



Data in Brief

Genome sequence of three *Psychrobacter* sp. strains with potential applications in bioremediation



Aide Lasa, Jesús L. Romalde *

Departamento de Microbiología y Parasitología, CIBUS-Facultad de Biología, Universidade de Santiago de Compostela, Campus Vida s/n. 15782, Santiago de Compostela, Spain

ARTICLE INFO

Article history:

Received 29 December 2016

Received in revised form 3 January 2017

Accepted 25 January 2017

Available online 14 February 2017

Keywords:

Psychrobacter

Genome sequencing

Mercury detoxification

Virulence factors

Phylogenetic analysis

ABSTRACT

To date, the genus *Psychrobacter* consists of 37 recognized species isolated from different sources, however they are more frequently found in cold and other non-polar environments of low water activity. Some strains belonging to the genus have shown different enzymatic activities with potential applications in bioremediation or food industry. In the present study, the whole genome sequences of three *Psychrobacter*-like strains (C 20.9, Cmf 22.2 and Rd 27.2) isolated from reared clams in Galicia (Spain) are described. The sequenced genomes resulted in an assembly size of 3,143,782 bp for C 20.9 isolate, 3,168,467 bp for Cmf 22.2 isolate and 3,028,386 bp for Rd 27.2 isolate. Among the identified coding sequences of the genomes, mercury detoxification and biogeochemistry genes were found, as well as genes related to heavy metals and antibiotic resistance. Also virulence-related features were identified such as the siderophore vibrioferrin or an aerobactin-like siderophore. The phylogenetic analysis of the 16S rRNA gene suggested that these strains may represent novel species of the *Psychrobacter* genus. The genome sequences of the *Psychrobacter* sp. strains have been deposited at DDBJ/EMBL/GenBank under the accession numbers MRYA00000000 (Cmf 22.2), MRYB00000000 (Rd 27.2) and MRYC00000000 (C 20.9), and the sequences could be found at the site <https://www.ncbi.nlm.nih.gov/bioproject/PRJNA353858>.

© 2017 The Authors. Published by Elsevier Inc. This is an open access article under the CC BY-NC-ND license (<http://creativecommons.org/licenses/by-nc-nd/4.0/>).

Specifications	
Organism/cell line/tissue	<i>Psychrobacter</i> spp.
Strain	C 20.9, Cmf 22.2, Rd 27.2
Sex	N/A
Sequencer or array type	Illumina paired-end
Data format	Analyzed
Experimental factors	Genomic DNA extracted from pure bacterial isolated from clams
Experimental features	Draft genome sequences of <i>Psychrobacter</i> sp. C 20.9, Cmf 22.2 and Rd 27.2, assembly and annotation
Consent	N/A
Sample source locations	Galicia, Spain. C 20.9 (8° 47' 0"N, 42° 37' 0" W), Cmf 22.2 (43° 07' 48, N, 9° 11' 06" W), Rd 27.2 (42° 17' 00" N, 8° 36' 00" W)

1. Direct link to deposited data

<https://www.ncbi.nlm.nih.gov/bioproject/PRJNA353858>

* Corresponding author.

E-mail address: jesus.romalde@usc.es (J.L. Romalde).

2. Introduction

The genus *Psychrobacter* was first proposed by Juni and Heym [9], with the description of *Psychrobacter immobilis* as the type species, to accommodate a non-motile and psychrotolerant bacterium with aerobic metabolism. To date, the genus *Psychrobacter* consists of 37 recognized species isolated from a great variety of sources including fish, poultry, meat products and human pathological specimens. Members of the genus have been detected in air samples collected from different geographical locations including north-western Colorado [3] and the Baltic Sea coast [6]. Although *Psychrobacter* species have a global distribution, they are most frequently found in cold and other non-polar environments of low water activity [18]. Thus, at least 18 species of the genus were isolated from low-temperature environments, including Antarctic glacier mud and sediment [5], Antarctic ornithogenic soils [4], sea ice [19], alpine soil [20], Siberian permafrost [1] and Arctic seawater [23].

Different strains of this genus are of industrial interest since they have shown different enzymatic activities with potential applications in bioremediation or food industry [17]. Microbially induced carbonate precipitation (MICP) is a recent well-recognized process that has the potential to precipitate heavy metals [10]. Some

Table 1
Genome statistics for the three clams isolates.

Attribute	C 20.9	Cmf 22.2	Rd 27.2
Genome size (bp)	3,143,782	3,168,467	3,028,386
Contigs	60	100	197
N50	151,201	640,377	182,392
G + C content	43.9	42.6	47.1
Coding sequences	2,777	2,652	2,596
Total RNA genes	52	47	46
tRNA genes	44	44	43
CRISPR repeats	–	–	7

Psychrobacter strains have shown valuable activities involved in bio-remediation by producing carbonic anhydrase enzyme [13]. On the other hand, the genome sequence of *P. alimentarius* displayed two interesting pathways involved in the biosynthesis of terpenoids and benzoate degradation [12].

In this study, we report the complete genome sequences of three strains (C 20.9, Cmf 22.2 and Rd 27.2) isolated from reared clams in Galicia (Spain) and designated as *Psychrobacter* spp., that will provide fundamental information for further research.

3. Methods

3.1. Sample collection and identification

Psychrobacter isolates C 20.9, Cmf 22.2 and Rd 27.2 were obtained between 2007 and 2008 during a sampling program of reared clams in Galicia (NW Spain). The isolates were cultured in Marine Agar (MA, Pronadisa) at 25 °C for 24 h and stored at –80°C in Marine Broth containing 15% glycerol. Phenotypic characterization and 16S rRNA gene sequence analysis allowed the identification of these strains as members of the *Psychrobacter* genus. C 20.9 strain is phylogenetically most closely related to *P. piscatorii* (98.1%), Cmf 22.2 to *P. maritimus* (98.1%) and Rd 27.2 to *P. celer* (98.2%).

3.2. Genome sequencing, assembly and annotation

High Pure PCR Template Preparation kit (Roche) was employed for isolation of genomic DNA for whole genome sequencing at Sistemas Genómicos (Valencia, Spain) using Illumina paired-end sequencing technology. The Illumina reads were analyzed for quality control using FASTQC (Brabraham Bioinformatics). Reads were trimmed and filtered to remove adapters and low quality bases, using Trimmomatic 0.32 [2] program. The remaining reads were used for the genome assembly, performed with the SPAdes 3.6.2 the novo assembler tool [15], and QUAST [8] software was used to evaluate the assembly.

The draft genomes of the three strains were annotated using the Rapid Annotations using Subsystems Technology (RAST) server [16] and tRNAs were identified by tRNAscan-SE v1.21 [14]. CRISPRfinder tool [7] was used to assess the presence of CRISPR repeats in the genomes of the clam isolates. The G + C content of the chromosomal DNA was calculated on the basis of its whole genome sequence.

3.3. Phylogenetic analysis

To evaluate the relatedness among the three *Psychrobacter* sp. strains and the closest relatives, 16S rRNA sequences were aligned using CLUSTALW tool [11], and phylogenetic trees were reconstructed using the neighbour-joining (NJ) algorithms in MEGA software package version 6.06 [22].

4. Results

The genome assembly of the *Psychrobacter* sp. strains resulted in a genome size of 3,143,782 bp for C 20.9 isolate, 3,168,467 bp for Cmf 22.2 isolate and 3,028,386 for Rd 27.2 isolate. The G + C content of C 20.9, Cmf 22.2 and Rd 27.2 strains was 43.9%, 42.6% and 47.1% respectively. The genomic features of the C 20.9 included 2777 coding sequences and 52 RNAs, of which 44 were transfer RNA sequences. Meanwhile, Cmf 22.2 genome contained 2652 coding sequences and 47 RNAs sequences, including 44 tRNA sequences. The Rd 27.2 genome displayed a total of 2596 coding sequences and 46 RNAs, of which 43 were tRNAs. CRISPR arrays were only found in the Rd 27.2 genome that included Cas1, Cas2, Cas3, Cas5e and Cse1–4 family proteins (Table 1).

According to the annotation results, the genomes of the clam isolates revealed the presence of genes responsible for resistance to antibiotics and toxic compounds, copper homeostasis, copper tolerance, cobalt-zinc-cadmium resistance, resistance to fluoroquinolones, arsenic resistance, and multidrug resistance efflux pump subsystems. In addition, the analysis identified mercuric reductase in the three strains involved in mercury detoxification and biogeochemistry. Also, C 20.9 displayed a mercury resistance operon (Fig. 1), which included a regulatory protein (MerR), transport proteins (MerT and MerC), a periplasmic mercury binding protein (MerP) and a mercury ion reductase.

Virulence-related proteins were found in the genomes that could play roles in bacterial pathogenicity and virulence. For instance, a cluster of five genes for biosynthesis of the siderophore vibrioferrin was found in Cmf 22.2 and Rd 27.2 strains. On the other hand, in C 20.9 strain an aerobactin-like siderophore was annotated.

Sequence similarity of the 16S rRNA gene of the *Psychrobacter* sp. strains isolated from clams is below the threshold (98.7%) proposed by Stackebrandt and Ebers [21] for delimitation of new bacterial

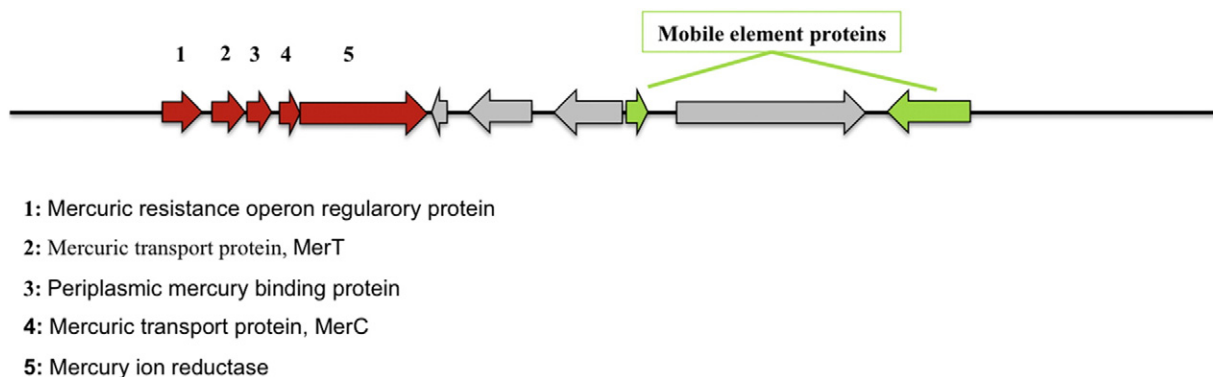


Fig. 1. Representation of the genomic region containing the mercuric resistance operon in the C 20.9 strain.

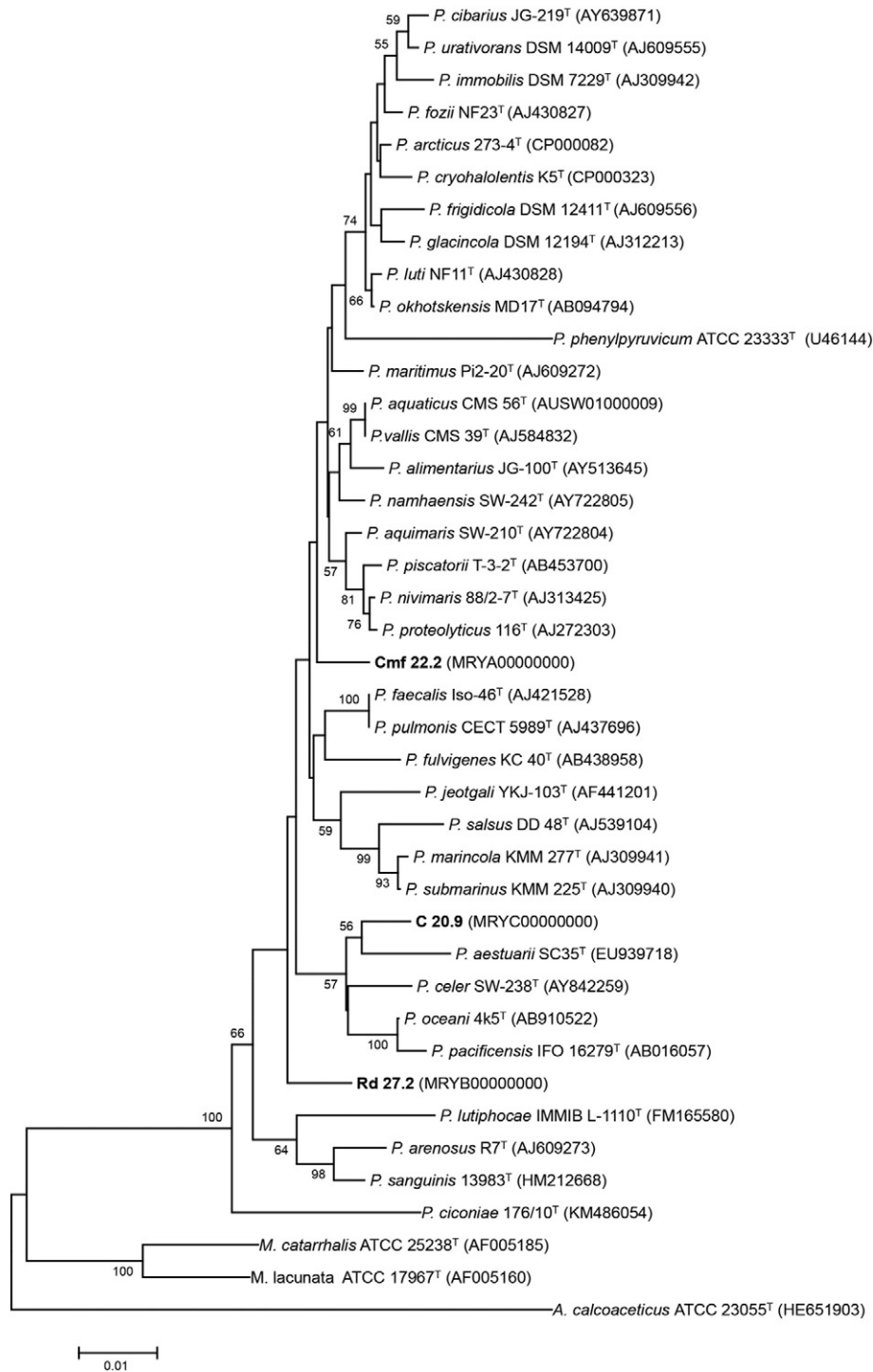


Fig. 2. Phylogenetic tree based on 16S rRNA gene sequences using the Neighbour-Joining algorithm, showing the relationships of *Psychrobacter* sp. strains within the genus. Bootstrap values (>50%) based on 1000 replications are shown at the nodes of the trees. Bar, substitutions per nucleotide position. Accession numbers for all *Psychrobacter* strains included in the study are given in parentheses.

species. The phylogenetic reconstruction based on the 16S rRNA gene sequences showed that the clam isolates can be distinguished among them and to the closest relatives (Fig. 2). These results suggest that each isolate may constitute a novel species within the *Psychrobacter* genus.

5. Nucleotide sequence accession number

This Whole Genome Shotgun projects has been deposited at DDBJ/ENA/GenBank under the accession numbers MRYA00000000 (Cmf 22.2),

MRYB00000000 (Rd 27.2) and MRYC00000000 (C 20.9). The versions described in this paper are version MRYA01000000, MRYB01000000 and MRYC01000000 respectively.

Conflict of interest statement

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Acknowledgements

This research was supported in part by grants AGL2013-42628-R and AGL2016-77539-R from Ministerio de Economía y Competitividad (Spain).

References

- [1] C. Bakermans, H.L. Ayala-del-Río, M.A. Ponder, T. Vishnivetskaya, D. Gilichinsky, M.F. Thomashow, J.M. Tiedje, *Psychrobacter cryohalolentis* sp. nov. and *Psychrobacter arcticus* sp. nov., isolated from Siberian permafrost. *Int. J. Syst. Evol. Microbiol.* 56 (2006) 1285–1291.
- [2] A.M. Bolger, M. Lohse, B. Usadel, Trimmomatic: a flexible trimmer for Illumina sequence data. *Bioinformatics* 30 (2014) 2114–2120.
- [3] R.M. Bowers, C.L. Lauber, C. Wiedinmyer, M. Hamady, A.G. Hallar, R. Fall, R. Knight, N. Fierer, Characterization of airborne microbial communities at a high elevation site and their potential to act as atmospheric ice nuclei. *Appl. Environ. Microbiol.* 75 (2009) 5121–5130.
- [4] J.P. Bowman, J. Cavanagh, J.J. Austin, K. Sanderson, Novel *Psychrobacter* species from Antarctic ormithogenic soils. *Int. J. Syst. Bacteriol.* 46 (1996) 841–848.
- [5] N. Bozal, M.J. Montes, E. Tudela, J. Guinea, Characterization of several *Psychrobacter* strains isolated from Antarctic environments and description of *Psychrobacter luti* sp. nov. and *Psychrobacter fozii* sp. nov. *Int. J. Syst. Evol. Microbiol.* 53 (2003) 1093–1100.
- [6] C. Fahlgren, A. Hagström, D. Nilsson, U.L. Zweifel, Annual variations in the diversity, viability, and origin of airborne bacteria. *Appl. Environ. Microbiol.* 76 (2010) 3015–3025.
- [7] I. Grissa, G. Vergnaud, C. Pourcel, CRISPRFinder: a web tool to identify clustered regularly interspaced short palindromic repeats. *Nucleic Acids Res.* 35 (2007) W52–W57.
- [8] A. Gurevich, V. Saveliev, N. Vyahhi, G. Tesler, QUASt: quality assessment tool for genome assemblies. *Bioinformatics* 29 (2013) 1072–1075.
- [9] E. Juni, G.A. Heym, *Psychrobacter immobilis* gen. nov., sp. nov.: genospecies composed of gram-negative, aerobic, oxidase-positive coccobacilli. *Int. J. Syst. Bacteriol.* 36 (1986) 388–391.
- [10] D. Kumari, X.Y. Qian, X. Pan, V. Achal, Q. Li, G.M. Gadd, Microbially induced carbonate precipitation for immobilization of toxic metals. *Adv. Appl. Microbiol.* 94 (2016) 79–108.
- [11] M.A. Larkin, G. Blackshields, N.P. Brown, R. Chenna, P.A. McGettigan, H. McWilliam, F. Valentin, I.M. Wallace, A. Wilm, R. Lopez, J.D. Thompson, T.J. Gibson, D.G. Higgins, Clustal W and Clustal X version 2.0. *Bioinformatics* 23 (2007) 2947–2948.
- [12] J. Lee, M. Kwon, J.Y. Yang, J. Woo, H.K. Lee, S.G. Hong, O.S. Kim, Complete genome sequence of *Psychrobacter alimentarius* PAMC 27889, a psychrophile isolated from an Antarctic rock sample. *Genome Announc.* 4 (4) (2016) e00704–e00716.
- [13] M. Li, X. Zhu, S. Wilkinson, M. Huang, V. Achal, Complete genome sequence of carbonic anhydrase producing *Psychrobacter* sp. SHUES1. *Front. Microbiol.* 7 (2016) 1442.
- [14] T.M. Lowe, S.R. Eddy, tRNAscan-SE: a program for improved detection of transfer RNA genes in genomic sequence. *Nucleic Acids Res.* 25 (5) (1997) 0955–0964.
- [15] S. Nurk, A. Bankevich, D. Antipov, A. Gurevich, A. Korobeynikov, A. Lapidus, A. Prjibelsky, A. Pyshkin, A. Sirotkin, Y. Sirotkin, R. Stepanauskas, J. McLean, R. Lasken, S.R. Clingenpeel, T. Woyke, G. Tesler, M.A. Alekseyev, P.A. Pevzner, Assembling genomes and mini-metagenomes from highly chimeric reads. *Lect. N. Bioinform.* 7821 (2013) 158–170.
- [16] R. Overbeek, R. Olson, G.D. Pusch, G.J. Olsen, J.J. Davis, T. Disz, R.A. Edwards, S. Gerdes, B. Parrello, M. Shukla, V. Vonstein, A.R. Wattam, F. Xia, R. Stevens, The SEED and the rapid annotation of microbial genomes using subsystems technology (RAST). *Nucleic Acids Res.* 42 (D206–D214 Genomics. 9) (2014) 75.
- [17] S. Ozturkoglu-Budak, A. Wiebenga, P.A. Bron, R.P. de Vries, Protease and lipase activities of fungal and bacterial strains derived from an artisanal raw ewe's milk cheese. *Int. J. Food Microbiol.* 237 (2016) 17–27.
- [18] D.F. Rodrigues, E. da C. Jesus, H.L. Ayala-Del-Río, V.H. Pellizari, D. Gilichinsky, L. Sepulveda-Torres, J.M. Tiedje, Biogeography of two cold-adapted genera: *Psychrobacter* and *Exiguobacterium*. *ISME J.* 3 (2009) 658–665.
- [19] L.A. Romanenko, A.M. Lysenko, M. Rohde, V.V. Mikhailov, E. Stackebrandt, *Psychrobacter maritimus* sp. nov. and *Psychrobacter arenosus* sp. nov., isolated from coastal sea ice and sediments of the Sea of Japan. *Int. J. Syst. Evol. Microbiol.* 54 (2004) 1741–1745.
- [20] T.N.R. Srinivas, S.M. Singh, S. Pradhan, M.S. Pratibha, K.H. Kishore, A.K. Singh, Z. Begum, S.R. Prabakaran, G.S.N. Reddy, S. Shivaji, Comparison of bacterial diversity in proglacial soil from Kafni Glacier, Himalayan Mountain ranges, India, with the bacterial diversity of other glaciers in the world. *Extremophiles* 15 (2011) 673–690.
- [21] E. Stackebrandt, J. Ebers, Taxonomic parameters revisited: tarnished gold standards. *Microbiol. Today* 33 (2006) 152–155.
- [22] K. Tamura, D. Peterson, N. Peterson, G. Stecher, M. Nei, S. Kumar, MEGA6: molecular evolutionary genetics analysis using maximum likelihood, evolutionary distance, and maximum parsimony methods. *Mol. Biol. Evol.* 28 (2011) 2731–2739.
- [23] Y.X. Zeng, Y. Yu, H.R. Li, W. Luo, *Psychrobacter fjordensis* sp. nov., a psychrotolerant bacterium isolated from an Arctic fjord in Svalbard. *Antonie Van Leeuwenhoek* 108 (2015) 1283–1292.