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1 **Genomic analyses of a novel bioemulsifier-producing *Psychrobacillus* strain**

2 **isolated from soil of King George Island, Antarctica**

3

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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 4,042 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.

50 **Introduction**

51 The species *Bacillus psychrotolerans* and *Bacillus psychrodurans* were described as
52 psychrotolerant species of the genus *Bacillus* in 2002 (Abd El-Rahman et al. 2002).
53 Some decades before, *Bacillus insolitus* was proposed as a psychrophilic species whose
54 strains were isolated from soil (Larkin and Stokes 1967). After a detailed polyphasic
55 taxonomic study of these *Bacillus* species – using the type strains *B. insolitus* DSM 5^{T},
56 *B. psychrotolerans* DSM 11706^{T} and *B. psychrodurans* DSM 11713^{T} – the three species
57 were considered distinct from other members of *Bacillus* rRNA group 2. As a result, the
58 genus *Psychrobacillus* was created in 2010, with *B. insolitus* as the type species of the
59 genus (Krishnamurthi et al. 2010). The new genus *Psychrobacillus* was described
60 harboring Gram-positive, endospore-forming motile rods and strictly aerobic bacteria.
61 Their G + C content of the genomic DNA ranged from 35.7 to 36.6 mol %, and the
62 three species shared high 16S rRNA gene sequence similarities among them (97.8–99.7
63 %) (Krishnamurthi et al. 2010). Later, new *Psychrobacillus* species were described:
64 *Psychrobacillus soli* (Pham et al. 2015), *Psychrobacillus lasiicapitis* (Shen et al. 2017),
65 *Psychrobacillus vulpis* (Rodríguez et al. 2020), and *Psychrobacillus glaciei* (Choi and
66 Lee 2020). Therefore, currently, the genus *Psychrobacillus* is composed of seven
67 validly published species (lpsn.dsmz.de/genus/psychrobacillus).

68 Strains belonging to different species of *Psychrobacillus* were isolated
69 worldwide from different kinds of soils (Krishnamurthi et al. 2010; Pham et al. 2015),
70 from feces of a red fox (Rodríguez et al. 2020), the head of an ant (Shen et al. 2017),
71 and an iceberg in Antarctica (Choi and Lee 2020). Vollú et al. (2014) described the
72 isolation of 80 spore-forming and cold-adapted bacterial strains from nine different soil
73 samples of King George Island, in maritime Antarctica, including different
74 *Psychrobacillus* strains.

75 It is widely known that spore-forming and cold-adapted bacterial strains are
76 resistant to harsh conditions, and they are also potentially valuable for biotechnological
77 applications involving the production of cold-active enzymes and bioproducts important
78 to food, pharmaceutical, cosmetics, fine chemical, and other industries (Margesin et al.
79 2005; Kuddus 2018; Al-Maqtari et al. 2019). Therefore, the interest in cold-adapted
80 microorganisms has increased in an attempt to contribute for a potential source of cold-
81 active biomaterials. For example, Vollú et al. (2014) determined the ability to produce
82 extracellular enzymes (esterase, caseinase, amylase and gelatinase), antimicrobial
83 substances (against *Staphylococcus aureus* and *Candida albicans*) and biosurfactants in
84 all spore-forming bacterial strains isolated from Antarctic soils.
85 One strain denoted as Val9 (Vollú et al. 2014) – previously identified as *Bacillus*
86 *psychrodurans* and later reclassified as *Psychrobacillus* sp. – was chosen for further
87 studies as it was able to produce a bioemulsifier (BE) in low temperatures, in laboratory
88 conditions. Bioemulsifiers derived from microbial sources can be used more efficiently
89 in the food and drug industries than synthetic emulsifiers, because of their nutritional
90 benefits (Alizadeh-Sani et al. 2018). Bioemulsifiers are considered high molecular
91 weight biopolymers or exopolysaccharides (EPS), constituted of complex mixtures of
92 heteropolysaccharides, lipopolysaccharides, lipoproteins, and/or proteins (Uzoigwe et
93 al. 2015). Alasan (Navon-Venezia et al. 1995), emulsan (Rosenberg et al. 1979) and
94 levan (Haddar et al. 2021) are examples of well-studied bioemulsifiers. Conversely,
95 studies of bioemulsifiers produced by cold-adapted bacteria are still incipient.
96 Therefore, a more in-depth study of strain Val9 may provide a new model strain for
97 basic and biotechnological research within the genus *Psychrobacillus*. Performing a
98 comparative analysis of the genomes of the different *Psychrobacillus* species, we can

99 contribute not only for the taxonomy but also for the biotechnological relevance of the
100 genus.

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

105

106 **Materials and methods**

107 **Bacterial strain, culture conditions and DNA extraction**

108 The bacterial strain studied here – Val9 – was isolated from Vale Ulman soil samples,
109 King George Island, Antarctica (Vollú et al. 2014). A map showing the location of the
110 study site is shown in Online Resource 1. Strain Val9 was stored in trypticase soy broth
111 (TSB) containing 20 % glycerol at -80 °C. The same medium was used for growth at
112 15 °C for 48 h.

113 DNA from strain Val9 was isolated according to the method described in Seldin
114 et al. (1998). Further purification steps were those described in Seldin and Dubnau
115 (1985). The DNA was quantified spectrophotometrically using a Qubit™ fluorimeter
116 (Thermo Fisher Scientific, MA, USA).

117

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

119 The gene encoding 16S rRNA from Val9 was amplified by PCR using the pair of
120 universal primers pA and pH and the conditions described in Massol-Deya et al. (1995),
121 and the products sequenced using Macrogen (South Korea) facilities. For phylogenetic
122 tree analysis, the sequences of closely related *Psychrobacillus* strains were recovered
123 from GenBank database and aligned to the sequence obtained in this study using the

124 online Multiple alignment program MAFFT version 7

125 (https://mafft.cbrc.jp/alignment/software/). The phylogenetic analyses were performed

126 using the RaxML-HPC2 model in CIPRES Science Gateway (Miller et al. 2010), with

127 the phylogenetic tree inference using maximum likelihood/rapid bootstrapping run. The

128 sequence generated in this study was deposited in NCBI GenBank under accession

129 number KF026354.1.

130

131 **Genome sequencing and assembly**

132 The amount of 5 µg µl^{-1} of gDNA was considered for the construction of paired-end

133 sequencing libraries (2 × 150 bp) of 450 bp insert length following the manufacturer's

134 protocol for the NEBNext® Fast DNA Fragmentation and Library Preparation Kit (New

135 England Biolabs Inc., MA, USA). Final library-quality analysis was performed via 2100

136 bioanalyzer (Agilent Technologies, CA, USA) with read length gDNA size control

137 using agarose gel electrophoresis. All samples were sequenced on the Illumina Hi-Seq

138 2500 platform as recommended by the manufacturer.

139 The genome assembly process started checking the quality of the reads through

140 FastQC (Andrews 2010) and Adapter Removal to remove the bases with quality below

141 Phred 20 (Lindgreen 2012) softwares. The estimated best five k-mers were selected by

142 KmerStream (Melsted and Halldórsson 2014) after checking the values from 7-mers to

143 127-mers, followed by the assembly using SPAdes with the five best *k*-mers (Bankevich

144 et al. 2012).

145

146 **Average Nucleotide Identity (ANI) and digital DNA–DNA hybridization (dDDH)**

147 The reference draft genomes of *P. psychrodurans* DSM 11713

148 (NZ_FOUN00000000.1), *P. psychrotolerans* DSM 11706 (NZ_FOXU00000000.1), *P.*

149 *glaciei* PB01 (NZ_CP031223.1), *P. soli* NHI-2 (NZ_VDGG00000000.1), *P. insolitus*
150 DSM 5 (NZ_QKZI00000000.1), *P. lasiicapitis* NEAU-3TG517
151 (NZ_VDGH00000000.1) and *P. vulpis* Z8 (NZ_VDGI00000000.1) were downloaded
152 from NCBI (www.ncbi.nlm.nih.gov/refseq). The Val9 genome was compared with the
153 seven related type strains using the JSpeciesWS database
154 (http://jspecies.ribohost.com/jspeciesws/) with two alignment algorithms: mummer
155 (ANIm) and blastn (ANIb).
156 DNA digital hybridization (dDDH) was performed using the Genome-to-
157 Genome Distance Calculator - GGDC 2.1 (Meier-Kolthoff et al. 2013) provided by
158 Leibniz on the DSMZ Institute website (http://ggdc.dsmz.de/distcalc2.php) with the
159 recommended parameters and/or default settings.
160
161 **Genome annotation**
162 The automatic annotation of the Val9 genome and related *Psychrobacillus* strains was
163 performed using the RAST online server (Aziz et al. 2008) and GOFEAT
164 (http://computationalbiology.ufpa.br/gofeat/). KEGG (www.genome.jp/kegg) and
165 Metacyc (https://metacyc.org/) databases were used for the manual annotation and the
166 construction of the metabolic pathways. The pathways according to genome annotation
167 of strain Val9 were created with BioRender.com.
168
169 **Comparative genomics**
170 A comparative genome map was plotted through a BLASTN-based ring generated by
171 BLAST Ring Image Generator (BRIG) version 0.95 (Alikhan et al. 2011) to compare
172 the draft genomes of the seven *Psychrobacillus* type strains. The *Psychrobacillus* strain
173 Val9 was used as reference. The prediction of orthologous genes among the

174 *Psychrobacillus* genomes was performed using the software program OrthoFinder
175 v2.5.4 (Emms and Kelly 2015). A manual annotation of proteins was also performed
176 using GO FEAT and BLASTp, and KEGG database (www.genome.jp/kegg) was used
177 to understand the possible metabolic pathways in which some proteins are embedded.
178

179 **Results**

180 **Phylogenetic analysis of 16S rRNA encoding gene**

181 Results of BLAST sequence analyses of the 16S rRNA encoding gene (1,474 bp)
182 indicated that the strain, previously isolated from Antarctic soil and named Val9 (Vollú
183 et al. 2014), is related to members of the genus *Psychrobacillus* (Fig. 1). Its closest
184 relatives were *P. psychrotolerans* DSM 11706^T, *P. psychrodurans* DSM 11713^T and *P.*
185 *glaciei* PB01^T, with 99.92, 99.79 and 99.25 % gene sequence similarities, respectively.
186

187 **Genome sequence analyses**

188 The draft genome sequence of strain Val9 was determined in this study, and the Whole
189 Genome Shotgun project has been deposited at DDBJ/ENA/GenBank under the
190 accession number JAIZDB000000000. The version described in this paper is version
191 JAIZDB010000000. The genome of strain Val9 reveals 3,986,526 bp with a G+C
192 content of 36.6 %, and a total of 4,042 coding DNA sequences (CDSs) were predicted.
193 The identified CDSs were classified into subsystems, such as carbohydrates (174
194 CDSs), amino acids and derivates (273 CDSs), protein metabolism (146 CDSs), RNA
195 metabolism (60 CDSs), and stress response (48 CDSs) (Online Resource 2).

196 In an attempt to phylogenetically classify the proteins encoded in the Val9
197 genome within the genus *Psychrobacillus*, the orthologous groups were predicted using
198 the seven type strain genomes available for the genus. The analyses revealed 265

199 proteins found exclusively in Val9, but 199 proteins showed to be hypothetical ones
200 (Online Resource 3).
201 To elucidate the taxonomic relatedness between Val9 and the other known
202 *Psychrobacillus* species, the average nucleotide identity (ANI) and digital DNA–DNA
203 hybridization (dDDH) values were determined between strain Val9 and the other seven
204 type genomes of the members of the genus *Psychrobacillus* (Table 1). The ANI values
205 varied between 75.95-85.46 % considering ANIb and 83.98-87.37 % in ANIm. These
206 values are considered below the accepted threshold for species delimitation using ANI
207 (95–96 %). Moreover, the *in silico* DDH results were in all cases lower than 70 %
208 which is the cutoff value for species delineation. The highest dDDH value was 37.30
209 (34.8-39.8) observed between Val9 and *P. psychrodurans* DSM 11713^{T} (Table 1). Both
210 ANI and DDH results suggest that strain Val9 could be considered as a new species of
211 the genus *Psychrobacillus*.
212
213 **Genome features**
214 ***Metabolism***
215 The analysis of the Val9 genome revealed the presence of some transporters, such as
216 PTS (Phosphoenolpyruvate-dependent sugar phosphotransferase system) and ABC
217 (ATP-binding Cassette) types, which act in the transport of several types of sugars such
218 as D-glucose (EC 2.7.1.-), D-fructose (EC 2.7.1.-), D-galactose (EC 7.5.2.11), maltose
219 (EC 7.5.2.1) and lactose (EC 7.5.2.2) (Fig. 2). In addition, Val9 utilizes sugars, such as
220 D-glucose and D-fructose, through the Embden-Meyerhoff glycolytic pathway and the
221 non-oxidative pentose phosphate pathway, generating pyruvic acid. As part of the
222 oxidative metabolism, Val9 can convert pyruvate into acetyl-coenzyme A, and it will be

223 converted into citrate through the enzyme citrate synthase (EC 2.3.3.1) to carry out the
224 tricarboxylic acid (TCA) cycle (Fig. 3).

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

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

232

233 ***Adaptations to cold environments***

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

238 A two-component signal transduction system was detected in strain Val9 related
239 to membrane plasmatic fluidity: DesK, a kinase sensor (EC 2.7.13.3) and DesR, a
240 response regulator (EC 2.7.13.3). DesR binds to the *des* gene and starts the transcription
241 of des-Δ5-lipid desaturase (EC 1.14.19.30). Furthermore, a fatty acid desaturase (EC
242 1.14.19) which catalyzes the insertion of a double bond at the delta position of fatty
243 acids and is also related to the increase of the fluidity of membranes was also identified
244 in Val9.

245 As a response to oxidative stress, strain Val9 produces the enzymes catalase (EC
246 1.11.1.6) and superoxide dismutase (EC 1.15.1.1). The enzyme catalase acts as an
247 antioxidant, which catalyzes the conversion of hydrogen peroxide (H_2O_2) into water

248 (H_2O) and molecular oxygen (O_2), neutralizing the toxic effects caused by hydrogen
249 peroxide on cells. Superoxide dismutase acts similarly to catalase, converting
250 superoxide radicals to molecular oxygen. A peptide methionine sulfoxide reductase (EC
251 1.8.4.12) encoded by the *msr*B gene was also found and might play an important role as
252 a repair enzyme for proteins that have been inactivated by oxidation. Furthermore, Val9
253 strain also showed a tellurite resistance protein (TerD).
254 Several genes encoding proteins involved in adaptation to osmotic stress are also
255 present in the Val9 genome. CDSs that encode types of transporter proteins for
256 osmolytes were found, with the function of acting as osmoprotectors. ABC-type
257 transporters have been identified for glycine-betaine (EC 7.6.2.9), involved in protection
258 in environments with high osmolarity. Under stress conditions, bacteria make use of this
259 transport system to accumulate glycine-betaine (OpuD), and other solutes that provide
260 osmoprotection. Besides, another transporter was also identified, BCCT
261 (Betaine/Carnitine/Choline Transporter), as well as potassium uptake proteins, TrkH
262 and TrkR, and a system transporter. The presence of genes encoding Na+/H+ antiporter
263 NhaC related to adaptation to alkaline pH was also detected.
264 Finally, the protein arginine kinase (EC 2.7.3.3) is present in strain Val9 and
265 catalyzes the specific phosphorylation of arginine residues in a large number of
266 proteins. The arginine kinase is part of the bacterial stress response system, and it is
267 involved in the regulation of many critical cellular processes.
268
269 ***Bioemulsifier production***
270 The genome analyses of the Val9 strain identified CDSs related to exopolysaccharides
271 (EPS) production. The synthesis of a precursor molecule is necessary for the stepwise
272 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)

298 linkages – production. These CDSs include the *sacB* gene that encodes the enzyme
299 levansucrase (EC 2.4.1.10) which synthesizes polymers of fructose through a
300 transfructosylation reaction using sucrose as a fructose donor. In this study,
301 levansucrase was found in none of the seven type strains of the *Psychrobacillus* species.

302 The similarity among regions involved in BE production between strain Val9
303 and related species is highlighted on the comparative genome map (Fig. 4). The regions
304 of UTP-glucose-1-phosphate and sugar transferase (EpsD) showed nucleotide similarity
305 higher than 50 % among the compared genomes. No similarity was found when
306 galactokinase (which catalyzes the first reaction in the galactose metabolism pathway,
307 the ATP-dependent phosphorylation of galactose, yielding galactose-1-phosphate) and
308 levansucrase (which catalyzes the conversion of sucrose to glucose) were compared
309 between the Val9 genome and those of strains *P. psychrodurans* DSM 11713^T, *P.*
310 *psychrotolerans* DSM 11706^T, *P. insolitus* DSM 5^T and *P. glaciei* PB01^T.
311

312 **Discussion**

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

320 Phylogenetic analysis of 16S rRNA encoding gene indicated that the strain is
321 related to members of the genus *Psychrobacillus*. This genus was created in 2010,
322 harboring some species of the genus *Bacillus* and considering *B. insolitus* as the type

323 species of the genus (Krishnamurthi et al. 2010). However, the average nucleotide
324 identity (ANI) and digital DNA–DNA hybridization (dDDH) values – determined
325 between strain Val9 and the other seven type genomes of the members of the genus
326 *Psychrobacillus* – suggested that strain Val9 could be considered as a new species of
327 the genus *Psychrobacillus*. The accepted threshold for species delimitation using ANI is
328 95–96 % (Richter and Rosselló-Móra 2009) and the highest ANI values obtained here
329 were about 85 % considering ANIb and 87 % in ANIm. Moreover, the in silico DDH
330 results were in all cases lower than 70 %, which is the cutoff value for species
331 delineation (Goris et al. 2007). Nonetheless, its physiological, biochemical, and
332 chemotaxonomic characterization are still necessary for describing new taxa of aerobic,
333 endospore-forming bacteria (Logan et al. 2009).

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

345 Cold environments pose physicochemical stresses to their
346 psychrophile/psychrotolerant habitants, such as low water activity, excessive UV
347 radiation, low solute diffusion, and low nutrient availability. Therefore,

348 psychrophiles/psychrotolerants have evolved adaptive mechanisms by changing their
349 genome content to gain high capacity for DNA repair, translation, and membrane
350 transport to cope with unfavorable environments (De Maayer et al. 2014; Choi and Lee
351 2020). As expected, different adaptive mechanisms to low temperatures were observed
352 in the Val9 genome. For example, CDSs codifying cold shock proteins (CSPs), the
353 CspA family, act as RNA chaperons destabilizing secondary structures (Cardoza and
354 Singh 2021). These cold shock proteins encoding genes were also found in *P. glaciei*
355 PB01 as a potential low-temperature adaptation strategy (Choi et al. 2020). It is also
356 suggested that the cold shock proteins bind to mRNA and regulate translation, the rate
357 of mRNA degradation, and transcription termination, functions that are important
358 during normal growth in cold temperatures (Keto-Timonen et al. 2016). Val9 genomic
359 analyses also identified DEAD-box RNA helicases (EC 3.6.4.13), responsible for
360 remodeling RNA molecules and in facilitating various RNA-protein interactions, and
361 important to cold and oxidative tolerance (Lehnik-Habrink et al. 2013). A fatty acid
362 desaturase (EC 1.14.19) which is also related to the increase of the fluidity of
363 membranes (Dhaulaniya et al. 2019) was also identified in Val9. Finally, strain Val9
364 produces the enzymes catalase (EC 1.11.1.6) and superoxide dismutase (EC 1.15.1.1),
365 also as a response to oxidative stress, and a tellurite resistance protein (TerD). Tellurite
366 is highly toxic to most bacteria due to its strong oxidative ability and ROS generation
367 (Nguyen et al. 2021).

368 Several genes encoding proteins involved in adaptation to osmotic stress were
369 found in the Val9 genome. For example, under high osmolarity, bacteria make use of
370 ABC-type transport system to accumulate glycine-betaine (OpuD) and other solutes that
371 provide osmoprotection. It has previously been demonstrated that glycine-betaine
372 uptake is accompanied by sodium cotransport (Na+) (Annamalai and Venkitanarayanan

373 2009). Moreover, as part of the bacterial stress response system, the Protein-arginine
374 kinase (EC 2.7.3.3) is present in strain Val9 and catalyzes the specific phosphorylation
375 of arginine residues in a large number of proteins. Protein-arginine kinase has a
376 physiologically important role as it is involved in the regulation of many critical cellular
377 processes, such as protein homeostasis, motility, competence, and stringent, and stress
378 responses by regulating gene expression and protein activity (Suskiewicz et al. 2019).

379 Bioemulsifier (BE) production in cold-adapted bacteria, especially
380 exopolysaccharides (EPS), provide certain properties and functions useful to the
381 microorganisms, such as production of aggregates, adhesion to surfaces, biofilm
382 formation, and emulsification of hydrophobic substrates (Poli et al. 2010; Wang et al.
383 2019). Because of these properties, BEs also provide a valuable resource for
384 biotechnological exploitation. Besides the fact they may not be found in traditional
385 polymers of plant origin or mesophilic bacteria, BEs produced by cold-adapted bacteria
386 (as Val9 strain) may remain functional at low temperatures, reducing the production
387 costs (Freitas et al. 2011; Rizzo and Lo Giudice 2020; Rizzo et al. 2020).

388 As previously observed the production of a bioemulsifier in laboratory
389 experiments, we identified CDSs related to exopolysaccharides (EPS) production –
390 more specifically to levan production – in the genome analyses of the Val9 strain.
391 Levan is a polysaccharide composed of (β2→6)-linked fructofuranosyl residues
392 branched through (β2→1) linkages. The enzyme levansucrase (EC 2.4.1.10), which
393 synthesizes polymers of fructose through a transfructosylation reaction using sucrose as
394 a fructose donor is encoded by the *sacB* gene (Xu et al. 2021). In this study,
395 levansucrase was found in none of the seven type strains of the *Psychrobacillus* species,
396 making it an exclusivity of Val9. Moreover, only few studies have already reported
397 levan production in cold-adapted bacteria, such *Bacillus licheniformis* ANT 179 (Xavier

398 et al. 2017) and *Pseudomonas extremaustralis* 2ASCA (Finore et al. 2020). The great
399 biotechnological interest in levan production is its wide use in many food products.
400 Levan provides emulsification, stabilization, and shows thickening properties due to its
401 high molecular weight, mechanical, and rheological properties (Esawy et al. 2013).
402 Nonetheless, we are aware that the presence of encoding genes related to levan
403 production in Val9 genome does not guarantee that they are being expressed, and that
404 levan is the bioemulsifier produced by Val9. Further studies will be developed to
405 characterize the chemical structure and the possible applications of this bioemulsifier,
406 contributing to a better understanding of the biotechnological potential of this
407 bioproduct.

408

409 **Conclusions**

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

416

417 **Data Availability Statement**

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

423 **Author Contribution Statement**

424 LS, AS and MBFS conceived and designed the study. MBFS, FFM and DJ conducted
425 the experiments. VACA, MMC, AG-N, RTJR and SCS contributed with the genomic
426 data analyses. MBFS and LS wrote the manuscript. All authors revised the manuscript,
427 provided comments and approved the final version of the manuscript.

428

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437

438 **Declarations Conflict of interest**

439 The authors declare that they have no conflicts of interest.

440

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615 **Figure legends**

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

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

629 **Fig. 3.** Biosynthesis of EPS, assembly and transportation in strain Val9. 1: Glucose-1-
630 phosphate thymidylyltransferase (EC 2.7.7.24); 2: dTDP-glucose 4,6-dehydratase (EC
631 4.2.1.46); 3: Bifunctional UDP-N-acetylglucosamine
632 diphosphorylase/acetylglucosamine 1-phosphate uridylyltransferase (EC 2.7.7.23).

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