Isolation and physiological characterization of psychrophilic denitrifying bacteria from permanently cold Arctic fjord sediments (Svalbard, Norway)

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ABSTRACT
A large proportion of reactive nitrogen loss from polar sediments is known to be mediated by denitrification. However, the microorganisms mediating denitrification in polar environments remain poorly characterized. A combined approach of MPN enumeration, cultivation, and physiological characterization was used to describe psychrophilic denitrifying bacterial communities in sediments of three Arctic fjords in Svalbard (Norway). The physiological response of representative isolates to temperature was examined by quantifying growth rates, nitrate depletion, and membrane lipid composition across a temperature gradient. A most probable number (MPN) assay showed the presence of $10^3$ - $10^6$ cells of psychrophilic denitrifying bacteria g$^{-1}$ of sediment. Seventeen denitrifying strains displaying wide phylogenetic affiliations within the Proteobacteria were isolated using a systematic enrichment approach with organic acids as an electron donor and nitrate as an electron acceptor. Phylogenetic characterization of 16S rRNA gene sequences indicated that the isolates belonged to five genera, including *Shewanella, Pseudomonas, Psychromonas* (Gammaproteobacteria), *Arcobacter* (Epsilonproteobacteria), and *Herminiimonas* (Betaproteobacteria). All the isolates were determined to be facultative anaerobes and complete denitrifiers, showing stoichiometric conversion of nitrate to gaseous end products. The growth response from 0 to 40°C indicated that all genera, except *Shewanella* were psychrophiles (optimal growth <15 °C). Adaptation to low temperature was confirmed as membrane fatty acid profiles showed a shift from primarily C16:0 saturated fatty acids to C16:1 monounsaturated fatty acids at lower temperatures. This study provides the first targeted enrichment and characterization of psychrophilic denitrifying bacteria from polar
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sediments, and two genera, *Arcobacter* and *Herminiimonas*, are isolated for the first time in permanently cold marine sediments.
INTRODUCTION

Nitrogen is a major limiting nutrient of biological productivity in the coastal ocean (Rabalais, 2002; Howarth and Marino, 2006). The response of the nitrogen cycle to anthropogenic disturbances is strongly influenced by the phylogenetic structure and associated function of microbial communities responsible for nitrogen loss in coastal marine ecosystems. Two microbially catalyzed respiration processes, denitrification and anammox, convert dissolved inorganic nitrogen (NO$_3^-$, NO$_2^-$, NH$_4^+$) to gaseous N$_2$ and comprise the largest sink of reactive nitrogen from the coastal ocean on a global scale. Up to 50% of marine N removal is estimated to occur by denitrification and anammox in continental shelf sediments (Codispoti, 2007). The relative contribution of sedimentary denitrification and anammox to N removal varies strongly with water column depth, but denitrification is generally considered the dominant pathway for N removal in shallow (<100m) shelf sediments (Dalsgaard et al., 2005).

The Arctic Ocean is the shallowest of the world’s ocean basins and is comprised of 50% continental shelf. Substantial denitrification and anammox rates have been measured on Arctic shelves, indicating that the Arctic basin has a significant role in global N removal (Devol et al., 1997; Rysgaard et al., 2004; Gihring et al., 2010). Future reductions in Arctic sea-ice cover may lead to diminished fluxes of organic matter to sediments, resulting in major shifts in the biogeochemical cycling of nitrogen (Piepenburg, 2005; Arrigo et al., 2008). Thus, an understanding of the diversity and physiology of denitrifying bacteria from polar sediments is integral to understanding climate change related effects on nitrogen cycling in the Arctic.
Though sedimentary denitrification comprises an important N sink in marine ecosystems on a global scale and the majority of the seafloor is cold (< 5 °C), few studies have addressed the physiological adaptation of denitrifiers to cold temperatures. Arctic shelf sediments are characterized by permanently cold conditions, but rates of microbial metabolism (e.g., hydrolysis, oxygen respiration, and sulfate reduction) from Arctic sediments largely overlap with those of temperate sediments (Arnosti et al., 1998; Thamdrup and Fleischer, 1998; Kostka et al., 1999). This apparent lack of temperature limitation has been ascribed to the fact that microbes in these sediments are psychrophilic (see Morita, 1975). The permanently cold conditions in Arctic sediments may exert a strong selection for psychrophilic bacteria, but isolation of aerobic bacteria from Arctic sediments has yielded a mix of psychrophilic and psychrotolerant bacteria (Groudieva et al., 2004; Helmke and Weyland, 2004; Srinivas et al., 2009). Denitrifying bacteria have been isolated from cold (≤ 4 °C) marine waters from temperate environments under anaerobic conditions with nitrate as an electron acceptor (Brettar et al., 2001), but to date, no study has systematically investigated psychrophilic denitrifying bacteria in permanently cold sediments.

Shallow sediments in the Arctic Ocean basin have been shown to be active sites of denitrification, but the microbial communities mediating this process are understudied. Cultivation-independent methods have been used to study the community structure of denitrifying bacteria in coastal marine sediments from primarily temperate ecosystems (Braker et al., 2001; Mills et al., 2008), but horizontal gene transfer events of denitrification genes make it difficult to reconstruct phylogenies (Heylen et al., 2006). Therefore, cultivation of representative denitrifying bacteria is a crucial component to
improving detection of environmentally relevant taxa by cultivation-independent approaches. A better understanding of the physiology of psychrophilic denitrifying bacteria is also a necessity to better predict the role of low temperature in controlling denitrification activity in polar sediments. In the present study, a primarily cultivation-based approach was used to investigate the phylogeny and physiology of psychrophilic denitrifying bacteria from Arctic fjord sediments. The objectives of this study were to: (i) isolate and phylogenetically characterize psychrophilic bacteria capable of denitrification; (ii) examine the physiology of cold adaptation in psychrophilic denitrifying isolates; and (iii) detect isolated taxa in sediment samples using molecular community fingerprinting.

MATERIALS AND METHODS

Sample sites and sampling procedures

Sediment cores were collected in August 2008 from three fjord sites within the Svalbard archipelago (Table 1). At the time of collection, sediment surface temperatures ranged from 1.3 - 6.5°C. Sediments from Smeerenburgfjorden (SM) were black clayey and rich with organic matter, while the sediments from Ymerbukta (YM) and Kongsfjorden (KF) were black sandy and reddish-brown loamy, respectively. Sediment cores were retrieved with a Haps corer, and subsamples from the upper 0-5 cm depth interval were collected aseptically into sterile conical tubes. Samples for cultivation were transported at in situ temperature and stored at 1.5 °C until processed. Samples for molecular characterization were frozen immediately and stored at -80 °C until further analysis.
Enrichment and isolation of denitrifying bacteria

A bicarbonate buffered minimal saltwater medium (MSW) was prepared and dispensed according to Widdel and Bak (1992), with the modifications of omitting sulfate, resazurin, selenite, and tungstate. The medium contained the following components per liter: NaCl (20 g), NH₄Cl (0.250 g), KH₂PO₄ (0.200 g), KCl (0.5 g), MgCl₂·6H₂O (3.0 g) and CaCl₂·2H₂O (0.150 g) NaHCO₃ (2.5 g), trace element solution (TES; 1 ml), vitamin B₁₂ (1 ml), vitamin mix (1 ml) and thiamine (1 ml). The medium was autoclaved and poured under strictly anoxic conditions with a N₂:CO₂ (80:20) headspace, resulting in a final pH of 7.0. All enrichments and physiological screening of the isolates was conducted in this medium with modifications to the electron donor and NO₃⁻ concentration as indicated.

Enrichment experiments were conducted with 1 mM NO₃⁻ as the electron acceptor and with either acetate (10mM), lactate (10mM), or a APB (acetate, propionate, butyrate, 10mM each) as the source of carbon and energy. Enrichments were inoculated with 10 % (w/v) sediment from each sample site and incubated in the dark at 1.5 °C. Enrichments were transferred to fresh medium every 10 days using a 10 % inoculum (v/v). After the second transfer, the concentration of NO₃⁻ was raised from 1 mM to 5 mM in order to prevent growth limitation by nitrate and cell lysis.

For isolation and purification, the MSW medium was supplemented with 10 mM HEPES (Fisher Scientific) and 1.8 % molecular grade agar (Sigma-Aldrich) as a buffering and solidifying agent, respectively. Streak plates were prepared and incubated at 1.5 °C under aerobic conditions. Morphologically distinct colonies were picked using sterile toothpicks and purified by multiple re-streakings onto fresh plates. The purity of
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each culture was reconfirmed by PCR amplification and sequencing of the small-subunit (SSU) rRNA gene. Culture stocks were preserved at -80 °C in 20 % glycerol.

Purified isolates were screened for nitrate depletion and gaseous nitrogen production under denitrifying conditions in anaerobic MSW medium amended with \(^{15}\text{N}\)-enriched \(\text{NO}_3^-\) (98 atom %; Cambridge Isotope Laboratories, Inc., Andover, MA).

Cultures and uninoculated controls were prepared in 10-ml Hungate tubes. At the initial time-point (immediately after inoculation) and after maximum cell density was achieved, growth was terminated in duplicate cultures by the addition of 1 % (wt/vol) \(\text{HgCl}_2\). Gas samples for \(\text{N}_2\text{O}\) analysis were extracted from the headspace through the rubber septa cap using a 100-µl gas-tight syringe and were immediately analyzed by gas chromatography using a Shimadzu GC-8A gas chromatograph equipped with a Porapak-Q column and an electron-capture detector. The production of \(\text{N}_2\) was determined by the accumulation of excess \(^{15}\text{N}\)-\(\text{N}_2\) using a membrane inlet mass spectrometer configured and calibrated according to An et al. (2001). Nitrate depletion was confirmed using a colorimetric method (Cataldo, 1975).

**Most probable number enumeration**

Psychrophilic denitrifying bacterial populations from Arctic fjords were enumerated by the three-tube most-probable-number (MPN) assay using 10-fold serial dilutions of fjord sediments in MSW growth medium. Tubes were incubated at ambient sediment temperature (1°C) for two months. Lactate was chosen as the electron donor for the MPN experiments, based on the vigorous growth and taxonomic coverage in initial lactate-amended enrichments. Growth of denitrifying bacteria was monitored by culture turbidity, depletion of added nitrate, and accumulation of \(\text{N}_2\text{O}\) in the vial.
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headsace as compared to nitrate-free controls. The MPN index was determined from statistical tables published by the American Public Health Association (1969). Isolates were obtained from the highest positive MPN dilutions and were subsequently identified by SSU rRNA gene sequences. However, no physiological tests were performed on these isolates.

**Total community profiling by TRFLP**

Genomic DNA from frozen sediment grabs was extracted in triplicate using a Mo-Bio Power Soil™ DNA kit (Mobio Laboratories, Carlsbad, CA, USA) according to the manufacturer’s instructions. The 16S rRNA gene was amplified by PCR using the 27F and 1492R general bacterial primers (Lane, 1991). The forward primer (27F) was fluorescently labeled with 6-carboxy fluorescine (FAM) for Terminal Restriction Fragment Length Polymorphism (TRFLP) profiling. PCR reactions were conducted using EconoTaq Plus 2X master mix (Lucigen Corporation, Middleton, WI). A final concentration of 0.33 µM and 0.25 µM was added for the forward and reverse primer, respectively. PCR yields were column purified using the UltraClean™ PCR clean-up kit (Mobio). A single enzyme digestion of PCR products was performed using the restriction enzyme Bsh (Fermentas, Glen Burnie, MD). Digestion reaction products were read by an ABI 310 genetic analyzer at the Florida State University sequencing facility (Tallahassee, USA). Processing of TRFLP profiles was performed using Gene Mapper software (Applied Biosystems, Foster City, CA). TRFLP profiles that had a total peak area of less than 1000 were not included in the analysis.

**Phylogenetic analyses**
Genomic DNA of the recovered isolates was extracted using the Mo Bio UltraClean™ Microbial DNA Isolation Kit (Mo Bio Laboratories, Carlsbad, CA, USA) according to the manufacturer’s instructions. For 16S rRNA gene amplification, the 27F and 1492R general bacterial primers (Lane, 1991) were used. PCR reactions were conducted using EconoTaq Plus 2X master mix (Lucigen Corporation, Middleton, WI). Primers were added to a final concentration of 0.5 μM, and the magnesium concentration was adjusted to 4.0 mM with the addition of magnesium chloride. The resulting PCR yield was column purified using the GenCatch (TM) PCR Cleanup Kit (Epoch Biolabs, www.epochbiolabs.com). In some cases, long rRNA gene sequences were generated using multiple sequencing reactions, and composite sequences were generated using the software package Sequencher (Gene Codes, Ann Arbor, MI). Low quality data were trimmed from the sequences prior to generating the composite sequences. Nearly full length 16S rRNA gene sequences were submitted to Genbank under the accession numbers [XXXX-YYYY].

Recovered sequences were aligned to known bacterial sequences using the “greengenes” 16S rRNA gene database and alignment tool (DeSantis et al., 2006). Aligned sequences and close relatives were imported and alignments were manually refined by visual inspection in the ARB software package (Ludwig et al., 2004). Sequences were exported from ARB using a bacterial 50 % conservation filter (excluding positions at which less than 50 % of the sequences had the same base). These filtered sequences were imported into the MEGA 4.0 software package (Tamura et al., 2007), and neighbor-joining phylogenetic trees were constructed using the maximum composite likelihood substitution model with complete deletion of gapped positions (946
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Informational positions. The robustness of inferred tree topologies was evaluated by 1,000 bootstrap re-samplings of the data. Additionally, Bayesian analyses were performed on the filtered sequence data (MrBayes ver. 3.1; Ronquist and Huelsenbeck, 2003) by running four simultaneous chains (3 heated, 1 cold) for four million generations, sampling every 1000 generations. The selected model was the general time reversible (GTR) using empirical base frequencies, and estimating the shape of the gamma distribution and proportion of invariant sites from the data. A resulting 50% majority-rule consensus tree (after discarding the burn-in of 25% of the generations) was determined to calculate the posterior probabilities for each node. The split differential between the two runs was below 0.01 after the completion of the run.

**Fatty acid methyl ester analysis**

The response of membrane-derived fatty acid composition to shifts in temperature was determined for a representative isolate of each genus under aerobic conditions at 1.5, 5 and 15 °C using the MSW medium supplemented with low levels of peptone (0.1 %), yeast extract (0.1 %) and beef extract (0.05 %) as a carbon source. Freeze-dried cells (60 to 90 mg) were extracted using a modified Bligh and Dyer procedure (methanol-chloroform-water, 10:5:4). The solid cellular residue was recovered by centrifugation and the solvent phase partitioned by addition of chloroform and water to a final ratio of 10:10:9. The lower chloroform layer containing the total lipid extract (TLE) was removed and dried under N₂. Fatty acid methyl esters (FAME) were prepared by treatment of the TLE by transesterification with freshly prepared 0.1 N methanolic NaOH for 60 min at 37 °C (White, 1979). FAME were identified by GC-MS as described by
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(Jahnke, 2004). The double-bond positions of FAME were determined by preparing

dimethyl disulfide adducts by heating at 35 °C for 35 min (Yamamoto, 1991).

**Nitrate Utilization and Optimum Growth Temperatures**

The growth rate and nitrate utilization potential were determined in batch culture

for representative isolates of each identified genus. A 5% (vol/vol) inoculum from mid-

log phase cultures was added to MSW media amended with 10 mM lactate and 5 mM

NO₃⁻ for all isolates. Triplicate cultures were incubated at 5°C in 160 mL serum bottles,

and nitrate-free controls were used to test for fermentative growth. Growth was

monitored as optical density at 600 nm using a Shimadzu UV-Vis spectrophotometer.

Nitrate + nitrite and nitrite were determined by chemiluminescence detection after

reduction with vanadium (Braman and Hendrix, 1989) or iodide (Garside, 1982).

Optimum growth temperatures were determined for representative isolates in a

temperature gradient block incubator. Isolates were grown under denitrifying conditions

in MSW with 10mM lactate and 5mM NO₃⁻ at 7-10 temperatures between 0 °C and 30

°C. Optical density at 600 nm was monitored twice daily in a Spectronic 21

spectrophotometer by placing an entire Balch tube into the instrument. Specific growth

rates (µ) were calculated as the slope of the linear portion of the plot of the natural log

(ln) of O.D. versus time.

**RESULTS**

**Characterization of in situ communities**

Cultivable denitrifying microorganisms were enumerated using an MPN serial

dilution assay at each site. MPN counts were 2.4 X 10³ cells/g sediment, 6.1 X 10⁵
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cells/g sediment, and $3.0 \times 10^6$ cells/g sediment at sites SM, KF, and YM, respectively (Table 1). Growth by denitrification in the MPN tubes was inferred from higher turbidity as compared to control tubes (lactate only), as well as depletion of nitrate and accumulation of N$_2$O. Isolation of bacteria from the highest positive dilutions, followed by 16S rRNA gene sequencing and BLAST alignment, indicated that the cultivatable denitrifier with the highest relative abundance at KF and YM was closely related to *Psychromonas* sp., while *Shewanella* sp. and *Psychomonas* sp. were highly enriched at SM.

DNA fingerprinting by TRFLP targeted to 16S rRNA gene sequences indicated that the SM and KF sediments had a highly similar community composition (Figure 1). For both sites, the most dominant peaks were seen at fragment sizes of 56, 103, 107, 210, 242, and 389 base pairs (bp). The 16S rRNA profile from the YM site showed a distinct community composition compared to the other two sites, and the most dominant peaks were at fragment sizes of 56, 109, 242, and 391 bp. An *in silico* digest of 16S rRNA gene sequences from the isolates obtained in this study (see following section) showed that peaks from all three sites at 210 and 389 bp matched the predicted fragment sizes from *Shewanella* and *Pseudomonas*. A peak at 395 bp corresponding to *Arcobacter* was observed at sites SM and YM (Figure 1).

**Isolation and phylogenetic characterization**

A systematic enrichment strategy was used to isolate denitrifying bacteria from one intertidal and two permanently cold sediments. The most rapid growth was observed in the serum vials amended with sediments from SM, followed by YM and KF. Visual observation of the plates indicated an abundance of slow growing, small colonies and
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fewer, fast growing, slightly pigmented colonies. More than 200 colonies were obtained from each enrichment on solid media plates, and based on colony morphology and growth pattern, a total of 17 colonies were selected for further screening.

Phylogenetic analysis of near full-length 16S rRNA gene sequences showed that the marine denitrifying isolates belonged to five genera within the Gamma-, Beta- and Epsilonproteobacteria (Figure 2). Isolates were classified within the genera *Arcobacter* (6 isolates), *Herminiimonas* (1 isolate), *Pseudomonas* (3 isolates), *Psychromonas* (3 isolates), and *Shewanella* (4 isolates) based on 16S rRNA gene sequence similarity (Table 2). *Arcobacter* isolates showed high sequence identity (>97% BLAST similarity) to either *Arcobacter* sp. KT0913 (Heylen, 2006) or *Arcobacter venerupis* F67-11 (Levican et al., 2012).

**Fatty acid methyl ester profiles**

Representative isolates were grown in MSW medium at 1.5°C, 5°C, and 15°C under aerobic conditions to examine the acclimation of membrane fatty acid composition to low temperature (Table 3). At all growth temperatures, the primary FAMEs detected in all isolates were C16:0, 16:1ω7c and 18:1 ω7c. These three fatty acids comprised greater than 95% of the total extracted fatty acids in strains Y2B (*Psychromonas*), SL-1 (*Pseudomonas*) and SPB (*Herminiimonas*). In addition to 16:1ω7c and 18:1 ω7c, strains YAS-1 and SAS1-1 (*Arcobacter*) also contained significant amounts of C14:0 (4 – 5%), 14:1ω7c (5 – 8%), and 16:1ω7t (5 – 10%). *Shewanella* strain YLB-1 had the most diverse fatty acid profile and was the only strain that contained branched fatty acids (20 – 28 %), as well as eicoaspenaenoic acid (20:5ω3).
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With decreasing growth temperatures, all strains except *Shewanella* YLB-1 exhibited a decrease in the relative abundance of the most abundant saturated fatty acid, C16:0. *Arcobacter* and *Herminiimonas* strains also exhibited a lower concentration of 18:1 \(\omega7c\) with lowered temperature. Concomitant with the relative decrease in saturated and long chain fatty acids at low temperature, increases in monounsaturated acids were observed that showed variation with respect to strain. *Psychromonas* Y2B and *Herminiimonas* SP-B exhibited an increase primarily in 16:1\(\omega7c\), while *Arcobacter* strains increased 14:1\(\omega7c\) and 16:1\(\omega7c\). *Pseudomonas* SL-1 increased 16:1 \(\omega7c\) and 18:1 \(\omega7c\) in response to lowered temperature. Consistent with its unique fatty acid profile, *Shewanella* YLB-1 exhibited unique shifts in fatty acids with lowered growth temperature, including increases in C16:0 and 17:1\(\omega8c\) and decreases in branched (i13:0 – i15:0) fatty acids and 14:1\(\omega7c\).

**Denitrification activity and optimal growth temperature**

Denitrification capacity was confirmed in all 16 isolates by higher biomass accumulation in nitrate-amended media as compared to nitrate-free controls, as well as near-stoichiometric conversion of nitrate to gaseous end products (\(N_2\) and \(N_2O\)). Strains were all facultative anaerobes, and produced either \(N_2O\) (*Shewanella* and *Psychromonas*) or \(N_2\) (*Arcobacter*, *Pseudomonas*, *Herminiimonas*) as the primary end-product of denitrification.

Based on the phylogenetic analysis, six strains were selected (SL-1, Y2B, YAS-1, SAS-1, YLB, SP-B), for further physiological characterization. The isolates were grown at 5 °C in MSW media with 5mM NO\(_3^-\) and 10mM lactate, and the complete depletion of nitrate concomitant with exponential growth was observed (Figure 3). Isolates from the
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the *Gammaproteobacteria* had the highest specific growth rates (Figure 3), with *Shewanella* sp. YLB-1 growing fastest ($\mu$, 0.54 d\(^{-1}\)), followed by *Pseudomonas* sp. SL-1 ($\mu$, 0.28 d\(^{-1}\)) and *Psychromonas* sp. Y2B ($\mu$, 0.23 d\(^{-1}\)). Growth rates for the *Herminiimonas* sp. SPB isolate (0.20 d\(^{-1}\)) and both *Arcobacter* isolates (0.14 – 0.17 d\(^{-1}\)) were lower than the Gammaproteobacteria isolates. Nitrate utilization, estimated by linear regression of nitrate depletion during exponential growth phase, was highest in *Arcobacter* sp. SAS-1, *Shewanella* sp. YLB, and *Arcobacter* sp. YAS-1. There was no strong correlation between growth rate and nitrate utilization rate. All strains had optimal growth temperatures of 15 °C or less, except *Shewanella* YLB-1, which had an optimal growth temperature of 18 °C (Figure 4). All strains maintained substantial growth rates near 0 °C that were between 25 – 50% of the optimal growth rate. Growth was not observed in any of the strains above 30 °C, and two strains, *Psychromonas* Y2B and *Herminiimonas* SP-B, did not grow above 25 °C.

**DISCUSSION**

Denitrification is well recognized as a dominant pathway for the removal of reactive nitrogen in marine sediments, including polar sediments. However, no prior cultivation based studies have targeted denitrifying bacteria in permanently cold marine sediments. Previous enrichment studies from Arctic sediments have often been conducted under aerobic conditions, using complex cultivation media, short incubation times, and incubation temperatures above *in situ* values (Srinivas et al., 2009; Kim et al., 2010a; Yu et al., 2010). In this study, denitrifying bacteria were anaerobically enriched in a minimal medium with defined electron donors. Enrichments were carefully
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maintained at in situ temperatures and incubation times were lengthened (> 30 days) to mimic in situ conditions. This approach allowed for the isolation of taxa whose role in denitrification may have previously been overlooked.

Characterization of in situ denitrifying communities

Most probable number (MPN) enumeration indicated the presence of $2 \times 10^3 - 3 \times 10^6$ cells of denitrifying bacteria $g^{-1}$ of sediment. Quantification of total bacterial abundance by direct counts in Svalbard surface sediments has shown the presence of $2 \times 10^8 - 3 \times 10^9$ cells $cm^{-3}$ of sediment, and site SM has been determined to have $2.1 - 4.7 \times 10^9$ cells $cm^{-3}$ (Sahm and Berninger, 1998; Ravenschlag, 2001). From these results, the relative abundance of denitrifying bacteria can be estimated to contribute between less than 0.01% to 1.5% of the total community. The relative abundance of denitrifying bacteria was similar (0.17%) for temperate estuarine sediments using a MPN-based approach, but the same study found up to two orders of magnitude more denitrifying bacteria using qPCR-based functional gene analysis (Michotey et al., 2000).

It is unclear why differences in denitrifying MPN cell numbers between sites did not correspond with reported denitrification rates. While site SM exhibited high rates of denitrification, it also had a lower number of denitrifying bacteria than site KF. The choice of lactate as an electron donor for the MPN experiment may have biased the growth in SM sediments, and also, the use of only an organic electron donor may have limited the growth of autotrophic denitrifying bacteria. Site YM had the highest number of denitrifying cells ($3.0 \times 10^6$), which may have been influenced by the input of
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macroalgal detritus in the intertidal zone. The C:N ratio of 19.9 ratio at site YM falls near the median value reported for macroalgae (Atkinson and Smith, 1983)

Based on an in silico digest of 16S rRNA gene sequences from our isolates, three isolates (Shewanella, Pseudomonas, Arcobacter) were putatively detected in the TRFLP profiles from the fjord sediments. All of the genera isolated in this study except Herminiimonas have been previously detected in polar marine sediments in 16S rRNA gene clone libraries. Bowman et al. (2003) found 5 -10 % Shewanella and 2 -5 % Psychromonas in clone libraries from the top 1cm of Antarctic coastal sediments. Members of Shewanella and Pseudomonas have also been detected in clone libraries from surficial sediments (0-5 cm) in the Beaufort Sea (Li, 2009). In Svalbard sediments, Pseudomonas has been detected at Hornsund (Ravenschlag et al., 1999) and Shewanella, Psychromonas, and Arcobacter have been detected near site KF in Kongsfjorden (Tian et al., 2009). These studies provide further evidence for the widespread presence of the genera isolated in this study in the surficial layers of permanently cold sediments.

However, further cultivation-independent studies are needed to confirm that the isolates from this study are the primary taxa that perform denitrification in situ.

Distribution of psychrophily and denitrification within the genera isolated

Members of the genus Shewanella have been isolated and described from a wide range of oceanic regions, including psychrophilic strains from deep-sea and polar sediments (Kato and Nogi, 2001). Shewanella species are capable of respiring a diverse set of electron acceptors, including metals (Fe, Mn), sulfur compounds, and nitrate (Hau and Gralnick, 2007). Complete denitrification has been confirmed for a few Shewanella isolates from the marine environment (Brettar et al., 2002; Zhao et al., 2006), but the
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presence of the marker gene for dissimilatory reduction of nitrate to ammonium (nrfA) in
the genomes of *Shewanella* species indicates that this nitrate respiration pathway may be
more common within the genus than denitrification (Simpson et al., 2010). For example,
the described organism that shows the highest SSU rRNA gene sequence similarity to
*Shewanella* YLB-1, *Shewanella frigidimarina*, is unable to reduce nitrite, lacks the key
denitrification genes (nirS/K and nosZ), and possesses the nrfA gene (Kato and Nogi,
2001; Markowitz et al., 2012). The isolation of N$_2$ gas producing *Shewanella* strains in
this study further strengthens the evidence for the contribution of *Shewanella* to
sedimentary denitrification in permanently cold sediments.

Nearly all described species of the genus *Psychromonas* are psychrophilic, as the
name implies. This genus (along with *Shewanella*) is found in the order
*Alteromonadales*, and is readily isolated under aerobic conditions from sea-ice, marine
water columns, and sediments (Groudieva et al., 2003; Auman et al., 2006; Nogi, 2007).
While nitrate reduction to nitrite is common within the genus, the only evidence for
complete denitrification is nitrite reduction by *Psychromonas hadalis* (Nogi, 2007) and
the presence of nitrous oxide reductase genes in *Psychromonas ingrahamii* (Markowitz et
al., 2012). The confirmation of gaseous nitrogen production in the isolate *Psychromonas*
Y2B from this study provides more conclusive evidence for denitrification within the
genus *Psychromonas*.

*Pseudomonas* is readily isolated from marine sediments, and the genus contains
many denitrifying representatives (Zumft, 1997). One marine strain, *Pseudomonas*
*stutzeri* ZoBell, has been used as a model organism for the study of denitrification
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(Lalucat et al., 2006). *Pseudomonas* is a ubiquitous denitrifying genus, and its occurrence in permanently cold marine sediments is not unexpected.

Isolates from the genus *Arcobacter* have been obtained from a variety of marine environments, including hydrothermal vents, tissue from mussels, and the water column off the coast of Europe and Africa (Eilers et al., 2000; Huber et al., 2003; Levican et al., 2012). Some strains of *Arcobacter* are able to oxidize sulfide to produce filamentous sulfur (Wirsen, 2002), which may be coupled to denitrification under anaerobic conditions (Lavik et al., 2009). Substantial rates of sulfate reduction have been measured in surface sediments at sites SM and YM (Arnosti and Jorgensen, 2006; Sawicka et al., 2010), which may supply sulfide for autotrophic denitrification by *Arcobacter* species.

Reduction of nitrate to nitrate is ubiquitous within the genus *Arcobacter*, and complete denitrification has been confirmed for *Arcobacter* isolates from activated sewage sludge (Heylen, 2006). However, no denitrifying strains from the marine environment have been described. The *Arcobacter* isolates from this study are the first confirmed denitrifying isolates from the marine environment, as well as the first reported psychrophilic strains.

The psychrophilic nature of *Herminiimonas* is not surprising given that isolates have been obtained from an Antarctic glacier (Garcia-Echauri et al., 2011), a deep (3042m) Greenland glacial ice core (Loveland-Curtze et al., 2009), and Greenland sea ice brine (Møller et al., 2011). At least two other isolates of *Herminiimonas* have been shown to reduce nitrate, and the *Herminiimonas arsenicoxydans* genome contains the nirK gene (Lang et al., 2007; Muller, 2006). The isolate *Herminiimonas* SP-B from this study is the first confirmed denitrifying *Herminiimonas* isolate from the marine
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sediments, which broadens the potential functional role of *Herminiimonas* in marine sediments.

**Adaptation of denitrifying bacteria to low temperatures**

The optimal growth temperature, Topt, has been established as the primary parameter to distinguish psychrophilic from psychrotolerant and mesophilic bacteria (Morita, 1975). The relatively high growth rates of psychrophilic bacteria at low temperature reflect the adaptations necessary to maintain cellular metabolism at low temperatures. These adaptations include expression of enzymes that are efficient at low temperatures, production of cryoprotectant molecules, and the ability to maintain membrane fluidity by altering lipid composition (D'Amico et al., 2006). In the present study, low temperature adaption was confirmed in psychrophilic denitrifying bacteria by growth, nitrate depletion, and by a comparison of membrane lipid composition at low temperature. The optimal growth temperatures and high rates of growth at 0°C (25-50% of Topt) of the current isolates reflect the highly psychrophilic nature of our isolates. For all isolates except *Shewanella* YLB-1, we observed optimum temperatures for growth (Topt) that were amongst the lowest reported for the genera (Table 4). Furthermore, we isolated the first confirmed psychrophilic *Arcobacter* strains, and we present the lowest Topt for *Herminiimonas*, a taxon that is often isolated from permanently cold habitats.

A comparison of the three most abundant fatty acids (C16:0, C16:1, C18:1) from our isolates to literature values show the highest values of C16:1 unsaturated fatty acids in our isolates grown at 5 °C (Table 4). Very few psychrophilic isolates have been grown at 5 °C or less for FAME analysis, which precludes a fair comparison between our strains and previously isolated psychrophiles. However, a decrease in C16:0 and an increase in
16:1\(\omega 7c\) with decreasing growth temperature was the main adaptation consistent amongst all isolates except *Shewanella* YLB-1 (Table 4). These results are consistent with previous research that demonstrated the importance of monounsaturated fatty acids for low temperature growth of *Photobacterium profundum* (Allen et al., 1999). The genus *Shewanella*, in contrast, uses a strategy that involves regulating branched fatty acids and eicosapentaenoic acid in addition to monounsaturated fatty acids (Wang et al., 2009).

**Conclusion**

A total of 17 strains of psychrophilic denitrifying bacteria were isolated from Arctic fjord sediments with varying depth and organic carbon content. This study reports the first systematic enrichment of psychrophilic bacteria under denitrifying conditions in permanently cold marine sediments. The taxa isolated in this study are routinely detected by cultivation-independent techniques in surficial sediments, but only *Pseudomonas* species have been previously recognized in marine sediments for their ability to denitrify. The genera *Arcobacter* and *Herminiimonas* have not been previously isolated from permanently cold marine sediments, and there are no reports of psychrophilic marine *Arcobacter* strains. Growth experiments revealed optimal temperatures for growth of the current isolates that were amongst the lowest reported for all genera, with the exception of *Shewanella*. Concordantly, monounsaturated fatty acids, necessary for low temperature growth, were higher than previously reported concentrations. These results confirm the strongly psychrophilic nature of the present isolates and corroborate the hypothesis that denitrification activity in permanently cold sediments is maintained at relatively high levels due to the activity of psychrophilic bacteria.
REFERENCES


Bozal, N., Montes, M.J., Miñana-Galbis, D., Manresa, A., and Mercadé, E. (2009) Shewanella vesiculosa sp. nov., a psychrotolerant bacterium isolated from an Antarctic...
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psychrophile isolated from pond sediment of the McMurdo Ice Shelf, Antarctica. *Archives of Microbiology* 169: 231-238.


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Denitrifying Bacteria in Arctic Sediments


Denitrifying Bacteria in Arctic Sediments

Table 1. Sample site descriptions and most probable number (MPN) estimates of denitrifying bacteria

<table>
<thead>
<tr>
<th>Sample Site (Abbr.)</th>
<th>Sample Site (Abbr.)</th>
<th>Latitude</th>
<th>Longitude</th>
<th>Depth</th>
<th>Sediment temperature</th>
<th>Sediment C:N</th>
<th>Denitrification Rate (µmol N m⁻² d⁻¹)</th>
<th>MPN (cells g⁻¹)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Kongsfjorden (KF)</td>
<td>78°59.43' N</td>
<td>12°17.87' E</td>
<td>51m</td>
<td>1.3°C</td>
<td>11*</td>
<td>N.D.</td>
<td>34 (± 12)*</td>
<td>6.1 X 10⁵</td>
</tr>
<tr>
<td>Smeerenbergfjorden (SM)</td>
<td>79°42.01' N</td>
<td>11°05.20' E</td>
<td>211m</td>
<td>1.6°C</td>
<td>7.2*</td>
<td>289 (± 5)*</td>
<td>2.4 X 10⁵</td>
<td></td>
</tr>
<tr>
<td>Ymerbukta (YM)</td>
<td>78°16.84' N</td>
<td>14°02.97' E</td>
<td>intertidal</td>
<td>6.5°C</td>
<td>19.9</td>
<td>N.D.</td>
<td>3.0 X 10⁶</td>
<td></td>
</tr>
</tbody>
</table>

*data from Gihring et al. (2010). Denitrification rates were measured by Isotope Pairing Technique (Nielsen, 1992). N.D., not determined.
### Table 2. Phenotypic and genotypic characterization of denitrifying isolates. (N.D., not determined; APB, combination of acetate, propionate and butyrate)

<table>
<thead>
<tr>
<th>Genus (phylum) and isolate</th>
<th>Sample site</th>
<th>Electron donor</th>
<th>Primary denitrification endproduct</th>
<th>Closest Isolate by BLAST (Accession Number)</th>
<th>BLAST % Similarity</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Herminiimonas</strong> (Betaproteobacteria)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>SP-B</td>
<td>SM</td>
<td>APB</td>
<td>N₂</td>
<td><em>Herminiimonas fonticola</em> CCQ (EU636040)</td>
<td>98%</td>
</tr>
<tr>
<td><strong>Arcobacter</strong> (Epsilonproteobacteria)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>KLS-1</td>
<td>KF</td>
<td>Lactate</td>
<td>N₂</td>
<td><em>Arcobacter</em> sp. KT0913 (AF235110)</td>
<td>97%</td>
</tr>
<tr>
<td>SAS-1</td>
<td>SM</td>
<td>Acetate</td>
<td>N₂</td>
<td><em>Arcobacter</em> sp. KT0913 (AF235110)</td>
<td>98%</td>
</tr>
<tr>
<td>SL-3</td>
<td>SM</td>
<td>Lactate</td>
<td>N₂</td>
<td><em>Arcobacter</em> sp. KT0913 (AF235110)</td>
<td>97%</td>
</tr>
<tr>
<td>Y2S</td>
<td>YM</td>
<td>APB</td>
<td>N₂</td>
<td><em>Arcobacter</em> venerupis F67-11 (HE565359)</td>
<td>97%</td>
</tr>
<tr>
<td>YAPB-1</td>
<td>YM</td>
<td>APB</td>
<td>N₂</td>
<td><em>Arcobacter</em> venerupis F67-11 (HE565359)</td>
<td>97%</td>
</tr>
<tr>
<td>YAS-1</td>
<td>YM</td>
<td>Acetate</td>
<td>N₂</td>
<td><em>Arcobacter</em> venerupis F67-11 (HE565359)</td>
<td>97%</td>
</tr>
<tr>
<td><strong>Pseudomonas</strong> (Gammaproteobacteria)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>SL-1</td>
<td>SM</td>
<td>Lactate</td>
<td>N₂</td>
<td><em>Pseudomonas frederikshagensis</em> (HQ242750)</td>
<td>97%</td>
</tr>
<tr>
<td>SLB-2</td>
<td>SM</td>
<td>Lactate</td>
<td>N₂</td>
<td><em>Pseudomonas</em> sp. ice-oil-499 (DQ521397)</td>
<td>99%</td>
</tr>
<tr>
<td>UL-1</td>
<td>SM</td>
<td>Lactate</td>
<td>N₂</td>
<td><em>Pseudomonas brenneri</em> (FM877472)</td>
<td>99%</td>
</tr>
<tr>
<td><strong>Psychromonas</strong> (Gammaproteobacteria)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>SL-2</td>
<td>SM</td>
<td>Lactate</td>
<td>N₂O</td>
<td><em>Psychromonas ingrahamii</em> (CP000510)</td>
<td>97%</td>
</tr>
<tr>
<td>YAB-1</td>
<td>YM</td>
<td>Acetate</td>
<td>N.D.</td>
<td><em>Psychromonas</em> sp. IC004 (U85849)</td>
<td>98%</td>
</tr>
<tr>
<td>Y2B</td>
<td>YM</td>
<td>APB</td>
<td>N₂O</td>
<td><em>Psychromonas</em> sp. IC004 (U85849)</td>
<td>96%</td>
</tr>
<tr>
<td><strong>Shewanella</strong> (Gammaproteobacteria)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>KLB-1</td>
<td>KF</td>
<td>Lactate</td>
<td>NA</td>
<td><em>Shewanella vesiculosa</em> (NR_042710)</td>
<td>99%</td>
</tr>
</tbody>
</table>
## Denitrifying Bacteria in Arctic Sediments

<table>
<thead>
<tr>
<th>Sample</th>
<th>Medium</th>
<th>Substrate</th>
<th>Product</th>
<th>Strain Name</th>
<th>Sequence</th>
</tr>
</thead>
<tbody>
<tr>
<td>SLB-1</td>
<td>SM</td>
<td>Lactate</td>
<td>N₂O</td>
<td><em>Shewanella</em> sp. gap-d-13</td>
<td>(DQ530458) 97%</td>
</tr>
<tr>
<td>YLB-1</td>
<td>YM</td>
<td>Lactate</td>
<td>N₂O</td>
<td><em>Shewanella</em> frigidimarina</td>
<td>(AJ300833) 99%</td>
</tr>
<tr>
<td>UA-1</td>
<td>SM</td>
<td>Acetate</td>
<td>N.D.</td>
<td>NA</td>
<td>NA</td>
</tr>
</tbody>
</table>
### Table 3. Temperature wise distributions (% distribution) of fatty acids in psychrophilic denitrifying bacteria isolated from Svalbard. Fatty acids that contributed less than 1% in all samples are not shown.

<table>
<thead>
<tr>
<th>Isolate</th>
<th>YLB-1</th>
<th>Y2B</th>
<th>YAS-1</th>
<th>SAS-1</th>
<th>SL-1</th>
<th>SP-B</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.5° C</td>
<td>5° C</td>
<td>15° C</td>
<td>1.5° C</td>
<td>5° C</td>
<td>15° C</td>
<td>1.5° C</td>
</tr>
<tr>
<td><strong>Saturated</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>C12:0</td>
<td>0.4</td>
<td>0.5</td>
<td>1.5</td>
<td>0.1</td>
<td>0.1</td>
<td>0.4</td>
</tr>
<tr>
<td>C14:0</td>
<td>3.5</td>
<td>2.7</td>
<td>3.7</td>
<td>4.6</td>
<td>5.3</td>
<td>4.4</td>
</tr>
<tr>
<td>C15:0</td>
<td>5.9</td>
<td>4.6</td>
<td>4.6</td>
<td>0.1</td>
<td>0.3</td>
<td>0.1</td>
</tr>
<tr>
<td>C16:0</td>
<td>15.7</td>
<td>12.5</td>
<td>12.6</td>
<td>15.4</td>
<td>17.7</td>
<td>23</td>
</tr>
<tr>
<td>C17:0</td>
<td>2.4</td>
<td>1.8</td>
<td>1.1</td>
<td>0.1</td>
<td>0.1</td>
<td>0.1</td>
</tr>
<tr>
<td><strong>Branched</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>i13:0</td>
<td>5.9</td>
<td>6.1</td>
<td>9.9</td>
<td>0.2</td>
<td>0.1</td>
<td>0.1</td>
</tr>
<tr>
<td>i14:0</td>
<td>0.7</td>
<td>3.3</td>
<td>3.6</td>
<td>0.1</td>
<td>0.1</td>
<td>0.1</td>
</tr>
<tr>
<td>i15:0</td>
<td>9.2</td>
<td>12.7</td>
<td>12.5</td>
<td>0.1</td>
<td>0.1</td>
<td>0.1</td>
</tr>
<tr>
<td><strong>Unsaturated</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>14:1ω7c</td>
<td>0.8</td>
<td>1.9</td>
<td>4.9</td>
<td>0.2</td>
<td>0.1</td>
<td>0.1</td>
</tr>
<tr>
<td>15:1ω8c</td>
<td>1.6</td>
<td>1.1</td>
<td>1.3</td>
<td>0.1</td>
<td>0.1</td>
<td>0.1</td>
</tr>
<tr>
<td>16:1ω9c</td>
<td>1.5</td>
<td>1.1</td>
<td>1</td>
<td>0.1</td>
<td>0.1</td>
<td>0.1</td>
</tr>
<tr>
<td>16:1ω7c</td>
<td>25.8</td>
<td>27.6</td>
<td>25.6</td>
<td>67.2</td>
<td>62.7</td>
<td>58.9</td>
</tr>
<tr>
<td>16:1ω7t</td>
<td>0.4</td>
<td>7.3</td>
<td>4.5</td>
<td>9.6</td>
<td>8.1</td>
<td>nd</td>
</tr>
<tr>
<td>16:1ω5c</td>
<td>0.2</td>
<td>2.5</td>
<td>2.1</td>
<td>1.7</td>
<td>2.5</td>
<td>2.1</td>
</tr>
<tr>
<td>17:1ω8c</td>
<td>9.9</td>
<td>8.1</td>
<td>5.6</td>
<td>0.1</td>
<td>0.2</td>
<td>0.1</td>
</tr>
<tr>
<td>18:1ω7c</td>
<td>4.4</td>
<td>5.3</td>
<td>4.4</td>
<td>15.8</td>
<td>16.6</td>
<td>15.9</td>
</tr>
<tr>
<td>b19:1ω6c</td>
<td>0.4</td>
<td>0.4</td>
<td>0.4</td>
<td>0.1</td>
<td>0.2</td>
<td>0.1</td>
</tr>
<tr>
<td>20:5</td>
<td>1.7</td>
<td>2</td>
<td>1.5</td>
<td>0.1</td>
<td>0.2</td>
<td>0.1</td>
</tr>
<tr>
<td><strong>∑X:1</strong></td>
<td>49.7</td>
<td>51.5</td>
<td>47.1</td>
<td>83.5</td>
<td>80.3</td>
<td>75.7</td>
</tr>
</tbody>
</table>
Table 4. Comparison of representative isolates from the present study to described isolates. (Topt, optimal growth temperature; FAME Temp, growth temperature for fatty acid analysis). [Note: References in table will be given a number abbreviation]

<table>
<thead>
<tr>
<th>Species/ Strain</th>
<th>Study</th>
<th>Environment</th>
<th>Topt</th>
<th>NO3 reduction</th>
<th>FAME Temp</th>
<th>C16:0</th>
<th>C16:1</th>
<th>C18:1</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Psychromonas profunda</em></td>
<td>Xu</td>
<td>Deep Atlantic Sediments (2770m)</td>
<td>4</td>
<td>+</td>
<td>4</td>
<td>31</td>
<td>44</td>
<td>NR</td>
</tr>
<tr>
<td><em>Psychromonas Y2B</em></td>
<td>This study</td>
<td>Ymerbukta</td>
<td>5</td>
<td>+</td>
<td>5</td>
<td>18</td>
<td>63</td>
<td>17</td>
</tr>
<tr>
<td><em>Psychromonas ingrahamii</em>*</td>
<td>Auman</td>
<td>Arctic Sea Ice (Point Barrow)</td>
<td>5</td>
<td>+</td>
<td>4</td>
<td>19</td>
<td>67</td>
<td>4</td>
</tr>
<tr>
<td><em>Psychromonas hadalis</em></td>
<td>Nogi</td>
<td>Japan Trench (7542m) sediments</td>
<td>6</td>
<td>+</td>
<td>NA</td>
<td>31</td>
<td>37</td>
<td>NR</td>
</tr>
<tr>
<td><em>Psychromonas kaikoae</em></td>
<td>Nogi</td>
<td>Japan Trench (7434m) sediments</td>
<td>10</td>
<td>+</td>
<td>10</td>
<td>15</td>
<td>52</td>
<td>2</td>
</tr>
<tr>
<td><em>Psychromonas boydii</em></td>
<td>Auman</td>
<td>Arctic Sea Ice (Point Barrow)</td>
<td>0-10</td>
<td>+</td>
<td>4</td>
<td>26</td>
<td>45</td>
<td>4</td>
</tr>
<tr>
<td><em>Psychromonas antarcticus</em></td>
<td>Mountfort</td>
<td>McMurdo Ice Shelf pond sediments</td>
<td>12</td>
<td>-</td>
<td>12</td>
<td>24</td>
<td>58</td>
<td>3</td>
</tr>
<tr>
<td><em>Psychromonas marina</em></td>
<td>Kawasaki</td>
<td>Okhotsk Sea WC</td>
<td>20</td>
<td>-</td>
<td>4</td>
<td>17</td>
<td>35</td>
<td>3</td>
</tr>
<tr>
<td><em>Psychromonas arctica</em></td>
<td>Mordieva</td>
<td>Svalbard Water Column</td>
<td>20</td>
<td>-</td>
<td>4</td>
<td>7-16</td>
<td>50</td>
<td>7-16</td>
</tr>
<tr>
<td><em>Psychromonas macrocephali</em></td>
<td>Miyazaki</td>
<td>Marine Sediments adajacent to whale carcass</td>
<td>20</td>
<td>+</td>
<td>20</td>
<td>27</td>
<td>51</td>
<td>4</td>
</tr>
<tr>
<td><em>Psychromonas ossibalaenae</em></td>
<td>Miyazaki</td>
<td>Marine Sediments adajacent to whale carcass</td>
<td>20</td>
<td>+</td>
<td>20</td>
<td>25</td>
<td>56</td>
<td>1</td>
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<tr>
<td><em>Psychromonas japonica</em></td>
<td>Miyazaki</td>
<td>Marine Sediments adajacent to whale carcass</td>
<td>21</td>
<td>+</td>
<td>21</td>
<td>22</td>
<td>53</td>
<td>3</td>
</tr>
<tr>
<td><em>Psychromonas agarivorans</em></td>
<td>Hosoya</td>
<td>Marine Sediments (Japan)</td>
<td>20-25</td>
<td>-</td>
<td>20</td>
<td>38</td>
<td>35</td>
<td>3</td>
</tr>
<tr>
<td><em>Psychromonas aquimarina</em></td>
<td>Miyazaki</td>
<td>Marine Sediments adajacent to whale carcass</td>
<td>20-25</td>
<td>+</td>
<td>20</td>
<td>29</td>
<td>49</td>
<td>2</td>
</tr>
<tr>
<td><em>Shewanella halifakensis</em></td>
<td>Zhao</td>
<td>Emerald Basin (215m) sediments</td>
<td>10</td>
<td>+</td>
<td>10</td>
<td>20</td>
<td>19</td>
<td>7</td>
</tr>
<tr>
<td><em>Shewanella sediminis</em></td>
<td>Zhao</td>
<td>Emerald Basin (215m) sediments</td>
<td>10</td>
<td>+</td>
<td>10</td>
<td>17</td>
<td>33</td>
<td>7</td>
</tr>
<tr>
<td><em>Shewanella psychrophila</em></td>
<td>Xiao</td>
<td>West Pacific (1914m) sediment</td>
<td>10-15</td>
<td>+</td>
<td>10</td>
<td>13</td>
<td>38</td>
<td>9</td>
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<tr>
<td><em>Shewanella gelidimarina</em></td>
<td>Bowman</td>
<td>Antarctic Sea Ice</td>
<td>16</td>
<td>+</td>
<td>10</td>
<td>6-11</td>
<td>27-37</td>
<td>1-8</td>
</tr>
<tr>
<td><em>Shewanella YLB</em></td>
<td>This study</td>
<td>Ymerbukta</td>
<td>18</td>
<td>+</td>
<td>5</td>
<td>13</td>
<td>28</td>
<td>7</td>
</tr>
<tr>
<td><em>Shewanella vesiculosa</em></td>
<td>Bozal</td>
<td>Shetland Island Antarctic marine sediments</td>
<td>15-20</td>
<td>+</td>
<td>20</td>
<td>10</td>
<td>25</td>
<td>0</td>
</tr>
<tr>
<td><em>Shewanella arctica</em></td>
<td>Kim</td>
<td>Tempelfjorden Svalbard sediment</td>
<td>20</td>
<td>+</td>
<td>20</td>
<td>17</td>
<td>NR</td>
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<tr>
<td><em>Shewanella frigidimarin</em></td>
<td>Bowman</td>
<td>Antarctic Sea Ice</td>
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<td>+</td>
<td>10</td>
<td>5-17</td>
<td>38-55</td>
<td>3-7</td>
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<td><em>Shewanella denitrificans</em></td>
<td>Brettar</td>
<td>Gotland Deep oxic-anoxic WC</td>
<td>20-25</td>
<td>+</td>
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<td>+</td>
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<td><em>Arcobacter YAS-1</em></td>
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<td>Ymerbukta</td>
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<td>Smeerenbergefjorden</td>
<td>10</td>
<td>+</td>
<td>5</td>
<td>11</td>
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### Denitrifying Bacteria in Arctic Sediments

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<tr>
<th>Genus/Moniker</th>
<th>Author</th>
<th>Habitat/Location</th>
<th>Min-Max</th>
<th>+</th>
<th>20</th>
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<tbody>
<tr>
<td>Arcobacter halophilus</td>
<td>Donachie</td>
<td>Hypersaline Hawaiian lagoon water</td>
<td>18-22</td>
<td>+</td>
<td>20</td>
<td>26</td>
<td>26</td>
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<td>Arcobacter venerupis</td>
<td>Levican</td>
<td>Mussels - Ebro delta Spain</td>
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<td>+</td>
<td>NR</td>
<td>NR</td>
<td>NR</td>
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<td>Arcobacter marinus</td>
<td>Kim</td>
<td>Seaweeds, starfish, and water - East Sea</td>
<td>30-37</td>
<td>+</td>
<td>37</td>
<td>26</td>
<td>28</td>
<td>24</td>
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<tr>
<td>Arcobacter nitrofigilis</td>
<td>McClung</td>
<td>Spartina Marsh sediments - Sapelo Island, GA</td>
<td>18-35‡</td>
<td>+</td>
<td>37</td>
<td>32</td>
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<td>+</td>
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<td><strong>Herminiimonas SPB</strong>*</td>
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<td>Fernandes</td>
<td>Spring water - Portugal</td>
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<td>-</td>
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<td>Greenland Ice Core</td>
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<td>Arsenic contaminated sludge</td>
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<td>12</td>
<td>48</td>
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<td>Weon</td>
<td>Beach Sand, Korea</td>
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<td>Bile</td>
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<td>Pseudomonas stutzeri*</td>
<td>Rossello-Mora</td>
<td>multiple strains (need access to paper)</td>
<td>+</td>
<td></td>
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</table>

* Complete Denitrification confirmed by gas production
** Complete Denitrification inferred by genome analysis
‡ No Topt reported, growth range given instead

References: (McClung et al., 1983; Rosselló-Mora et al., 1994; Mountfort et al., 1998; Brettar et al., 2001; Yumoto et al., 2001; Brettar et al., 2002; Kawasaki et al., 2002; Nogi et al., 2002; Groudieva et al., 2003; Xu et al., 2003; Reddy et al., 2004; Donachie et al., 2005; Fernandes et al., 2005; Zhao et al., 2005; Auman et al., 2006; Köpf et al., 2006; Weon et al., 2006; Zhao et al., 2006; Lang et al., 2007; Nogi, 2007; Xiao et al., 2007; Miyazaki et al., 2008; Romanenko et al., 2008; Bozal et al., 2009; Hosoya et al., 2009; Loveland-Curtze et al., 2009; Auman et al., 2010; Kim et al., 2010b; Kim et al., 2012; Levican et al., 2012; Bowman, 1997; Heylen, 2006; Muller, 2006)
Figure Legends

Figure 1. TRFLP profiles of the 16S rRNA gene from surficial (0-5 cm) sediments. The Shannon Index (H) is given for each site. Peaks that were tentatively matched to isolated strains included *Shewanella* sp. (210), *Pseudomonas sp.* (389), and *Arcobacter* sp. (395).

Figure 2. 16S rRNA gene sequence based phylogenetic tree of isolated psychrophillic denitrifiers from fjord of Svalbard showing relatedness of isolated strains with previously characterized clones and isolated representative of database. Tree was generated by neighbor-joining method and tested with bootstrap (1000). Nodes supported by bootstrap values greater than 70% are indicated by numeric values. The scale bar represents 0.02 substitutions per nucleotide position. [add brackets for phyla and remove extra isolates not in the paper, also reqsequencing will be done for UL-1, SL-2, SLB-1, Y2B before new tree is made]

Figure 3. Growth and nitrate utilization of the selected psychrophilic denitrifiers at 5°C under denitrifying conditions (10mM lactate, 5mM NO$_3^-$). The average specific growth rate (µ) and nitrate utilization rate (mM L$^{-1}$ d$^{-1}$) are given to the right of the figure.

Figure 4. Temperature response of growth under denitrifying conditions (10 mM lactate, 5 mM NO$_3^-$). Error bars represent the standard deviation of triplicate measurements.
Figure 1.
Figure 3.

- *Shewanella* sp. YLB
  - $\mu = 0.54$ d$^{-1}$; $NO_3 = 1.1$ mM d$^{-1}$
- *Pseudomonas* sp. SL-1
  - $\mu = 0.28$ d$^{-1}$; $NO_3 = 0.6$ mM d$^{-1}$
- *Psychromonas* sp. Y2B
  - $\mu = 0.23$ d$^{-1}$; $NO_3 = 0.4$ mM d$^{-1}$
- *Herminiimonas* sp. SPB
  - $\mu = 0.20$ d$^{-1}$; $NO_3 = 0.7$ mM d$^{-1}$
- *Arcobacter* sp. SAS-1
  - $\mu = 0.17$ d$^{-1}$; $NO_3 = 0.9$ mM d$^{-1}$
- *Arcobacter* sp. YAS-1
  - $\mu = 0.14$ d$^{-1}$; $NO_3 = 1.3$ mM d$^{-1}$
Figure 4.