Polar Biology (2022) 45:691–701
https://doi.org/10.1007/s00300-022-03028-1

ORIGINAL PAPER

Genomic analyses of a novel bioemulsifier-producing *Psychrobacillus* strain isolated from soil of King George Island, Antarctica

Mariana Barbalho Farias da Silva[1] · Fabio Faria da Mota[2] · Diogo Jurelevicius[1] · Vasco Ariston de Carvalho Azevedo[3] · Mateus Matiuzzi da Costa[4] · Aristóteles Góes-Neto[3] · Rommel Thiago Jucá Ramos[5] · Siomar de Castro Soares[6] · Alexandre Soares Rosado[7] · Lucy Seldin[1]

Received: 15 November 2021 / Revised: 2 February 2022 / Accepted: 14 February 2022 / Published online: 25 February 2022

Abstract

Cold-adapted bacterial strains are potentially valuable for biotechnological applications involving the production of cold-active enzymes and bioproducts important to various industries. A psychrotolerant, aerobic, Gram-positive, endospore-forming, bioemulsifier-producing strain, named Val9, was isolated from Vale Ulman soil samples, King George Island, Antarctica and identified as a member of the genus *Psychrobacillus*. To better characterize this novel strain, its whole genome was sequenced revealing a size of 3,986,526 bp with a G + C content of 36.6%, and 4042 predicted coding DNA sequences (CDSs). Digital DNA–DNA hybridization (dDDH) and average nucleotide identity (ANI) analyses between strain Val9 and the type strains of the seven *Psychrobacillus* species revealed that the highest values were observed with *Psychrobacillus psychrodurans* DSM11713^T but below the conventional thresholds of 70% dDDH and 95% ANI for bacterial species assignment, suggesting that strain Val9 could represent a distinct species. As potential low-temperature adaptation strategies, genes encoding cold shock proteins, transporters for glycine-betaine, carnitine and choline, and enzymes acting against oxidative stress were found in Val9 genome. DEAD-box RNA helicases, important for cold and oxidative tolerance, and a two-component signal transduction system related to plasmatic membrane fluidity as well as biotechnologically important CDSs, related to levan production, were detected. The *sacB* gene encoding the enzyme levansucrase was exclusive for Val9 and it was not found in the other *Psychrobacillus* type strains. Altogether, the comparative genomic analyses presented here highlight important metabolic pathways and the biotechnological potential of this novel strain.

Keywords *Psychrobacillus* · Genome · Antarctica · Bioemulsifier · Low-temperature adaptation

✉ Lucy Seldin
lseldin@micro.ufrj.br

1 Laboratório de Genética Microbiana, Departamento de Microbiologia Geral, Instituto de Microbiologia Paulo de Góes (IMPPG), Centro de Ciências da Saúde (CCS), Bloco I, Universidade Federal do Rio de Janeiro, Ilha do Fundão, Rio de Janeiro CEP 21941-902, Brazil

2 Instituto Oswaldo Cruz, Rio de Janeiro, Brazil

3 Instituto de Ciências Biológicas, Universidade Federal de Minas Gerais, Belo Horizonte, MG, Brazil

4 Universidade Federal do Vale do São Francisco, Petrolina, Brazil

5 Instituto de Ciências Biológicas, Universidade Federal do Pará, Belém, PA, Brazil

6 Instituto de Ciências Biológicas e Naturais, Universidade Federal do Triângulo Mineiro, Uberaba, MG, Brazil

7 KAUST, King Abdullah University of Science and Technology, Thuwal, Kingdom of Saudi Arabia

Introduction

The species *Bacillus psychrotolerans* and *Bacillus psychrodurans* were described as psychrotolerant species of the genus *Bacillus* in 2002 (Abd El-Rahman et al. 2002). Some decades before, *Bacillus insolitus* was proposed as a psychrophilic species whose strains were isolated from soil (Larkin and Stokes 1967). After a detailed polyphasic taxonomic study of these *Bacillus* species—using the type strains *B. insolitus* DSM 5^T, *B. psychrotolerans* DSM 11706^T, and *B. psychrodurans* DSM 11713^T—the three species were considered distinct from other members of *Bacillus* rRNA group 2. As a result, the genus *Psychrobacillus* was created in 2010, with *B. insolitus* as the type species of the genus (Krishnamurthi et al. 2010). The new genus *Psychrobacillus* was described harboring Gram-positive, endospore-forming motile rods, and strictly aerobic bacteria. Their G + C

content of the genomic DNA ranged from 35.7 to 36.6 mol%, and the three species shared high 16S rRNA gene sequence similarities among them (97.8–99.7%) (Krishnamurthi et al. 2010). Later, new *Psychrobacillus* species were described: *Psychrobacillus soli* (Pham et al. 2015), *Psychrobacillus lasiicapitis* (Shen et al. 2017), *Psychrobacillus vulpis* (Rodríguez et al. 2020), and *Psychrobacillus glaciei* (Choi and Lee 2020). Therefore, currently, the genus *Psychrobacillus* is composed of seven validly published species (lpsn.dsmz.de/genus/psychrobacillus). Strains belonging to different species of *Psychrobacillus* were isolated worldwide from different kinds of soils (Krishnamurthi et al. 2010; Pham et al. 2015), from feces of a red fox (Rodríguez et al. 2020), the head of an ant (Shen et al. 2017), and an iceberg in Antarctica (Choi and Lee 2020). Vollú et al. (2014) described the isolation of 80 spore-forming and cold-adapted bacterial strains from nine different soil samples of King George Island, in maritime Antarctica, including different *Psychrobacillus* strains.

It is widely known that spore-forming and cold-adapted bacterial strains are resistant to harsh conditions, and they are also potentially valuable for biotechnological applications involving the production of cold-active enzymes and bioproducts important to food, pharmaceutical, cosmetics, fine chemical, and other industries (Margesin et al. 2005; Kuddus 2018; Al-Maqtari et al. 2019). Therefore, the interest in cold-adapted microorganisms has increased in an attempt to contribute for a potential source of cold-active biomaterials. For example, Vollú et al. (2014) determined the ability to produce extracellular enzymes (esterase, caseinase, amylase, and gelatinase), antimicrobial substances (against *Staphylococcus aureus* and *Candida albicans*), and biosurfactants in all spore-forming bacterial strains isolated from Antarctic soils.

One strain denoted as Val9 (Vollú et al. 2014)—previously identified as *Bacillus psychrodurans* and later reclassified as *Psychrobacillus* sp.—was chosen for further studies as it was able to produce a bioemulsifier (BE) in low temperatures, in laboratory conditions. Bioemulsifiers derived from microbial sources can be used more efficiently in the food and drug industries than synthetic emulsifiers, because of their nutritional benefits (Alizadeh-Sani et al. 2018). Bioemulsifiers are considered high molecular weight biopolymers or exopolysaccharides (EPS), constituted of complex mixtures of heteropolysaccharides, lipopolysaccharides, lipoproteins, and/or proteins (Uzoigwe et al. 2015). Alasan (Navon-Venezia et al. 1995), emulsan (Rosenberg et al. 1979), and levan (Haddar et al. 2021) are examples of well-studied bioemulsifiers. Conversely, studies of bioemulsifiers produced by cold-adapted bacteria are still incipient. Therefore, a more in-depth study of strain Val9 may provide a new model strain for basic and biotechnological research within the genus *Psychrobacillus*. Performing a comparative analysis of the genomes of the different *Psychrobacillus* species, we can contribute not only for the taxonomy but also for the biotechnological relevance of the genus.

Herein, we report the genomic characterization of the psychrotolerant strain Val9, which was isolated from soil collected in Vale Ulman, King George Island, Antarctica, highlighting important metabolic pathways and pieces of evidence that suggest its identification as a novel *Psychrobacillus* species.

Materials and methods

Bacterial strain, culture conditions, and DNA extraction

The bacterial strain studied here—Val9—was isolated from Vale Ulman soil samples, King George Island, Antarctica (Vollú et al. 2014). A map showing the location of the study site is shown in Online Resource 1. Strain Val9 was stored in trypticase soy broth (TSB) containing 20% glycerol at − 80 °C. The same medium was used for growth at 15 °C for 48 h.

DNA from strain Val9 was isolated according to the method described in Seldin et al. (1998). Further purification steps were those described in Seldin and Dubnau (1985). The DNA was quantified spectrophotometrically using a Qubit™ fluorimeter (Thermo Fisher Scientific, MA, USA).

Sequencing of 16S rRNA encoding gene from strain Val9 and phylogenetic analysis

The gene encoding 16S rRNA from Val9 was amplified by PCR using the pair of universal primers pA and pH and the conditions described in Massol-Deya et al. (1995), and the products sequenced using Macrogen (South Korea) facilities. For phylogenetic tree analysis, the sequences of closely related *Psychrobacillus* strains were recovered from GenBank database and aligned to the sequence obtained in this study using the online Multiple alignment program MAFFT version 7 (https://mafft.cbrc.jp/alignment/software/). The phylogenetic analyses were performed using the RaxML-HPC2 model in CIPRES Science Gateway (Miller et al. 2010), with the phylogenetic tree inference using maximum likelihood/rapid bootstrapping run. The sequence generated in this study was deposited in NCBI GenBank under accession number KF026354.1.

Genome sequencing and assembly

The amount of 5 µg μl^{-1} of gDNA was considered for the construction of paired-end sequencing libraries (2 × 150 bp) of 450 bp insert length following the manufacturer's protocol

for the NEBNext® Fast DNA Fragmentation and Library Preparation Kit (New England Biolabs Inc., MA, USA). Final library-quality analysis was performed via 2100 bioanalyzer (Agilent Technologies, CA, USA) with read length gDNA size control using agarose gel electrophoresis. All samples were sequenced on the Illumina Hi-Seq 2500 platform as recommended by the manufacturer.

The genome assembly process started checking the quality of the reads through FastQC (Andrews 2010) and Adapter Removal to remove the bases with quality below Phred 20 (Lindgreen 2012) softwares. The estimated best five k-mers were selected by KmerStream (Melsted and Halldórsson 2014) after checking the values from 7-mers to 127-mers, followed by the assembly using SPAdes with the five best *k*-mers (Bankevich et al. 2012).

Average nucleotide identity (ANI) and digital DNA–DNA hybridization (dDDH)

The reference draft genomes of *P. psychrodurans* DSM 11713 (NZ_FOUN00000000.1), *P. psychrotolerans* DSM 11706 (NZ_FOXU00000000.1), *P. glaciei* PB01 (NZ_CP031223.1), *P. soli* NHI-2 (NZ_VDGG00000000.1), *P. insolitus* DSM 5 (NZ_QKZI00000000.1), *P. lasiicapitis* NEAU-3TG517 (NZ_VDGH00000000.1), and *P. vulpis* Z8 (NZ_VDGI00000000.1) were downloaded from NCBI (www.ncbi.nlm.nih.gov/refseq). The Val9 genome was compared with the seven related type strains using the JSpeciesWS database (http://jspecies.ribohost.com/jspeciesws/) with two alignment algorithms: mummer (ANIm) and blastn (ANIb).

DNA digital hybridization (dDDH) was performed using the Genome-to-Genome Distance Calculator—GGDC 2.1 (Meier-Kolthoff et al. 2013) provided by Leibniz on the DSMZ Institute website (http://ggdc.dsmz.de/distcalc2.php) with the recommended parameters and/or default settings.

Genome annotation

The automatic annotation of the Val9 genome and related *Psychrobacillus* strains was performed using the RAST online server (Aziz et al. 2008) and GOFEAT (http://computationalbiology.ufpa.br/gofeat/). KEGG (www.genome.jp/kegg) and Metacyc (https://metacyc.org/) databases were used for the manual annotation and the construction of the metabolic pathways. The pathways according to genome annotation of strain Val9 were created with BioRender.com.

Comparative genomics

A comparative genome map was plotted through a BLASTN-based ring generated by BLAST ring image generator (BRIG) version 0.95 (Alikhan et al. 2011) to compare the draft genomes of the seven *Psychrobacillus* type strains. The *Psychrobacillus* strain Val9 was used as reference. The prediction of orthologous genes among the *Psychrobacillus* genomes was performed using the software program OrthoFinder v2.5.4 (Emms and Kelly 2015). A manual annotation of proteins was also performed using GO FEAT and BLASTp, and KEGG database (www.genome.jp/kegg) was used to understand the possible metabolic pathways in which some proteins are embedded.

Results

Phylogenetic analysis of 16S rRNA encoding gene

Results of BLAST sequence analyses of the 16S rRNA encoding gene (1474 bp) indicated that the strain, previously isolated from Antarctic soil and named Val9 (Vollú et al. 2014), is related to members of the genus *Psychrobacillus* (Fig. 1). Its closest relatives were *P. psychrotolerans* DSM 11706^T, *P. psychrodurans* DSM 11713^T, and *P. glaciei* PB01^T, with 99.92, 99.79, and 99.25% gene sequence similarities, respectively.

Genome sequence analyses

The draft genome sequence of strain Val9 was determined in this study, and the Whole Genome Shotgun project has been deposited at DDBJ/ENA/GenBank under the accession number JAIZDB000000000. The version described in this paper is version JAIZDB010000000. The genome of strain Val9 reveals 3,986,526 bp with a G + C content of 36.6%, and a total of 4042 coding DNA sequences (CDSs) were predicted. The identified CDSs were classified into subsystems, such as carbohydrates (174 CDSs), amino acids and derivatives (273 CDSs), protein metabolism (146 CDSs), RNA metabolism (60 CDSs), and stress response (48 CDSs) (Online Resource 2).

In an attempt to phylogenetically classify the proteins encoded in the Val9 genome within the genus *Psychrobacillus*, the orthologous groups were predicted using the seven type strain genomes available for the genus. The analyses revealed 265 proteins found exclusively in Val9, but 199 proteins showed to be hypothetical ones (Online Resource 3).

To elucidate the taxonomic relatedness between Val9 and the other known *Psychrobacillus* species, the ANI and dDDH values were determined between strain Val9 and the other seven type genomes of the members of the genus *Psychrobacillus* (Table 1). The ANI values varied between 75.95 and 85.46% considering ANIb and 83.98–87.37% in ANIm. These values are considered below the accepted threshold for species delimitation using ANI (95–96%). Moreover, the in silico DDH results were in all cases lower

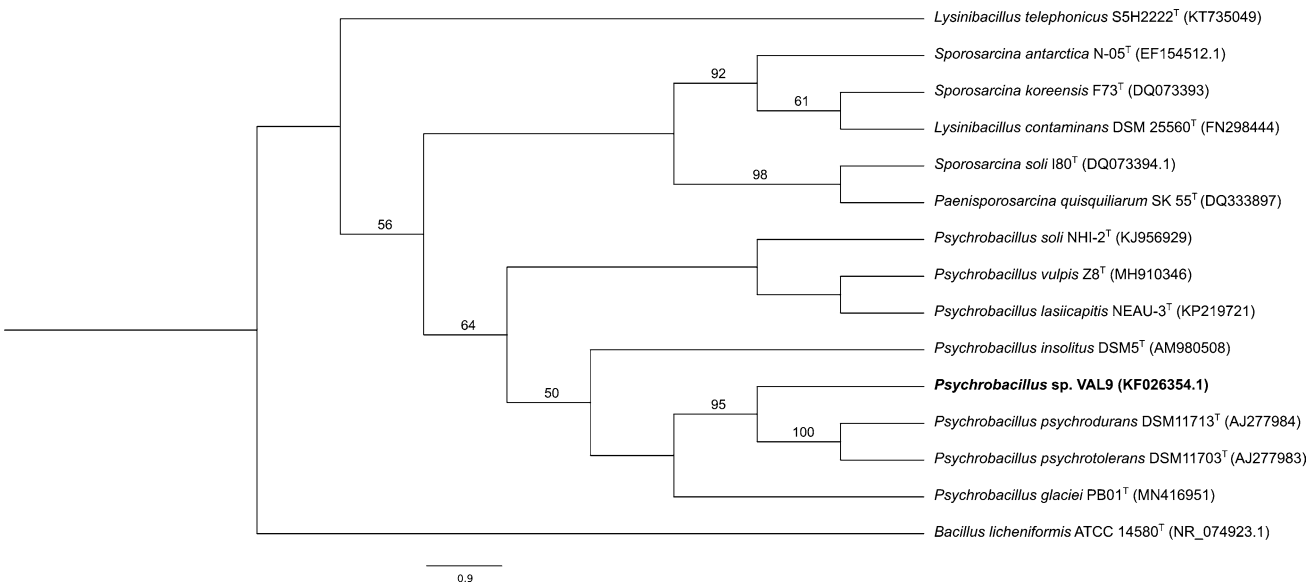


Fig. 1 Multiple alignment of the 16S rRNA encoding gene of *Psychrobacillus* sp. Val9 and related species. The maximum likelihood tree was constructed based on GTRGAMMA distribution. GenBank accession number of each sequence is shown in parenthesis. Bootstrap values are expressed as percentages of 1000 replications, and are shown at branch points. *Bacillus licheniformis* ATCC 14580^{T} was used as outgroup. *Bar* substitutions per nucleotide position

Table 1 dDDH and ANI values between Val9 and closely related species. Numbers between brackets after dDDH values are the confidence intervals

Reference genomes	Accession number	DDH (%)	ANIb	ANIm
Psychrobacillus psychrodurans DSM 11713	FOUN01000007.1	37.30 [34.8–39.8]	85.46 (61.55)	87.37 (60.74)
Psychrobacillus psychrotolerans DSM 11706	FOXU01000007.1	36 [33.6–38.6]	85.25 (62.61)	86.98 (61.19)
Psychrobacillus vulpis Z8	VDGI01000018.1	26.50 [24.2–29]	76.01 (51.83)	83.98 (13.32)
Psychrobacillus lasiicapitis NEAU-3TGS17	VDGH01000003.1	26.40 [24–28.9]	76.24 (55.62)	84.00 (15.11)
Psychrobacillus soli NHI-2	VDGG01000009.1	29.90 [27.5–32.4]	76.67 (54.03)	84.10 (15.11)
Psychrobacillus insolitus DSM 5	QKZI01000006.1	25.70 [23.4–28.2]	76.32 (45.69)	85.01 (11.69)
Psychrobacillus glaciei PB01	NZ_CP031223.1	30.50 [28.54–31.9]	75.95 (51.79)	84.12 (12.64)

The numbers between parentheses after values of ANIb and ANIm are % of conserved aligned DNA between two genomes

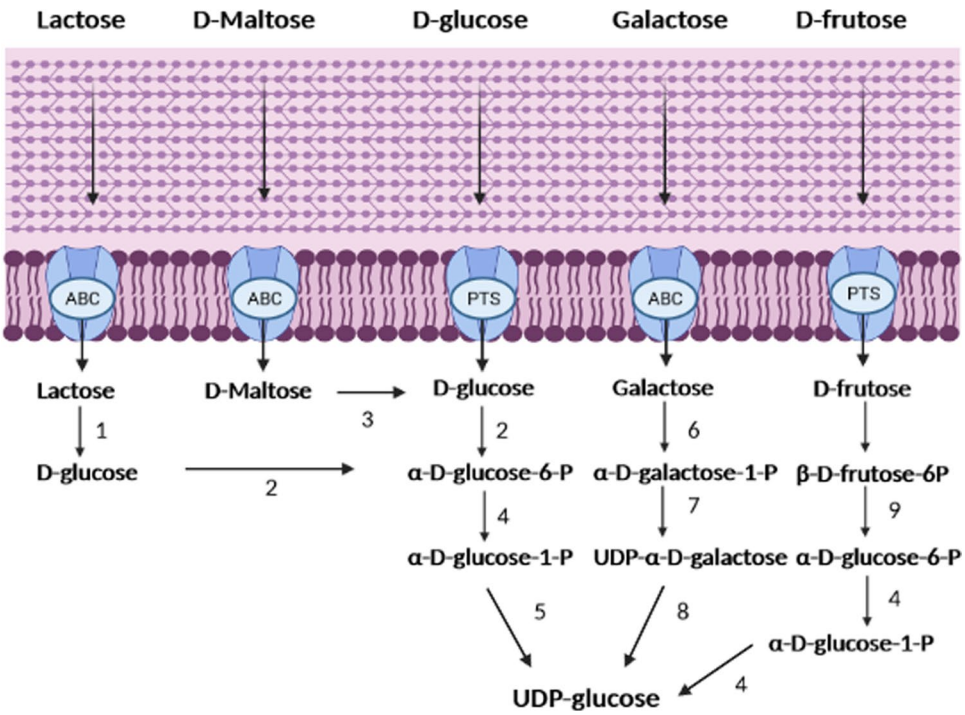
than 70% which is the cutoff value for species delineation. The highest dDDH value was 37.30 (34.8–39.8) observed between Val9 and *P. psychrodurans* DSM 11713^{T} (Table 1). Both ANI and DDH results suggest that strain Val9 could be considered as a new species of the genus *Psychrobacillus*.

Genome features

Metabolism

The analysis of the Val9 genome revealed the presence of some transporters, such as PTS (Phosphoenolpyruvate-dependent sugar phosphotransferase system) and ATP-binding Cassette (ABC) types, which act in the transport of several types of sugars such as D-glucose (EC 2.7.1.-), D-fructose (EC 2.7.1.-), D-galactose (EC 7.5.2.11), maltose (EC 7.5.2.1), and lactose (EC 7.5.2.2) (Fig. 2). In addition, Val9 utilizes sugars, such as D-glucose and D-fructose, through the Embden-Meyerhoff glycolytic pathway and the non-oxidative pentose phosphate pathway, generating pyruvic acid. As part of the oxidative metabolism, Val9 can convert pyruvate into acetyl-coenzyme A, and it will be converted into citrate through the enzyme citrate synthase (EC 2.3.3.1) to carry out the tricarboxylic acid (TCA) cycle (Fig. 3).

The presence of two enzymes related to an alternative way of the TCA cycle—succinyl-CoA/3-ketoacid CoA transferase (EC 2.8.3.5) and malate/quinone oxidoreductase (EC 1.1.5.4)—were found in Val9 genome analyses.



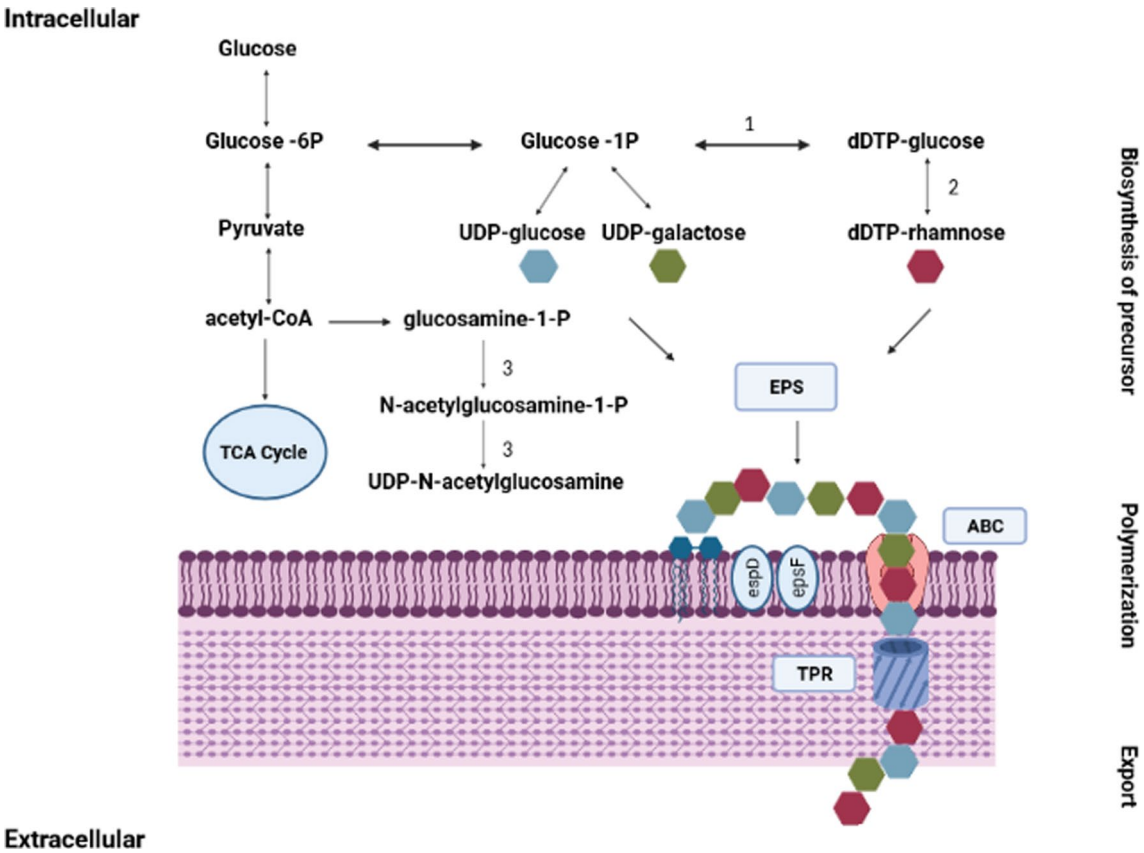


Fig. 2 Export and biosynthesis of some nucleotide sugars in strain Val9. The strain Val9 possesses the following enzymes according to genome analyses: 1: β-galactosidase (EC 3.2.1.23); 2: Glucokinase (EC 2.7.1.2); 3: α-glucosidase (EC 3.2.1.20); 4: Phosphoglucomutase (EC 5.4.2.2); 5: UTP–glucose-1-phosphate uridylyltransferase (EC 2.7.7.9); 6: Galactokinase (EC 2.7.1.6); 7: UTP-hexose-1-phosphate uridylyltransferase (EC 2.7.7.10); 8: UDP-glucose 4-epimerase (EC 5.1.3.2); 9: Glucose-6-phosphate isomerase (EC 5.3.1.9)

Fig. 3 Biosynthesis of EPS, assembly and transportation in strain Val9. 1: Glucose-1-phosphate thymidylyltransferase (EC 2.7.7.24); 2: dTDP-glucose 4,6-dehydratase (EC 4.2.1.46); 3: Bifunctional UDP-N-acetylglucosamine diphosphorylase/acetylglucosamine 1-phosphate uridylyltransferase (EC 2.7.7.23)

Finally, oxaloacetate generated in the TCA cycle can be converted into phosphoenolpyruvate in gluconeogenesis, generating glucose. The electrons generated in glycolysis and in TCA cycle are directed to the electron transport chain, divided into four complexes. In the end, O_2 is used as the final acceptor and ATP is produced.

Adaptations to cold environments

Different adaptive mechanisms to low temperatures were observed in the Val9 genome. First, CDSs codifying cold shock proteins (CSPs), the CspA family, were found. Val9 genomic analyses also identified DEAD-box RNA helicases (EC 3.6.4.13), important to cold and oxidative tolerance.

A two-component signal transduction system was detected in strain Val9 related to membrane plasmatic fluidity: DesK, a kinase sensor (EC 2.7.13.3) and DesR, a response regulator (EC 2.7.13.3). DesR binds to the *des* gene and starts the transcription of des-Δ5-lipid desaturase (EC 1.14.19.30). Furthermore, a fatty acid desaturase (EC 1.14.19) which catalyzes the insertion of a double bond at the delta position of fatty acids and is also related to the increase of the fluidity of membranes was also identified in Val9.

As a response to oxidative stress, strain Val9 produces the enzymes catalase (EC 1.11.1.6) and superoxide dismutase (EC 1.15.1.1). The enzyme catalase acts as an antioxidant, which catalyzes the conversion of hydrogen peroxide (H_2O_2) into water (H_2O) and molecular oxygen (O_2), neutralizing the toxic effects caused by hydrogen peroxide on cells. Superoxide dismutase acts similarly to catalase, converting superoxide radicals to molecular oxygen. A peptide methionine sulfoxide reductase (EC 1.8.4.12) encoded by the *msr*B gene was also found and might play an important role as a repair enzyme for proteins that have been inactivated by oxidation. Furthermore, Val9 strain also showed a tellurite resistance protein (TerD).

Several genes encoding proteins involved in adaptation to osmotic stress are also present in the Val9 genome. CDSs that encode types of transporter proteins for osmolytes were found, with the function of acting as osmoprotectors. ABC-type transporters have been identified for glycine-betaine (EC 7.6.2.9), involved in protection in environments with high osmolarity. Under stress conditions, bacteria make use of this transport system to accumulate glycine-betaine (OpuD), and other solutes that provide osmoprotection. Besides, another transporter was also identified, BCCT (Betaine/Carnitine/Choline Transporter), as well as potassium uptake proteins, TrkH and TrkR, and a system transporter. The presence of genes encoding Na + /H + antiporter NhaC related to adaptation to alkaline pH was also detected.

Finally, the protein arginine kinase (EC 2.7.3.3) is present in strain Val9 and catalyzes the specific phosphorylation of arginine residues in a large number of proteins. The arginine kinase is part of the bacterial stress response system, and it is involved in the regulation of many critical cellular processes.

Bioemulsifier production

The genome analyses of the Val9 strain identified CDSs related to exopolysaccharides (EPS) production. The synthesis of a precursor molecule is necessary for the stepwise elongation of the polymer strands. This step happens with various enzymatic transformations inside the cell. The step of precursor starts when glucose-6-phosphate is converted into glucose-1-phosphate, which generates the intermediates UDP-glucose and UDP-galactose, including UDP-glucose 4-epimerase (GalE) (EC 5.1.3.2) and UTP-glucose-1-phosphate uridylyltransferase (EC 2.7.7.9) for biosynthesis. The acetyl-CoA is converted to UDP-N-acetylglucosamine (UDP-GlcNAc), another intermediate of EPS biosynthesis, by bifunctional protein UDP-N-acetylglucosamine pyrophosphorylase/glucosamine-1-phosphate N-acetyltransferase (GlmU) (EC 2.7.7.23). These enzymes were also found in the other seven *Psychrobacillus* type strains in accordance with their genome annotation, suggesting a complete biosynthetic way to EPS production (Fig. 4).

The second step is the polymerization of EPS chain occurs in intramembrane space by the action of some glycosyltransferases (EC 2.4.1.-), which can transfer the additional monosaccharides to the nascent polysaccharide chain linked on undecaprenol intermediate. The Val9 strain possesses the enzymes diacylglycerol kinase (EC 2.7.1.107) and undecaprenol kinase (EC 2.7.1.66) for undecaprenol synthesis. Its genome also showed the presence of sugar transferases encoded by *eps*F and *eps*D genes, possibly involved in EPS chain length determination. Because the absence of genes that encode for sucrase enzymes (EC 2.4.1.362), we believe that EPS biosynthesis occurs in intracellular medium. The export across plasmatic membrane to the extracellular medium is the third step on EPS biosynthesis. Some ABC-transporters evolved in EPS export were found, such as Carbohydrate Uptake Transporter-1 Family (TC 3.A.1.1.-), indicating that follow the ABC transporter-dependent pathway, and translocation across the periplasm through tetratricopeptide repeat (TPR) (Fig. 3).

The Val9 genome analyses showed CDSs related to levan—a polysaccharide composed of (β2 → 6)-linked fructofuranosyl residues branched through (β2 → 1) linkages—production. These CDSs include the *sacB* gene that encodes the enzyme levansucrase (EC 2.4.1.10) which synthesizes polymers of fructose through a transfructosylation reaction using sucrose as a fructose donor. In this study, levansucrase was found in none of the seven type strains of the *Psychrobacillus* species.

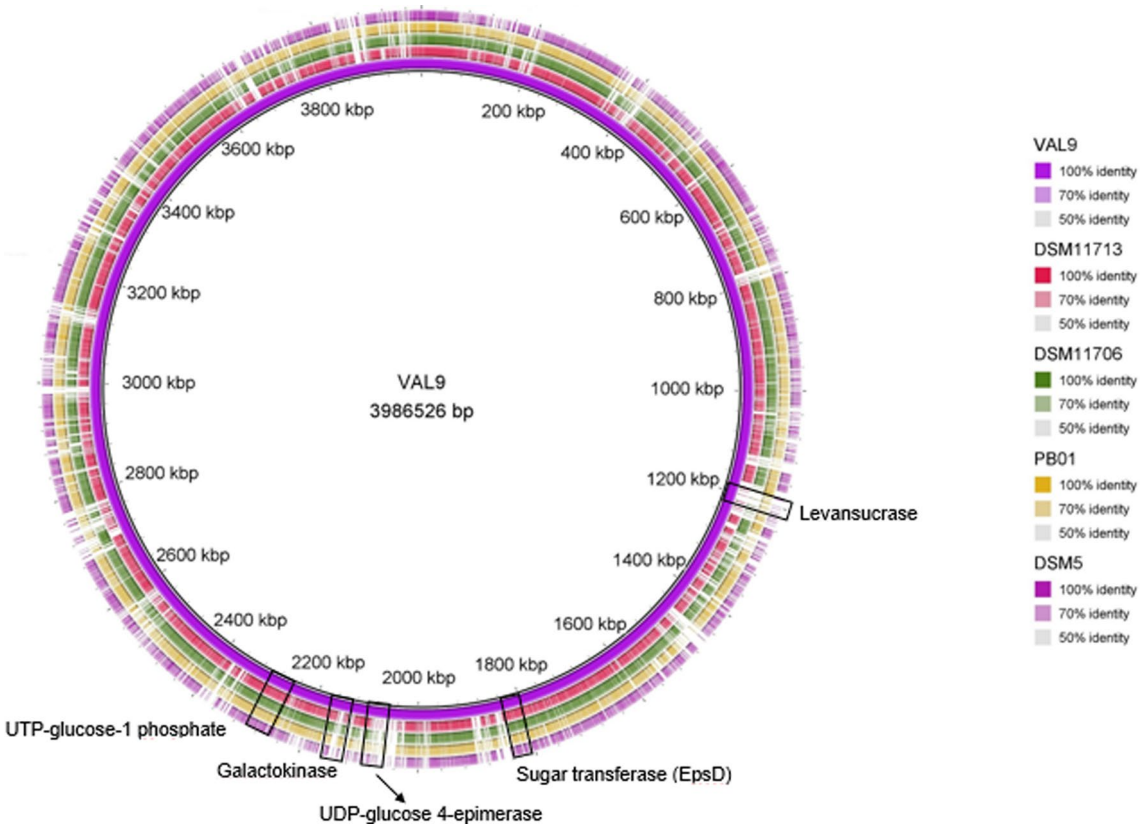


Fig. 4 Genomic context of different genes related to bioemulsifier production. *Psychrobacillus* sp. Val9 (inner circle) was used as a reference for multiple alignment. Colored regions represent similarities higher than 50% determined by BLASTn

The similarity among regions involved in BE production between strain Val9 and related species is highlighted on the comparative genome map (Fig. 4). The regions of UTP-glucose-1-phosphate and sugar transferase (EpsD) showed nucleotide similarity higher than 50% among the compared genomes. No similarity was found when galactokinase (which catalyzes the first reaction in the galactose metabolism pathway, the ATP-dependent phosphorylation of galactose, yielding galactose-1-phosphate) and levansucrase (which catalyzes the conversion of sucrose to glucose) were compared between the Val9 genome and those of strains *P. psychrodurans* DSM 11713^{T}, *P. psychrotolerans* DSM 11706^{T}, *P. insolitus* DSM 5^{T} and *P. glaciei* PB01^{T}.

Discussion

Psychrophilic and/or psychrotolerant bacteria are considered as a promising source for novel products such as bioactive compounds and other industrially relevant substances/compounds (Al-Maqtari et al. 2019; Dhakar and Pandey 2020). Strain Val9, a spore-forming and psychrotolerant bacterial strain isolated from an Antarctic soil (Vollú et al. 2014), was considered potentially valuable for biotechnological applications. This strain produced a bioemulsifier (BE) in low temperatures, in laboratory conditions, what motivated its better taxonomic and genetic characterization.

Phylogenetic analysis of 16S rRNA encoding gene indicated that the strain is related to members of the genus *Psychrobacillus*. This genus was created in 2010, harboring some species of the genus *Bacillus* and considering *B. insolitus* as the type species of the genus (Krishnamurthi et al. 2010). However, the ANI and dDDH values—determined between strain Val9 and the other seven type genomes of the members of the genus *Psychrobacillus*—suggested that strain Val9 could be considered as a new species of the genus *Psychrobacillus*. The accepted threshold for species delimitation using ANI is 95–96% (Richter and Rosselló-Móra 2009) and the highest ANI values obtained here were about 85% considering ANIb and 87% in ANIm. Moreover, the in silico DDH results were in all cases lower than 70%, which is the cutoff value for species delineation (Goris et al. 2007). Nonetheless, its physiological, biochemical, and chemotaxonomic characterization are still necessary for describing new taxa of aerobic, endospore-forming bacteria (Logan et al. 2009).

Strains belonging to *Psychrobacillus* are strictly aerobic according to the genus description by Krishnamurthi

et al. (2010). Val9 genome annotation showed that it can convert pyruvate into acetyl-coenzyme A, as part of the oxidative metabolism. Citrate will be formed through the enzyme citrate synthase (EC 2.3.3.1) to carry out the tricarboxylic acid (TCA) cycle. In silico studies of *P. glaciei* strain PB01^T demonstrated the presence of three enzymes related to an alternative way of the TCA cycle: ferredoxin-dependent 2-oxoglutarate oxidoreductase (EC 1.2.7.11), succinyl-CoA/3-ketoacid CoA transferase (EC 2.8.3.5), and malate/quinone oxidoreductase (EC 1.1.5.4) (Choi et al. 2020). Although the authors considered the presence of these enzymes in the other *Psychrobacillus* type strains, only succinyl-CoA/3-ketoacid CoA transferase and malate/quinone oxidoreductase were found in Val9 genome analyses.

Cold environments pose physicochemical stresses to their psychrophile/psychrotolerant habitants, such as low water activity, excessive UV radiation, low solute diffusion, and low nutrient availability. Therefore, psychrophiles/psychrotolerants have evolved adaptive mechanisms by changing their genome content to gain high capacity for DNA repair, translation, and membrane transport to cope with unfavorable environments (De Maayer et al. 2014; Choi and Lee 2020). As expected, different adaptive mechanisms to low temperatures were observed in the Val9 genome. For example, CDSs codifying cold shock proteins (CSPs), the CspA family, act as RNA chaperons destabilizing secondary structures (Cardoza and Singh 2021). These cold shock proteins encoding genes were also found in *P. glaciei* PB01 as a potential low-temperature adaptation strategy (Choi et al. 2020). It is also suggested that the cold shock proteins bind to mRNA and regulate translation, the rate of mRNA degradation, and transcription termination, functions that are important during normal growth in cold temperatures (Keto-Timonen et al. 2016). Val9 genomic analyses also identified DEAD-box RNA helicases (EC 3.6.4.13), responsible for remodeling RNA molecules and in facilitating various RNA–protein interactions, and important to cold and oxidative tolerance (Lehnik-Habrink et al. 2013). A fatty acid desaturase (EC 1.14.19) which is also related to the increase of the fluidity of membranes (Dhaulaniya et al. 2019) was also identified in Val9. Finally, strain Val9 produces the enzymes catalase (EC 1.11.1.6) and superoxide dismutase (EC 1.15.1.1), also as a response to oxidative stress, and a tellurite resistance protein (TerD). Tellurite is highly toxic to most bacteria due to its strong oxidative ability and ROS generation (Nguyen et al. 2021).

Several genes encoding proteins involved in adaptation to osmotic stress were found in the Val9 genome. For example, under high osmolarity, bacteria make use of ABC-type transport system to accumulate glycine-betaine (OpuD) and other solutes that provide osmoprotection. It has previously been demonstrated that glycine-betaine uptake is accompanied by sodium cotransport (Na +) (Annamalai and Venkitanarayanan 2009). Moreover, as part of the bacterial stress response system, the Protein-arginine kinase (EC 2.7.3.3) is present in strain Val9 and catalyzes the specific phosphorylation of arginine residues in a large number of proteins. Protein-arginine kinase has a physiologically important role as it is involved in the regulation of many critical cellular processes, such as protein homeostasis, motility, competence, and stringent, and stress responses by regulating gene expression and protein activity (Suskiewicz et al. 2019).

Bioemulsifier (BE) production in cold-adapted bacteria, especially exopolysaccharides (EPS), provide certain properties and functions useful to the microorganisms, such as production of aggregates, adhesion to surfaces, biofilm formation, and emulsification of hydrophobic substrates (Poli et al. 2010; Wang et al. 2019). Because of these properties, BEs also provide a valuable resource for biotechnological exploitation. Besides the fact they may not be found in traditional polymers of plant origin or mesophilic bacteria, BEs produced by cold-adapted bacteria (as Val9 strain) may remain functional at low temperatures, reducing the production costs (Freitas et al. 2011; Rizzo and Lo Giudice 2020; Rizzo et al. 2020).

As previously observed the production of a bioemulsifier in laboratory experiments, we identified CDSs related to exopolysaccharides (EPS) production—more specifically to levan production—in the genome analyses of the Val9 strain. Levan is a polysaccharide composed of (β2 → 6)-linked fructofuranosyl residues branched through (β2 → 1) linkages. The enzyme levansucrase (EC 2.4.1.10), which synthesizes polymers of fructose through a transfructosylation reaction using sucrose as a fructose donor is encoded by the *sacB* gene (Xu et al. 2021). In this study, levansucrase was found in none of the seven type strains of the *Psychrobacillus* species, making it an exclusivity of Val9. Moreover, only few studies have already reported levan production in cold-adapted bacteria, such *Bacillus licheniformis* ANT 179 (Xavier et al. 2017) and *Pseudomonas extremaustralis* 2ASCA (Finore et al. 2020). The great biotechnological interest in levan production is its wide use in many food products. Levan provides emulsification, stabilization, and shows thickening properties due to its high molecular weight, mechanical, and rheological properties (Esawy et al. 2013).

Nonetheless, we are aware that the presence of encoding genes related to levan production in Val9 genome does not guarantee that they are being expressed, and that levan is the bioemulsifier produced by Val9. Further studies will be developed to characterize the chemical structure and the possible applications of this bioemulsifier, contributing to a better understanding of the biotechnological potential of this bioproduct.

Conclusion

This study contributes to the knowledge of a novel psychrotolerant strain belonging to the genus *Psychrobacillus* isolated from Antarctic soil. Different genes assigned to strain Val9 and presented herein suggest that they play critical roles in adapting this strain to extreme environments. Furthermore, the presence of predicted CDSs related to levan production highlights its potential for biotechnological purposes.

Supplementary Information The online version contains supplementary material available at https://doi.org/10.1007/s00300-022-03028-1.

Acknowledgements This study was supported by grants from Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq), Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES) and Fundação de Amparo à Pesquisa do Estado do Rio de Janeiro (FAPERJ). Thanks are due to RECOM ("Rede de Ciências Ômicas") for exchanging scientific knowledge among its participants, and to the reviewers (Dr. Carmen Rizzo, Dr. Annarita Poli, and Dr. Marco Fondi) for the improvement of the manuscript.

Author contributions LS, AS, and MBFS conceived and designed the study. MBFS, FFM, and DJ conducted the experiments. VACA, MMC, AGN, RTJR, and SCS contributed with the genomic data analyses. MBFS and LS wrote the manuscript. All authors revised the manuscript, provided comments, and approved the final version of the manuscript.

Data availability All data and materials cited in the manuscript are freely available for the scientific community. The draft genome sequence of strain Val9 was determined in this study, and the Whole Genome Shotgun project has been deposited at DDBJ/ENA/GenBank under the accession number JAIZDB000000000. The version described in this paper is version JAIZDB010000000.

Declarations

Conflict of interest The authors declare that they have no conflict of interest.

References

Abd El-Rahman HA, Fritze D, Spröer C, Claus D (2002) Two novel psychrotolerant species, *Bacillus psychrotolerans* sp. nov. and *Bacillus psychrodurans* sp. nov., which contain ornithine in their cell walls. Int J Syst Evol Microbiol 52:2127–2133. https://doi.org/10.1099/00207713-52-6-2127

Al-Maqtari QA, AL-Ansi W, Mahdi AA (2019) Cold-active enzymes and their applications in industrial fields—a review. Int J Res Agric Sci 6:2348–3997

Alikhan NF, Petty NK, Ben Zakour NL, Beatson SA (2011) BLAST ring image generator (BRIG): simple prokaryote genome comparisons. BMC Genomics 12:402. https://doi.org/10.1186/1471-2164-12-402

Alizadeh-Sani M, Hamishehkar H, Khezerlou A, Azizi-Lalabadi M, Azadi Y, Nattagh-Eshtivani E, Fasihi M, Ghavami A, Aynehchi A, Ehsani A (2018) Bioemulsifiers derived from microorganisms: applications in the drug and food industry. Adv Pharm Bull 8:191–199

Andrews S (2010) FastQC: a quality control tool for high throughput sequence data [Online]. http://www.bioinformatics.babraham.ac.uk/projects/fastqc. Accessed Feb 2020

Annamalai T, Venkitanarayanan K (2009) Role of *proP* and *proU* in betaine uptake by *Yersinia enterocolitica* under cold and osmotic stress conditions. Appl Environ Microbiol 75:1471–1477. https://doi.org/10.1128/AEM.01644-08

Aziz RK, Bartels D, Best AA, DeJongh M, Disz T, Edwards RA, Formsma K, Gerdes S, Glass EM, Kubal M, Meyer F, Olsen GJ, Olson R, Osterman AL, Overbeek RA, McNeil LK, Paarmann D, Paczian T, Parrello B, Pusch GD, Reich C, Stevens R, Vassieva O, Vonstein V, Wilke A, Zagnitko O (2008) The RAST Server: rapid annotations using subsystems technology. BMC Genomics 9:75. https://doi.org/10.1186/1471-2164-9-75

Bankevich A, Nurk S, Antipov D, Gurevich AA, Dvorkin M, Kulikov AS, Lesin VM, Nikolenko SI, Pham S, Prjibelski AD, Pyshkin AV, Sirotkin AV, Vyahhi N, Tesler G, Alekseyev MA, Pevzner PA (2012) SPAdes: a new genome assembly algorithm and its applications to single-cell sequencing. J Comput Biol 19:455–477. https://doi.org/10.1089/cmb.2012.0021

Cardoza E, Singh H (2021) Involvement of CspC in response to diverse environmental stressors in *Escherichia coli*. J Appl Microbiol 132:785–801. https://doi.org/10.1111/jam.15219

Choi JY, Lee PC (2020) *Psychrobacillus glaciei* sp. nov., a psychrotolerant species isolated from an Antarctic iceberg. Int J Syst Evol Microbiol 70:1947–1952. https://doi.org/10.1099/ijsem.0.003998

Choi JY, Kim SC, Lee PC (2020) Comparative genome analysis of *Psychrobacillus* strain PB01, isolated from an iceberg. J Microbiol Biotechnol 30:237–243. https://doi.org/10.4014/jmb.1909.09008

De Maayer P, Anderson D, Cary C, Cowan DA (2014) Some like it cold: understanding the survival strategies of psychrophiles. EMBO Rep 15:508–517. https://doi.org/10.1002/embr.201338170

Dhakar K, Pandey A (2020) Microbial ecology from the Himalayan cryosphere perspective. Microorganisms 8:257. https://doi.org/10.3390/microorganisms8020257

Dhaulaniya AS, Balan B, Kumar M, Agrawal PK, Singh DK (2019) Cold survival strategies for bacteria, recent advancement and potential industrial applications. Arch Microbiol 201:1–16. https://doi.org/10.1007/s00203-018-1602-3

Esawy MA, Amer H, Gamal-Eldeen AM, El Enshasy HA, Helmy WA, Abo-Zeid MAM, Malek R, Ahmed EF, Awad GEA (2013) Scaling up, characterization of levan and its inhibitory role in carcinogenesis initiation stage. Carbohydr Polym 95:578–587. https://doi.org/10.1016/j.carbpol.2013.02.079

Emms DM, Kelly S (2015) OrthoFinder: solving fundamental biases in whole genome comparisons dramatically improves orthogroup inference accuracy. Genome Biol 16:157. https://doi.org/10.1186/s13059-015-0721-2

Finore I, Vigneron A, Vincent WF, Leone L, Di Donato P, Schiano Moriello A, Nicolaus B, Poli A (2020) Novel psychrophiles and exopolymers from permafrost thaw lake sediments. Microorganisms 8:1282. https://doi.org/10.3390/microorganisms8091282

Freitas F, Alves VD, Reis MA (2011) Advances in bacterial exopolysaccharides: from production to biotechnological applications. Trends Biotechnol 29:388–398. https://doi.org/10.1016/j.tibtech.2011.03.008

Goris J, Konstantinidis KT, Klappenbach JA, Coenye T, Vandamme P, Tiedje JM (2007) DNA–DNA hybridization values and their relationship to whole-genome sequence similarities. Int J Syst Evol Microbiol 57:81–91. https://doi.org/10.1099/ijs.0.64483-0

Haddar A, Hamed M, Bouallegue A, Bastos R, Coelho E, Coimbra MA (2021) Structural elucidation and interfacial properties

of a levan isolated from *Bacillus mojavensis*. Food Chem 343:128456. https://doi.org/10.1016/j.foodchem.2020.128456
Keto-Timonen R, Hietala N, Palonen E, Hakakorpi A, Lindström M, Korkeala H (2016) Cold shock proteins: a minireview with special emphasis on Csp-family of enteropathogenic *Yersinia*. Front Microbiol 7:1151. https://doi.org/10.3389/fmicb.2016.01151
Krishnamurthi S, Ruckmani A, Pukall R, Chakrabarti T (2010) *Psychrobacillus* gen. nov. and proposal for reclassification of *Bacillus insolitus* Larkin & Stokes, 1967, *B. psychrotolerans* Abd-El Rahman et al., 2002 and *B. psychrodurans* Abd-El Rahman et al., 2002 as *Psychrobacillus insolitus* comb. nov., *Psychrobacillus psychrotolerans* comb. nov. and *Psychrobacillus psychrodurans* comb. nov. Syst Appl Microbiol 33:367–373. https://doi.org/10.1016/j.syapm.2010.06.003
Kuddus M (2018) Cold-active enzymes in food biotechnology: an updated mini review. J Appl Biol Biotechnol 6:58e63. https://doi.org/10.7324/jabb.2018.60310
Larkin JM, Stokes JL (1967) Taxonomy of psychrophilic strains of *Bacillus*. J Bacteriol 94:889–895. https://doi.org/10.1128/jb.94.4.889-895.1967
Lehnik-Habrink M, Rempeters L, Kovács ÁT, Wrede C, Baierlein C, Krebber H, Kuipers OP, Stülke J (2013) DEAD-box RNA helicases in *Bacillus subtilis* have multiple functions and act independently from each other. J Bacteriol 195:534–544. https://doi.org/10.1128/JB.01475-12
Lindgreen (2012) AdapterRemoval: easy cleaning of next-generation sequencing reads. BMC Res Notes 5:337. https://doi.org/10.1186/1756-0500-5-337
Logan NA, Berge O, Bishop AH, Busse H-J, De Vos P, Fritze D, Heyndrickx M, Kämpfer P, Rabinovitch L, Salkinoja-Salonen MS, Seldin L, Ventosa A (2009) Proposed minimal standards for describing new taxa of aerobic, endospore-forming bacteria. Int J Syst Evol Microbiol 59:2114–2121. https://doi.org/10.1099/ijs.0.013649-0
Margesin R, Fauster V, Fonteyne P-A (2005) Characterization of cold-active pectate lyases from psychrophilic *Mrakia frigida*. Lett Appl Microbiol 40:453–459. https://doi.org/10.1111/j.1472-765X.2005.01704.x
Massol-Deya AA, Odelson DA, Hichey RP, Tiedje JM (1995) Bacterial community fingerprinting of amplified 16S and 16S–23S ribosomal DNA gene sequences and restriction endonuclease analysis (ARDRA). In: Akkermans ADL, Van Elsas JD, De Bruijn FJ (eds) Molecular microbial ecology manual. Springer, Dordrecht. https://doi.org/10.1007/978-94-011-0351-0_20
Meier-Kolthoff JP, Auch AF, Klenk HP, Goker M (2013) Genome sequence-based species delimitation with confidence intervals and improved distance functions. BMC Bioinform 14:60. https://doi.org/10.1186/1471-2105-14-60
Melsted P, Halldórsson BV (2014) KmerStream: streaming algorithms for k-mer abundance estimation. Bioinformatics 30:3541–3547. https://doi.org/10.1093/bioinformatics/btu713
Miller MA, Pfeiffer W, Schwartz T (2010) Creating the CIPRES science gateway for inference of large phylogenetic trees. In: Gateway Computing Environments Workshop (GCE), pp 1–8. https://doi.org/10.1109/GCE.2010.5676129
Navon-Venezia S, Zosim Z, Gottlieb A, Legmann R, Carmeli S, Ron EZ, Rosenberg E (1995) Alasan, a new bioemulsifier from *Acinetobacter radioresistens*. Appl Environ Microbiol 61:3240–3244. https://doi.org/10.1128/aem.61.9.3240-3244.1995
Nguyen TTH, Kikuchi T, Tokunaga T, Lyoda S, Iguchi A (2021) Diversity of the tellurite resistance gene operon in *Escherichia coli*. Front Microbiol 12:681175. https://doi.org/10.3389/fmicb.2021.681175
Pham VH, Jeong SW, Kim J (2015) *Psychrobacillus* soli sp. nov., capable of degrading oil, isolated from oil-contaminated soil. Int J Syst Evol Microbiol 65:3046–3052. https://doi.org/10.1099/ijs.0.000375
Poli A, Anzelmo G, Nicolaus B (2010) Bacterial exopolysaccharides from extreme marine habitats: production, characterization and biological activities. Mar Drugs 8:1779–1802. https://doi.org/10.3390/md8061779
Richter M, Rosselló-Móra R (2009) Shifting the genomic gold standard for the prokaryotic species definition. PNAS 106:19126–19131. https://doi.org/10.1073/pnas.0906412106
Rizzo C, Lo Giudice A (2020) The variety and inscrutability of polar environments as a resource of biotechnologically relevant molecules. Microorganisms 8:1422. https://doi.org/10.3390/microorganisms8091422
Rizzo C, Conte A, Azzaro M, Papale M, Rappazzo AC, Battistel D, Roman M, Lo Giudice A, Guglielmin M (2020) Cultivable bacterial communities in brines from perennially ice-covered and pristine Antarctic lakes: ecological and biotechnological implications. Microorganisms 8:819. https://doi.org/10.3390/microorganisms8060819
Rodríguez M, Reina JC, Béjar V, Llamas I (2020) *Psychrobacillus vulpis* sp. nov., a new species isolated from faeces of a red fox in Spain. Int J Syst Evol Microbiol 70:882–888. https://doi.org/10.1099/ijsem.0.003840
Rosenberg E, Perry A, Gibson DT, Gutnick DL (1979) Emulsifier of *Arthrobacter* RAG-1: specificity of hydrocarbon substrate. Appl Environ Microbiol 37:409–413. https://doi.org/10.1128/aem.37.3.409-413.1979
Seldin L, Dubnau D (1985) DNA homology among *Bacillus polymyxa, Bacillus macerans, Bacillus azotofixans* and others nitrogen fixing *Bacillus* strains. Int Syst Bacteriol 35:151–154. https://doi.org/10.1099/00207713-35-2-151
Seldin L, Rosado AS, Cruz DW, Nobrega A, van Elsas JD, Paiva E (1998) Comparison of *Paenibacillus azotofixans* strains isolated from rhizoplane, rhizosphere and non-rhizosphere soil from maize planted in two different Brazilian soils. Appl Environ Microbiol 64:3860–3868. https://doi.org/10.1128/AEM.64.10.3860-3868.1998
Shen Y, Fu Y, Yu Y, Zhao J, Li J, Li Y, Wang X, Zhang J, Xiang W (2017) *Psychrobacillus lasiicapitis* sp. nov., isolated from the head of an ant (*Lasius fuliginosus*). Int J Syst Evol Microbiol 67:4462–4467. https://doi.org/10.1099/ijsem.0.002315
Suskiewicz MJ, Hajdusits B, Beveridge R, Heuck A, Vu LD, Kurzbauer R, Hauer K, Thoeny V, Rumpel K, Mechtler K, Meinhart A, Clausen T (2019) Structure of McsB, a protein kinase for regulated arginine phosphorylation. Nat Chem Biol 15:510–518. https://doi.org/10.1038/s41589-019-0265-y
Uzoigwe C, Burgess JG, Ennis CJ, Rahman PKSM (2015) Bioemulsifiers are not biosurfactants and require different screening approaches. Front Microbiol 6:245. https://doi.org/10.3389/fmicb.2015.00245
Vollú RE, Jurelevicius D, Ramos LR, Peixoto RS, Rosado AS, Seldin L (2014) Aerobic endospore-forming bacteria isolated from Antarctic soils as producers of bioactive compounds of industrial interest. Polar Biol 37:1121–1131. https://doi.org/10.1007/s00300-014-1505-y
Wang J, Salem DR, Sani R (2019) Extremophilic exopolysaccharides: a review and new perspectives on engineering strategies and applications. Carbohydr Polym 205:8–26. https://doi.org/10.1016/j.carbpol.2018.10.011
Xavier JR, Ramana KV (2017) Optimization of levan production by cold-active *Bacillus licheniformis* ANT 179 and fructooligosaccharide synthesis by its levansucrase. Appl Biochem Biotechnol 181:986–1006. https://doi.org/10.1007/s12010-016-2264-8
Xu M, Zhang L, Zhao F, Wang J, Zhao B, Zhou Z, Han Y (2021) Cloning and expression of levansucrase gene of *Bacillus*

velezensis BM-2 and enzymatic synthesis of levan. Processes 9:317. https://doi.org/10.3390/pr9020317