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ORIGINAL ARTICLE

# Biogeography of two cold-adapted genera: *Psychrobacter* and *Exiguobacterium*

Debora F Rodrigues[1], Ederson da C Jesus[1,2], Hector L Ayala-del-Río[3], Vivian H Pellizari[4], David Gilichinsky[5], Lycely Sepulveda-Torres[6] and James M Tiedje[1]

[1]*Center for Microbial Ecology, Michigan State University, East Lansing, MI, USA;* [2]*Department of Soil and Sciences, Federal University of Lavras, Minas Gerais, Brazil;* [3]*Department of Microbiology, University of Puerto Rico, Humacao, Puerto Rico;* [4]*Department of Microbiology, University of Sao Paulo, Sao Paulo, Brazil;* [5]*Institute for Physicochemical and Biological Problems in Soil Science, Russian Academy of Sciences, Moscow Region, Russia and* [6]*Department of Civil and Environmental Engineering, Stanford University, Stanford, CA, USA*

**The genera *Exiguobacterium* and *Psychrobacter* have been frequently detected in and isolated from polar permafrost and ice. These two genera have members that can grow at temperatures as low as −5 and −10 °C, respectively. We used quantitative PCR (Q-PCR) to quantify members of these genera in 54 soil or sediment samples from polar, temperate and tropical environments to determine to what extent they are selected by cold environments. These results were further analyzed by multiple linear regression to identify the most relevant environmental factors corresponding to their distribution. *Exiguobacterium* was detected in all three climatic zones at similar densities, but was patchier in the temperate and tropical samples. *Psychrobacter* was present in almost all polar samples, was at highest densities in Antarctica sediment samples, but was in very low densities and infrequently detected in temperate and tropical soils. Clone libraries, specific for the 16S rRNA gene for each genus, were constructed from a sample from each climatic region. The clone libraries were analyzed for α and β diversities, as well as for variation in population structure by using analysis of molecular variance. Results confirm that both genera were found in all three climatic zones; however, *Psychrobacter* populations seemed to be much more diverse than *Exiguobacterium* in all three climatic zones. Furthermore, *Psychrobacter* populations from Antarctica are different from those in Michigan and Puerto Rico, which are similar to each other.**

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## Introduction

Ubiquitous distribution of extremophiles has been suggested to be implausible because of barriers to their surviving dispersal (Whitaker *et al.*, 2003; Martiny *et al.*, 2006). Most studies focusing on bacterial biogeography from extreme environments such as hot springs or geothermal springs have shown endemism in these environments (Papke *et al.*, 2003; Whitaker *et al.*, 2003). A few studies with sea ice bacteria indicate that members of some genera occur at both poles; however, cosmopolitan species of cold-adapted genera have not yet been described (Staley and Gosink, 1999).

Among the cold habitats on our planet, microorganisms inhabiting permafrost are strong candidates for biogeography studies because this habitat underlies 20–25% of the terrestrial surface, the mean annual temperature is between −10 and −12 °C in the Arctic and between −18 and −27 °C in the Antarctic, and the environment has been constant and isolated for periods up to 3 million years (Vishnivetskaya and Kathariou, 2005).

Earlier studies with Siberian permafrost soils yielded isolates of *Exiguobacterium* and *Psychrobacter* from geological strata frozen for 20 thousand to 3 million years (Vishnivetskaya *et al.*, 2000; Bakermans *et al.*, 2006; Rodrigues *et al.*, 2006), and demonstrated ubiquity of the *Exiguobacterium* genus in this habitat (Rodrigues and Tiedje, 2007). In addition to Siberian permafrost, species of *Exiguobacterium* and *Psychrobacter* have also been

Correspondence: DF Rodrigues, Department of Microbiology and Molecular Genetics, Michigan State University, 540 Plant and Soil Sciences Bldg, East Lansing, MI 48824, USA.
E-mail: rodri257@msu.edu
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isolated from several other cold environments such as Antarctica soil, sea ice and Himalayan mountain soil (Bowman *et al.*, 1997; Shivaji *et al.*, 2005; Chaturvedi and Shivaji, 2006; Shravage *et al.*, 2007). The fact that members of these genera seem to be successful in cold environments, yet have very distinct physiologies as one is Gram-positive (*Exiguobacterium*) (Ponder *et al.*, 2005; Rodrigues *et al.*, 2008) and the other is Gram-negative (*Psychrobacter*) (Bergholz *et al.*, 2009), make them good candidates to test Baas Becking's statement 'everything is everywhere, the environment selects' for cold-adapted microorganisms. Results suggest that these cold-adapted microorganisms are not restricted to cold environments but are also found in temperate and tropical soils, although *Psychrobacter* especially is only marginally successful in the non-polar habitats.

## Materials and methods

*Sample collection and chemical analyses*
A total of six sediments and forty-eight soil samples were collected from a wide range of ecosystems with diverse climates and site characteristics (Supplementary Table S1). All samples were stored frozen at −20 °C until processed. The soil chemical analyses were performed by the Soil and Plant Nutrient Laboratory at Michigan State University.

*DNA extraction*
Total community genomic DNA was extracted using the Fast DNA SPIN kit for soil (Bio 101, Qbiogene, Inc., Carlsbad, CA, USA) with some modifications, namely the first step of the extraction was a grinding step using liquid nitrogen followed by treatment with 100 µl of lysozyme (50 µg µl$^{-1}$) at room temperature before using the kit. The quality of the extracted DNA was analyzed by electrophoresis on a 1% agarose gel. DNA concentrations were measured by absorbance at 260 nm.

*Quantitative real-time PCR assays (Q-PCR)*
The primer sets for *rpo*B, *gyr*B and a hypothetical gene, as well as the quantitative real-time PCR conditions for *Exiguobacterium* spp. were described earlier (Rodrigues and Tiedje, 2007). Detection was only considered positive when at least two genes were amplified by these primer sets. Data are presented as copy number averages and s.d. The primers used for *Psychrobacter* spp. quantification targeted the 16S rRNA gene and were 432-F (5′-GCACTTTAAGCAGTGAAGAAGA-3′) and 476-R (5′-TATTCTGCAGCTAATGTCATCG-3′). The reaction mixture contained 1X SYBR green PCR buffer, 1.75 mM $MgCl_2$, 0.2 mM dNTPs, 0.05 U µl$^{-1}$ Amplitaq Gold, 0.15 µM (Applied Biosystems, Foster City, CA, USA) and 0.4 mg ml$^{-1}$ of bovine serum albumin (BSA) (Roche Applied Science, Indianapolis, IN, USA). The amplification settings were the standard for real-time PCR with annealing and extension at 59 °C for 1 min. The real-time PCR assays and primer optimizations for both genera were done as described earlier (Rodrigues and Tiedje, 2007). The increase in fluorescence emission was monitored during PCR amplification using the 7700 Sequence Detector (PE Applied Biosystems, Foster City, CA, USA). The $C_t$ values obtained from each sample were compared with the standard curve to determine the initial copy number of the target gene. As all *Psychrobacter*, so far analyzed, have four 16S rRNA gene copies, we adjusted the standard curve to reflect this difference. The *Exiguobacterium* genes (rpoB, gyrB and a hypothetical gene) used in this study have only one copy per genome in the two Exiguobacterium closed genomes. So, there was no need to adjust the standard curves for each of these genes.

**Table 1** Results obtained from the simple and multiple linear regressions with Q-PCR results for each genus as a function of environmental variables

| *Bacterium* | *Significant Variables* | $R^2$ |
|---|---|---|
| *Simple linear regression* | | |
| *Psychrobacter* | $K^+$ | 36.6%** |
| | pH | 32.4%** |
| | Salinity | 23.2%* |
| *Exiguobacterium* | pH | 14.7%*** |
| | $Cu^{2+}$ | 34.7%** |
| *Multiple linear regression* | | |
| *Psychrobacter* | $K^+$, $Mg^{2+}$, salinity and pH | 90.5%** |
| *Exiguobacterium* | $K^+$, $Cu^{2+}$, salinity and pH | 63.4%** |

*$P<0.05$; **$P<0.01$; ***$P=0.08$.

Statistical analyses were performed with 22 samples from different geographic regions with distinct climates, namely Puerto Rico (P3, P5), Hawaii (HW), Brazil (B1, B2, B3, B4, B5, B6), Michigan (M1, M2, M3, M4, M5, M6, M7), Iowa (IA), Antarctica (A3, A5) and Siberia (S1, S4 and S23) (Supplementary Table S1). Simple and multiple linear regressions were used to investigate correlations between the abundance of gene copies of each genus and environmental factors (Table 1). The relevant environmental variables used for multiple regressions were chosen by stepwise selection among the 14 measured variables; these were phosphorus, potassium, magnesium, calcium, cation exchange capacity, copper, manganese, zinc, iron, pH, organic matter, moisture, salinity and average annual temperature. In total, five environmental variables were selected for further analysis with the Q-PCR data for each genus, namely, potassium, magnesium, pH, salinity and copper. The program XLSTAT was used for the statistical analyses (XLSTAT, 2006).

*Genus-specific clone library construction and sequencing*
Primers for conserved regions of the 16S rRNA genes for the genera *Exiguobacterium* spp. and

*Psychrobacter* spp. were designed using ARB, based on alignments of reference strains for each genus and 50 other Gram-positive and Gram-negative microorganisms (Ludwig *et al.*, 2004). The annealing temperatures for *Exiguobacterium* spp. and *Psychrobacter* spp. primer sets were 59 and 57 °C, respectively. Amplification for all primers was performed by standard procedures, except for the $MgCl_2$ concentration, which was used at 1.75 mM for *Psychrobacter* spp. and 1.5 mM for *Exiguobacterium* spp., respectively (Eden *et al.*, 1991). The sequences of the primers used for 16S rRNA gene were 432-F and 823-R (5′-TCAAGGGACCCAACGACTAGTA-3′) for *Psychrobacter* spp. and Exi16S-F (5′-GATGAA AGGCGCTYCGGCG-3′) and Exi16S-R (5′-CGGTCAR GGGGATGTCAAGAGTT-3′) for *Exiguobacterium* spp. Amplicon sizes were approximately 400 bp and 800 bp, respectively.

The specific 16S rRNA gene amplicons for each genus were cloned using the TOPO TA Cloning kit for sequencing (Invitrogen Life Technologies, Inc., Carlsbad, CA, USA) and sent to Macrogen Inc. (Seoul, Korea; www.Macrogen.com) for sequencing with primers for the cloning vector (M13F). The sequences with unique operational taxonomic units (OTUs) generated in this study were deposited in GenBank (accession numbers EU735449 to EU735531).

*Clone library analysis*

The 16S rRNA gene sequences from the clone libraries were processed by using the pipeline quality filter tools on the Ribosomal Database Project (RDP-II) website (Cole *et al.*, 2005) (http://rdp.cme.msu.edu). The Classifier tool provided by RDP-II was used to assign the 16S rRNA gene sequences to the taxonomic hierarchy. Aligned sequences assigned to *Exiguobacterium* or *Psychrobacter* were downloaded from RDP-II for further analyses. The aligned sequences were uploaded to FastGroupII program (Yu *et al.*, 2006) to cluster sequences with 99% similarity including gaps. One representative of each cluster with identical sequences was used to construct the phylogenetic tree within the MEGA 3.1 environment (Kumar *et al.*, 2004) by the neighbor-joining method. The robustness of the inferred tree was evaluated by applying 1000 bootstrap re-samplings.

Distance matrix files for each clone library were downloaded from the RDP-II pipeline to determine α diversity through the Distance-Based Operational Taxonomic Unit and Richness (DOTUR) program (Schloss and Handelsman, 2005), and to compare clone library similarity through statistical tests provided by ∫-libshuff and Unifrac programs for β diversity (Schloss *et al.*, 2004; Lozupone and Knight, 2005; Lozupone *et al.*, 2006). Libraries were also compared by the analysis of molecular variance (AMOVA), as implemented by the program Arlequin (Excoffier *et al.*, 2005).

## Results

*Distribution and abundance of* Exiguobacterium *and* Psychrobacter *in different environments*

The DNA extracted from the 54 samples was subjected to Q-PCR analysis with four specific primer sets (3 protein-coding marker genes for *Exiguobacterium* and the 16S rRNA gene for *Psychrobacter*) to establish the presence, distribution and abundance of the target organisms. *Psychrobacter* spp. was present at higher densities in Antarctica sediments than *Exiguobacterium* spp., and higher than *Psychrobacter* spp. in the Siberian permafrost (Figure 1). In temperate (Michigan and Iowa) and in tropical (Brazil, Puerto Rico and Hawaii) environments, the distribution and abundance of *Psychrobacter* spp. was patchy and minimal. The *Exiguobacterium* spp. distribution was also patchy but when present the abundance was similar to that found in colder habitats.

*The role of environmental factors in the selection of* Exiguobacterium *and* Psychrobacter

The unusual distribution and abundance of *Exiguobacterium* and *Psychrobacter* in diverse soils led us to investigate environmental variables to search for common factors that could select for these cold-adapted species. Among 14 variables, simple and multiple regression analyses were performed to find environmental variables that significantly correlate with the abundance of the two genera. The results show that several environmental variables significantly correlate with the abundance of the two genera (Table 1), indicating that environmental factors play an important role in the abundance and distribution of both *Exiguobacterium* and *Psychrobacter* species in different environments. The abundances of *Exiguobacterium* spp. and *Psychrobacter* spp. populations significantly correlated with pH, salinity, as well as potassium ($K^+$). Furthermore, *Exiguobacterium* and *Psychrobacter* abundance also significantly correlated with copper ($Cu^{2+}$) and magnesium ($Mg^{+2}$) concentrations, respectively.

*Diversity of* Exiguobacterium *and* Psychrobacter *populations in diverse geographic locations*

The fact that these microorganisms were found in diverse geographic locations does not imply that their populations were similar, especially because their densities were different in different regions or habitats. Hence, we analyzed 16S rRNA gene libraries from one site in each climatic region, that is, Antartica, Michigan and Puerto Rico, that had high population densities for each genus (Tables 2 and 3). The number of clones sequenced was determined by rank abundance curves to insure that most of the phylotypes were sampled in each site.

Phylogenetic analysis of *Psychrobacter* spp. and *Exiguobacterium* spp. was done with one represen-

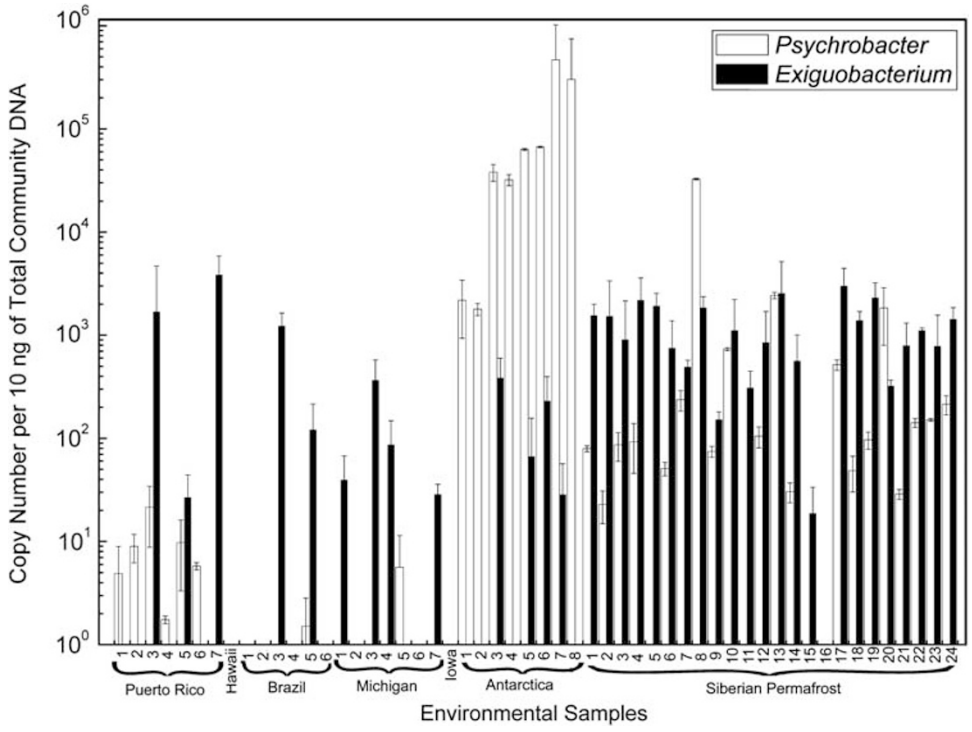


**Figure 1** *Exiguobacterium* spp. and *Psychrobacter* spp. abundance in various habitats as measured by Q-PCR amplification. The sample numbers shown on the graphic corresponds to the sample numbers presented in Supplementary Table S1.

**Table 2** Individual comparisons of *Exiguobacterium* and *Psychrobacter* 16S rRNA clone libraries obtained from different environments

| *Analyses* | *Psychrobacter spp.* | | | *Exiguobacterium spp.* | | |
|---|---|---|---|---|---|---|
| | *A3* | *M5* | *P5* | *A5* | *M7* | *P3* |
| Number of sequences | 129 | 150 | 137 | 149 | 109 | 135 |
| Number of OTUs | 21 | 14 | 11 | 7 | 4 | 1 |
| Shannon Index (H) | 2.60 | 2.22 | 1.84 | 1.53 | 0.34 | 0 |
| Simpson Index (D) | 0.09 | 0.13 | 0.21 | 0.26 | 0.84 | 1 |
| Chao 1 estimator | 21.43 | 17.00 | 11.50 | 7 | 5 | 1 |
| AMOVA population-specific ($F_{st}$) (%) | 5.41 | 5.29 | 5.33 | 58.25 | 58.53 | 58.77 |

Abbreviations: A, Antarctica; M, Michigan; P, Puerto Rico.
The numbers following the letters correspond to the sampling sites in Supplementary Table S1.

**Table 3** Pairwise 16S rRNA clone library comparisons between each pair of environments for each genus

| *Analyses* | *Psychrobacter spp.* | | | *Exiguobacterium spp.* | | |
|---|---|---|---|---|---|---|
| | *A3:M5* | *M5:P5* | *A3:P5* | *A5:M7* | *M7:P5* | *A5:P3* |
| $\int$-Libshuff (*P*-value) | 0.000 | 0.1578 | 0.0234 | 0.000 | 0.000 | 0.000 |
| Unifrac (*P*-value) | $\leqslant$0.003 | 0.573 | $\leqslant$0.003 | $\leqslant$0.003 | $\leqslant$0.003 | $\leqslant$0.003 |
| AMOVA within population (%) | 94.66 | | | 41.49 | | |

Abbreviations: A, Antarctica; M, Michigan; P, Puerto Rico.
The numbers following the letters correspond to the sampling sites Supplementary Table S1.

tative of each cluster of identical sequences. The phylogenetic trees show that new species are still to be described, especially in Antarctica (Figures 2 and 3). The $\alpha$ diversity, that is, species diversity within each location, differed between sampling sites as shown by the diversity index

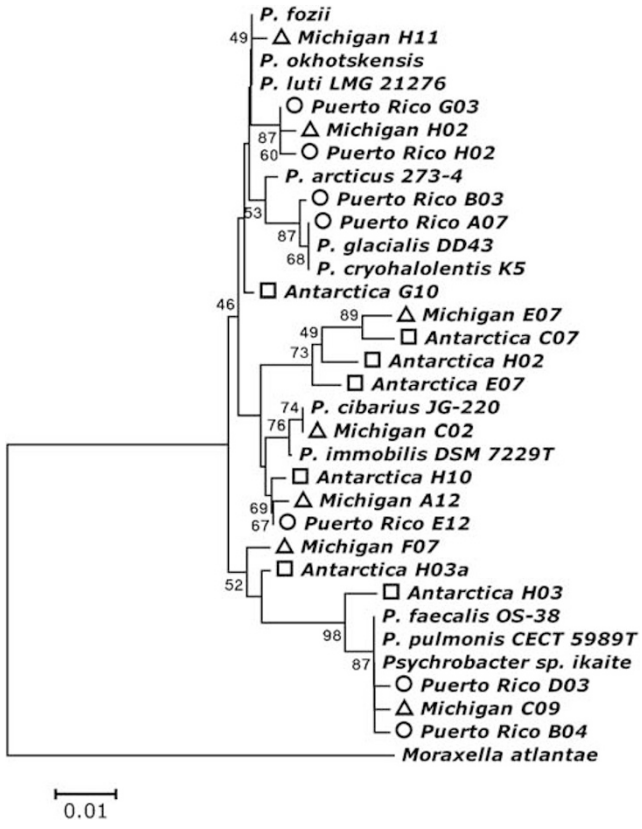


**Figure 2** Phylogenetic tree based on 16S rRNA gene sequences of *Psychrobacter* strains and the top seven dominant OTUs in the clone libraries from Michigan (△), Puerto Rico (○) and Antarctica sediment (□). The trees were produced by the neighbor-joining method and rooted using the 16S rRNA gene from *M. atlantae*. The nodes with less than 45% bootstrap values were not added to the tree. The scale bar represents changes per nucleotide.

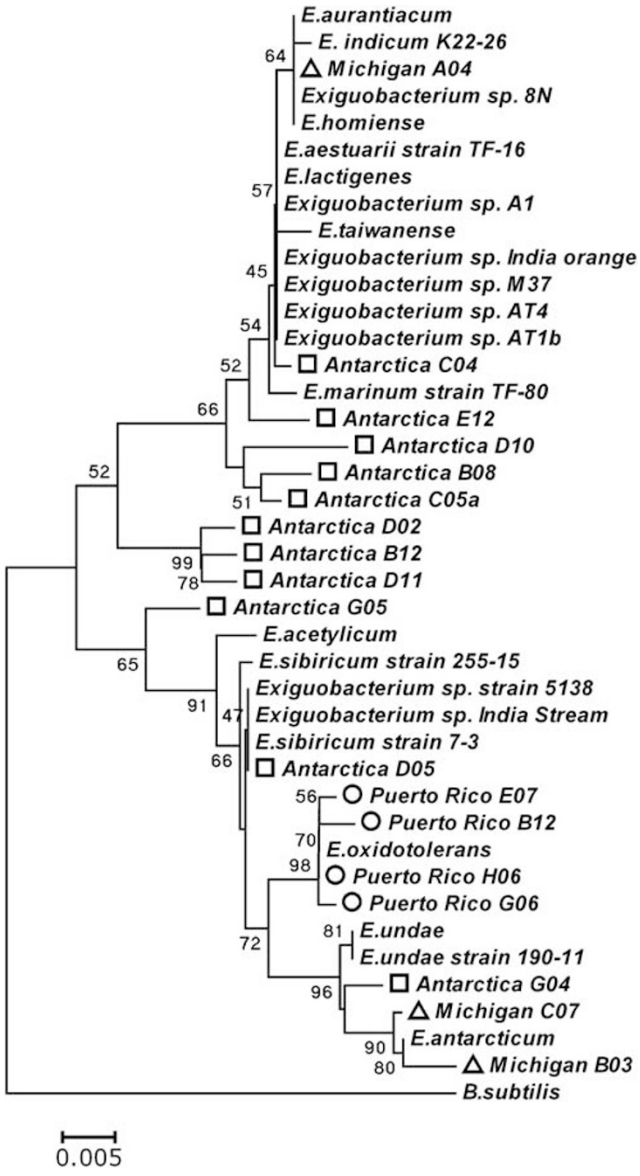


**Figure 3** Phylogenetic tree based on 16S rRNA gene sequences of *Exiguobacterium* strains and clone libraries from Michigan (△), Puerto Rico (○) and Antarctica sediment (□). The trees were produced by the neighbor-joining method and rooted using the 16S rRNA gene from *B. subtilis*. The nodes with less than 45% bootstrap values were not added to the tree. The scale bar represents changes per nucleotide.

values and OTU abundances (Table 2). *Psychrobacter* spp. and *Exiguobacterium* spp. were more diverse in Antarctica and had reduced diversity in Puerto Rico. *Psychrobacter* exhibited more diversity than *Exiguobacterium* in all sites (Table 2).

∫-libshuff analysis determines the β diversity by verifying whether two samples are drawn from the same population or whether one is a subset of the other. The Puerto Rican and Michigan clone libraries for *Psychrobacter* displayed significantly different communities from those from Antarctica (Table 3), but shared many OTUs with each other. All the *Exiguobacterium* from the three sites had significantly different populations (OTUs). Similar results were observed when AMOVA and the Unifrac tests were applied to these data.

The AMOVA evaluates the variance in genetic diversity within and between populations or communities by calculating the $F_{ST}$ value (Excoffier *et al.*, 2005). UniFrac measures β diversity by analyzing the differences in population composition among environments and by taking into account the different evolutionary relationships (Lozupone and Knight, 2005). According to the AMOVA results most of the phylogenetic variation for *Psychrobacter* was observed within populations (94.7%). The variation among populations was low ($F_{ST}$ 5.34%) but significant, showing that the *Psychrobacter* populations in the different environments were phylogenetically close, but still had significant genetic variation. All *Psychrobacter* populations presented different genetic structure with the exception of the populations from Michigan and Puerto Rico. Unifrac also showed non-significant *P*-values for the populations in these two environments (Table 3). More genetic variation was found among

*Exiguobacterium* populations than among *Psychrobacter* populations in the different environments, as indicated by its $F_{ST}$ value (58.3%), which was also statistically significant. For *Exiguobacterium*, all pairwise $F_{ST}$ values were significantly different.

## Discussion

The Q-PCR results show that *Exiguobacterium* spp. and *Psychrobacter* spp. were more commonly found in Antarctica and in the Siberian permafrost than in any of the other studied sites (Figure 1). Notably, *Psychrobacter* spp. seemed to be more successful in Antarctica than *Exiguobacterium* spp., whereas in the Siberian permafrost the result was opposite. The Antarctica samples were all non-frozen marine, salty or fresh water sediments, whereas the permafrost samples were subzero with low water activity. Hence, growth in the cold unfrozen Antarctic sediments could have accounted for the much higher population densities of *Psychrobacter* there than found elsewhere. The much lower densities of *Psychrobacter* in the Puerto Rican mangrove sediments suggest that their growth is favored by the cold temperatures, as the Antarctic and Puerto Rican sediments are otherwise similar. Strains of *Psychrobacter* have been shown to grow at temperatures as low as $-10\,^{\circ}C$ and have reasonable generation time of 2.5 days at $0\,^{\circ}C$ (Bakermans and Nealson, 2004).

In temperate (Michigan and Iowa) and tropical (Brazil, Puerto Rico and Hawaii) environments, *Psychrobacter* spp. and *Exiguobacterium* spp. populations exhibited a patchy distribution, but when present, *Psychrobacter* spp. was in very low density, whereas *Exiguobacterium* spp. abundance was similar to that found in colder habitats (Figure 1). The fact that both genera were not detected in 58% of the non-polar soil samples suggests that they are not cosmopolitan in the strict sense; however, they are not restricted to the poles as are some polar bacteria (Staley and Gosink, 1999).

The distribution of these microorganisms correlated with some physico-chemical factors of their environment (Table 1), such as salinity, pH, potassium and the micronutrient copper. The effect of salinity on the abundance of these genera can be explained by their adaptation to low water activity, whether it is due to subzero temperatures or salt. Studies with *Psychrobacter* and *Exiguobacterium* spp. isolated from the Siberian permafrost have shown that these genera can tolerate increased osmolarity and show associated changes in membrane composition, cell morphology and size (Ponder *et al.*, 2005). Furthermore, all isolates from *Exiguobacterium* spp. and *Psychrobacter* spp. can grow with additional NaCl in their medium (Romanenko *et al.*, 2004; Shivaji *et al.*, 2005; Rodrigues *et al.*, 2006; Crapart *et al.*, 2007). This evidence suggests that these genera are adapted to salinity, which would explain the strong correlation of the abundance of these genera to moderate to high salinity habitats, that is, Antarctic marine sediments, the Puerto Rican mangroves and the Siberian permafrost, the latter due to its limited but salty liquid phase (low water activity) (Ponder *et al.*, 2005).

The presence of these genera also showed strong correlation with pH. The ability of the cell to maintain the pH homeostasis is very important for cell survival. Although several species of *Exiguobacterium* have been described as alkalophilic (Collins *et al.*, 1983; Yumoto *et al.*, 2004), both *Exiguobacterium* spp. and *Psychrobacter* spp. seem to prefer pH closer to neutrality. Potassium is the principal cation in bacteria, and it not only plays a role as a cofactor for many enzymes and ribosomes, but is also responsible for pH and osmotic homeostasis (White, 2000). Environments with pH 6–8, which seems to be the optimum environmental pH range for these genera, triggers $K^+$ influx to raise the intracellular pH (White, 2000). This physiological need for $K^+$ to maintain the cell pH homeostasis, could explain their preference for higher environmental concentrations of $K^+$. Therefore, the environmental analysis combined to the Q-PCR showed that the Baas Becking hypothesis applies to these two cold adapted microorganisms.

Although these two genera were found in different geographic locations, their microbial diversities, that is, $\alpha$ diversity, were distinct. The recovered *Exiguobacterium* seemed to be much less diverse than *Psychrobacter*. Furthermore, in some sites the quantity of *Exiguobacterium* spp. was higher than that of *Psychrobacter* spp. (Figure 1), but the diversity within *Exiguobacterium* was never higher than within *Psychrobacter* (Table 2). The diversity results also indicate an apparent latitudinal gradient in diversity at the species level (99% 16S rRNA cutoff) (Konstantinidis and Tiedje, 2005) for both genera: *Psychrobacter* spp. and *Exiguobacterium* spp. were more diverse in the Antarctica samples with less diversity in Michigan and even less diversity in Puerto Rican samples (Table 2). The higher abundance and diversity of these genera at the poles than in the other regions suggest that the preferred habitats for these genera are the cold, polar environments.

The $\beta$ diversity analyses, that is, the pairwise 16S rRNA clone library comparisons between each pair of environments for each genus, showed distinct populations among the different environments (Table 3). *Psychrobacter* populations from the different sites were phylogenetically closer to each other than *Exiguobacterium*. For instance, the *Psychrobacter* populations from Puerto Rico were a subset of the Michigan populations, that is, species found in Puerto Rico were similar to species found in Michigan, whereas several species found in Michigan were not found in Puerto Rico, as indicated by $\int$-Libshuff, Unifrac and phylogenetic analyses (Table 3 and Figure 2). The *Psychrobacter* population

from Antarctica, on the other hand, was completely different from the populations from the other two environments. The *Exiguobacterium* populations from the three different sites were completely different from each other as shown in Table 3. These results suggested that the populations of both genera were genetically different or became genetically different as a result of different selective pressures to adapt to particular temperature or environmental conditions. The fact that these microbes have been shown to be ubiquitous in Siberian permafrost soils as old as 3 million years (Rodrigues *et al.*, 2008) and that microorganisms can be dispersed by dust an live for centuries and survive intercontinental travel (Gorbushina *et al.*, 2007) would suggest that these cold-adapted populations could have been dispersed at a much earlier time to other regions and (perhaps) diverged through local adaptation or random genetic drift yielding the warmer region species we have today. The presence of similar populations in different geographic regions, as seen in the *Psychrobacter* populations from Puerto Rico and Michigan (Table 3), would corroborate their common origin.

The phylogenetic trees from *Psychrobacter* spp. and *Exiguobacterium* spp. show that new species are yet to be described, especially in Antarctica, because there are many clusters of clones that do not contain any described species (Figures 2 and 3). The *Psychrobacter* spp. tree (Figure 2) confirms the results from $\int$-libshuff that Michigan and Puerto Rico share similar OTUs, because several clusters contain clones from both habitats. In addition, some Puerto Rican clones also contain OTUs with high similarity to isolates from the Siberian permafrost, such as *P. cryohalolentis* K5 (Bakermans *et al.*, 2006), but also to isolates found in warmer habitats, such as *P. pulmonis* (Vela *et al.*, 2003) that was isolated from lamb lungs.

In the case of *Exiguobacterium* spp. (Figure 3) all the Puerto Rican clones clustered with *Exiguobacterium oxidotolerans* isolated from a fish processing plant, a strain that tolerates 12% of NaCl and pH values from 7 to 10 (Yumoto *et al.*, 2004). One group of clones from Antarctica had identical sequences to several isolates from the Siberian permafrost, such as *Exiguobacterium sibiricum* strains 7–3 and 255–15, and *Exiguobacterium* sp. strain 5138 (Rodrigues *et al.*, 2006), confirming that both poles share similar phylotypes. The *Exiguobacterium* phylogenetic tree also confirmed the results from $\int$-libshuff, as the clones from the diverse geographic locations never clustered with one another.

Ecologists describing microbial biogeography typically invoke Bass Becking's statement that: 'Everything is everywhere, but the environment selects', that is, although all microbial life is distributed worldwide, in a given environmental setting most of the microbial species are only latently present (de Wit and Bouvier, 2006). This study shows that *Exiguobacterium* spp. and *Psychrobacter* spp. are more commonly found and have higher densities in the polar regions, but they can be detected in temperate and tropical sites. The latter is more likely the case when physicochemical conditions such as salinity, $K^+$ and pH are more similar to these in their polar sites. In the context of the Baas-Becking statement, our data suggest that *Exiguobacterium* and *Psychrobacter* have been widely distributed and colonized diverse sites but especially cold and other non-polar but low water activity environments.

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