.38	3.57	2.32	2.44	5.95	1.32	5.39	2.69	4.39	1.57	1.32	1.63	2.69	5.58	11.28	10.71	15.98	0.63	1.69	2.13	4.51	3.63	2.88	2.63	5.90
p_*Desulfobacterota*	0.25	0.00	0.13	0.06	2.32	0.56	0.56	1.94	0.88	0.81	0.13	0.56	0.19	0.06	0.06	0.06	2.38	2.94	0.38	0.81	0.06	0.63	6.65	12.34	2.94	2.07	0.54	3.12
p_*Acidobacteriota*	3.76	2.94	2.26	1.57	4.64	3.82	5.89	2.94	5.20	4.07	2.32	3.20	4.70	3.57	3.82	1.88	7.27	5.26	8.71	8.27	5.14	2.01	4.89	4.14	2.51	3.32	3.54	5.15
p_NB1-j	0.69	0.50	0.56	0.75	3.32	0.75	1.82	2.26	0.56	0.88	0.75	1.50	0.19	0.38	0.50	0.25	1.88	1.88	1.50	2.19	0.50	0.69	3.07	3.07	3.01	1.00	0.98	1.88
p_*Verrucomicrobiota*	1.00	0.19	0.19	0.31	1.32	0.56	0.94	1.00	0.88	0.69	0.25	1.13	0.81	0.44	0.50	0.38	3.20	1.75	2.01	4.07	0.31	0.69	0.88	2.26	1.38	0.81	0.66	1.74
p_*Dependentiae*	0.44	0.25	0.31	0.19	0.38	0.63	0.44	0.88	0.25	0.63	0.13	0.31	0.13	0.19	0.25	0.19	0.75	1.57	2.26	4.57	0.31	2.13	0.63	1.38	0.44	0.31	0.35	1.43
p_*Chloroflexi*	2.69	1.69	2.82	5.26	1.32	3.82	3.07	4.20	3.88	3.70	3.38	3.07	4.89	10.21	7.77	9.52	2.44	1.38	7.14	3.95	2.82	3.88	1.57	0.88	2.01	2.88	4.46	2.89
p_*Dadabacteria*	2.82	4.32	4.14	3.51	2.82	6.58	4.57	5.89	2.69	3.32	3.38	3.38	4.64	9.84	8.21	12.34	3.63	2.13	7.33	3.88	7.89	9.02	2.82	2.38	1.94	2.76	5.15	4.38
p_*Patescibacteria*	1.00	0.94	0.63	0.19	0.38	0.50	1.13	0.25	1.07	0.38	0.50	0.50	1.00	0.94	0.63	0.31	1.25	1.19	1.69	1.25	2.26	2.69	2.88	2.94	0.13	0.75	0.65	1.70
p_*Myxococcota*	0.19	0.13	0.06	0.13	0.81	0.13	0.31	0.81	0.63	0.94	0.38	0.31	0.19	0.25	0.38	0.19	0.69	0.63	0.63	0.94	0.19	0.31	0.38	0.56	1.25	0.50	0.36	0.61
p_*Marinimicrobia* (SAR406 clade)	0.00	0.00	0.00	0.00	0.06	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	1.25	0.25	0.00	0.00	0.00	0.15
p_Bacteria_Unclassified	2.19	2.01	3.13	0.94	3.57	1.75	4.14	2.44	3.20	2.44	1.50	1.82	1.07	1.69	2.51	0.63	1.75	1.82	1.75	2.07	2.13	1.32	1.32	2.69	2.38	2.69	2.19	1.99

c_Nitrososphaeria	40.91	60.03	71.62	74.31	24.31	43.48	31.27	34.40	44.36	49.69	61.53	52.44	11.09	35.59	46.18	52.69	8.27	2.44	20.99	9.27	41.10	43.23	16.99	5.58	33.77	37.91	45.87	21.96
c_Acidimicrobiia	2.26	0.88	0.69	2.13	3.13	2.13	2.07	4.82	0.75	4.51	2.26	3.51	1.19	1.13	1.57	2.19	4.20	8.65	9.77	13.47	0.44	1.38	1.44	2.94	2.88	2.32	2.20	4.75
c_Dadabacteriia	2.82	4.32	4.14	3.51	2.82	6.58	4.57	5.89	2.69	3.32	3.38	3.38	4.64	9.84	8.21	12.34	3.63	2.13	7.33	3.88	7.89	9.02	2.82	2.38	1.94	2.76	5.15	4.38
c_Planctomycetes	28.01	15.73	6.52	4.32	17.11	16.35	23.81	8.83	21.18	12.84	9.40	10.84	52.76	22.68	14.04	9.65	21.62	27.01	17.23	18.55	21.12	9.71	22.45	23.56	7.14	18.80	17.13	18.72
c_Alphaproteobacteria	2.26	1.63	0.63	1.13	4.51	3.57	3.70	8.96	2.57	2.94	2.88	3.88	1.75	2.57	1.50	2.01	11.47	13.35	6.77	10.28	3.76	5.76	6.52	10.28	8.02	5.26	2.91	8.15
c_Gammaproteobacteria	4.51	4.01	3.57	2.76	17.04	7.64	7.52	11.97	4.76	6.64	6.08	7.33	4.70	5.33	4.95	3.82	14.91	15.23	6.52	8.52	4.95	10.78	6.90	9.71	18.67	10.40	6.41	10.66
c_Desulfuromonadia	0.13	0.00	0.13	0.06	2.26	0.50	0.38	1.82	0.69	0.75	0.13	0.56	0.00	0.06	0.06	0.06	1.38	2.51	0.25	0.56	0.06	0.63	4.58	8.58	2.32	1.75	0.47	2.26
c_Acidobacteriota_Unclassified	1.63	1.25	1.38	0.75	1.57	1.44	2.76	1.07	1.88	1.38	0.75	1.50	0.88	1.38	1.82	0.94	1.32	1.25	5.20	4.89	1.00	0.56	0.31	0.38	0.00	1.07	1.40	1.60
c_Bacteria_Unclassified	2.19	2.01	3.13	0.94	3.57	1.75	4.14	2.44	3.20	2.44	1.50	1.82	1.07	1.69	2.51	0.63	1.75	1.82	1.75	2.07	2.13	1.32	1.32	2.69	2.38	2.69	2.19	1.99
c_TK17	0.19	0.19	0.31	0.56	0.56	1.13	0.56	1.19	1.19	1.00	1.13	0.44	0.31	2.44	1.63	2.88	1.13	0.31	1.00	0.31	0.88	1.57	0.38	0.00	0.94	1.00	0.98	0.75
c_KD4-96	1.32	0.31	0.13	0.19	0.13	0.25	0.50	0.38	0.25	0.44	0.00	0.56	1.44	1.50	0.13	0.13	0.50	0.50	1.32	1.07	0.38	0.06	0.25	0.19	0.06	0.81	0.48	0.51
c_JG30-KF-CM66	0.56	0.50	1.57	3.32	0.44	1.44	1.38	2.01	1.38	1.57	1.69	1.38	1.44	3.20	3.51	4.95	0.56	0.19	2.44	1.07	0.88	1.63	0.44	0.13	0.50	0.56	1.90	0.84
c_NB1-j	0.69	0.50	0.56	0.75	3.32	0.75	1.82	2.26	0.56	0.88	0.75	1.50	0.19	0.38	0.50	0.25	1.88	1.88	1.50	2.19	0.50	0.69	3.07	3.07	3.01	1.00	0.98	1.88
c_Bacteroidia	1.82	0.88	0.06	0.50	4.45	2.26	2.44	2.82	1.44	1.69	1.25	2.57	3.26	1.32	2.51	1.19	6.08	3.01	0.31	1.13	1.75	2.82	3.57	3.32	5.58	2.32	1.90	2.99
c_Thermoleophilia	0.31	0.19	0.00	0.25	0.44	0.19	0.31	1.13	0.56	0.75	0.44	0.88	0.31	0.19	0.06	0.44	1.38	2.38	0.88	2.44	0.13	0.25	0.38	1.00	0.75	0.50	0.40	1.01
c_Subgroup_22	0.44	0.19	0.38	0.31	0.44	0.50	0.69	0.44	0.44	0.75	0.44	0.44	0.38	0.31	0.38	0.19	1.38	1.82	1.82	1.69	0.31	0.06	0.13	0.06	0.69	0.50	0.42	0.85
c_Chloroflexi_Unclassified	0.25	0.56	0.13	0.31	0.00	0.56	0.19	0.31	0.31	0.25	0.13	0.38	0.31	1.69	1.13	0.69	0.00	0.06	0.75	0.25	0.06	0.00	0.13	0.06	0.25	0.13	0.45	0.17
c_Phycisphaerae	2.26	1.32	1.32	0.31	3.57	2.38	2.63	1.19	2.63	0.81	1.13	0.88	3.38	0.94	1.38	0.81	2.19	2.38	1.44	2.19	2.63	0.88	8.21	4.70	1.07	1.69	1.68	2.74
c_Aminicenantia	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.06	0.00	0.00	0.00	0.00	0.00	3.13	2.19	0.00	0.00	0.00	0.54
c_Vicinamibacteria	1.13	1.00	0.38	0.25	1.63	1.25	1.44	0.75	2.13	1.25	0.75	0.75	2.63	1.38	1.19	0.69	3.01	1.07	0.88	0.94	2.32	0.75	0.56	0.19	1.07	1.13	1.16	1.19
c_Babeliae	0.44	0.25	0.31	0.19	0.38	0.63	0.44	0.88	0.25	0.63	0.13	0.31	0.13	0.19	0.25	0.19	0.75	1.57	2.26	4.57	0.31	2.13	0.63	1.38	0.44	0.31	0.35	1.43

c_Desulfobulbia	0.00	0.00	0.00	0.00	0.06	0.00	0.06	0.06	0.19	0.00	0.00	0.00	0.19	0.00	0.00	0.00	0.69	0.31	0.00	0.00	0.00	0.00	0.82	1.82	0.56	0.31	0.04	0.45
c_Thermoanaerobaculia	0.13	0.19	0.00	0.13	0.38	0.19	0.31	0.25	0.00	0.38	0.13	0.13	0.06	0.19	0.06	0.00	0.44	0.38	0.25	0.38	0.06	0.13	0.50	1.07	0.31	0.13	0.16	0.36
c_Saccharimonadia	0.38	0.56	0.00	0.06	0.13	0.06	0.25	0.06	0.19	0.13	0.19	0.00	0.38	0.38	0.13	0.00	0.75	0.75	0.38	0.13	1.50	2.07	0.69	0.88	0.06	0.25	0.18	0.75
c_Planctomycetota_Unclassified	0.00	0.06	0.06	0.06	0.19	0.50	0.06	0.31	0.38	0.19	0.31	0.19	0.38	0.13	0.00	0.06	0.63	0.31	0.06	0.19	0.13	0.06	1.07	0.81	0.13	0.56	0.18	0.39
c_Polyangia	0.19	0.00	0.06	0.13	0.75	0.13	0.25	0.81	0.56	0.75	0.38	0.25	0.19	0.19	0.31	0.19	0.69	0.63	0.56	0.88	0.19	0.31	0.38	0.56	1.13	0.50	0.32	0.58
c_Rhodothermia	0.31	0.13	0.06	0.00	0.50	0.00	0.13	1.00	0.06	0.06	0.06	0.25	0.19	0.19	0.25	0.13	0.19	0.31	0.25	0.31	0.00	0.19	0.19	0.06	0.00	0.06	0.21	0.16
c_Marinimicrobia_(SAR406_clade)	0.00	0.00	0.00	0.00	0.06	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	1.25	0.25	0.00	0.00	0.00	0.15
c_Desulfobacteria	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.31	0.13	0.00	0.00	0.00	0.00	1.19	1.82	0.06	0.00	0.00	0.35
c_OM190	1.07	0.88	0.38	0.31	1.13	0.88	1.19	0.56	0.63	0.38	0.31	0.50	1.38	0.56	0.94	0.19	0.88	1.00	0.56	0.19	0.63	0.56	0.25	0.44	0.44	0.69	0.70	0.56
c_Chlamydiae	0.75	0.19	0.13	0.19	0.88	0.44	0.56	0.69	0.63	0.50	0.19	0.94	0.38	0.25	0.19	0.19	2.07	1.32	1.88	3.88	0.25	0.63	0.50	1.32	1.07	0.63	0.44	1.35
c_Gracilibacteria	0.44	0.13	0.44	0.13	0.13	0.31	0.31	0.06	0.38	0.19	0.19	0.38	0.38	0.50	0.50	0.19	0.31	0.25	0.56	0.38	0.13	0.44	1.57	1.57	0.00	0.13	0.29	0.53
o_Nitrosopumilales	40.91	60.03	71.55	74.12	24.31	43.48	31.27	34.34	44.36	49.69	61.53	52.44	11.09	35.59	46.18	0.81	8.27	2.44	20.99	9.27	41.10	43.23	16.99	5.58	33.77	37.91	42.61	21.96
o_Pirellulales	24.31	13.85	5.58	3.88	14.91	14.04	21.12	8.08	18.17	11.34	7.89	10.15	44.55	19.36	11.53	52.69	18.73	24.25	14.60	16.29	18.55	8.33	20.25	22.06	6.08	16.17	17.59	16.53
o_Actinomarinales	1.50	0.56	0.63	1.69	2.38	1.38	1.82	3.70	0.44	3.95	1.94	2.94	0.81	1.00	1.32	8.46	2.94	6.58	9.15	12.84	0.13	0.38	1.25	2.44	1.75	1.69	2.16	3.92
o_Gammaproteobacteria_Unclassified	1.44	1.07	1.19	0.88	6.39	2.82	2.57	3.95	2.01	1.94	2.63	2.63	1.63	1.57	1.44	0.75	5.01	4.51	2.19	3.01	1.75	3.32	1.25	1.69	7.58	4.45	2.18	3.48
o_Rhizobiales	1.13	0.50	0.25	0.81	1.38	1.07	1.38	3.32	1.50	1.32	0.88	2.01	0.38	0.81	0.38	0.00	4.82	5.95	3.82	5.26	1.13	2.94	4.14	5.39	3.76	2.57	1.07	3.98
o_Babeliales	0.44	0.25	0.31	0.19	0.38	0.63	0.44	0.88	0.25	0.63	0.13	0.31	0.13	0.19	0.25	1.63	0.75	1.57	2.26	4.57	0.31	2.13	0.63	1.38	0.44	0.31	0.44	1.43
o_Kiloniellales	0.44	0.19	0.13	0.06	1.69	1.19	1.19	3.76	0.44	0.94	1.07	0.81	0.56	0.81	0.63	1.00	4.82	4.20	2.26	3.70	1.13	0.94	1.57	4.01	1.75	1.19	0.93	2.56
o_Chlamydiales	0.75	0.19	0.13	0.19	0.88	0.44	0.56	0.69	0.63	0.50	0.19	0.94	0.31	0.25	0.19	0.13	2.07	1.32	1.88	3.88	0.19	0.63	0.38	1.19	1.07	0.56	0.43	1.32
o_NB1-j	0.69	0.50	0.56	0.75	3.32	0.75	1.82	2.26	0.56	0.88	0.75	1.50	0.19	0.38	0.50	0.00	1.88	1.88	1.50	2.19	0.50	0.69	3.07	3.07	3.01	1.00	0.96	1.88
o_Microtrichales	0.75	0.19	0.06	0.44	0.75	0.75	0.25	1.00	0.31	0.56	0.31	0.50	0.38	0.13	0.25	0.25	1.19	2.07	0.63	0.63	0.31	1.00	0.19	0.50	1.07	0.56	0.43	0.81

o_Dadabacteriales	2.82	4.32	4.14	3.51	2.82	6.58	4.57	5.89	2.69	3.32	3.38	3.38	4.64	9.84	8.21	0.13	3.63	2.13	7.33	3.88	7.89	9.02	2.82	2.38	1.94	2.76	4.39	4.38
o_Rhodobacterales	0.31	0.44	0.00	0.00	0.75	0.56	0.25	0.63	0.44	0.19	0.31	0.69	0.31	0.38	0.13	1.44	1.07	1.94	0.19	0.38	0.81	1.07	0.38	0.56	1.19	0.69	0.43	0.83
o_Flavobacteriales	1.50	0.56	0.06	0.19	2.57	1.44	1.94	1.44	0.94	0.88	0.75	1.63	2.51	1.13	1.69	0.81	4.26	2.13	0.06	0.69	1.25	2.44	1.63	1.75	4.01	1.69	1.25	1.99
o_Solirubrobacterales	0.06	0.13	0.00	0.13	0.13	0.13	0.13	0.56	0.31	0.44	0.25	0.44	0.06	0.00	0.06	0.00	0.88	1.25	0.75	1.63	0.00	0.13	0.31	0.69	0.63	0.31	0.18	0.66
o_Planctomycetales	3.20	1.69	0.81	0.19	2.01	2.19	2.57	0.63	2.63	1.07	1.50	0.63	7.83	2.94	2.26	0.19	2.63	2.76	2.32	1.69	2.26	1.19	1.94	1.32	0.88	2.63	2.02	1.96
o_Acidobacteriota_Unclassified	1.63	1.25	1.38	0.75	1.57	1.44	2.76	1.07	1.88	1.38	0.75	1.50	0.88	1.38	1.82	0.69	1.32	1.25	5.20	4.89	1.00	0.56	0.31	0.38	0.00	1.07	1.38	1.60
o_Bacteria_Unclassified	2.19	2.01	3.13	0.94	3.57	1.75	4.14	2.44	3.20	2.44	1.50	1.82	1.07	1.69	2.51	0.19	1.75	1.82	1.75	2.07	2.13	1.32	1.32	2.69	2.38	2.69	2.16	1.99
o_Gaiellales	0.25	0.06	0.00	0.13	0.25	0.06	0.13	0.56	0.06	0.19	0.19	0.38	0.25	0.19	0.00	0.00	0.50	1.13	0.13	0.81	0.13	0.13	0.06	0.25	0.13	0.19	0.17	0.34
o_Vicinamibacterales	1.13	1.00	0.31	0.25	1.32	1.19	1.38	0.75	2.01	1.25	0.69	0.69	2.38	1.25	1.13	0.19	2.88	1.00	0.75	0.88	2.32	0.63	0.50	0.19	1.00	1.07	1.06	1.12
o_Steroidobacterales	0.63	0.31	0.44	0.63	1.50	0.56	0.81	1.25	0.50	0.94	0.06	1.00	0.31	0.25	0.25	0.19	1.00	0.94	0.50	1.19	0.06	0.50	0.69	0.75	1.25	0.63	0.60	0.75
o_AT-s2-59	0.31	0.38	0.38	0.50	0.88	0.50	1.32	1.69	0.56	0.44	0.38	0.56	0.50	0.06	0.56	0.63	1.13	1.44	1.69	2.07	0.06	0.06	0.19	0.25	1.32	0.38	0.60	0.86
o_Gammaproteobacteria_Incertae_Sedis	0.75	0.88	0.25	0.13	3.57	1.32	1.25	1.75	0.75	1.50	1.19	1.63	1.19	1.25	0.50	0.00	3.20	5.14	1.07	0.44	1.13	2.57	2.32	4.70	3.63	1.94	1.12	2.61
o_Subgroup_22	0.44	0.19	0.38	0.31	0.44	0.50	0.69	0.44	0.44	0.75	0.44	0.44	0.38	0.31	0.38	0.06	1.38	1.82	1.82	1.69	0.31	0.06	0.13	0.06	0.69	0.50	0.41	0.85
o_Thermoanaerobaculales	0.13	0.19	0.00	0.13	0.38	0.19	0.31	0.25	0.00	0.38	0.13	0.13	0.06	0.19	0.06	12.34	0.44	0.38	0.25	0.38	0.06	0.13	0.50	1.07	0.31	0.13	0.93	0.36
o_Planctomycetota_Unclassified	0.00	0.06	0.06	0.06	0.19	0.50	0.06	0.31	0.38	0.19	0.31	0.19	0.38	0.13	0.00	0.13	0.63	0.31	0.06	0.19	0.13	0.06	1.07	0.81	0.13	0.56	0.18	0.39
o_Cellvibrionales	0.38	0.44	0.06	0.00	1.19	0.63	0.31	0.81	0.31	0.38	0.25	0.38	0.31	0.50	0.56	0.06	1.25	0.75	0.00	0.06	0.38	1.38	0.63	0.50	1.50	0.75	0.41	0.72
o_Saccharimonadales	0.38	0.56	0.00	0.06	0.13	0.06	0.25	0.06	0.19	0.13	0.19	0.00	0.38	0.38	0.13	0.06	0.75	0.75	0.38	0.13	1.50	2.07	0.69	0.88	0.06	0.25	0.18	0.75
o_TK17	0.19	0.19	0.31	0.56	0.56	1.13	0.56	1.19	1.19	1.00	1.13	0.44	0.31	2.44	1.63	0.63	1.13	0.31	1.00	0.31	0.88	1.57	0.38	0.00	0.94	1.00	0.84	0.75
o_JG30-KF-CM66	0.56	0.50	1.57	3.32	0.44	1.44	1.38	2.01	1.38	1.57	1.69	1.38	1.44	3.20	3.51	0.13	0.56	0.19	2.44	1.07	0.88	1.63	0.44	0.13	0.50	0.56	1.59	0.84
o_OM190	1.07	0.88	0.38	0.31	1.13	0.88	1.19	0.56	0.63	0.38	0.31	0.50	1.38	0.56	0.94	0.00	0.88	1.00	0.56	0.19	0.63	0.56	0.25	0.44	0.44	0.69	0.69	0.56
o_KD4-96	1.32	0.31	0.13	0.19	0.13	0.25	0.50	0.38	0.25	0.44	0.00	0.56	1.44	1.50	0.13	0.00	0.50	0.50	1.32	1.07	0.38	0.06	0.25	0.19	0.06	0.81	0.47	0.51

o_Phycisphaerales	2.07	1.07	1.19	0.31	1.69	0.94	1.63	0.50	1.32	0.56	0.88	0.50	2.88	0.63	1.19	0.00	0.75	1.00	1.07	1.57	2.19	0.75	2.32	0.75	0.56	0.88	1.08	1.18
o_Alteromonadales	0.06	0.00	1.00	0.38	0.50	0.19	0.44	0.00	0.13	0.06	0.06	0.06	0.06	0.13	0.38	0.94	0.25	0.19	0.00	0.06	0.38	0.19	0.06	0.00	0.38	0.25	0.27	0.18
o_Chloroflexi_Unclassified	0.25	0.56	0.13	0.31	0.00	0.56	0.19	0.31	0.31	0.25	0.13	0.38	0.31	1.69	1.13	2.88	0.00	0.06	0.75	0.25	0.06	0.00	0.13	0.06	0.25	0.13	0.59	0.17
o_Cytophagales	0.19	0.19	0.00	0.06	0.94	0.50	0.25	0.94	0.31	0.44	0.19	0.31	0.56	0.13	0.63	0.31	1.07	0.38	0.19	0.31	0.31	0.13	0.63	0.19	0.75	0.19	0.37	0.41
o_Desulfobacterales	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.69	0.31	0.13	0.00	0.00	0.00	0.00	1.19	1.82	0.06	0.00	0.04	0.35
o_Rickettsiales	0.06	0.06	0.06	0.00	0.00	0.00	0.13	0.00	0.00	0.00	0.06	0.06	0.00	0.00	0.00	0.00	0.00	0.06	0.00	0.00	0.13	0.06	0.00	0.00	0.00	0.06	0.03	0.03
o_Marinimicrobia_(SAR406_clade)	0.00	0.00	0.00	0.00	0.06	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.13	0.00	0.00	0.00	0.00	0.00	0.00	1.25	0.25	0.00	0.00	0.01	0.15
o_Aminicenantales	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.25	0.06	0.00	0.00	0.00	0.00	0.00	3.13	2.19	0.00	0.00	0.02	0.54
o_Desulfobulbales	0.00	0.00	0.00	0.00	0.06	0.00	0.06	0.06	0.19	0.00	0.00	0.00	0.19	0.00	0.00	4.95	0.69	0.31	0.00	0.00	0.00	0.00	0.82	1.82	0.56	0.31	0.34	0.45
o_MSBL9	0.00	0.13	0.06	0.00	1.32	1.32	0.69	0.50	1.00	0.13	0.00	0.06	0.19	0.13	0.13	0.00	0.88	1.07	0.19	0.13	0.38	0.06	5.33	3.70	0.31	0.63	0.35	1.27
o_Sva1033	0.00	0.00	0.00	0.00	1.94	0.50	0.19	1.69	0.19	0.06	0.00	0.13	0.00	0.06	0.00	0.69	1.13	2.19	0.00	0.00	0.06	0.44	4.51	8.52	1.75	1.19	0.34	1.98
o_Thiohalorhabdales	0.13	0.13	0.00	0.06	0.94	0.69	0.38	0.56	0.13	0.44	0.13	0.25	0.25	0.38	0.19	0.00	0.81	0.25	0.00	0.31	0.38	0.88	0.19	0.19	1.07	0.38	0.29	0.44
o_Bacteroidales	0.00	0.00	0.00	0.00	0.06	0.00	0.06	0.00	0.00	0.00	0.00	0.00	0.06	0.00	0.00	4.89	0.25	0.06	0.00	0.00	0.00	0.00	1.00	1.00	0.00	0.00	0.32	0.23
f_Nitrosopumilaceae	40.91	60.03	71.55	74.12	24.31	43.48	31.27	34.34	44.36	49.69	61.53	52.44	11.09	35.59	46.18	52.69	8.27	2.44	20.99	9.27	41.10	43.23	16.99	5.58	33.77	37.91	45.85	21.96
f_Actinomarinales_uncultured	1.50	0.56	0.63	1.69	2.38	1.38	1.82	3.70	0.44	3.95	1.94	2.94	0.81	1.00	1.32	1.44	2.94	6.58	9.15	12.84	0.13	0.38	1.25	2.44	1.75	1.69	1.72	3.92
f_Dadabacteriales	2.82	4.32	4.14	3.51	2.82	6.58	4.57	5.89	2.69	3.32	3.38	3.38	4.64	9.84	8.21	12.34	3.63	2.13	7.33	3.88	7.89	9.02	2.82	2.38	1.94	2.76	5.15	4.38
f_Pirellulaceae	24.31	13.85	5.58	3.88	14.91	14.04	21.12	8.08	18.17	11.34	7.89	10.15	44.55	19.36	11.53	8.46	18.73	24.25	14.60	16.29	18.55	8.33	20.25	22.06	6.08	16.17	14.83	16.53
f_Kiloniellaceae	0.44	0.19	0.06	0.06	1.69	1.19	1.13	3.63	0.44	0.94	1.00	0.75	0.50	0.81	0.63	0.63	4.82	4.14	2.19	3.63	1.13	0.94	1.57	4.01	1.75	1.07	0.88	2.53
f_Gammaproteobacteria_Unclassified	1.44	1.07	1.19	0.88	6.39	2.82	2.57	3.95	2.01	1.94	2.63	2.63	1.63	1.57	1.44	1.63	5.01	4.51	2.19	3.01	1.75	3.32	1.25	1.69	7.58	4.45	2.24	3.48
f_Sva1033	0.00	0.00	0.00	0.00	1.94	0.50	0.19	1.69	0.19	0.06	0.00	0.13	0.00	0.06	0.00	0.00	1.13	2.19	0.00	0.00	0.06	0.44	4.51	8.52	1.75	1.19	0.30	1.98
f_Methyloligellaceae	0.50	0.25	0.25	0.19	0.94	0.88	1.00	2.19	0.75	0.81	0.63	1.38	0.13	0.50	0.38	0.38	3.45	3.63	3.07	4.26	0.50	1.75	3.45	4.51	2.32	1.69	0.70	2.86

f_Gammaproteobacteria_Incertae_Sedis_Unknown_Family	0.75	0.88	0.25	0.13	3.57	1.32	1.25	1.75	0.75	1.50	1.19	1.63	1.19	1.25	0.50	1.00	3.20	5.14	1.07	0.44	1.13	2.57	2.32	4.70	3.63	1.94	1.18	2.61
f_Acidobacteriota_Unclassified	1.63	1.25	1.38	0.75	1.57	1.44	2.76	1.07	1.88	1.38	0.75	1.50	0.88	1.38	1.82	0.94	1.32	1.25	5.20	4.89	1.00	0.56	0.31	0.38	0.00	1.07	1.40	1.60
f_Bacteria_Unclassified	2.19	2.01	3.13	0.94	3.57	1.75	4.14	2.44	3.20	2.44	1.50	1.82	1.07	1.69	2.51	0.63	1.75	1.82	1.75	2.07	2.13	1.32	1.32	2.69	2.38	2.69	2.19	1.99
f_AT-s2-59	0.31	0.38	0.38	0.50	0.88	0.50	1.32	1.69	0.56	0.44	0.38	0.56	0.50	0.06	0.56	0.25	1.13	1.44	1.69	2.07	0.06	0.06	0.19	0.25	1.32	0.38	0.58	0.86
f_Woeseiaceae	0.63	0.31	0.44	0.63	1.50	0.56	0.81	1.25	0.50	0.94	0.06	1.00	0.31	0.25	0.25	0.06	1.00	0.94	0.50	1.19	0.06	0.50	0.69	0.75	1.25	0.63	0.60	0.75
f_TK17	0.19	0.19	0.31	0.56	0.56	1.13	0.56	1.19	1.19	1.00	1.13	0.44	0.31	2.44	1.63	2.88	1.13	0.31	1.00	0.31	0.88	1.57	0.38	0.00	0.94	1.00	0.98	0.75
f_Mitochondria	0.00	0.00	0.00	0.00	0.00	0.00	0.06	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.06	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.06	0.00	0.01
f_KD4-96	1.32	0.31	0.13	0.19	0.13	0.25	0.50	0.38	0.25	0.44	0.00	0.56	1.44	1.50	0.13	0.13	0.50	0.50	1.32	1.07	0.38	0.06	0.25	0.19	0.06	0.81	0.48	0.51
f_JG30-KF-CM66	0.56	0.50	1.57	3.32	0.44	1.44	1.38	2.01	1.38	1.57	1.69	1.38	1.44	3.20	3.51	4.95	0.56	0.19	2.44	1.07	0.88	1.63	0.44	0.13	0.50	0.56	1.90	0.84
f_NB1-j	0.69	0.50	0.56	0.75	3.32	0.75	1.82	2.26	0.56	0.88	0.75	1.50	0.19	0.38	0.50	0.25	1.88	1.88	1.50	2.19	0.50	0.69	3.07	3.07	3.01	1.00	0.98	1.88
f_Ilumatobacteraceae	0.50	0.00	0.00	0.06	0.19	0.19	0.06	0.25	0.06	0.06	0.00	0.13	0.13	0.00	0.00	0.19	0.31	0.81	0.19	0.25	0.00	0.56	0.06	0.31	0.50	0.13	0.11	0.31
f_Flavobacteriaceae	1.25	0.50	0.06	0.19	2.32	1.44	1.82	1.32	0.56	0.69	0.69	1.32	2.38	1.00	1.32	0.81	3.26	1.82	0.06	0.69	0.88	2.19	1.13	1.19	3.76	1.50	1.10	1.65
f_Gaiellales_uncultured	0.25	0.06	0.00	0.13	0.25	0.06	0.13	0.56	0.06	0.19	0.19	0.38	0.25	0.19	0.00	0.13	0.50	1.13	0.13	0.81	0.13	0.13	0.06	0.25	0.13	0.19	0.18	0.34
f_Subgroup_22	0.44	0.19	0.38	0.31	0.44	0.50	0.69	0.44	0.44	0.75	0.44	0.44	0.38	0.31	0.38	0.19	1.38	1.82	1.82	1.69	0.31	0.06	0.13	0.06	0.69	0.50	0.42	0.85
f_Chloroflexi_Unclassified	0.25	0.56	0.13	0.31	0.00	0.56	0.19	0.31	0.31	0.25	0.13	0.38	0.31	1.69	1.13	0.69	0.00	0.06	0.75	0.25	0.06	0.00	0.13	0.06	0.25	0.13	0.45	0.17
f_Hyphomicrobiaceae	0.19	0.00	0.00	0.50	0.25	0.13	0.19	0.63	0.50	0.31	0.06	0.38	0.00	0.06	0.00	0.13	0.44	0.88	0.56	0.63	0.00	0.25	0.31	0.44	0.69	0.38	0.21	0.46
f_Solirubrobacterales_67-14	0.06	0.13	0.00	0.13	0.13	0.13	0.13	0.50	0.31	0.44	0.25	0.44	0.06	0.00	0.06	0.31	0.88	1.25	0.75	1.57	0.00	0.13	0.31	0.69	0.63	0.31	0.19	0.65
f_Rhodobacteraceae	0.31	0.44	0.00	0.00	0.75	0.56	0.25	0.63	0.44	0.19	0.31	0.69	0.31	0.38	0.13	0.13	1.07	1.94	0.19	0.38	0.81	1.07	0.38	0.56	1.19	0.69	0.34	0.83
f_Phycisphaeraceae	2.07	1.07	1.19	0.31	1.63	0.94	1.38	0.50	1.32	0.50	0.81	0.50	2.88	0.63	1.19	0.69	0.69	0.88	1.07	1.57	2.13	0.75	1.07	0.25	0.50	0.88	1.10	0.98
f_MSBL9_SG8-4	0.00	0.00	0.06	0.00	1.00	1.07	0.50	0.50	0.81	0.13	0.00	0.06	0.13	0.00	0.00	0.00	0.75	1.00	0.06	0.06	0.38	0.06	4.33	3.07	0.31	0.63	0.27	1.07
f_Aminicenantales	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.06	0.00	0.00	0.00	0.00	0.00	3.13	2.19	0.00	0.00	0.00	0.54

f_Rhizobiaceae	0.38	0.13	0.00	0.00	0.13	0.06	0.06	0.31	0.25	0.13	0.06	0.19	0.25	0.19	0.00	0.19	0.75	1.19	0.13	0.31	0.63	0.88	0.13	0.38	0.38	0.25	0.14	0.50
f_Vicinamibacterales_uncultured	1.00	0.75	0.19	0.19	1.00	0.94	1.00	0.63	1.25	0.56	0.44	0.63	2.07	1.00	0.81	0.44	2.26	0.63	0.56	0.44	1.07	0.63	0.50	0.19	0.94	0.75	0.81	0.80
f_Babeliales	0.19	0.06	0.06	0.06	0.00	0.06	0.06	0.44	0.06	0.25	0.06	0.00	0.00	0.06	0.13	0.06	0.19	0.50	1.00	1.38	0.00	0.63	0.06	0.06	0.13	0.13	0.10	0.41
f_Halieaceae	0.31	0.31	0.06	0.00	1.07	0.56	0.25	0.63	0.25	0.38	0.19	0.25	0.19	0.13	0.38	0.13	1.07	0.69	0.00	0.00	0.13	1.25	0.38	0.31	1.00	0.50	0.32	0.53
f_Thiohalorhabdaceae	0.13	0.13	0.00	0.06	0.94	0.69	0.38	0.56	0.13	0.44	0.13	0.25	0.25	0.38	0.19	0.06	0.81	0.25	0.00	0.31	0.38	0.88	0.19	0.19	1.07	0.38	0.29	0.44
f_Planctomycetales_uncultured	0.63	0.50	0.56	0.00	0.50	0.50	0.88	0.13	1.00	0.38	0.81	0.13	2.26	0.63	0.50	0.19	1.07	0.44	1.07	0.56	0.56	0.13	0.38	0.31	0.25	0.50	0.60	0.53
f_Gimesiaceae	0.94	0.94	0.25	0.13	1.13	0.88	1.13	0.19	0.69	0.06	0.25	0.06	3.13	0.94	0.81	0.44	0.94	1.57	1.00	0.56	0.69	0.44	0.75	0.69	0.44	0.81	0.75	0.79
f_Microtrichaceae	0.06	0.13	0.06	0.31	0.38	0.44	0.06	0.69	0.25	0.50	0.25	0.31	0.19	0.06	0.19	0.38	0.56	0.69	0.25	0.31	0.19	0.44	0.00	0.06	0.44	0.25	0.27	0.32
f_Desulfocapsaceae	0.00	0.00	0.00	0.00	0.06	0.00	0.06	0.06	0.19	0.00	0.00	0.00	0.19	0.00	0.00	0.00	0.56	0.19	0.00	0.00	0.00	0.00	0.19	0.75	0.44	0.19	0.04	0.23
f_Vermiphilaceae	0.06	0.00	0.13	0.06	0.06	0.00	0.13	0.00	0.06	0.06	0.00	0.06	0.13	0.00	0.00	0.00	0.00	0.06	0.44	1.32	0.00	0.00	0.06	0.06	0.00	0.06	0.05	0.20
f_Thermoanaerobaculaceae	0.13	0.19	0.00	0.13	0.38	0.19	0.31	0.25	0.00	0.38	0.13	0.13	0.06	0.19	0.06	0.00	0.44	0.38	0.25	0.38	0.06	0.13	0.50	1.07	0.31	0.13	0.16	0.36
f_Planctomycetales_Unclassified	1.19	0.19	0.00	0.00	0.25	0.69	0.50	0.31	0.75	0.50	0.25	0.44	1.69	0.63	0.75	0.19	0.31	0.56	0.25	0.44	0.56	0.31	0.63	0.31	0.13	0.88	0.52	0.44
f_Saccharimonadales	0.38	0.56	0.00	0.06	0.13	0.06	0.25	0.06	0.19	0.13	0.19	0.00	0.38	0.38	0.13	0.00	0.75	0.75	0.38	0.13	1.50	2.07	0.69	0.88	0.06	0.19	0.18	0.74
f_Planctomycetota_Unclassified	0.00	0.06	0.06	0.06	0.19	0.50	0.06	0.31	0.38	0.19	0.31	0.19	0.38	0.13	0.00	0.06	0.63	0.31	0.06	0.19	0.13	0.06	1.07	0.81	0.13	0.56	0.18	0.39
f_Rhodothermaceae	0.31	0.13	0.06	0.00	0.50	0.00	0.13	1.00	0.06	0.06	0.06	0.25	0.19	0.19	0.25	0.13	0.19	0.31	0.25	0.31	0.00	0.19	0.19	0.06	0.00	0.06	0.21	0.16
f_Vicinamibacterales_Unclassified	0.13	0.19	0.13	0.06	0.31	0.25	0.38	0.13	0.75	0.56	0.25	0.00	0.13	0.13	0.00	0.06	0.56	0.38	0.19	0.31	1.19	0.00	0.00	0.00	0.00	0.19	0.22	0.28
f_Desulfosarcinaceae	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.25	0.13	0.00	0.00	0.00	0.00	0.75	1.75	0.00	0.00	0.00	0.29
f_Cyclobacteriaceae	0.19	0.19	0.00	0.06	0.88	0.38	0.19	0.88	0.31	0.44	0.13	0.31	0.56	0.13	0.50	0.13	1.00	0.38	0.19	0.31	0.25	0.13	0.63	0.19	0.69	0.06	0.33	0.38
f_OM190	1.07	0.88	0.38	0.31	1.13	0.88	1.19	0.56	0.63	0.38	0.31	0.50	1.38	0.56	0.94	0.19	0.88	1.00	0.56	0.19	0.63	0.56	0.25	0.44	0.44	0.69	0.70	0.56
f_Babeliales_Unclassified	0.13	0.13	0.13	0.06	0.31	0.56	0.25	0.44	0.13	0.31	0.06	0.25	0.00	0.13	0.06	0.13	0.50	0.94	0.75	1.63	0.31	1.44	0.44	1.13	0.25	0.13	0.19	0.75
f_Desulfobulbaceae	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.06	0.13	0.00	0.00	0.00	0.00	0.63	1.07	0.13	0.13	0.00	0.21

f_Chlamydiales_Unclassified	0.63	0.13	0.06	0.13	0.19	0.19	0.31	0.38	0.38	0.31	0.06	0.44	0.19	0.06	0.00	0.13	0.81	0.56	0.81	1.69	0.00	0.25	0.13	0.69	0.81	0.31	0.22	0.61
f_AKAU3564_sediment_group	0.00	0.00	0.00	0.00	0.06	0.00	0.25	0.00	0.00	0.06	0.06	0.00	0.00	0.00	0.00	0.00	0.06	0.13	0.00	0.00	0.06	0.00	1.25	0.50	0.06	0.00	0.03	0.21
g_Nitrosopumilaceae	17.36	20.43	35.46	37.28	3.20	5.95	5.76	4.70	10.53	14.54	13.47	12.41	0.31	2.69	2.07	0.13	1.69	0.25	3.07	1.69	2.19	2.07	1.94	0.50	3.45	4.01	16.27	7.02
g_Nitrosopumilaceae_Unclassified	23.56	39.60	36.09	36.84	21.12	37.53	25.50	29.64	33.77	35.15	47.87	40.04	10.78	32.89	44.11	0.63	6.58	2.19	17.92	7.58	38.91	41.17	15.05	5.08	30.08	33.90	31.23	30.65
g_Actinomarinales_uncultured	1.50	0.56	0.63	1.69	2.38	1.38	1.82	3.70	0.44	3.95	1.94	2.94	0.81	1.00	1.32	3.57	2.94	6.58	9.15	12.84	0.13	0.38	1.25	2.44	1.75	1.69	1.71	2.00
g_Pirellulaceae_Unclassified	8.40	5.51	1.75	1.19	7.21	6.33	8.65	4.14	6.39	5.76	3.76	4.26	16.79	8.02	4.07	0.94	7.96	8.71	4.07	5.20	7.33	3.20	6.46	5.08	3.38	8.08	5.40	6.25
g_Pir4_lineage	8.21	4.01	2.76	2.07	4.76	5.39	7.71	2.01	7.89	3.57	2.76	3.07	15.79	7.71	4.89	0.00	6.77	10.28	8.21	8.02	8.08	2.82	7.96	10.09	1.44	4.76	4.61	5.71
g_Gammaproteobacteria_Unclassified	1.44	1.07	1.19	0.88	6.39	2.82	2.57	3.95	2.01	1.94	2.63	2.63	1.63	1.57	1.44	0.19	5.01	4.51	2.19	3.01	1.75	3.32	1.25	1.69	7.58	4.45	2.54	1.75
g_Kiloniellaceae_uncultured	0.44	0.19	0.06	0.06	1.69	0.88	1.07	3.63	0.44	0.94	1.00	0.75	0.50	0.81	0.63	0.00	4.76	4.07	2.19	3.57	1.13	0.94	1.57	4.01	1.69	1.07	1.00	0.63
g_Blastopirellula	6.08	2.76	0.38	0.31	1.69	1.19	2.19	0.44	1.63	0.75	0.75	1.32	6.89	2.32	1.38	0.06	1.63	1.44	0.19	0.38	0.69	0.88	1.50	1.88	0.63	1.75	1.88	1.89
g_NB1-j	0.69	0.50	0.56	0.75	3.32	0.75	1.82	2.26	0.56	0.88	0.75	1.50	0.19	0.38	0.50	0.44	1.88	1.88	1.50	2.19	0.50	0.69	3.07	3.07	3.01	1.00	1.33	0.65
g_Dadabacteriales	2.82	4.32	4.14	3.51	2.82	6.58	4.57	5.89	2.69	3.32	3.38	3.38	4.64	9.84	8.21	1.44	3.63	2.13	7.33	3.88	7.89	9.02	2.82	2.38	1.94	2.76	4.33	4.61
g_Chlamydiales_Unclassified	0.63	0.13	0.06	0.13	0.19	0.19	0.31	0.38	0.38	0.31	0.06	0.44	0.19	0.06	0.00	0.19	0.81	0.56	0.81	1.69	0.00	0.25	0.13	0.69	0.81	0.31	0.25	0.20
g_Solirubrobacterales_67-14	0.06	0.13	0.00	0.13	0.13	0.13	0.13	0.50	0.31	0.44	0.25	0.44	0.06	0.00	0.06	0.13	0.88	1.25	0.75	1.57	0.00	0.13	0.31	0.69	0.63	0.31	0.15	0.21
g_Acidobacteriota_Unclassified	1.63	1.25	1.38	0.75	1.57	1.44	2.76	1.07	1.88	1.38	0.75	1.50	0.88	1.38	1.82	0.31	1.32	1.25	5.20	4.89	1.00	0.56	0.31	0.38	0.00	1.07	1.48	1.24
g_Bacteria_Unclassified	2.19	2.01	3.13	0.94	3.57	1.75	4.14	2.44	3.20	2.44	1.50	1.82	1.07	1.69	2.51	0.00	1.75	1.82	1.75	2.07	2.13	1.32	1.32	2.69	2.38	2.69	2.52	1.78
g_Gaiellales_uncultured	0.25	0.06	0.00	0.13	0.25	0.06	0.13	0.56	0.06	0.19	0.19	0.38	0.25	0.19	0.00	0.38	0.50	1.13	0.13	0.81	0.13	0.13	0.06	0.25	0.13	0.19	0.18	0.20
g_Babeliales_Unclassified	0.13	0.13	0.13	0.06	0.31	0.56	0.25	0.44	0.13	0.31	0.06	0.25	0.00	0.13	0.06	0.13	0.50	0.94	0.75	1.63	0.31	1.44	0.44	1.13	0.25	0.13	0.25	0.13
g_Hyphomicrobiaceae_uncultured	0.00	0.00	0.00	0.19	0.19	0.00	0.00	0.38	0.38	0.00	0.06	0.38	0.00	0.06	0.00	0.69	0.31	0.50	0.50	0.38	0.00	0.00	0.13	0.38	0.25	0.19	0.09	0.20
g_Methyloligellaceae_Unclassified	0.25	0.00	0.19	0.00	0.38	0.31	0.19	1.38	0.00	0.31	0.31	0.63	0.13	0.25	0.19	4.95	0.75	1.57	1.63	3.26	0.00	0.25	0.75	1.00	0.63	0.63	0.34	0.85
g_Babeliales	0.19	0.06	0.06	0.06	0.00	0.06	0.06	0.44	0.06	0.25	0.06	0.00	0.00	0.06	0.13	2.88	0.19	0.50	1.00	1.38	0.00	0.63	0.06	0.06	0.13	0.13	0.12	0.43

g_Vicinamibacterales_uncultured	1.00	0.75	0.19	0.19	1.00	0.94	1.00	0.63	1.25	0.56	0.44	0.63	2.07	1.00	0.81	0.13	2.26	0.63	0.56	0.44	1.07	0.63	0.50	0.19	0.94	0.75	0.71	0.86
g_Ilumatobacter	0.50	0.00	0.00	0.06	0.19	0.19	0.06	0.25	0.06	0.06	0.00	0.13	0.13	0.00	0.00	12.34	0.31	0.81	0.19	0.25	0.00	0.56	0.06	0.31	0.50	0.13	0.16	1.59
g_Rhodobacteraceae_Unclassified	0.31	0.25	0.00	0.00	0.25	0.25	0.06	0.25	0.19	0.06	0.06	0.44	0.00	0.31	0.13	0.13	0.50	1.19	0.19	0.00	0.50	0.38	0.00	0.00	0.38	0.38	0.17	0.16
g_AT-s2-59	0.31	0.38	0.38	0.50	0.88	0.50	1.32	1.69	0.56	0.44	0.38	0.56	0.50	0.06	0.56	0.06	1.13	1.44	1.69	2.07	0.06	0.06	0.19	0.25	1.32	0.38	0.74	0.39
g_Woeseia	0.63	0.31	0.44	0.63	1.50	0.56	0.81	1.25	0.50	0.94	0.06	1.00	0.31	0.25	0.25	0.00	1.00	0.94	0.50	1.19	0.06	0.50	0.69	0.75	1.25	0.63	0.77	0.42
g_Subgroup_22	0.44	0.19	0.38	0.31	0.44	0.50	0.69	0.44	0.44	0.75	0.44	0.44	0.38	0.31	0.38	0.00	1.38	1.82	1.82	1.69	0.31	0.06	0.13	0.06	0.69	0.50	0.42	0.39
g_Pseudahrensia	0.38	0.13	0.00	0.00	0.06	0.06	0.06	0.25	0.25	0.06	0.06	0.19	0.06	0.19	0.00	0.00	0.63	0.88	0.13	0.31	0.63	0.81	0.13	0.38	0.25	0.25	0.12	0.10
g_Gimesiaceae_uncultured	0.88	0.88	0.25	0.13	1.13	0.81	1.07	0.19	0.63	0.00	0.25	0.06	3.07	0.88	0.81	0.00	0.94	1.50	1.00	0.56	0.69	0.44	0.75	0.69	0.44	0.81	0.67	0.71
g_Methyloceanibacter	0.13	0.06	0.00	0.06	0.00	0.44	0.63	0.56	0.38	0.25	0.06	0.06	0.00	0.06	0.06	0.25	2.57	1.94	0.88	0.88	0.44	1.25	2.63	3.45	1.44	0.88	0.23	0.14
g_Vermiphilaceae	0.06	0.00	0.13	0.06	0.06	0.00	0.13	0.00	0.06	0.06	0.00	0.06	0.13	0.00	0.00	0.00	0.00	0.06	0.44	1.32	0.00	0.00	0.06	0.06	0.00	0.06	0.05	0.04
g_Planctomycetota_Unclassified	0.00	0.06	0.06	0.06	0.19	0.50	0.06	0.31	0.38	0.19	0.31	0.19	0.38	0.13	0.00	0.06	0.63	0.31	0.06	0.19	0.13	0.06	1.07	0.81	0.13	0.56	0.16	0.20
g_Gammaproteobacteria_Incertae_Sedis_uncultured	0.50	0.81	0.25	0.13	2.88	1.19	1.07	1.13	0.50	1.19	0.75	1.38	0.56	1.13	0.44	0.19	2.69	5.14	0.81	0.31	0.81	2.32	2.19	4.70	2.38	1.25	0.99	0.77
g_Flavobacteriaceae_Unclassified	0.50	0.44	0.06	0.19	1.69	0.81	1.07	0.56	0.50	0.25	0.19	0.63	1.57	0.38	0.69	0.00	1.32	0.75	0.00	0.56	0.25	1.63	0.25	0.19	2.82	0.88	0.67	0.52
g_TK17	0.19	0.19	0.31	0.56	0.56	1.13	0.56	1.19	1.19	1.00	1.13	0.44	0.31	2.44	1.63	0.00	1.13	0.31	1.00	0.31	0.88	1.57	0.38	0.00	0.94	1.00	0.59	1.02
g_Saccharimonadales	0.38	0.56	0.00	0.06	0.13	0.06	0.25	0.06	0.19	0.13	0.19	0.00	0.38	0.38	0.13	0.19	0.75	0.75	0.38	0.13	1.50	2.07	0.69	0.88	0.06	0.19	0.19	0.20
g_JG30-KF-CM66	0.56	0.50	1.57	3.32	0.44	1.44	1.38	2.01	1.38	1.57	1.69	1.38	1.44	3.20	3.51	0.25	0.56	0.19	2.44	1.07	0.88	1.63	0.44	0.13	0.50	0.56	1.40	1.80
g_KD4-96	1.32	0.31	0.13	0.19	0.13	0.25	0.50	0.38	0.25	0.44	0.00	0.56	1.44	1.50	0.13	3.88	0.50	0.50	1.32	1.07	0.38	0.06	0.25	0.19	0.06	0.81	0.40	1.03
g_OM190	1.07	0.88	0.38	0.31	1.13	0.88	1.19	0.56	0.63	0.38	0.31	0.50	1.38	0.56	0.94	0.88	0.88	1.00	0.56	0.19	0.63	0.56	0.25	0.44	0.44	0.69	0.80	0.70
g_Chloroflexi_Unclassified	0.25	0.56	0.13	0.31	0.00	0.56	0.19	0.31	0.31	0.25	0.13	0.38	0.31	1.69	1.13	3.26	0.00	0.06	0.75	0.25	0.06	0.00	0.13	0.06	0.25	0.13	0.29	0.93
g_Rhodopirellula	0.56	0.63	0.13	0.13	0.31	0.31	0.50	0.75	0.88	0.31	0.19	0.38	2.01	0.50	0.88	0.19	0.31	0.69	0.44	0.56	0.31	0.38	0.06	0.69	0.31	0.19	0.42	0.67
g_Pirellulaceae_uncultured	0.75	0.38	0.25	0.06	0.38	0.69	1.07	0.50	1.07	0.63	0.31	0.88	1.82	0.63	0.00	0.00	0.50	1.19	1.13	1.25	0.44	0.13	2.19	2.69	0.06	0.88	0.51	0.67

g_Planctomycetales_uncultured	0.63	0.50	0.56	0.00	0.50	0.50	0.88	0.13	1.00	0.38	0.81	0.13	2.26	0.63	0.50	0.19	1.07	0.44	1.07	0.56	0.56	0.13	0.38	0.31	0.25	0.50	0.46	0.74
g_Lutimonas	0.06	0.00	0.00	0.00	0.19	0.25	0.06	0.38	0.00	0.00	0.00	0.00	0.06	0.00	0.00	0.19	0.00	0.13	0.00	0.00	0.00	0.00	0.69	0.75	0.13	0.00	0.12	0.03
g_Halieaceae_Unclassified	0.31	0.19	0.06	0.00	0.88	0.50	0.13	0.63	0.19	0.31	0.13	0.25	0.19	0.13	0.31	0.31	0.63	0.56	0.00	0.00	0.00	0.63	0.38	0.31	0.56	0.31	0.34	0.23
g_Rhodothermaceae_uncultured	0.31	0.13	0.06	0.00	0.50	0.00	0.13	1.00	0.06	0.06	0.06	0.25	0.19	0.19	0.25	0.19	0.19	0.31	0.25	0.31	0.00	0.19	0.19	0.06	0.00	0.06	0.27	0.16
g_Planctomycetales_Unclassified	1.19	0.19	0.00	0.00	0.25	0.69	0.50	0.31	0.75	0.50	0.25	0.44	1.69	0.63	0.75	0.63	0.31	0.56	0.25	0.44	0.56	0.31	0.63	0.31	0.13	0.88	0.39	0.70
g_Limibaculum	0.00	0.00	0.00	0.00	0.38	0.13	0.13	0.25	0.13	0.13	0.19	0.06	0.00	0.06	0.00	0.13	0.19	0.31	0.00	0.13	0.00	0.13	0.38	0.56	0.63	0.19	0.11	0.09
g_Urania-1B-19_marine_sediment_group	0.69	0.25	0.50	0.13	0.31	0.13	0.13	0.13	0.50	0.19	0.25	0.31	0.56	0.25	0.06	0.25	0.38	0.13	0.13	0.44	0.63	0.00	0.38	0.13	0.13	0.19	0.28	0.30
g_Aminicenantales	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.13	0.06	0.00	0.00	0.00	0.00	0.00	3.13	2.19	0.00	0.00	0.00	0.02
g_Vicinamibacterales_Unclassified	0.13	0.19	0.13	0.06	0.31	0.25	0.38	0.13	0.75	0.56	0.25	0.00	0.13	0.13	0.00	0.13	0.56	0.38	0.19	0.31	1.19	0.00	0.00	0.00	0.00	0.19	0.20	0.24
g_Flavobacteriaceae_uncultured	0.44	0.06	0.00	0.00	0.06	0.06	0.38	0.00	0.00	0.06	0.19	0.13	0.25	0.19	0.50	0.13	0.44	0.00	0.00	0.13	0.13	0.25	0.00	0.00	0.13	0.13	0.13	0.18
g_Mitochondria	0.00	0.00	0.00	0.00	0.00	0.00	0.06	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.06	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.06	0.01	0.00
g_Thermoanaerobaculaceae_Subgroup_23	0.00	0.00	0.00	0.00	0.00	0.06	0.00	0.06	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.06	0.06	0.00	0.00	0.00	0.00	0.38	1.00	0.00	0.06	0.02	0.00
g_Desulfobulbaceae_uncultured	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	1.63	0.06	0.13	0.00	0.00	0.00	0.00	0.56	1.00	0.13	0.13	0.00	0.20
g_Desulfocapsaceae_Unclassified	0.00	0.00	0.00	0.00	0.00	0.00	0.06	0.00	0.19	0.00	0.00	0.00	0.00	0.00	0.00	0.25	0.06	0.19	0.00	0.00	0.00	0.00	0.06	0.19	0.13	0.06	0.01	0.05
g_Sva1033	0.00	0.00	0.00	0.00	1.94	0.50	0.19	1.69	0.19	0.06	0.00	0.13	0.00	0.06	0.00	0.06	1.13	2.19	0.00	0.00	0.06	0.44	4.51	8.52	1.75	1.19	0.54	0.06
g_SG8-4	0.00	0.00	0.06	0.00	1.00	1.07	0.50	0.50	0.81	0.13	0.00	0.06	0.13	0.00	0.00	1.00	0.75	1.00	0.06	0.06	0.38	0.06	4.33	3.07	0.31	0.63	0.39	0.27
g_AKAU3564_sediment_group	0.00	0.00	0.00	0.00	0.06	0.00	0.25	0.00	0.00	0.06	0.06	0.00	0.00	0.00	0.00	0.06	0.06	0.13	0.00	0.00	0.06	0.00	1.25	0.50	0.06	0.00	0.04	0.02
g_Phycisphaeraceae_uncultured	0.69	0.56	0.31	0.00	0.75	0.56	0.69	0.19	0.31	0.06	0.31	0.06	1.50	0.19	0.75	0.06	0.06	0.31	0.19	0.50	0.63	0.25	0.25	0.06	0.25	0.31	0.47	0.41
g_Rubripirellula	0.13	0.31	0.31	0.13	0.56	0.13	0.75	0.13	0.19	0.25	0.06	0.19	0.69	0.13	0.19	0.13	1.50	1.69	0.44	0.56	1.57	0.63	2.01	1.50	0.19	0.44	0.31	0.23
g_Thiohalorhabdaceae_uncultured	0.13	0.13	0.00	0.06	0.94	0.69	0.38	0.56	0.13	0.44	0.13	0.25	0.25	0.38	0.19	6.58	0.81	0.25	0.00	0.31	0.38	0.88	0.19	0.19	1.07	0.38	0.36	1.04

Table S2.3. Summarized PICRUSt2.0 results in all samples at MetaCyc level 2 found in the Amundsen Sea and the Ross Sea.

Level 1	Level 2	Relative abundance (%)																									
		A1-1	A1-2	A1-3	A1-4	A2-1	A2-2	A2-3	A2-4	A3-1	A3-2	A3-3	A3-4	A4-1	A4-2	A4-3	A4-4	R1-1	R1-2	R2-1	R2-2	R3-1	R3-2	R4-1	R4-2	R5-1	R5-2
Biosynthesis	Nucleoside and Nucleotide Biosynthesis	20.9739	23.0555	24.7376	25.1875	18.4258	20.7556	19.9198	19.1604	21.0214	21.0795	22.7726	21.2639	19.3762	21.0072	21.8914	22.9736	17.3868	16.7510	19.0812	17.7981	20.6306	20.3310	18.6707	17.4232	18.7176	19.6335
	Amino Acid Biosynthesis	18.7987	19.6552	20.2130	20.2819	17.4188	18.6029	18.3913	17.6659	18.5677	18.5896	19.0267	18.5959	18.5757	18.7621	18.9686	19.4819	16.8496	16.8306	17.6399	16.8503	18.7464	18.2192	17.4737	16.7981	17.2105	17.9617
	Cofactor, Prosthetic Group, Electron Carrier, and Vitamin Biosynthesis	16.1426	16.1754	16.4998	17.1072	16.6750	16.2869	15.7481	17.1256	16.3800	16.6999	16.7741	16.7895	14.9011	15.9158	16.1742	16.4388	16.2723	16.1312	16.3765	16.4199	16.3789	16.9606	16.2431	16.3269	17.1619	16.2748
	Fatty Acid and Lipid Biosynthesis	7.4506	5.6460	4.4929	4.1713	9.4407	7.4486	8.2666	8.5590	7.3804	6.9745	6.1074	6.8881	9.0683	7.6488	6.7611	6.0323	10.4838	10.9270	8.7076	9.8562	7.4094	7.5377	9.0591	10.4535	9.1517	8.4965
	Carbohydrate Biosynthesis	6.0230	6.1837	6.4968	6.2978	5.9397	6.0131	6.1858	5.8591	6.1826	6.1434	6.1431	6.0698	5.9374	5.9616	6.1753	5.8975	5.6563	5.4687	5.9142	5.6567	6.1577	5.9256	5.9850	5.7095	5.8124	5.8678
	Secondary Metabolite Biosynthesis	2.7837	2.6312	2.4438	2.2855	2.5352	2.5699	2.7154	2.4604	2.6971	2.5318	2.4424	2.4654	2.9927	2.7252	2.6195	2.5213	2.5767	2.5137	2.6811	2.6388	2.6727	2.4023	2.8530	2.7067	2.3703	2.5221
	Cell Structure Biosynthesis	2.5615	2.1758	1.8709	1.7570	3.2908	2.7279	2.9604	3.0523	2.5920	2.6013	2.2580	2.5303	2.9487	2.7177	2.6547	2.3848	3.4782	3.4716	3.3188	3.5569	2.6293	2.6559	3.2642	3.5102	3.1798	2.7934
	Aromatic Compound Biosynthesis	1.7475	1.8505	1.8997	1.8993	1.4897	1.6720	1.6235	1.5161	1.7164	1.6895	1.7873	1.6870	1.6598	1.7200	1.7362	1.8091	1.3956	1.3676	1.5437	1.4612	1.6519	1.5754	1.4645	1.3997	1.4728	1.5931
	Aminoacyl-tRNA Charging	0.7909	0.7951	0.7334	0.5808	0.6856	0.7219	0.7431	0.6439	0.7765	0.7018	0.6857	0.6764	0.7569	0.7228	0.7294	0.6375	0.6343	0.6199	0.6596	0.6376	0.7444	0.6512	0.7144	0.6669	0.6510	0.7029
	Amine and Polyamine Biosynthesis	0.2442	0.2257	0.1796	0.1610	0.4765	0.3514	0.3269	0.4236	0.2636	0.2734	0.2915	0.3104	0.2356	0.2299	0.1934	0.1971	0.5664	0.6326	0.3471	0.3685	0.3304	0.4356	0.4479	0.5646	0.5423	0.4688
	Metabolic Regulator Biosynthesis	0.0481	0.0377	0.0190	0.0238	0.0845	0.0558	0.0615	0.1129	0.0609	0.0552	0.0722	0.0786	0.0480	0.0538	0.0438	0.0492	0.1448	0.1567	0.0984	0.1487	0.0804	0.1249	0.1043	0.1304	0.1199	0.1052
Degradation/Utilization/Assimilation	C1 Compound Utilization and Assimilation	2.1667	2.3504	2.4659	2.4785	2.3575	2.4098	2.4066	2.3534	2.3318	2.3960	2.3151	2.3057	2.3319	2.4160	2.4852	2.4849	2.3492	2.3085	2.5345	2.2996	2.3275	2.4681	2.8509	2.7374	2.3863	2.4172
	Inorganic Nutrient Metabolism	1.6425	1.6576	1.5400	1.5075	1.4283	1.5109	1.5063	1.4138	1.5448	1.4832	1.5532	1.5534	1.6185	1.3882	1.3883	1.3405	1.4533	1.5651	1.3124	1.3407	1.5622	1.4758	1.4408	1.4152	1.4384	1.5510
	Nucleoside and Nucleotide Degradation	1.4536	1.1457	0.8159	0.6734	1.6526	1.3507	1.5954	1.5844	1.4967	1.5162	1.1746	1.4012	1.6946	1.4143	1.2169	1.0786	2.0426	2.1664	2.1444	2.5044	1.5699	1.3901	1.4251	1.6556	1.4844	1.6252
	Carbohydrate Degradation	0.7300	0.5923	0.4245	0.3100	0.7420	0.6758	0.7439	0.5689	0.6688	0.5312	0.5199	0.5389	0.9804	0.6768	0.6366	0.5694	0.8174	0.8168	0.6358	0.6319	0.7823	0.6553	0.8956	0.7854	0.5943	0.6629
	Fatty Acid and Lipid Degradation	0.5810	0.4763	0.4014	0.3866	0.4989	0.4924	0.5552	0.5038	0.5554	0.5188	0.4446	0.4605	0.6116	0.5459	0.5175	0.4889	0.5421	0.5617	0.5068	0.5244	0.5086	0.4521	0.4312	0.4734	0.4681	0.5135
	Polymeric Compound Degradation	0.4936	0.3917	0.2998	0.3526	0.6931	0.5573	0.6276	0.6602	0.4881	0.5792	0.4694	0.5598	0.5881	0.4920	0.4912	0.4475	0.7816	0.7795	0.7423	0.8294	0.5106	0.5359	0.7637	0.7685	0.6145	0.6036
	Secondary Metabolite Degradation	0.3680	0.2785	0.1649	0.1674	0.5173	0.3887	0.4279	0.4577	0.3530	0.3689	0.3080	0.3535	0.4403	0.3242	0.3107	0.2581	0.6672	0.6815	0.3936	0.5038	0.4240	0.4632	0.5847	0.6083	0.4521	0.4732
	Amino Acid Degradation	0.2353	0.1766	0.1644	0.1169	0.3553	0.2703	0.3009	0.3812	0.2354	0.2737	0.2088	0.2702	0.2494	0.2082	0.2443	0.1521	0.4475	0.4717	0.3402	0.4372	0.2775	0.3129	0.2990	0.3260	0.3787	0.3301
	Carboxylate Degradation	0.0662	0.0695	0.0723	0.0428	0.1580	0.1057	0.1047	0.1574	0.0727	0.0954	0.0868	0.1056	0.0784	0.0797	0.0767	0.0615	0.1812	0.2060	0.1048	0.0992	0.1005	0.1558	0.1964	0.2658	0.1905	0.1440
	Aromatic Compound Degradation	0.0580	0.0328	0.0283	0.0284	0.0937	0.0634	0.0599	0.1343	0.0614	0.0940	0.0438	0.0983	0.0713	0.0825	0.0510	0.0367	0.1129	0.1556	0.0750	0.1298	0.0740	0.1120	0.0578	0.0948	0.1310	0.1141

	Amine and Polyamine Degradation	0.0546	0.0520	0.0019	0.0088	0.0420	0.0532	0.0361	0.0644	0.0528	0.0314	0.0331	0.0514	0.0425	0.0489	0.0316	0.0264	0.0900	0.1308	0.0474	0.0740	0.0786	0.1194	0.0263	0.0399	0.0872	0.0713
	Degradation/Utilization/Assimilation - Other	0.0223	0.0245	0.0176	0.0060	0.0402	0.0406	0.0230	0.0300	0.0226	0.0207	0.0142	0.0322	0.0415	0.0248	0.0251	0.0129	0.0567	0.0671	0.0081	0.0192	0.0415	0.0591	0.0457	0.0254	0.0544	0.0495
	Alcohol Degradation	0.0008	0.0000	0.0000	0.0000	0.0024	0.0000	0.0030	0.0028	0.0021	0.0057	0.0000	0.0025	0.0024	0.0002	0.0000	0.0005	0.0021	0.0042	0.0011	0.0033	0.0020	0.0034	0.0047	0.0092	0.0022	0.0046
	Aldehyde Degradation	0.0000	0.0000	0.0000	0.0000	0.0002	0.0000	0.0000	0.0001	0.0000	0.0000	0.0000	0.0000	0.0006	0.0001	0.0000	0.0000	0.0000	0.0000	0.0000	0.0003	0.0000	0.0000	0.0000	0.0000	0.0000	0.0003
Generation of Precursor Metabolite and Energy	Fermentation	3.2091	3.2489	3.1969	3.2922	2.8678	3.1669	3.0818	3.0659	3.1383	3.1989	3.1221	3.2027	3.0929	3.3115	3.2094	3.3815	2.9615	3.1190	3.2991	3.3187	3.1825	3.1359	3.0802	3.0121	3.0205	3.0992
	TCA cycle	3.0364	2.9349	2.8965	2.8428	3.2447	3.0650	3.0553	3.3806	3.0396	3.1348	3.0788	3.2699	3.0001	2.9983	3.0455	2.9854	3.1994	3.1502	3.0459	3.1296	2.9739	3.2806	3.0451	3.0860	3.5077	3.2142
	Respiration	2.5284	2.8030	3.0538	3.1302	2.1519	2.4633	2.3404	2.2318	2.4889	2.5398	2.7595	2.5588	2.3127	2.4510	2.5535	2.6873	2.0053	1.9925	2.2373	2.1531	2.3952	2.3220	2.0668	1.9760	2.1773	2.3328
	Pentose Phosphate Pathways	1.6391	1.7699	1.8412	1.8782	1.3936	1.6056	1.5161	1.4359	1.6250	1.5799	1.6994	1.6020	1.6004	1.6972	1.6866	1.8108	1.3493	1.3317	1.5160	1.4213	1.6118	1.5714	1.4377	1.3435	1.3802	1.5190
	Glycolysis	1.4128	1.3065	1.2809	1.4134	1.7419	1.5931	1.5909	1.6877	1.4985	1.5373	1.4068	1.5027	1.6063	1.7104	1.6689	1.6691	1.6693	1.7340	1.6824	1.6917	1.4024	1.5851	1.7231	1.8627	1.7385	1.6024
	Photosynthesis	0.1971	0.1438	0.1402	0.1176	0.2516	0.2245	0.2296	0.2239	0.1973	0.2138	0.1701	0.2034	0.2098	0.1756	0.1629	0.1552	0.2891	0.3309	0.2486	0.2803	0.1836	0.1959	0.2835	0.3106	0.2363	0.2506
	glyoxylate cycle	0.0980	0.0826	0.0840	0.0715	0.2650	0.1554	0.1543	0.2381	0.1148	0.1593	0.1506	0.1802	0.0924	0.1188	0.1073	0.0899	0.2472	0.2535	0.1554	0.2063	0.1129	0.1939	0.1621	0.2341	0.3027	0.1912
	ethylmalonyl-CoA pathway	0.0233	0.0197	0.0072	0.0152	0.0359	0.0419	0.0318	0.0692	0.0313	0.0294	0.0300	0.0416	0.0163	0.0252	0.0153	0.0262	0.0942	0.1010	0.0529	0.0728	0.0340	0.0570	0.0722	0.1047	0.0771	0.0519
	1,5-anhydrofructose degradation	0.0042	0.0014	0.0000	0.0000	0.0026	0.0000	0.0011	0.0015	0.0032	0.0000	0.0024	0.0013	0.0031	0.0031	0.0000	0.0013	0.0048	0.0030	0.0000	0.0028	0.0065	0.0079	0.0022	0.0020	0.0053	0.0020
	methyl ketone biosynthesis (engineered)	0.0023	0.0020	0.0000	0.0000	0.0069	0.0046	0.0011	0.0045	0.0012	0.0064	0.0013	0.0018	0.0021	0.0011	0.0018	0.0039	0.0054	0.0053	0.0010	0.0034	0.0011	0.0034	0.0010	0.0049	0.0059	0.0081
	isopropanol biosynthesis (engineered)	0.0000	0.0013	0.0015	0.0000	0.0012	0.0012	0.0011	0.0083	0.0023	0.0035	0.0000	0.0048	0.0021	0.0023	0.0000	0.0026	0.0018	0.0082	0.0000	0.0019	0.0011	0.0080	0.0042	0.0033	0.0147	0.0047
	methylaspartate cycle	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0011	0.0000	0.0000	0.0000	0.0091	0.0000	0.0116	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0072	0.0000	0.0000
Glycan Pathways	Glycan Biosynthesis	0.5998	0.4773	0.3156	0.2815	0.5707	0.5213	0.6033	0.4362	0.5372	0.4817	0.3938	0.4623	0.7502	0.5867	0.4948	0.4030	0.5834	0.5767	0.5679	0.5853	0.5171	0.4333	0.5599	0.5788	0.4594	0.5207
Macromolecule Modification	Nucleic Acid Processing	0.5104	0.3956	0.3586	0.2907	0.6057	0.5157	0.5621	0.5243	0.4764	0.4455	0.3641	0.3949	0.6531	0.4715	0.4628	0.3131	0.6817	0.6958	0.5277	0.6436	0.4934	0.4908	0.7033	0.7782	0.5596	0.5044
Superpathways	superpathway of histidine, purine, and pyrimidine biosynthesis	0.3190	0.2893	0.1334	0.1669	0.3428	0.3185	0.3165	0.3628	0.3284	0.3130	0.2880	0.3203	0.3301	0.2652	0.2524	0.2329	0.3537	0.3210	0.2702	0.3088	0.3624	0.3632	0.3425	0.3638	0.3455	0.3635
	superpathway of glycolysis and the Entner-Doudoroff pathway	0.2862	0.2280	0.1904	0.2265	0.3970	0.3316	0.3435	0.3756	0.2942	0.3096	0.2652	0.3192	0.3298	0.3086	0.2839	0.2728	0.4230	0.4483	0.3801	0.4008	0.2904	0.3444	0.3702	0.4255	0.3922	0.3525
	aspartate superpathway	0.2662	0.2456	0.1994	0.1755	0.3756	0.3234	0.3172	0.3546	0.2577	0.2923	0.2650	0.3003	0.2906	0.2702	0.2492	0.2299	0.3816	0.3874	0.2893	0.3408	0.2882	0.3359	0.2882	0.3267	0.3626	0.3379
	superpathway of S-adenosyl-L-methionine biosynthesis	0.2025	0.1896	0.1537	0.1256	0.3190	0.2598	0.2507	0.2654	0.1917	0.2189	0.2009	0.2356	0.2339	0.1937	0.1716	0.1562	0.3003	0.3046	0.1941	0.2446	0.2177	0.2698	0.2169	0.2475	0.2847	0.2766
	superpathway of glycolysis, pyruvate dehydrogenase, TCA, and glyoxylate bypass	0.1832	0.1739	0.1621	0.1345	0.3681	0.2686	0.2584	0.3935	0.2226	0.2684	0.2489	0.2953	0.1895	0.2318	0.2045	0.1851	0.4062	0.4172	0.3060	0.3584	0.2289	0.3438	0.2908	0.3938	0.4252	0.3306
	adenosylcobalamin biosynthesis I (anaerobic)	0.0164	0.0000	0.0000	0.0000	0.0000	0.0029	0.0012	0.0146	0.0088	0.0000	0.0000	0.0000	0.0063	0.0000	0.0000	0.0000	0.0130	0.0000	0.0000	0.0135	0.0000	0.0087	0.0079	0.0000	0.0076	0.0159
	superpathway of hexuronide and hexuronate degradation	0.0160	0.0074	0.0000	0.0018	0.0134	0.0089	0.0088	0.0176	0.0108	0.0131	0.0113	0.0129	0.0091	0.0071	0.0069	0.0119	0.0247	0.0315	0.0109	0.0157	0.0056	0.0185	0.0110	0.0140	0.0179	0.0181

adenosylcobalamin biosynthesis II (aerobic)	0.0123	0.0000	0.0000	0.0000	0.0000	0.0026	0.0011	0.0124	0.0076	0.0000	0.0000	0.0000	0.0051	0.0000	0.0000	0.0000	0.0115	0.0000	0.0000	0.0118	0.0000	0.0078	0.0066	0.0000	0.0068	0.0134
superpathway of chorismate metabolism	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0026	0.0000	0.0000	0.0000	0.0000	0.0000	0.0028	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0077
superpathway of D-glucarate and D-galactarate degradation	0.0000	0.0000	0.0014	0.0037	0.0000	0.0000	0.0000	0.0011	0.0000	0.0000	0.0000	0.0000	0.0010	0.0011	0.0000	0.0000	0.0027	0.0009	0.0007	0.0014	0.0000	0.0000	0.0228	0.0257	0.0000	0.0014
superpathway of fucose and rhamnose degradation	0.0000	0.0000	0.0000	0.0000	0.0009	0.0000	0.0000	0.0003	0.0000	0.0000	0.0004	0.0017	0.0000	0.0001	0.0000	0.0007	0.0021	0.0017	0.0000	0.0015	0.0000	0.0000	0.0000	0.0043	0.0000	0.0007

Table S4.1. Taxonomic assignments of the bacterial isolates.

Species name	Similarity (%)	No. of strains
Actinobacteria (5.5%)		
Nesterenkonia jeotgali	99.5	1
Nocardioides spp.	98.5-98.8	3
Bacteroidetes (6.8%)		
Lacinutrix venerupis	98.4-98.6	5
Alphaproteobacteria (6.8%)		
Erythrobacter citreus	99.9	1
Sphingorhabdus flavimaris	99.7	1
Sulfitobacter pontiacus	99.8	3
Gammaproteobacteria (80.8%)		
Alcanivorax borkumensis	99.3-99.6	8
Halomonas aquamarina	99.7-99.9	10
Marinobacter psychrophilus	99.7-99.9	7
Paraglaciecola spp.	99.2-100	12
Photobacterium frigidiphilum	99.9-100	2
Pseudoalteromonas spp.	99.6-99.9	2
Shewanella spp.	**98.8-98.9**	**13**
Spongiibacter tropicus	99.4	5
Sum		73

Table S4.2. Description of sampling stations, prokaryotic abundance (PA) and colony-forming units (prokaryotic) in sediment samples. PA were analyzed in duplicate and CFU were calculated in duplicate under 2 media (marine agar and saline Reasoner's 2A agar) and 3 temperatures (4, 15 and 20 °C). The value means average of duplicate measure value ± standard deviation.

Sample names	Date of sampling	Latitude	Longitude	Bottom Depth (m)	PA (x 10^8 cells/g of wet sediment; n=2)	CFU (x 10^6/g of sediment; n=12)	% of culturable bacteria
R2	12-Jan-2015	75°39.57'S	165°28.98'E	848	1.81 ± 0.51	0.01 ± 0.01	0.01 ± 0.00
A1	16-Jan-2016	72°05.03'S	118°53.05'W	745	1.46 ± 0.04	4.09 ± 4.66	2.80 ± 3.20
A2	18-Jan-2016	73°02.40'S	115°43.50'W	710	2.19 ± 0.13	2.89 ± 1.09	1.32 ± 0.50
A3	20-Jan-2016	74°10.29'S	112°31.79'W	1034	1.33 ± 0.57	0.60 ± 0.64	0.45 ± 0.48

Table S4.3. Numbers of gene copy for a given set of 120 bacterial marker genes [1] in the 30 genome sequences of *Shewanella* spp. The 10 genes with strikethrough lines were excluded in phylogenomic analyses due to their presence at multiple loci of genome sequence(s). Taxa numbers correspond to the numbers in Supplementary Table S4.1.

No.	Name	Length	Taxa																													
		(bp)	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30
1	Ribosomal_S9	121	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
2	Ribosomal_S8	129	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
3	Ribosomal_L10	100	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
4	GrpE	166	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
5	DUF150	141	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
6	PNPase	83	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
7	TIGR00006	310	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
8	prfA	361	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
9	prfB	365	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
10	S20	87	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	0	1	1	1	1	1	1	1	1	1	1	1	1	1	1
11	TIGR00043	111	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
~~12~~	~~TIGR00054~~	~~421~~	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	2	1	1
13	L17	112	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
14	L21	101	1	1	1	1	1	1	0	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
15	ftsY	279	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1

~~16~~	~~ftsZ~~	~~353~~	1	1	1	1	1	1	1	1	1	1	2	2	2	2	1	1	2	1	1	1	1	1	1	1	1	1	1	2	1	1
17	rbfA	115	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
18	ribF	290	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
19	ruvA	192	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
20	smpB	144	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
21	trmD	233	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
22	rsfS_iojap_ybeB	99	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
23	TIGR00092	368	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
24	TIGR00095	194	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
25	tig	410	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
26	tsf	293	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
27	rsmG_gidB	183	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
28	L9	148	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
29	S6	95	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
30	infC	165	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
31	rRNA_methyl_3	240	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
32	uvrC	574	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
33	RNAse_H_YqgF	130	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
34	PyrG	526	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
35	alaS	847	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
36	DnaA	437	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
~~37~~	~~clpX~~	~~414~~	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2

38	ileS	861	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
39	leuS_bact	843	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
40	metG	530	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
41	serS	418	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
42	sms	454	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
43	trmU	351	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
44	TruB	210	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
45	cysS	466	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
46	era	270	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
~~47~~	~~hisS~~	~~406~~	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	2	1	1	1	1	1	1	1	1	1	1
48	mraY	321	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
49	argS	569	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
50	aspS_bact	586	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
51	fmt	315	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
52	pheS	324	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
53	pheT_bact	798	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
54	IF-2	587	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
55	frr	176	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
~~56~~	~~hemN_rel~~	~~361~~	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	2
57	mfd	923	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
58	pola	890	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
59	recR	196	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1

~~60~~	~~uvrb~~	~~658~~	1	1	1	1	1	1	1	1	1	1	1	1	1	2	2	1	1	1	1	1	1	1	2	1	1	1	1	1	1	1
61	recN	563	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
62	ruvB	305	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
63	recG	629	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
64	dnan	367	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
65	rpsA	516	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
66	ksgA	256	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
67	secG	73	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
68	nusG	172	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
69	purB	436	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	0	1	1	1	1	1	1	1	1	1	1
70	ffh	428	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
71	secA	787	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
72	secE_bact	57	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
73	3a0501s007	414	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
74	rpsC_bact	212	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
75	rpsB_bact	225	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
76	rpsD_bact	200	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
77	rpsE_bact	156	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
78	rpsG_bact	154	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
79	rplT_bact	114	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
~~80~~	~~atpD~~	~~462~~	1	1	1	1	2	2	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
81	rplV_bact	103	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1

82	gyrB	639	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
83	gyrA	800	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	0	1	1	1	1
84	rplM_bact	141	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	0	1	1	1	1	1	1	1	1	1	1	1	1
85	rplO_bact	144	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
86	rplX_bact	104	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	0	1	1	1	1	1	1	1	1	1	1
87	murC	449	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
88	murD	441	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
89	holA	314	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
~~90~~	~~ATPsyn_F1gamma~~	~~286~~	1	1	1	1	2	2	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
91	rplP_bact	126	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
92	rplA_bact	227	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
93	rplB_bact	275	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
94	IMP_dehydrog	450	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
95	dnaG	414	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
~~96~~	~~lepA~~	~~595~~	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	2	1	1	1	1	1	1	1	1	1	1
~~97~~	~~TypA_BipA~~	~~594~~	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	2	1	1	1	1
98	coaD_prev_kdtB	155	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
99	L11_bact	140	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
100	nusB	131	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
101	NusA	340	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
102	tigrfam_recA	321	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
103	rpoB	1238	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1

104	rpoA	298	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
105	pyrH_bact	233	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
106	RNaseIII	219	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
107	16S_RimM	166	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
108	prok_dnaK	596	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
109	rpoC_TIGR	1147	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
110	dnaX_nterm	355	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
111	lysidine_TilS_N	189	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
112	Obg_CgtA	329	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
113	guanyl_kin	180	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
114	GTPase_EngA	432	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
115	L3_bact	202	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
116	uS11_bact	117	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	0	1	1	1	1	1	1	1	1	1	1	1	1
117	L6_bact	175	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
118	T6A_TsaD_YgjD	314	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	0	1	1	1	1	1	1	1	1	1	1
119	T6A_YeaZ	212	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
120	rplD_bact	188	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1

Table S4.4. Characteristics of *Shewanella frigidimarina* KCCM 41815^T and *Shewanella arctica* KCTC 23109^T.

Taxa: 1, *S. frigidimarina* KCCM 41815^T; 2, *S. arctica* KCTC 23109^T. Data were obtained from this study. Differential phenotypic features are highlighted. +, Positive; –, negative.

Characteristic	**1**	**2**
Oxidase	+	+
Catalase	+	+
Hydrolysis of		
Starch	–	–
Tween 80	+	+
Casein	+	+
DNA	–	–
H_2S production	+	+
Iron reduction	+	+
API ZYM		
N-Acetyl-ß-glucosaminidase	+	+
Acid phosphatase	+	+
Alkaline phosphatase	+	+
α-Chymotrypsin	–	–
Cystine arylamidase	+	+
Esterase (C4)	+	+
Esterase lipase (C8)	–	–
α-Fucosidase	–	–
α-Galactosidase	–	–
ß-Galactosidase	+	+
α-Glucosidase	+	+
ß-Glucosidase	–	–
ß-Glucuronidase	–	–
Leucine arylamidase	–	–
Lipase (C14)	+	+
Naphthol-AS-BI-phosphohydrolase	–	–
α-Mannosidase	–	–
Trypsin	–	–

Valine arylamidase	–	–
API 20NE		
Nitrate reduction	+	+
Indole production	–	–
Hydrolysis of gelatin	–	+
Fermentation (glucose)	–	–
Arginine dihydrolase	–	–
Urease	–	–
ß-Glucosidase	+	+
ß-Galactosidase	–	–
API 50CH		
Glycerol	–	–
Erythritol	–	–
D-Arabinose	–	–
L-Arabinose	–	–
D-Ribose	+	+
D-Xylose	–	–
L-Xylose	–	–
D-Adonitol	–	–
Methyl-ß-D-xylopyranoside	–	–
D-Galactose	–	–
D-Glucose	+	+
D-Fructose	+	–
D-Mannose	–	–
L-Sorbose	–	–
L-Rhamnose	–	–
Dulcitol	–	–
Inositol	–	–
D-Mannitol	+	–
D-Sorbitol	–	–
Methyl α-D-mannopyranoside	–	–
Methyl α-D-glucopyranoside	–	–
N-Acetylglucosamine	–	–
Amygdalin	–	–
Arbutin	–	–
Esculine ferric citrate	+	+
Salicin	–	–
D-Cellobiose	+	+

D-Maltose	+	+
D-Lactose	–	–
D-Melibiose	–	–
Sucrose	+	+
D-Trehalose	–	–
Inulin	–	–
D-Melezitose	–	–
D-Raffinose	–	–
Starch	+	–
Glycogen	–	–
Xylitol	–	–
Gentiobiose	–	–
D-Turanose	–	–
D-Lyxose	–	–
D-Tagatose	–	–
D-Fucose	–	–
L-Fucose	–	–
D-Arabitol	–	–
L-Arabitol	–	–
Potassium gluconate	–	–
Potassium 2-ketogluconate	–	–
Potassium 5-ketogluconate	–	–
Utilization of sole carbon source		
Glycerol	+	+
Starch	+	+
D-Galactose	+	+
Maltose	–	–
Cellobiose	+	+
L-Arabinose	+	+
Sucrose	+	–
L-Proline	+	+
L-Lactate	+	+
Butyrate	+	+
Fumarate	+	+
Propionate	+	+
D-Ribose	–	–
D-Fructose	+	+

D-Xylose	+	+
D-Glucose	+	+
Lactose	+	+
Melibiose	+	+
D-Sorbitol	+	+
D-Malic acid	–	–
D-Mannitol	+	+
D-Trehalose	–	+
D-Lyxose	+	+
N-acetyl-D-glucosamine	+	+
Quinone (%)		
MK-7	26.4	27.2
Q-7	9.6	36.3
Q-8	19.1	28.4
MMK-7	44.9	8.1

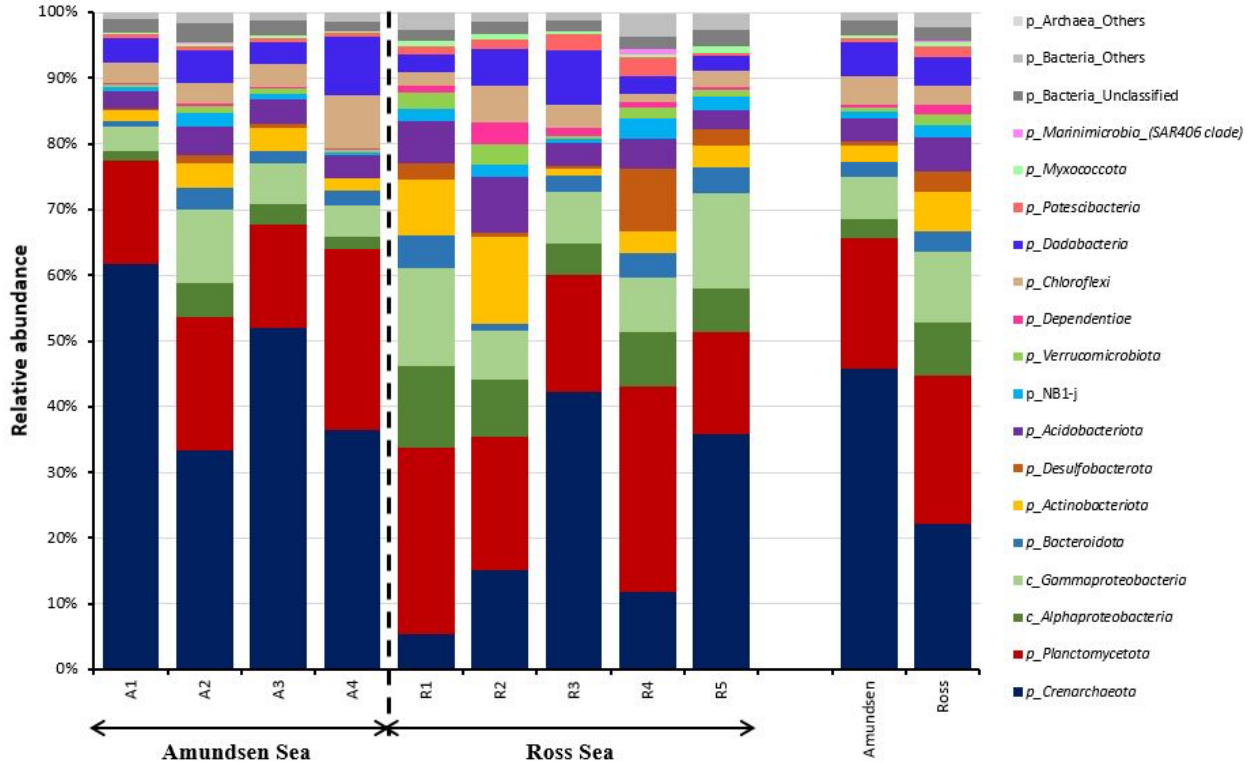

Figure S2.1. Taxonomic composition of bacterial and archaeal phyla that appeared at ≥ 1% relative abundance at any of the samples. Mean relative abundance of each sample is shown and the description of sample names is the same as in Table 2.1.

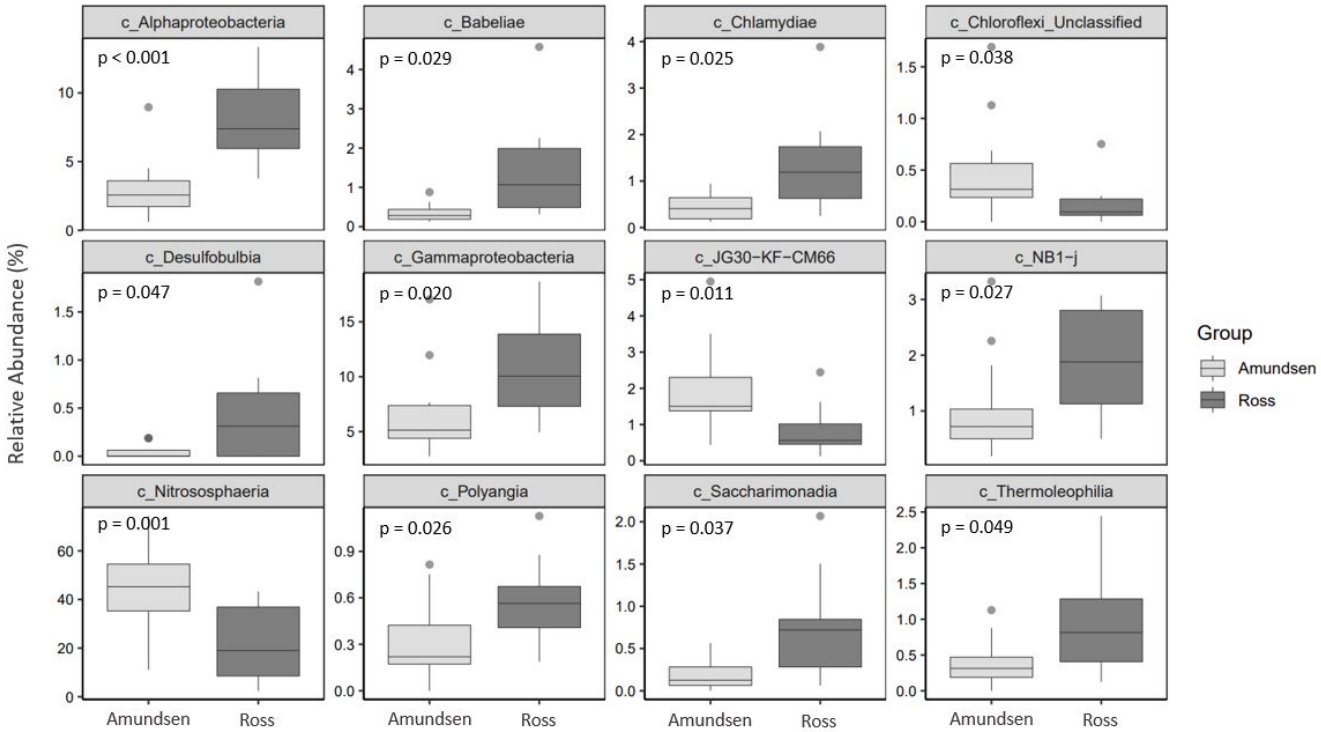

Figure S2.2. Boxplots of significant difference class between in the Amundsen Sea and in the Ross Sea.

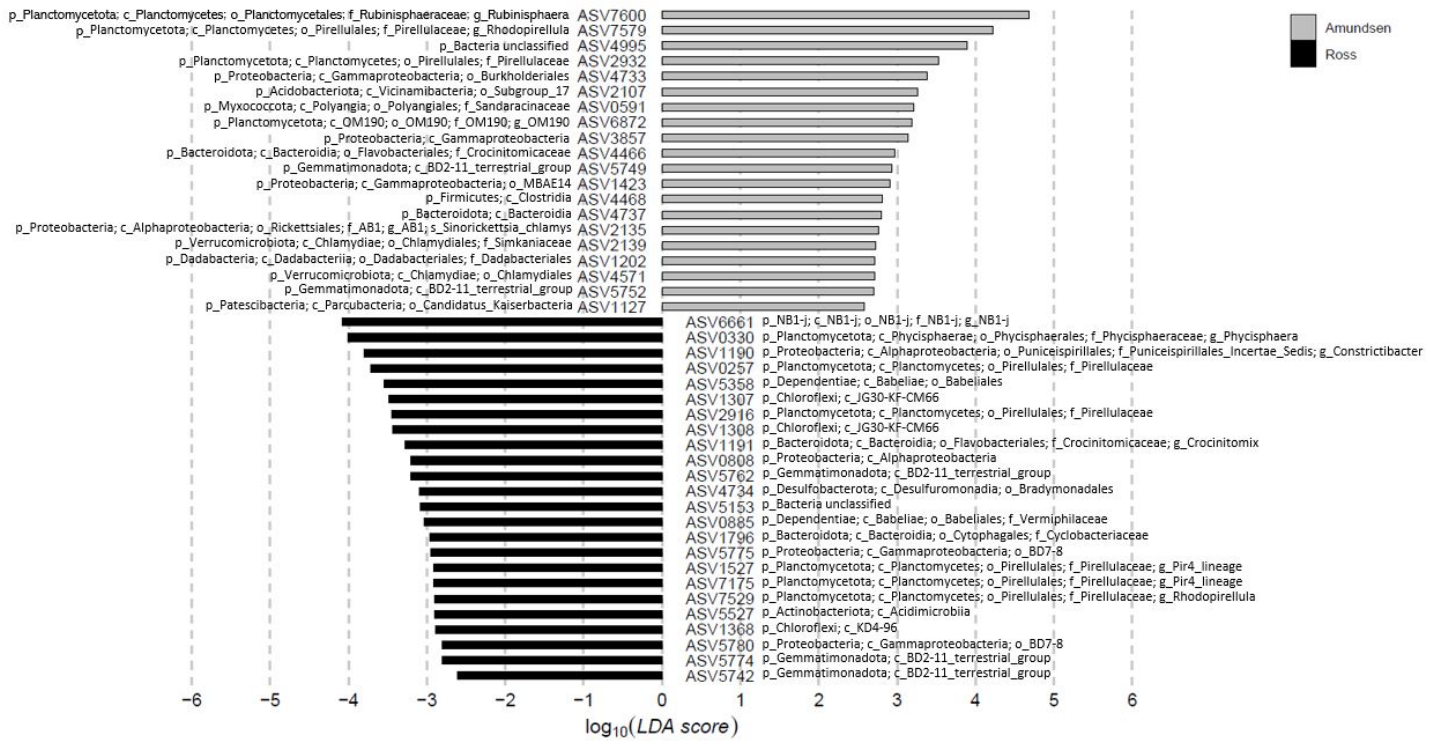

Figure S2.3. Calculation of the distinct ASVs between in the Amundsen Sea and in the Ross Sea using LEfSe analysis. LDA scores > 2.0 was used to distinguish.

국문초록 (Abstract in KOREA)

남극의 환경은 대기권, 수권, 암석권, 극저온권, 생물권과 밀접하게 연관되어 있다. 이러한 환경은 빙하가 녹는 것과 같은 극적인 변화가 발생하는 지역이기 때문에 지구의 물리적, 생물학적 시스템에서 매우 중요한 부분이다. 또한 남극의 저서 환경은 지역에 따라 높은 압력, 낮은 온도, 낮은 영양 가용성으로 인해 원핵생물에게 생존하기 어려운 조건이다.

원핵생물은 바이오매스 측면에서 우세하며 해양 생태계에서 유기물과 영양분의 생물지구화학적 흐름에서 중요한 역할을 한다. 원핵생물들은 혹독한 환경에서 성장하고 생존하기 위해 변화를 겪었고 따라서 연구를 위한 엄청난 유전적 다양성을 보여준다. 따라서 남극에서 이러한 미생물 군집 구조와 대사 기능을 이해하는 것은 빠르게 변화하는 남극 환경에 대한 미생물 군집의 변화에 대해 예상 할 수 있을 뿐만 아니라 생명공학 응용을 위한 우수한 유전학적 자원을 제공 할 수 있다. 그러나 서남극 지역에서는 원핵생물 군집의 다양성과 생태학적 기능에 대한 이해가 여전히 부족하다. 본 연구에서는 지역에 따라 원핵생물 군집이 어떻게 다른지, 표층 퇴적물의 물리적·화학적 특성이 서남극 지역의 원핵생물의 다양성 및 그 기능과 어떤 관련이 있는지를 연구하였다. 본 연구에서는 서남극 퇴적물의 생태계를 보다 잘 이해하기 위한 저서 원핵생물의 다양성과 그 대사기능을 연구하기 위해 16SrDNA 파이로 시퀀싱과 샷건시퀀싱을 적용하였다.

원핵생물의 16S rRNA 유전자의 V5-V8 영역의 파이로 시퀀싱을 통해 원핵생물 군집 분포가 지리적으로 뚜렷한 차이를 나타내었다. 아문젠 해역에서는 크렌아키오타가 더 우세한 것으로 밝혀진 반면, 알파와 감마프로테오박테리아 는 로스 해역에서 상대적으로 더 풍부했다. 게다가, 주로 암모니아 산화 고세균 니트로소스페리아형 크렌아키오타는 아문젠 해의 비 폴리냐 지역보다 폴리냐 지역에 덜 풍부했고, 온도와는 양의 상관관계를 전 유기탄소와는 음의 상관관계를 보였다. 반면, 프로테오박테리아는 온도와 음의 상관관계를 보였지만, 로스해의 전 질소 및 전 유기탄소와는 양의 상관관계를 보였다. 이 자료는 환경 조건이 원핵생물의 다양성과 군집 구조에 영향을

미친다는 것을 시사한다. 약간 높은 온도, 낮은 전 유기탄소와 및 낮은 $\delta^{13}C$ 는 아문젠 해에서 암모니아 산화 고세균 니트로소스페리아형 크렌아키오타가 번성할 수 있게 하고, 온도가 낮고 불안정한 질소와 탄소가 로스 해에서 프로테오박테리아가 우세할 수 있게 한다.

세 개의 원양 퇴적물의 샷건 시퀀싱 자료를 분석한 결과 세 개의 정점에는 유사한 분류군이 존재하였지만, 원핵생물 군집 구조는 정점마다 달랐다. 비록 세 정점 모두에서 프로테오박테리아가 가장 풍부한 문이었지만, 정점 R6 에서는 감마프로테오박테리아가 가장 풍부한 문이었고, 정점 R7 과 R8 에서는 알파프로테오박테리아가 가장 풍부한 문이었다. 고세균은 모든 정점에서 덜 풍부했고, 현장에서 저서 원핵생물 군집에 대한 기여도가 상당히 적었다. 정점 R6 에서는 정점 R7 과 R8 보다 타움고센균이 덜 풍부했다. 암모니아 산화물인 타움고센균은 과영양 해양 환경에서 살고 있기 때문에, 더 많은 내화질소와 탄소(예: 더 높은 $\delta^{15}N$, 더 낮은 $\delta^{13}C$) 조건이 이러한 유기체들이 정점 R7 과 R8 에 더 풍부하게 존재할 수 있도록 하는 것으로 나타났다. 따라서 대륙붕 내부와 대륙붕 외부 사이에는 미생물 군집 구조와 기능적 잠재력에 차이가 있으며, 이 차이는 뚜렷한 환경 조건에 의해 크게 영향을 받으며, 유기물 활용은 로스해의 원핵생물 군집을 형성하는 중요한 요소이다.

서남극 저서 퇴적물로 배양 가능한 박테리아의 발견과 특성화 연구를 수행하였다. 4 개의 퇴적물 샘플에서 73 개의 박테리아 균주를 분리하여 확인하였으며, 그 중 슈와넬라속의 2 개의 균주 $M2^T$ 와 R106 의 생리학적, 화학적, 계통발생학적, 게놈적 특성을 분석하였다. 슈와넬라속에 속하는 2 종의 새로운 종들은 선택적으로 혐기성이며, 성장을 위한 최적의 온도가 가장 낮았다. 또한, 새로운 종은 철 수송에 관여하는 두 세트의 feoA-feoB 오페론을 가지고 있었다. 따라서, 이러한 결과는 $M2^T$ 가 그들의 가장 가까운 계통발생학적 슈와넬라 종들 사이에서 다른 전략을 사용하여 분리된 환경에 적응한다는 것을 나타낸다. 파이로 시퀀싱과 샷건 시퀀싱 분석을 통해 슈와넬라 종들이 연구 지역의 저서 퇴적물에서 우점하고 있다는 것도 확인되었다. 본 연구는 다단계적 접근법을 통해 얻은 자료를 바탕으로 연구 지역에서 슈와넬라 종이 철 순환에 중요한 역할을 한다는 것을 밝혀냈다.

전반적으로, 이 연구는 원핵생물이 서남극의 저서 환경에서 중요한 역할을 한다고 제안한다. 그리고 이러한 발견은 서남극에 있는 원핵생물의 생태학적 기능에 대한 우리의 지식을 증가시킬 뿐만 아니라, 환경 변화에 대한 그들의 적응 능력을 예측하는 데 도움이 될 것이다.

주요어: 서남극, 해양 퇴적물, 저서 원핵생물 군집, 454 파이로 시퀀싱, 샷건 시퀀싱, 메타지노믹스
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