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Advances in Polar Science

doi: 10.3724/SP.J.1085.2011.00025

March 2011 Vol.22 No.1: 25–34

Relationships between two *Pseudoalteromonas* strains isolated from the Canada Basin and the Southern Ocean using a polyphasic approach

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Received September 10, 2010; accepted October 10, 2010

Abstract The evolutionary relationships and taxonomic position of two marine planktonic bacterial strains BSw20211 and BSw10014, isolated from the Canada Basin and from the Southern Ocean, respectively, were determined using a polyphasic taxonomic approach. There was a close phylogenetic relationship between the two strains and most phenotypic properties were shared. Nonetheless, they were found to belong to different species of the genus *Pseudoalteromonas* on the basis of genotypic analyses. Findings were consistent with the suggestion that *gyrB* gene sequence comparison and DNA-DNA relatedness might better define phylogenetic relationships of bacteria at the species level. However, a cut-off value of 90% *gyrB* gene sequence similarity was not reliable for the differentiation of species within the genus *Pseudoalteromonas*.

Keywords ***Pseudoalteromonas*, phylogenetic relationship, 16S rRNA, *gyrB*, DNA relatedness**

Citation: Zeng Y X, Zheng T L. Relationships between two *Pseudoalteromonas* strains isolated from the Canada Basin and the Southern Ocean using a polyphasic approach. Adv Polar Sci，2011,22：25—34，doi: 10.3724/SP.J.1085.2011.00025

0 Introduction

Species of the genus *Pseudoalteromonas* are common in aquatic environments and are frequently isolated from marine ecosystems[1-2]. This genus has attracted significant interest because it has often been found in close association with marine eukaryotic hosts. In add ition, many members produce bioactive compounds, including extracellular enzymes, toxins and extracellular polysaccharides[1-4]. A number of species have the capacity to form high molecular mass compounds with antibiotic properties[5]. These compounds have potential for use in biotechnological and antifouling applications[4].

Pseudoalteromonas species are characteristically gram-negative, rod-shaped, heterotrophic bacteria which are motile by means of one or two polar flagella[1]. The genus *Pseudoalteromonas* currently contains 43 species (NCBI Taxonomy Browser, May 2009) and the type species is *Pseudoalteromonas haloplanktis*[6]. Species of the genus *Pseudoalteromonas* are frequently isolated from marine waters around the world[7], suggesting that they represent model microorganisms for studying bacterial biogeography in the marine environment.

Although microbes have biogeographies[8], little effort has been made in the study of microbial biogeography[9]. All microbes are potentially everywhere because of their small size, great abundance and easy dispersal[10]. Cosmopolitan bacteria have been found in tropical and polar deep-sea sediments[11]. *Shewanella hanedai* ACAM 544^T isolated from sediment in Arctic Ocean and *Shewanella hanedai* ACAM 585 isolated from Antarctic sea-ice sample have a DNA-DNA hybridization value of 70%[12], supporting that these two bacteria belong to the same species. Members of the species *Shewanella*

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frigidimarine have been observed in seawater from the Greenland Sea, Canada Basin and Antarctic Prydz Bay[13]. At the same time, evidence for endemism is found among bacteria inhabiting sea ice where the barriers to surviving dispersal are high. This includes gas vacuolated sea ice bacteria[14]. It suggests that microbial communities may consist of a mixture of species, some of which are endemic and some of which are cosmopolitan[14].

We have isolated hundreds of *Pseudoalteromonas* strains from Arctic and Antarctic seawater. Two *Pseudoalteromonas* strains (BSw20211 and BSw10014) isolated from the Canada Basin and the Southern Ocean, respectively, show 99.7% 16S rRNA gene sequence similarity to each other. This suggests that the two bacteria may belong to the same species. In this study, the evolutionary relationship and taxonomic position of the two bacteria were examined using polyphasic taxonomic analysis.

1 Materials and methods

1.1 Bacterial strains and growth conditions

The two planktonic bacterial strains investigated are listed in Table 1. The strains were isolated using the spread plate method from seawater that had been collected from different regions. These strains were grown on marine agar 2216 (MA; Difco) at 4℃ for 10 days and stored frozen at − 80℃ in marine broth 2216 (MB; Difco) supplemented with 30% (v/v) glycerol. The cultures were incubated for 2 days at 12℃. To obtain large numbers of cells, cultures were incubated in MB at 120 r /min on a gyratory shaker.

Table 1 Planktonic bacterial strains investigated in this study

Strain	Sampling date	Water depth	Origin	GenBank accession number		
				16S rRNA	*gyrB*	ITS
BSw10014	01/12/2001	0—1 m	The Southern Ocean (40°07'01" S, 173°30'10" E)	EF375559	FJ416136	FJ501589 (large fragment); FJ501590 (small fragment)
BSw20211	23/08/2002	5 m	The Canada Basin (72°33'29"' N, 140°59'49"W)	EF437162	FJ416137	FJ501592 (large fragment); FJ501591 (small fragment)

1.2 Phenotypic analysis

Cell morphology was examined using a JEM 2100HC transmission electron microscope (JEOL, Tokyo, Japan). The presence or absence of flagella was determined using cells from exponentially growing cultures. Tolerance towards NaCl was studied in Luria-Bertani (LB) media supplemented with 0%, 1.0%, 3.0%, 7.0%, 10.0% and 15.0% (w/v) NaCl, at pH 7.2. Growth was measured photometrically at 600 nm.Growth at various temperatures (4℃—37℃) was measured from cultures on MA. All physiological tests were performed aerobically at 12℃, except for temperature range determinations. Catalase activity was determined using bubble production in 3% (v/v) hydrogen peroxide solution[15]. Hydrolysis of casein was determined as described by Lee et al.[16]. Other physiological and biochemical tests were performed using API 20E, API 20NE and API ZYM test kits (bioMérieux, Marcy l'Etoile, France), which were prepared according to the manufacturer's specifications, except that bacterial strains were suspended in 3% sea salt (Sigma, St. Louis, MO, USA) solution.

1.3 Fatty acid methyl ester analysis

Cellular fatty acids were determined using a culture grown on MA at 12℃ for 2 days and were extracted, methylated and analyzed using the standard MIDI system (Microbial ID Inc., Newark, Delaware, USA) as described by Sasser[17].

1.4 Genetic analysis

DNA was extracted and purified as described by Sambrook et al.[18]. DNA G + C content (mol%) was determined by thermal denaturation with *Escherichia coli* K-12 as the reference strain. The experiment was carried out using a DU800 spectrophotometer (Beckman Coulter, Fullerton, CA, USA). DNA-DNA hybridization was performed using the method of Seldin and Dubnau [19]. Probe labeling was conducted using the non-radioactive DIG high prime system (Roche, Penzberg, Germany). Hybridized DNA was visualized using the DIG luminescent detection kit (Roche, Mannheim, Germany). DNA-DNA relatedness was quantified using a densitometer (Bio-Rad, Hercules, CA, USA).

1.5 DNA extraction, 16S rRNA gene amplification and sequencing

Bacterial DNAs for PCR were prepared using the MiniBest bacterial genomic DNA extraction kit (TaKaRa, Dalian, China) according to the manufacturer's instructions. Nearly complete 16S rRNA genes were PCR-amplified in 50 μL reactions using 20—50 ng of a genomic DNA template, 0.2 μm of the forward 8F (5′ to 3′ AGAGTTTGATCCTGGCTCAG) and reverse 1492R (5′ to 3′ GGTTACCTTGTTACGACTT) primers[20], 50 μm dNTPs, 1× PCR buffer and 1 U *Taq* DNA polymerase (TaKaRa, Dalian, China). Amplification was performed with an Eppendorf Mastercycler Gradient (Eppendorf, Hamburg, Germany). The PCR conditions were 95℃ for 4min followed by 25 cycles of 95℃ for 45 sec, 50℃ for 45 sec, 72℃ for 1.5 min, followed by 72℃ for 10 min. The PCR products were purified using a TaKaRa agarose gel DNA purification kit following the manufacturer's protocols (TaKaRa, Dalian, China). The purified PCR products were ligated into pMD18-T Vector (TaKaRa, Dalian, China) and transformed into competent *Escherichia coli* DH5α cells using standard protocols. The cloned 16S rRNAs were sequenced using M13 primers with an ABI Prism 3730 DNA analyzer (PE Applied Biosystems, Foster City, USA). The accession numbers of the 16S rRNA gene sequences were listed in Table 1.

1.6 *GyrB* gene amplification and sequencing

The 1.2 kb nucleotide sequences of the *gyrB* genes of bacterial strains were amplified using PCR with universal primer sets UP1 and UP2r (5′ to 3′ GAAGTCATCATGA CCGTTCTGCACAYGCNGGNGGNAARTTYGA and AGCAGGGTACGGATGTGCGAGCCCCRTCNACRTC NGCRTCNGTCAT) as descibed by Yamamoto and Harayama[21]. Polymerase chain reactions were performed in a total volume of 25 μL containing the primer set: 20—50 ng DNA template, 0.4 μm each UP1 and UP2r, 100 μm dNTPs, 1× PCR buffer and 1 U *Taq* DNA polymerase (TaKaRa, Dalian, China). The PCR conditions were as follows: 95℃ for 3 min followed by 35 cycles of 94℃ for 1 min, 58℃ for 1 min, 72℃ for 1 min, followed by 72℃ for 10 min. PCR products were purified and sequenced as described above using M13 forward and reverse primers. The accession numbers of the *gyrB* gene sequences are listed in Table 1.

1.7 Amplification and sequencing of the 16 S-23 S rRNA ITS (intergenic transcribed spacer)

Two sets of primers were designed to amplify the 16S-23S rRNA ITS region using sequences in the 16S and 23S rRNA genes. These primer sets were: (1) 1405F and 456R (5′ to 3′ TGCACACACCGCCCGT and CCTTTCC CTCACGGTACTG) as described by Gürtler and Stanisich [22]; and (2) 16S14F and 23S1R (5′ to 3′ CTTGTACA CACCGCCCGTC and GGGTTTCCCCA TTCGGAAATC) as described by Zavaleta et al.[23]. Polymerase chain reactions were performed in a total volume of 25 μL that contained primer set 20—50 ng DNA template, 0.4 μm concentrations of each forward and reverse primer, 100 μm dNTPs, 1× PCR buffer and 1 U *Taq* DNA polymerase (TaKaRa, Dalian, China). The PCR conditions were as follows: 95℃ for 3 min followed by 35 cycles of 94℃ for 1 min, 60℃ for 2.5 min, 68℃ for 1 min, followed by 72℃ for 10 min. PCR products were purified and sequenced as described above using M13 forward and reverse primers. GenBank accession numbers for the 16 S-23 S rRNA ITS sequences are listed in Table 1.

1.8 Phylogenetic analysis

Sequences obtained in this study were compared with those in GenBank using BLAST search (http://www.ncbi.nlm.nih.gov). The closest matches were aligned together with the clone sequences using the CLUSTALX 1.81 program[24]. A phylogenetic tree was inferred using the PHYLIP v.3.5c software package[25]. Evolutionary distance matrices, generated using DNADIST, were constructed using the method of Kimura[26]. The matrices were used to infer dendrograms using the neighbor-joining method[27]. Bootstrap values were obtained for a consensus based on 100 randomly generated trees using SEQBOOT and CONSENSE. Tree figures were generated using NJplot version 2.1[28].

2 Results

2.1 Morphological characterization

Bacterial strains BSw10014 and BSw20211 exhibited quite similar morphological characteristics (Figure 1): The cells of the two strains were gramnegative, non-sporeforming, straight or curved rods that were 0.8 to 1.1 μm by 1.8 to 3.0 μm. The cells were motile by means of single unsheathed polar flagella. On marine agar 2216 (MA; Difco) incubated at 12℃ for 2 days, young colonies of the two isolates were circular, smooth, convex, slightly mucoid and creamy white in color, with a diameter of 1 mm to 2 mm. The morphological characteristics of the two strains were consistent with the previous observations of *Pseudoalteromonas* species[6, 29−30].

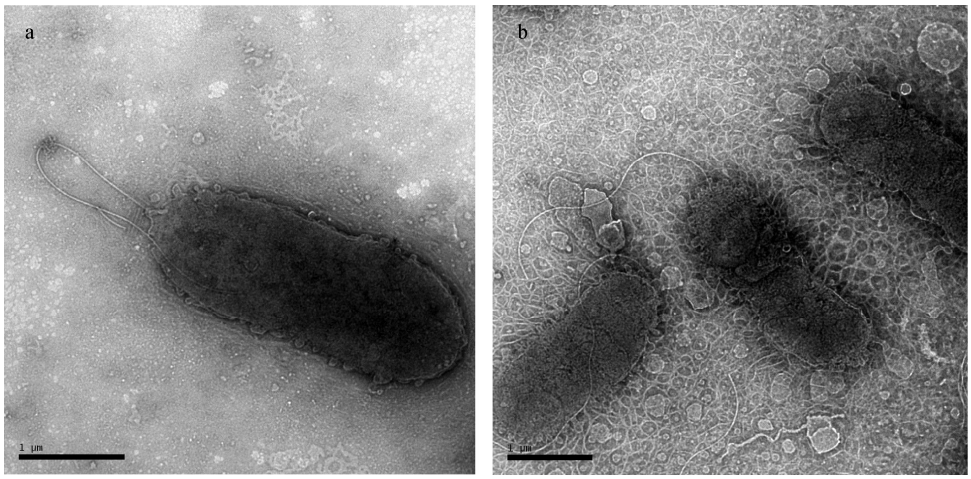

Figure 1 Transmission electron micrographs of two *Pseudoalteromonas* strains grown on MA for 2 days at 4℃. (a) Strain BSw10014 from the Southern Ocean. (b) Strain BSw20211 from the Canada Basin.

2.2 Phenotypic characterization

The physiological and biochemical properties of the two strains are summarized in Table 2. Both strains grew at temperatures between 4℃ and 35℃. Arctic strain BSw20211 could not grow at 37℃. No growth was detected in the absence of sodium ions, while no growth occurred when NaCl concentration reached up to 15% (wt/vol). Both strains had negative Voges-Proskauer and indole reactions. None of the strains produced pigments. They were chemoorganotrophic and capable of respiratory but not fermentative metabolism. Strain BSw10014 was capable of producing hydrogen sulfide from thiosulfate but negative for α-glucosidase activity. On the basis of the phenotypic characterization, bacterial strains BSw10014 and BSw20211 were phenotypically similar to the genus *Pseudoalteromonas*[6, 29−31], although some differences in phenotypic properties were observed between the two strains.

2.3 Cellular fatty acid composition

The fatty acid compositions of the two *Pseudoalteromonas* strains are shown in Table 3. Both strains presented high amounts of summed feature 3 (32.13% for strain BSw10014 and 45.42% for BSw20211, respectively) consisting of monounsaturated 16:1ω7*c* and the terminally branched saturate 15:0 iso 2OH.

These values were similar to those found for other *Pseudoalteromonas* species[1, 30−31], which contained a dominant fatty acid of monounsaturated 16:1ω7*c*. Other predominant fatty acids, including 17:1ω8*c*, 16:00, 15:1ω8*c*, 15:00 and 12:0 3OH, were detected in strain BSw10014. Fatty acids including 16:00, 17:1ω8*c*, 18:1ω7*c*, 12:0 3OH and summed feature 7 (5.09%) consisting of an unknown, 18.846 and 19:1ω6*c*, were found to be rich in strain BSw20211. These properties allowed the two isolates to be assigned to the genus *Pseudoalteromonas*. However, the similar fatty acid composition inferred that fatty acid methyl ester profiles could not be used to differentiate between the *Pseudoalteromonas* species.

2.4 DNA base composition and DNA-DNA hybridization

The DNA G+C contents of strains BSw10014 and BSw20211 were 43.7 mol % and 41.4 mol %, respectively. This was similar to the reported G+C contents of *Pseudoalteromonas haloplanktis* (41%—45%) and *Pseudoalteromonas tetraodonis* (41%—42%)[1,29,31]. However, the result of DNA-DNA hybridization indicated that bacterial strains BSw10014 and BSw20211 were 63% related to each other, which was less than the 70% required to consider two strains to be members of the same species[32−33].

2.5 16S rRNA phylogenetic analysis

16S rRNA phylogenetic studies showed that strain BSw10014 shared 99.7% sequence similarity with the Canadian Basin strain BSw20211. Sequences of the two strains were compared with the sequences of other *Pseudoalteromonas* species obtained from the GenBank database. The two strains showed 99.7%—99.8% similarity to *Pseudoalteromonas tetraodonis* KMM 458 (AF21 4729). The phylogenetic tree constructed by the neighbor joining methods (Figure 2) showed that the two strains were grouped into one cluster represented by *Pseudoalteromonas tetraodonis* KMM 458. These results suggested that the two strains might belong to the species *Pseudoalteromonas tetraodonis*.

Table 2 Selected phenotypic properties of the two bacterial isolates and other *Pseudoalteromonas* species

Characteristic	BSw 10014	BSw 20211	*P.haloplanktis* IAM 12915 [a–b]	*P.tetraodonis* KMM 458 [a–c]	*P.peptidolytica* F12−50−A1[d]
Growth at:					
4℃—35℃	+	+	+	+	+
37℃	+	-	ND	ND	+
Growth in:					
0% NaCl	-	-	ND	-	-
10% NaCl	+	+	ND	+	+
H_2S production	+	-	ND	ND	-
Reduction of NO_3^- to NO_2^-	-	-	-	+	-
Oxidative acid production from:					
Glucose, sucrose	+	+	ND	+	ND
Hydrolysis of:					
Gelatin	+	+	+	+	+
Casein, esculin	+	+	ND	ND	ND
Utilization of:					
D-glucose	-	-	+	+	+
D-mannose	-	-	+	+	-
D-mannitol	-	-	V	-	-
Malic acid, D-arabinose, gluconate	-	-	-	+	ND
N-acetyl-glucosamine	-	-	-	+	+
Caprate	-	-	ND	+	ND
Adipate, phenylacetate,	-	-	ND	ND	ND
D-maltose	+	-	+	+	+
Citrate	+	-	+	+	-
Enzymatic activity:					
Cytochrome oxidase, catalase	+	+	ND	+	+
Alkaline and acid phosphatase, esterase (C4), esterase lipase (C8), leucine arylamidase, valine arylamidase,trypsin,naphthol-AS-BI-phosphohydrolase,	+	+	ND	ND	ND
Arginine dihydrolase	-	-	ND	-	-
β -glucosidase, lysine decarboxylase, ornithine decarboxylase,	-	-	ND	ND	-
Urease	-	-	+	-	ND
Tryptophane deaminase, lipase (C14), cystine arylamidase, α-chymotrypsin, α-galactosidase, β-glucuronidase,β-glucosidase,N-acetyl-β-glucosaminidase, α-mannosidase, α-fucosidase	-	-	ND	ND	ND
α-glucosidase	-	+	ND	ND	ND

+, Positive reaction; −, negative reaction; v, variable; ND, no data.
a, b, c, d: Data from references [6, 29–31], respectively.

Table 3 Fatty acid composition of the two bacterial isolates and other *Pseudoalteromonas* species

Fatty acid	BSw10014	BSw20211	*P.haloplanktis* IAM 12915 [a]	*P.tetraodonis* KMM 458 [a]	*P.peptidolytica* F12-50-A1 [b]
10:0	0.16	0.38			0.93
11:0	0.37	0.11			
12:0	3.03	3.23	2.0	1.5	1.85
10:0 3OH	1.01	1.25			2.16
11:0 2OH	0.15	0.13			
11:0 3OH	3.21	0.68	0.3	0.4	
Unknown 11.799	1.48	1.34			1.64
11:0 iso 3OH	0.44	0.17			0.12
13:0	0.69	0.11	0.2	0.5	
12:0 3OH	5.47	5.34	1.9	0.9	6.14
12:0 iso 3OH	1.27	0.56			0.22
14:0	1.06	1.03	2.0	2.0	3.11
13:0 2OH	0.16	0.14			
13:0 iso 3OH	0.09				
15:0 iso	0.12		0	0	
15:0 Anteiso	0.18		0.2	0.4	
15:1 ω8*c*	7.12	1.4	2.3	6.3	0.35
15:1 ω6*c*	0.91	0.18	0.2	0.6	
15:0	6.64	1.7	3.3	8.0	
16:0 iso	0.88	0.61	0.2	1.4	0.12
16:0	7.07	10.54	30.1	18.2	17.46
16:1 ω5*c*	0.08		0	0.2	
16:1 ω9*c*	0.74		0	0	0.87
17:0	3.06	1.91	3.9	5.5	
17:0 iso	0.37	0.36	0	0.3	
17:0 Anteiso	0.24	0.54	0	0.6	
17:1 ω8*c*	14.4	7.63	6.0	10.7	0.19
17:1 ω6*c*	1.27	1.27	0.2	0.8	
18:0	0.59	0.65	1.9	0.6	
18:0 iso		0.21	0	0.2	
18:1 ω9*c*	0.24	0.34	0.2	0.2	0.39
18:1 ω7*c*	2.7	6.62	2.4	2.5	3.03
Unknown 18.814		0.8			
Summed feature 1	1.17	0.25			
Summed feature 3	32.13	45.42	40.5	35.0	
Summed feature 7	1.51	5.09			

a and b: Data from references [30-31], respectively.

Summed feature 1, fatty acids 15:1 iso H and 13:0 3OH could not be separated by GC with the MIDI system and therefore were considered together; summed feature 3, 16:1 ω7*c* and 15:0 iso 2OH; summed feature 7, unknown 18.846 (a fatty acid whose identity is unknown and whose equivalent chain−length is 18.846) and 19:1 ω6*c*.

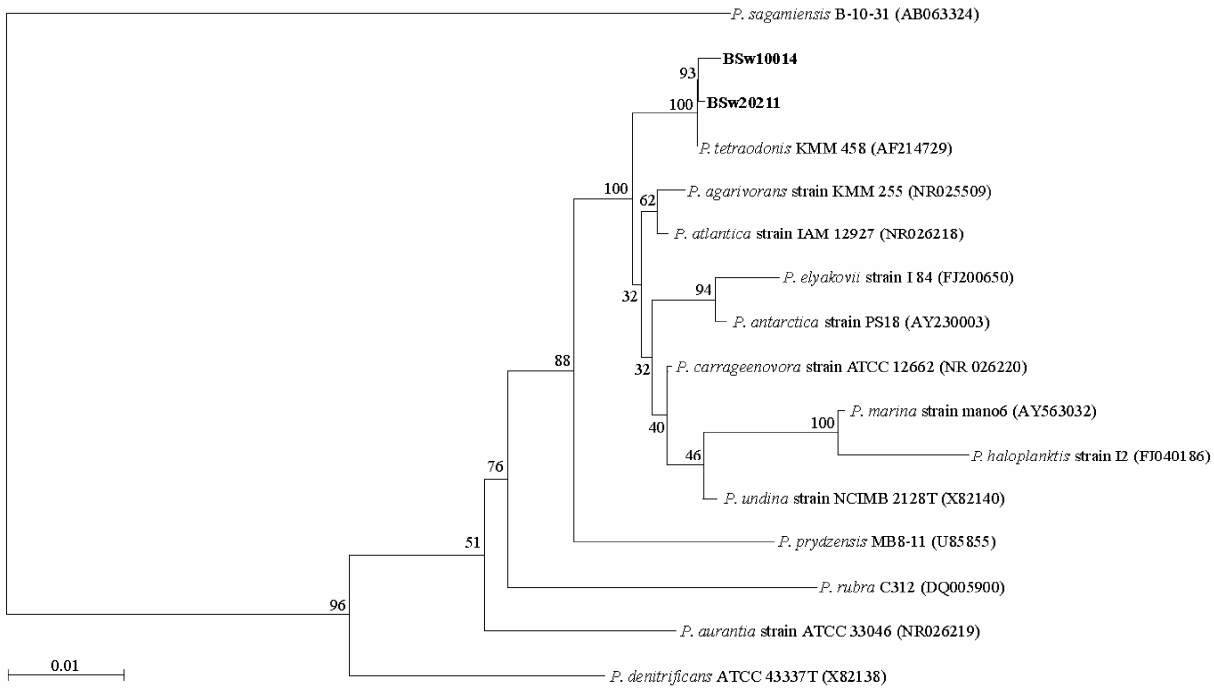

Figure 2 Phylogenetic tree obtained using neighbor-joining analysis of 16S rRNA gene sequences showing the positions of the two isolated strains, and related *Pseudoalteromonas* strains. Strain and accession numbers are indicated. Bootstrap values (100 replications) are shown as percentages at each node. Bar, 1 substitution per 100 nt.

2.6 Phylogenetic analysis of the *gyrB* gene

In comparing the *gyrB* gene sequences, strains BSw10014 and BSw20211 shared 97% and 93% sequence similarity to *Pseudoalteromonas haloplanktis* ATCC 14393^T (AF007279) respectively, higher than the species cut-off value of 90% [34]. At the same time, strains BSw10014 and BSw20211 showed 92% *gyrB* gene sequence similarity to each other. The two isolated strains only showed 89% and 88% *gyrB* gene sequence similarity to *Pseudoalteromonas tetraodonis* NCIMB 13 177^T (AF007 283), supporting species differentiation. The phylogenetic tree (Figure 3) showed that the two isolated strains formed a distinct cluster with *Pseudoalteromonas haloplanktis*, suggesting that strains BSw10014 and BSw20211 should be included in the species *Pseudoalteromonas haloplanktis*, instead of the species *Pseudoalteromonas tetraodonis* based on 16S rRNA gene sequences.

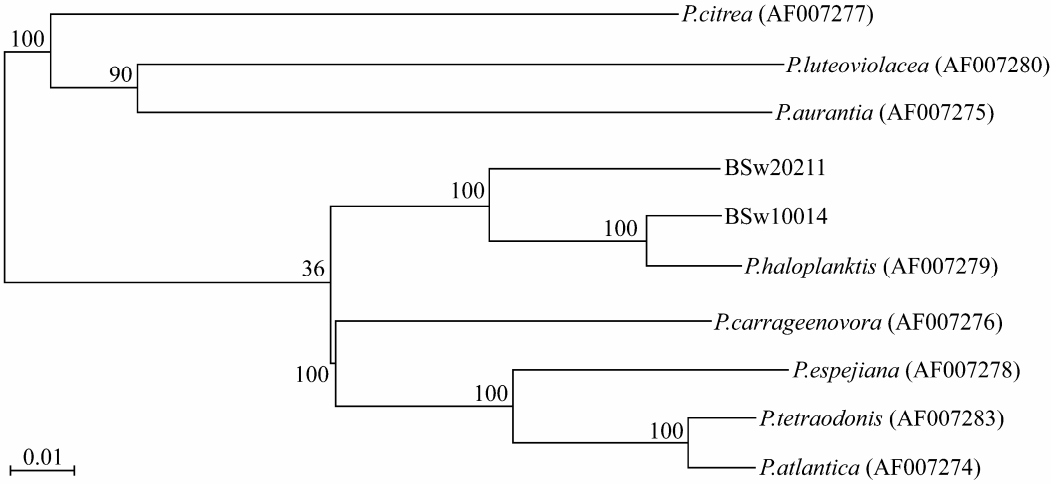

Figure 3 hylogenetic tree obtained using neighbor-joining analysis of *gyrB* gene sequences showing the positions of the two isolated strains, and related *Pseudoalteromonas* strains. Strain and accession numbers are indicated. Bootstrap values (100 replications) are shown as percentages at each node. Bar, 1 substitution per 100 nt.

2.7 Phylogenetic analysis of 16S-23S rRNA ITS

Two sets of primers were designed to amplify the 16S-23S rRNA ITS region using sequences in the 16S and 23S rRNA genes. Thus, two sequences with different length (580 bp and 908 bp) for each bacterial isolate were obtained from the PCR products. The sequence of the large fragment from strain BSw10014 showed 98.7% and 96.8% similarity to strain BSw20211 and *Pseudoalteromonas haloplanktis* TAC125 (CR954246), respectively. As for the small fragment, strain BSw10014 showed 96.9% and 94.7% sequence similarity to strain BSw20211 and *Pseudoalteromonas haloplanktis* TAC125, respectively. Results were consistent with the result based on *gyrB* gene analysis, suggesting that the two isolated strains were most similar to *Pseudoalteromonas haloplanktis*.

3 Discussion

Among hundreds of *Pseudoalteromonas* strains isolated from the Arctic and Antarctic seawater, strains BSw20211 from the Canada Basin and BSw10014 from the Southern Ocean showed significant 16S rRNA gene sequence similarity to each other, suggesting that the two bacterial strains might belong to the same species. However, analysis at the highly conservative gene level of 16S rRNA is not sufficient to determine the bacterial bipolar distribution at the species level, since 16S rRNA evolves so slowly that the specificity of probes based on 16S rRNA sequences may not always be high enough to distinguish closely related strains [21,30]. Although characterization of phenotypic properties, cellular fatty acid composition and DNA base composition supported that these two strains belonged to the genus *Pseudoalteromonas* and were closely related to each other, other analytical methods that could overcome the limitation of 16S rRNA gene sequence in the phylogenetic resolution of close taxa and elucidate diversity at the species and strain level, including DNA-DNA hybridization and protein coding gene analysis, were necessary to determine interspecies relationships between the two isolated strains.

Lack of congruence was observed between the phylogenetic trees based on 16S rRNA gene and *gyrB* gene (encoding DNA gyrase B subunit) sequences. Although the two strains showed 99.7%—99.8% 16S rRNA gene sequence similarity to *Pseudoalteromonas tetraodonis*, the 11%—12% variation seen in *gyrB* gene sequences between the two isolates and *Pseudoalteromonas tetraodonis* supports species differentiation. The base substitution frequency of *gyrB* is much higher than that of the 16S rRNA gene, making it a more appropriate choice for differentiating close phylogenetic relationships[30]. The *gyrB* gene sequences of *Pseudoalteromonas* sp. BSw10014 and BSw20211 showed significant similarities to type strain *Pseudoalteromonas haloplanktis* ATCC 14393^T, indicating that the two bacteria belong to the species *Pseudoalteromonas haloplanktis*. This was further supported by the sequence analysis of 16S-23S rRNA ITS, which evolves 10 times greater than 16S rRNA gene[35] and has become a powerful and reliable supplementary tool for intrageneric and intraspecific determination of phylogenetic relationships that are almost invisible in a 16S rRNA-based phylogeny[36]. However, the application of this potential phylogenetic marker is hampered by the limited number of 16S-23S rRNA ITS sequences deposited in GenBank. Compared with 16S rRNA gene (99.7%) and 16S-23S rRNA ITS (98.7% and 96.9% for large and small fragments, respectively), *gyrB* genes of strains BSw10014 and BSw20211 showed a lower sequence similarity (92%) to each other, suggesting that *gyrB* gene is a reliable molecular marker with higher resolution to define the phylogenetic relationships at the species level.

Strains BSw10014 and BSw20211 were classified as different species of the genus *Pseudoalteromonas* on the basis of a cut-off value of 70% DNA-DNA relatedness for a single species, although they showed 92% *gyrB* gene sequence similarity to each other. The classification achieved by *gyrB* sequence analysis is usually in agreement with results obtained with DNA-DNA hybridization[37]. Venkateswaran et al.[34] proposed a species cut-off value of 90% for *gyrB* sequences based on the polyphasic taxonomy of the genus *Shewanella*. However, comparison of *gyrB* gene sequences and DNA-DNA hybridization in the *Bacillus subtilis* group shows that strains with approximately 95% or higher *gyrB* gene sequence similarity exhibit DNA-DNA relatedness of > 70%[37], suggesting that the cut-off value of 90% *gyrB* gene sequence similarity for a species may be too low to determine interspecies relationships of the genus *Pseudoalteromonas*. The type species of the genus *Pseudoalteromonas* is *Pseudoalteromonas haloplanktis*, which includes two subspecies, *P. haloplanktis* subsp. *haloplanktis* and *P. haloplanktis* subsp. *tetraodonis* [6].

However, Ivanova et al.[31] reported that the type stains IAM 12 915^T and IAM 14 160^T of these two subspecies are only 48%—49% related to each other, and therefore they propose that the two type strains comprise two separate species, *P. haloplanktis* and *P. tetraodonis*. Based on the sequences of *gyrB* gene and 16 S-23 S rRNA ITS, strains BSw10014 should be classified as *P.*

haloplanktis. According to DNA-DNA relatedness, strain BSw20211 may belong to a single *Pseudoalteromonas* species different from strain BSw10014, although it shows close phylogenetic relationships to strain BSw10014 and *P. haloplanktis* members. Further data regarding the DNA-DNA hybridization with type strains of *Pseudoalteromonas* species is required to determine the phylogenetic position and taxonomic classification of the two isolated bacteria BSw10014 and BSw20211 at the species level.

Acknowledgments The authors appreciate the assistance of the Chinese Arctic and Antarctic Administration(CAA), SOA that organized the Chinese Arctic and Antarctic Expeditions, and thank Prof. John Hodgkiss of The University of Hong Kong for language improvement on this paper. This work was supported by the National Natural Science Foundation of China (Grant nos. 40676002, 40876097, 41076131), the National High Technology Research and Development Program of China (Grant no. 2008AA09Z408), the Program for Changjiang Scholars and Innovative Research Team in the University (Grant no.40821063), and China's Action Plan for the International Polar Year (IPY).

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