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**International Journal of Systematic and Evolutionary Microbiology**  
**Planococcus versutus sp. nov., Isolated from Antarctic soil**  
 --Manuscript Draft--

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<b>Abstract:</b>	A taxonomic study was performed on a novel Gram-staining-positive, cocci-shaped, orange-pigmented motile bacterium, designated strain L10.15T. The organism was isolated from a soil sample collected on Lagoon Island (close to Adelaide Island, western Antarctic Peninsula) using a quorum quenching enrichment medium. Growth occurred at 4-30 °C, pH 6-11, and at moderately high salinity (0-15 ‰), with optimal growth at 26 °C, at pH 7-8 and 6% NaCl. The 16S rRNA gene sequence analysis showed that strain L10.15T belonged to the genus Planococcus and was closely related to <i>P. halocryophilus</i> Or1T (99.3 ‰), <i>P. donghaensis</i> JH 1T (99.0 ‰), <i>P. antarcticus</i> DSM 14505T (98.3 ‰), <i>P. plakortidis</i> AS/ASP6 (II)T (97.6 ‰), <i>P. maritimus</i> TF-9T (97.5 ‰), <i>P. salinavum</i> ISL-6 T (97.5 ‰), and <i>P. kocurii</i> NCIMB 629T (97.5 ‰). However, the ANI-MUMmer (ANIm) analysis showed low genomic relatedness values of 71.1-81.7% to the type strains of these closely related species of the genus Planococcus. The principal fatty acids were anteiso-C15 : 0, C16 : 1 ω7c, and anteiso-C17 : 0 and the major menaquinones of strain L10.15T were MK-5 (48 ‰), MK-6 (6 ‰) and MK-7 (44 ‰). Polar lipid analysis revealed presence of phosphatidylethanolamine, phosphatidylglycerol, diphosphatidylglycerol and aminophospholipid. DNA G+C content was 39.4 mol%. The phenotypic and genotypic data indicate that strain L10.15T represents a novel species of the genus Planococcus, for which the name <i>Planococcus versutus</i> sp. nov. is proposed. The type strain is L10.15T (=DSM 101994T = KACC 18918T).

1 ***Planococcus versutus* sp. nov., isolated from Antarctic soil**

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17 Running title: *Planococcus versutus* sp. nov.

18 Subject category: New Taxa, *Firmicutes* and related organisms.

19 Keywords: *Planococcus versutus*, quorum quenching; Antarctic soil

20 The GenBank/EMBL/DDBJ accession number for the 16S rRNA and complete genome  
21 sequence of the novel strain L10.15<sup>T</sup> are KX516729 and CP016540-CP16542, respectively.

22 The genome accession numbers for *Planococcus* species that are used in this study are: *P.*  
23 *donghaensis* DSM 22276<sup>T</sup> (CP016543-CP016544), *P. plakortidis* DSM 23997<sup>T</sup> (CP016539),  
24 *P. maritimus* DSM 17275<sup>T</sup> (CP016538), *P. halocryphilus* DSM 24743<sup>T</sup> (CP016537), *P.*

25 *antarcticus* DSM 14505<sup>T</sup> (CP016534- CP016536), and *P. salinarum* DSM 23820<sup>T</sup>  
26 (MBQG000000000).

27 **A taxonomic study was performed on a novel Gram-staining-positive, cocci-shaped,**  
28 **orange-pigmented motile bacterium, designated strain L10.15<sup>T</sup>. The organism was**  
29 **isolated from a soil sample collected on Lagoon Island (close to Adelaide Island, western**  
30 **Antarctic Peninsula) using a quorum quenching enrichment medium. Growth occurred**  
31 **at 4-30 °C, pH 6-11, and at moderately high salinity (0-15 %), with optimal growth at 26**  
32 **°C, at pH 7-8 and 6% NaCl. The 16S rRNA gene sequence analysis showed that strain**  
33 **L10.15<sup>T</sup> belonged to the genus *Planococcus* and was closely related to *P. halocryophilus***  
34 **Or1<sup>T</sup> (99.3 %), *P. donghaensis* JH 1<sup>T</sup> (99.0 %), *P. antarcticus* DSM 14505<sup>T</sup> (98.3 %), *P.***  
35 ***plakortidis* AS/ASP6 (II)<sup>T</sup> (97.6 %), *P. maritimus* TF-9<sup>T</sup> (97.5 %), *P. salinavum* ISL-6<sup>T</sup>**  
36 **(97.5 %), and *P. kocurii* NCIMB 629<sup>T</sup> (97.5 %). However, the ANI-MUMmer (ANIm)**  
37 **analysis showed low genomic relatedness values of 71.1-81.7% to the type strains of**  
38 **these closely related species of the genus *Planococcus*. The principal fatty acids were**  
39 **anteiso-C<sub>15</sub> : 0, C<sub>16</sub> : 1 ω7c, and anteiso-C<sub>17</sub> : 0 and the major menaquinones of strain**  
40 **L10.15<sup>T</sup> were MK-5 (48 %), MK-6 (6 %) and MK-7 (44 %). Polar lipid analysis**  
41 **revealed presence of phosphatidylethanolamine, phosphatidylglycerol,**  
42 **diphosphatidylglycerol and aminophospholipid. DNA G+C content was 39.4 mol%. The**  
43 **phenotypic and genotypic data indicate that strain L10.15<sup>T</sup> represents a novel species of**  
44 **the genus *Planococcus*, for which the name *Planococcus versutus* sp. nov. is proposed.**  
45 **The type strain is L10.15<sup>T</sup> (=DSM 101994<sup>T</sup> = KACC 18918<sup>T</sup>).**

46 The genus *Planococcus* was proposed by Migula (1894) to accommodate aerobic, Gram-  
47 stain-positive, motile, cocci- or rod-shaped bacteria. In 2001, five *Planococcus* species were  
48 transferred to the newly proposed genus *Planomicrobium* to differentiate rod shaped, motile,  
49 non-sporogenous and low G+C content bacterial species within the original genus

50 *Planococcus* (Yoon *et al.*, 2001). These two genera can be differentiated through their 16S  
51 rRNA gene sequences, which were shown to have sequence signatures at positions 183 (T for  
52 *Planococcus* and C for *Planomicrobium*) and 190 (A for *Planococcus* and G for  
53 *Planomicrobium*), following the 16S rRNA gene sequence numbering of *E. coli*. To date,  
54 according to the *List of Prokaryotic with Standing in Nomenclature*  
55 (<http://www.bacterio.net/planococcus.html>), there are 12 species described in the genus  
56 *Planococcus*. Although 18 species are cited in the files of the genus *Planococcus* in LPSN,  
57 six of these have been reclassified to the genera *Planomicrobium* or *Marinococcus*.

58 Members of *Planococcaceae* are able to survive extreme environments having been isolated  
59 from a wide range of sources include deep sea sediments, marine solar salterns, glaciers,  
60 permafrost, Antarctic deserts, faeces, cyanobacterial mats and sea ice brine (Kim *et al.*, 2015;  
61 Margolles *et al.*, 2012; Pearson & Noller, 2011; Reddy *et al.*, 2002). All members of  
62 *Planococcus* are able to grow at moderately low temperatures (psychrotrophic) and are  
63 moderately halotolerant (halophilic). The type strain of *Planococcus halocryophilus*, which  
64 was isolated from Arctic permafrost, was reported to grow and divide even at extremely low  
65 temperature (-15 °C) (Mykytczuk *et al.*, 2013). Members of *Planococcus* can be exploited in  
66 the field of biotechnological and industrial applications, for instance through their production  
67 of carotenoids, thermophilic and alkaline/salt-tolerant xylanases and biosynthesis of butanol  
68 (See-Too *et al.*, 2016; Huang *et al.*, 2015; Unverferth *et al.*, 2014; Kim *et al.*, 2015). Here, we  
69 provide a detailed taxonomic characterization of a novel species of the genus *Planococcus*,  
70 strain L10.15<sup>T</sup>, which was recently isolated from Antarctic soil samples.

71 In this study, strain L10.15<sup>T</sup> was isolated during an ecological survey of the quorum  
72 quenching (QQ) soil bacteria in Antarctic soil samples using QQ bacteria enrichment  
73 medium (Chan *et al.*, 2009). The soil sample was collected from an elephant seal wallow on

74 Lagoon Island, close to Adelaide Island, off the west coast of the Antarctic Peninsula (67°  
75 35.689'S 068° 14.495'E). Briefly, around 1 g of soil sample and 5 ml sterile QQ bacteria  
76 enrichment medium with the sole carbon source of 100 µg synthetic C<sub>6</sub>-HSL was added to a  
77 sterile 50 ml polypropylene conical tube and incubated at 4 °C with 150 rpm agitation. A  
78 total of 100 µL of the bacterial suspension was transferred into new QQ bacteria enrichment  
79 medium including C<sub>6</sub>-HSL after 1 week of incubation. This step was repeated three times  
80 and, finally, 100 µl of bacterial suspension was plated onto Luria-Bertani (LB) agar. An  
81 orange-pigmented isolate, strain L10.15<sup>T</sup>, was recovered. The cell suspensions were kept in  
82 20 % w/v glycerol stock for long-term storage at -80 °C. Strain L10.15<sup>T</sup> was then routinely  
83 cultured aerobically in LB broth or LB agar at 26 °C (optimum growth temperature). As this  
84 is the first reported *Planococcus* species with QQ activity, we sequenced its complete  
85 genome using Pacific Biosciences (PacBio) RSII to facilitate our investigation.

86 Colony morphology of strain L10.15<sup>T</sup> was orange-pigmented, circular, entire, smooth,  
87 convex and 1-2 mm in size on LB agar after 48 h incubation at 26 °C. Gram-staining was  
88 performed using Difco Gram stain set and observed using a Leica DM 750  
89 microscope (Leica Microsystems). Cells of strain L10.15<sup>T</sup> were observed to be motile and  
90 Gram-positive with no spore formation. Electron micrographs were obtained using a table top  
91 scanning electron microscope (SEM, TM3030; Hitachi, Japan) and a scanning transmission  
92 electron microscope (STEM, LIBRA 120; Carl Zeiss AG, Germany). For SEM, a sample was  
93 prepared as described by Vali *et al.* (2004). For STEM, overnight suspension cells were  
94 stained using 1% phosphotungstic acid on a Formvar grid and observed at an operating  
95 voltage of 80 kV. Cells of strain L10.15<sup>T</sup> were coccoid, typically 1.0-1.5 µm in diameter,  
96 mostly arranged as diplococci, but cells in single coccoid or tetrad were also observed (Fig.  
97 1). A catalase test was conducted using 3 % (v/v) H<sub>2</sub>O<sub>2</sub> and determined by observing the  
98 production of copious bubbles. Oxidase activity was determined using 1 % (w/v) *N,N,N',N'*-

99 tetramethyl 1,4-phenylenediamine (bioMérieux) as described by Smibert & Krieg (1994).  
100 API ZYM and Biolog GEN III Microplates were prepared according to the manufacturer's  
101 instructions. The activities of various enzymes were determined by using the API ZYM after  
102 incubation for 24 h. Antibiotic susceptibility was tested by using ATB PSE 5 strips  
103 (bioMérieux) and disc diffusion assay following the manufacturer's instructions. All tests  
104 were performed at 26 °C and in triplicate. The temperature range for growth was determined  
105 by plating on LBA and incubation at 4-37 °C with increments of 1 or 2 °C over 14 d. The pH  
106 range for growth of strain L10.15<sup>T</sup> was determined on LBA plates adjusted to various pH  
107 values between 4 to 12 with 1 pH unit increments. Tolerance of salt was determined by  
108 growing on LBA media supplemented with 0-25 % (w/v) NaCl at increments of 1%. Both  
109 salt tolerance and pH range tests were conducted by incubating the LBA plates at 26 °C for  
110 up to 14 d. All results of physiological tests of strain L10.15<sup>T</sup>, and comparison with closely  
111 related species, are presented in Table 1.

112 Genomic DNA of L10.15<sup>T</sup> was extracted from an overnight cell suspension culture using the  
113 MasterPure™ Gram-positive DNA purification kit (Epicentre Technologies). A 20-kb  
114 SMRTbell template library was then constructed using the extracted genomic DNA. The  
115 whole genome sequencing was performed using Pacific Biosciences (PacBio) RSII  
116 sequencing platform with C4 chemistry in two single molecule real time (SMRT) cells. The  
117 complete genome of strain L10.15<sup>T</sup> has been sequenced, enabling the discovery of the gene  
118 responsible for QQ activity (See-Too et al., unpublished data). To determine the identity of  
119 strain L10.15<sup>T</sup>, the 16S rRNA partial gene sequence was amplified from the extracted DNA  
120 obtained as described above by using primers 27F and 1492R (Lane, 1991) and analyzed  
121 using the Ex-Taxon database (Kim *et al.*, 2012). Pairwise similarity analysis demonstrated  
122 that strain L10.15<sup>T</sup> is a member of the genus *Planococcus*, with *P. halocryophilus* Or1<sup>T</sup> (99.3  
123 %), *P. donghaensis* JH 1<sup>T</sup> (99.0 %), *P. antarticus* DSM 14505<sup>T</sup> (98.3 %), *P. plakortidis*

124 AS/ASP6 (II)<sup>T</sup> (97.6 %), *P. maritimus* TF-9<sup>T</sup> (97.5 %), *P. salinavum* ISL-6<sup>T</sup> (97.5 %) and *P.*  
125 *kocurii* NCIMB 629<sup>T</sup> (97.5 %) as the closest relatives present in the database. Phylogenetic  
126 analyses of the 16S rRNA was carried out using the full 16S rRNA gene sequence (1538 bp)  
127 retrieved from complete genome sequence. MEGA 6.0 software (Tamura *et al.*, 2013) was  
128 used to performed the alignment using the MUSCLE algorithm (Edgar, 2004) and the  
129 phylogenies were constructed using default settings of neighbour-joining (NJ, Fig. 2),  
130 maximum likelihood (ML, Supplementary Fig. S1) and maximum parsimony (MP,  
131 Supplementary Fig. S2) algorithms. The 16S rRNA gene sequence of L10.15<sup>T</sup> contained the  
132 signature nucleotides of *Planococcus*, T and A, respectively at positions 183 and 190  
133 (*Escherichia coli* 16S rRNA gene sequence numbering) and thus clustered separately from  
134 the related genus *Planomicrobium* (Dai *et al.*, 2005). All 16S rRNA phylogenies  
135 concordantly demonstrated that strain L10.15<sup>T</sup> clustered within *Planococcus*, but formed a  
136 distinct branch separate from *P. halocryophilus* Or1<sup>T</sup>, *P. donghaensis* JH1<sup>T</sup>, *P. antarcticus*  
137 DSM 14505<sup>T</sup>, *P. plakortidis* AS/ASP6 (II)<sup>T</sup>, *P. maritimus* TF-9<sup>T</sup>, *P. salinavum* ISL-6<sup>T</sup>, and *P.*  
138 *kocurii* NCIMB 629<sup>T</sup>. The G+C content of strain L10.15<sup>T</sup> was 39.4 mol% as determined from  
139 the complete genome sequence.

140 Average nucleotide identity (ANI) analysis was performed using JSpecies Web Service  
141 (JSpeciesWS; <http://jspecies.ribohost.com/jspeciesws/>) (Richter *et al.*, 2015) in which strain  
142 L10.15<sup>T</sup> demonstrated ANI-MUMmer (ANIm) values of between 71 % and 82 % similarity  
143 against all close relatives (*P. halocryophilus* Or1<sup>T</sup> (81.2%), *P. donghaensis* JH 1<sup>T</sup> (80.8 %),  
144 *P. antarcticus* DSM 14505<sup>T</sup> (79.6 %), *P. plakortidis* AS/ASP6 (II)<sup>T</sup> (71.1 %), *P. maritimus*  
145 TF-9<sup>T</sup> (72.0 %), *P. salinavum* ISL-6<sup>T</sup> (73.0 %), and *P. kocurii* NCIMB 629<sup>T</sup> (81.7 %))  
146 (Supplementary Table S1). ANI-Blast (ANIB) values in comparison with all close relatives  
147 indicated 84 % to 88 % similarity (*P. halocryophilus* Or1<sup>T</sup> (84.8 %), *P. donghaensis* JH1<sup>T</sup>  
148 (84.8 %), *P. antarcticus* DSM 14505<sup>T</sup> (84.3 %), *P. plakortidis* AS/ASP6 (II)<sup>T</sup> (85.0 %), *P.*



149 *maritimus* TF-9<sup>T</sup> (84.6 %), *P. salinavum* ISL-6<sup>T</sup> (88.2%), and *P. kocurii* NCIMB 629<sup>T</sup> (86.1  
150 %)) (Supplementary Table S2). The results were similar with OrthoANI analysis (Lee *et al.*,  
151 2016), which giving OrthoANI values ranging from 71.5 % to 82.2 % (*P. halocryophilus*  
152 Or1<sup>T</sup> (81.4 %), *P. donghaensis* JH1<sup>T</sup> (81.3 %), *P. antarcticus* DSM 14505<sup>T</sup> (79.9 %), *P.*  
153 *plakortidis* AS/ASP6 (II)<sup>T</sup> (72.9 %), *P. maritimus* TF-9<sup>T</sup> (72.0 %), *P. salinavum* ISL-6<sup>T</sup>  
154 (71.5%), and *P. kocurii* NCIMB 629<sup>T</sup> (82.2 %)) (Supplementary Fig. S4). Richter *et al.*  
155 (2009) proposed a threshold of 94–96 % for species delimitation, with our analyses therefore  
156 indicating that strain L10.15<sup>T</sup> does not belong to any of these related species.

157 The isoprenoid quinones were extracted using petroleum ether as described by Minnikin *et*  
158 *al.* (1984) and subsequently identified by HPLC (Shimadzu; Nexera-X2). The isoprenoid  
159 quinone profile of strain L10.15<sup>T</sup> was characterized by the predominance of the  
160 menaquinones MK-5 (48 %), MK-6 (6 %) and MK-7 (44 %). The polar lipids of strain  
161 L10.15<sup>T</sup> were extracted and analyzed by two-dimensional TLC following Embley & Wait  
162 (1994). Molybdophosphoric acid was used for the detection of total polar lipids, ninhydrin for  
163 amino lipids, molybdenum blue for phospholipids, Dragendorff reagent for choline-  
164 containing lipids and  $\alpha$ -naphthol/sulphuric acid reagent for glycolipids. Strain L10.15<sup>T</sup>  
165 exhibited a complex polar lipid profile consisting of phosphatidylethanolamine,  
166 phosphatidylglycerol, diphosphatidylglycerol, an unidentified aminophospholipid, two  
167 unidentified lipids and four unidentified aminolipids (Supplementary Fig. S3). The  
168 predominant polar lipids of strain L10.15<sup>T</sup> were phosphatidylethanolamine,  
169 phosphatidylglycerol, diphosphatidylglycerol and aminophospholipid. This result is  
170 consistent with the description of *Planococcus plakortidis* (Kaur *et al.*, 2012).

171 Cellular fatty acid profiles were determined following the standard protocol of the  
172 MIDI/Hewlett Packard Microbial Identification System (Pandey *et al.*, 2002). Fatty acids

173 were extracted and fatty acid methyl esters were prepared and analyzed in the Microbial  
174 Identification System (MIDI). Briefly, overnight cultures of strain L10.15<sup>T</sup> were harvested  
175 from LBA determined previously to be in the mid-exponential growth phase at 26°C. The  
176 fatty acids were separated using an Agilent GC (model 6890N) and were identified using  
177 Sherlock version 6.0 via the RTSBA6 database. The fatty acid profile of strain L10.15<sup>T</sup>  
178 comprised (each constituting  $\geq 0.5$  % of the total): saturated fatty acids C<sub>14:0</sub> (0.6 %), C<sub>15:0</sub>  
179 (1.5 %), C<sub>16:0</sub> (4.0 %), C<sub>17:0</sub> (0.7 %) and C<sub>18:0</sub> (1.0 %), branched fatty acids anteiso-C<sub>13:0</sub>  
180 (0.6 %), anteiso-C<sub>15:0</sub> (46.2 %), anteiso-C<sub>17:0</sub> (10.7 %), iso-C<sub>14:0</sub> (3.4 %), iso-C<sub>15:0</sub> (1.9  
181 %), iso-C<sub>16:0</sub> (5.5 %), iso-C<sub>17:0</sub> (1.9 %), Iso-C<sub>17:1</sub>  $\omega$ 10c (1.3 %) and iso-C<sub>18:0</sub> (0.7 %);  
182 unsaturated fatty acids C<sub>16:1</sub>  $\omega$ 7c alcohol (6.5 %), C<sub>16:1</sub>  $\omega$ 11c alcohol (5.6 %), C<sub>17:1</sub>  $\omega$ 9c  
183 alcohol (0.8 %) and C<sub>18:1</sub>  $\omega$ 9c alcohol (0.7 %); summed feature 3 (iso-C<sub>15:0</sub> 2OH and/or  
184 anteiso-C<sub>17:1</sub>; 0.6 %) and summed feature 4 (iso-C<sub>17:1</sub> and/or C<sub>16:1</sub>  $\omega$ 7c; 6.0 %). This  
185 profile is similar to those of recognized *Planococcus* species, although there were differences  
186 in the proportions of some fatty acids. Table 2 presents the fatty acids of strain L10.15<sup>T</sup> and  
187 closely related species. The fatty acid profile of strain L10.15<sup>T</sup> was similar to those of  
188 members of the genus *Planococcus* and contained anteiso-C<sub>15:0</sub> and anteiso-C<sub>17:0</sub> as the major  
189 fatty acids. The distinctive characteristic of L10.15<sup>T</sup> compared to other member of the genus  
190 *Planococcus* lies in the menaquinone profile, in which the predominant menaquinones are  
191 MK-5, MK-6 and MK-7 instead of MK-6, MK-7 and MK-8. L10.15<sup>T</sup> is also the only strain  
192 sensitive to fusidic acid of the reference strains tested.

### 193 **Description of *Planococcus versutus* sp. nov.**

194 *versutus* (ver.su'tus. L. masc. adj. *versutus* adroit, shrewd, ingenious)

195 The cells of L10.15<sup>T</sup> are aerobic, Gram-stain-positive cocci, motile, and non-sporulating.  
196 Colonies on LB agar are orange-colored, circular, entire, smooth, convex and 1.0–2.0 mm in  
197 diameter. Strain L10.15<sup>T</sup> grows at temperatures between 4 and 30 °C (optimum, 25 °C) and  
198 pH 6.0–11.0 (optimum, pH 7.0–8.0). Growth is observed between 0 and 14 % NaCl  
199 (optimum, 6 %). Tests positive for catalase, but negative for amylase. Strain L10.15<sup>T</sup> is  
200 positive in assimilation of *N*-acetyl-D-glucosamine, *N*-acetyl neuraminic acid, *N*-acetyl  
201 neuraminic acid,  $\alpha$ -D-glucose, inosine, D-mannitol, glycerol, D-fructose- 6-PO<sub>4</sub>, glycyl-L-  
202 proline, L-alanine, L-aspartic acid, L-glutamic acid, L-pyroglutamic acid, L-serine, L-  
203 galactonic acid lactone, D-gluconic acid, D-glucuronic acid, mucic acid, D-saccharic acid, D-  
204 lactic acid methyl ester,  $\alpha$ -keto-glutaric acid, D-malic acid, L-malic acid, tween 40,  $\beta$ -  
205 hydroxy-D,L-butyric acid, acetoacetic acid, acetic acid and formic acid, dextrin, D-fructose,  
206 D-glucose- 6-PO<sub>4</sub>, L-alanine, L-glutamic acid, pectin, D-galacturonic acid, glucuronamide,  
207 dextrin, D-fructose, D-glucose- 6-PO<sub>4</sub>, L-alanine, L-glutamic acid, pectin, D-galacturonic  
208 acid, and glucuronamide L10.15<sup>T</sup>. It is negative in assimilation of D-turanose, stachyose, D-  
209 mannose, 3-methyl glucose, D-sorbitol, citric acid, bromo-succinic acid, *N*-Acetyl- $\beta$ -D-  
210 mannosamine, *N*-acetyl-D-galactosamine, D-galactose, D-fucose, L-fucose, L-rhamnose, D-  
211 arabitol, myo-inositol, D-aspartic acid, D-serine, gelatin, L-arginine, L-histidine, quinic acid,  
212 *p*-hydroxy-phenylacetic acid, methyl pyruvate, L-lactic acid,  $\gamma$ -amino-butryric acid,  $\alpha$ -  
213 hydroxy-butyric acid,  $\alpha$ -keto-butyric acid and propionic acid. In the chemical sensitivity test,  
214 strain L10.15<sup>T</sup> was resistant to D-serine, lincomycin, guanidine HCl, tetrazolium blue,  
215 potassium tellurite, 1 % sodium lactate, aztreonam and sodium butyrate, slightly resistant to  
216 tetrazolium violet and sodium bromate and sensitive to fusidic acid, nalidixic acid, lithium  
217 chloride, vancomycin, niaproof 4, troleandomycin, rifamycin SV and minocycline. The DNA  
218 G+C content of the type strain is 39.4 mol%. The respiratory menaquinones are MK-5, MK-6  
219 and MK-7. Major fatty acids are anteiso-C<sub>15</sub>:0, C<sub>16</sub>:1  $\omega$ 7c, and anteiso-C<sub>17</sub>:0. The

220 predominant polar lipids are phosphatidylethanolamine (PE), phosphatidylglycerol (PG),  
221 diphosphatidylglycerol, and aminophospholipids.

222 The type strain, strain L10.15<sup>T</sup> (=DSM 101994<sup>T</sup> = KACC 18918<sup>T</sup>), was isolated from a soil  
223 sample collected from an elephant seal wallow on Lagoon Island (close to Adelaide Island,  
224 western Antarctic Peninsula).

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319 **Fig. 1.** Scanning (a) and scanning transmission (b) electron micrographs of cells of strain  
320 L10.15<sup>T</sup> grown at 26 °C. Most of the cells are observed as diplococci and cell division septa at  
321 different stages were also observed. Scale bars: a, 5 µm; b, 0.5 µm.

322 **Fig. 2.** Phylogenetic tree constructed by neighbour-joining analysis based on 16S rDNA  
323 sequences, depicting the phylogenetic relationship of strain L10.15<sup>T</sup> with related type species  
324 of the genus *Planococcus*. Scale bar represents evolutionary distance as 0.005 change per  
325 nucleotide position. Bootstrap values (%) > 50 % from 1,000 replicates are shown.

**Table 1.** Differential phenotypic characteristics of *P. versutus* L10.15<sup>T</sup> and its phylogenetically closest related species. Strains: 1, L10.15<sup>T</sup>; 2, *P. donghaensis* JH1<sup>T</sup>; 3, *P. halocryphilus* Or1<sup>T</sup>; 4, *P. antarcticus* DSM 14505<sup>T</sup>; 5, *P. kocurii* DSM 20747<sup>T</sup>; 6, *P. maritimus* JCM 11543<sup>T</sup>; 7, *P. plakortidis* DSM 23997<sup>T</sup> and 8, *P. salinarum* ISL-16<sup>T</sup>. All strains are positive for the utilization of dextrin, D-fructose, D-glucose- 6-PO<sub>4</sub>, L-alanine, L-glutamic acid, pectin, D-galacturonic acid, and glucuronamide. All strains are negative for utilization of D-turanose, stachyose, D-mannose, 3-methyl glucose, D-sorbitol, citric acid, and bromo-succinic acid. In chemical sensitivity assay, all strains are able to growth in 1 % sodium lactate, aztreonam and sodium butyrate, but not in vancomycin, niaproof 4, troleandomycin, rifamycin SV and minocycline. All data were obtained in this study.

Characteristics	1	2	3	4	5	6	7	8
Growth:								
at pH	6.0-12	6.0-10	6.0-11	6.0-12	6.0-12	5.0-8	6-10	5.5-12
NaCl tolerance (% , w/v)	15	12	19	12	8	17	9	13
up to °C	30	37	37	28	37	41	37	38
<b>From GenIII plate</b>								
<b>Assimilation of:</b>								
D-Maltose	-	+	+	+	+	-	-	+
D-Trehalose	-	+	-	+	-	-	-	-
D-Cellobiose	-	-	-	+	-	-	-	-
Gentiobiose	-	+	-	-	-	-	-	-
Sucrose	-	+	-	-	-	-	-	-
D-Raffinose	-	-	-	+	-	-	-	-
α-D-Lactose	-	-	-	-	+	+	-	-
D-Melibiose	-	-	-	-	+	+	-	-
β-Methyl-D- Glucoside	-	+	+	+	-	-	-	-
D-Salicin	-	+	+	-	-	+	-	-
N-Acetyl-D- Glucosamine	+	+	+	-	+	+	-	-
N-Acetyl- β-D- Mannosamine	-	+	+	-	+	+	-	-
N-Acetyl-D- Galactosamine	-	-	-	+	-	+	-	-
N-Acetyl Neuraminic Acid	+	-	+	-	-	-	-	-
α-D-Glucose	+	+	+	-	+	-	-	-
D-Galactose	-	-	-	-	+	+	-	-
D-Fucose	-	+	-	-	-	-	-	-
L-Fucose	-	-	-	+	-	-	-	-

L-Rhamnose	-	-	-	-	-	+	-	-
Inosine	+	+	+	-	+	+	+	-
D-Mannitol	+	+	+	+	+	+	-	-
D-Arabitol	-	-	-	+	-	-	-	+
myo-Inositol	-	-	-	+	-	-	-	-
Glycerol	+	+	+	+	+	+	-	-
D-Fructose-6-PO <sub>4</sub>	+	+	+	-	+	+	+	-
D-Aspartic Acid	-	+	+	+	-	+	-	-
D-Serine	-	-	+	+	-	-	-	+
Gelatin	-	+	+	+	+	+	-	-
Glycyl-L-Proline	+	+	+	+	+	+	-	-
L-Arginine	-	+	+	+	+	+	-	+
L-Aspartic Acid	+	+	-	+	+	+	-	+
L-Histidine	-	+	+	+	-	-	-	-
L-Pyrogutamic Acid	+	+	+	-	+	+	-	+
L-Serine	+	+	+	-	+	+	+	-
L-Galactonic Acid Lactone	+	+	+	-	+	+	+	+
D-Gluconic Acid	+	+	+	+	+	+	-	+
D-Glucuronic Acid	+	+	-	-	+	+	+	+
Mucic Acid	+	+	+	+	+	+	-	+
Quinic Acid	-	+	+	+	+	+	-	+
D-Saccharic Acid	+	+	+	-	+	+	-	-
<i>p</i> -Hydroxy- Phenylacetic Acid	-	-	-	+	-	-	-	-
Methyl Pyruvate	-	-	-	+	-	-	-	-
D-Lactic Acid Methyl Ester	+	+	+	-	+	-	-	+
L-Lactic Acid	-	+	+	+	-	+	-	-
$\alpha$ -Keto-Glutamic Acid	+	+	+	-	+	+	-	+
D-Malic Acid	+	+	+	+	+	+	-	+
L-Malic Acid	+	+	+	-	+	+	-	+
Tween 40	+	+	+	+	+	+	+	+
$\gamma$ -Amino-Butyric Acid	-	-	-	+	-	-	-	-
$\alpha$ -Hydroxy- Butyric Acid	-	-	+	+	-	+	-	-
$\beta$ -Hydroxy-D,L- Butyric Acid	+	+	+	-	+	+	+	+
$\alpha$ -Keto-Butyric Acid	-	-	+	-	-	+	+	-
Acetoacetic Acid	+	+	-	+	+	-	+	+
Propionic Acid	-	-	-	+	-	+	+	-
Acetic Acid	+	+	+	-	+	+	+	+
Formic Acid	+	+	+	-	+	-	-	+
<b>Chemical Sensitivity:</b>								
Fusidic Acid	+	-	-	-	-	-	-	-
D-Serine	-	-	+	-	-	-	-	-
Lincomycin	-	-	-	+	-	-	-	-
Guanidine HCl	-	-	-	+	-	-	+	-

Tetrazolium Violet	W	+	w	w	+	+	+	+
Tetrazolium Blue	-	-	-	w	-	-	-	-
Nalidixic Acid	+	+	-	w	+	-	+	+
Lithium Chloride	+	+	+	-	+	+	+	+
Potassium Tellurite	-	+	-	-	+	+	+	+
Sodium Bromate	W	w	-	+	-	-	+	-
<b>API ZYM test:</b>								
Alkaline phosphatase	-	+	-	-	+	+	+	+
Esterase	-	w	-	+	w	+	+	+
Leucine arylamidase	+	w	-	+	+	+	+	+
Valine arylamidase	-	+	-	w	+	+	w	+
Cystine arylamidase	+	-	-	+	+	+	+	+
$\alpha$ -chymotrypsin	+	+	-	w	-	-	-	+
$\beta$ - galactosidase	-	w	-	+	+	+	-	-
$\beta$ -glucosidase	-	+	+	-	-	-	-	-
<b>Genome feature:</b>								
Genome size (Mb)	3.37	3.32	3.42	3.83	3.49	3.29	3.28	NA
DNA G+C content (mol %)	39.4	40.1	40.1	43.2	40.9	47.2	50.0	NA
Number of genes #	4639	4417	4598	5040	4631	4609	4889	NA
Number of coding sequences #	4425	4196	4276	4811	4460	4365	4718	NA

**Table 2.** Cellular fatty acid profile of strain L10.15<sup>T</sup> and close related species.

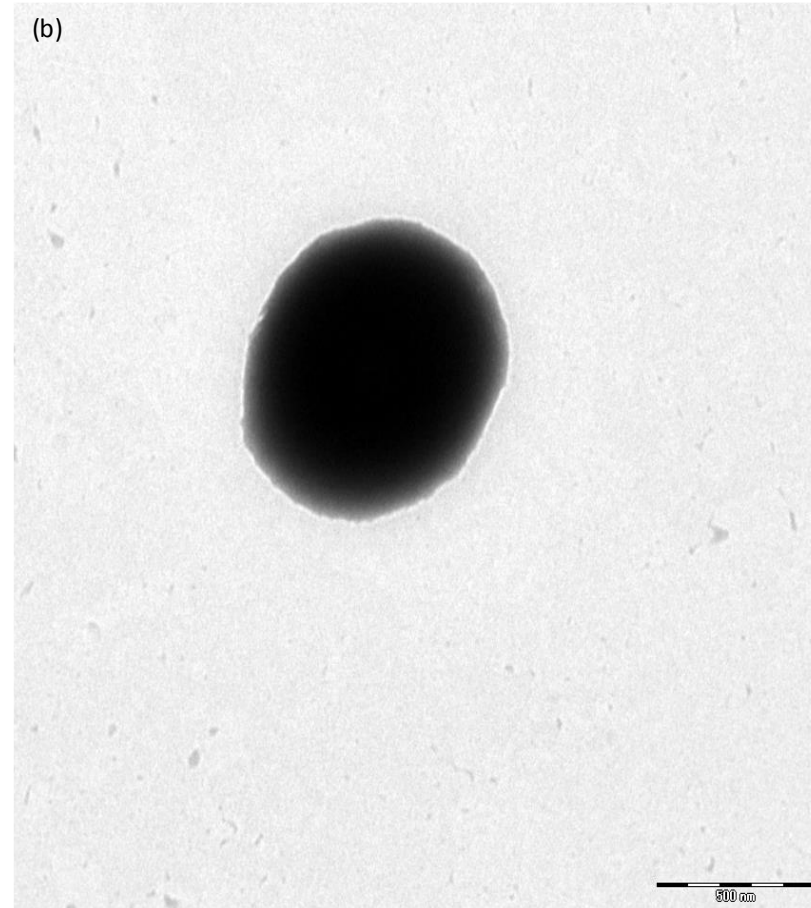
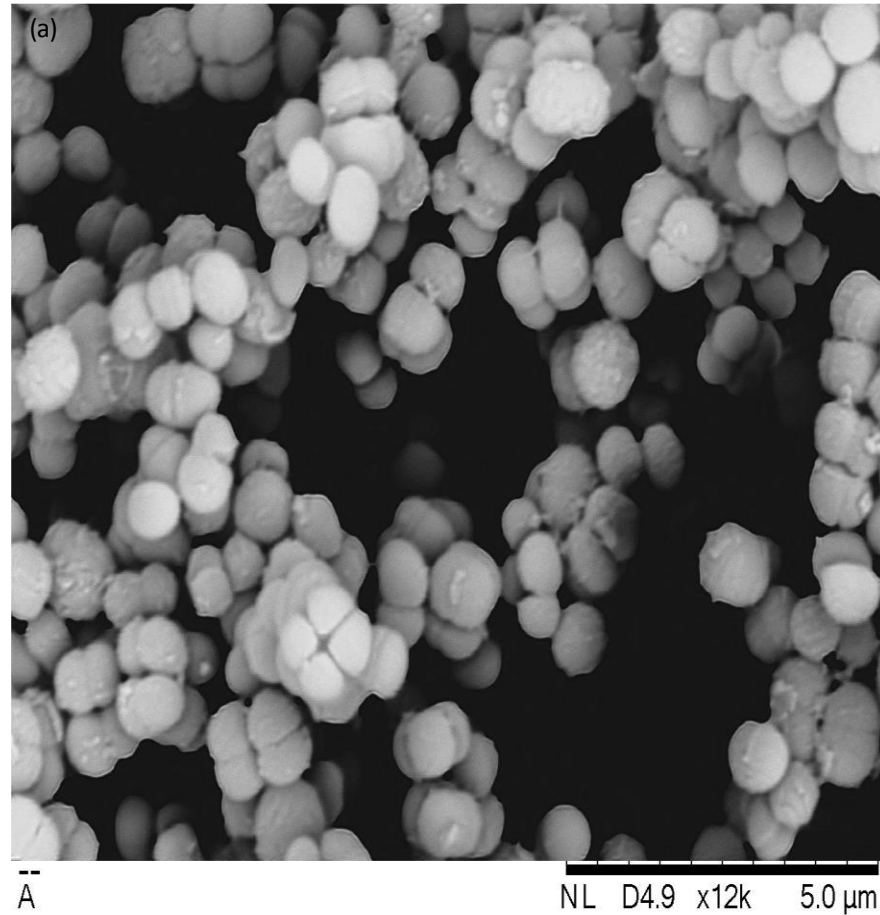
Strains: 1, *P. versutus* sp. nov. L10.15<sup>T</sup>; 2, *P. donghaensis* JH1<sup>T</sup>; 3, *P. halocryphilus* Or1<sup>T</sup>; 4, *P. antarcticus* DSM 14505<sup>T</sup>; 5, *P. kocurii* DSM 20747<sup>T</sup>; 6, *P. maritimus* JCM 11543<sup>T</sup>; 7, *P. plakortidis* DSM 23997<sup>T</sup> and 8, *P. salinarum* ISL-16<sup>T</sup>. Values are percentages of the total fatty acids and only fatty acids comprising 0.5 % are shown. 2, ND-Not detected. All data were obtained in this study.

Fatty acid	1	2	3	4	5	6	7	8
<b>Straight chain</b>								
C <sub>14:0</sub>	0.6	1.2	0.5	0.7	0.6	-	0.6	1.2
C <sub>15:0</sub>	1.2	0.9	0.6	1.6	4.1	1.1	1.9	-
C <sub>16:0</sub>	4.0	<b>12.6</b>	6.8	4.1	2.6	1.5	4.4	3.5
C <sub>17:0</sub>	0.7	1.9	0.5	1.2	2.9	1.9	-	0.8
C <sub>18:0</sub>	1.0	4.8	0.9	1.4	0.6	1.2	1.9	1.8
<b>Branched chain</b>								
anteiso-C <sub>13:0</sub>	0.6	-	0.5	-	-	-	-	-
iso-C <sub>14:0</sub>	3.4	2.4	2.2	1.5	2.1	3.4	2.4	3.2
iso-C <sub>15:0</sub>	1.9	2.3	2.5	3.6	3.6	9.8	5.2	2.5
anteiso-C <sub>15:0</sub>	<b>46.2</b>	<b>35.0</b>	<b>44.4</b>	<b>44.7</b>	<b>43.0</b>	<b>32.3</b>	<b>43.4</b>	<b>32.1</b>
iso-C <sub>16:0</sub>	5.5	4.6	4.9	3.7	4.0	4.2	6.5	3.7
iso-C <sub>17:0</sub>	1.9	3.2	3.6	7.5	5.3	5.5	-	2.9
iso-C <sub>17:1</sub> <sup>ω<sup>10c</sup></sup>	1.3	0.9	-	3.5	2.7	4.1	-	3.3
anteiso-C <sub>17:0</sub>	<b>10.7</b>	<b>14.1</b>	<b>15.7</b>	<b>11.9</b>	9.6	5.9	-	9.3
iso-C <sub>18:0</sub>	0.7	1.0	0.4	-	0.6	4.7	1.5	-
<b>Unsaturated</b>								
C <sub>16:1</sub> <sup>ω<sup>7c</sup></sup> alcohol	6.5	1.8	2.9	2.2	2.9	6.6	4.8	<b>10.1</b>
C <sub>16:1</sub> <sup>ω<sup>11c</sup></sup> alcohol	5.6	5.8	4.6	2.9	3.8	1.5	2.8	1.8
C <sub>17:1</sub> <sup>ω<sup>7</sup></sup>	0.8	1.1	0.3	0.7	3.0	4.1	-	-
C <sub>18:1</sub> <sup>ω<sup>9c</sup></sup>	0.7	2.1	0.8	0.9	1.0	1.6	1.8	0.8
Summed feature 3 <sup>†</sup>	0.6	-	-	-	0.4	-	-	1.0
Summed feature 4 <sup>††</sup>	6.0	3.3	6.1	5.3	6.0	5.4	2.9	8.6

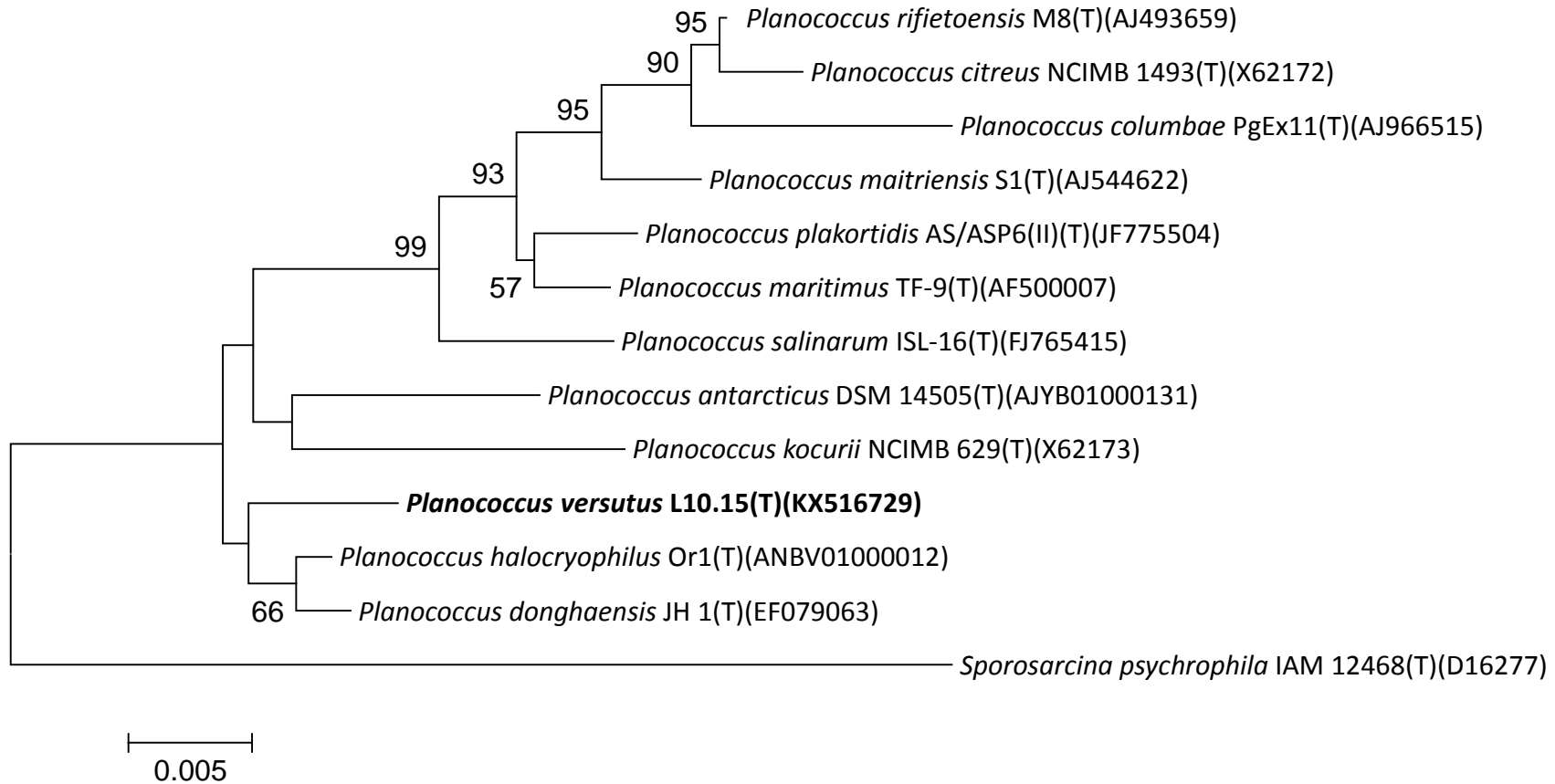
<sup>†</sup>Summed feature 3 contains C<sub>16:1</sub> <sup>ω<sup>7c</sup></sup> and/or C<sub>16:1</sub>, which could not be separated by GC with the MIDI system.

<sup>††</sup>Summed feature 4 contains iso-C<sub>17:1</sub> and/or anteiso-C<sub>17:1</sub>, which could not be separated by GC with the MIDI system.

Fig. 1



# Fig.2



***Planococcus versutus* sp. nov., isolated from Antarctic soil**

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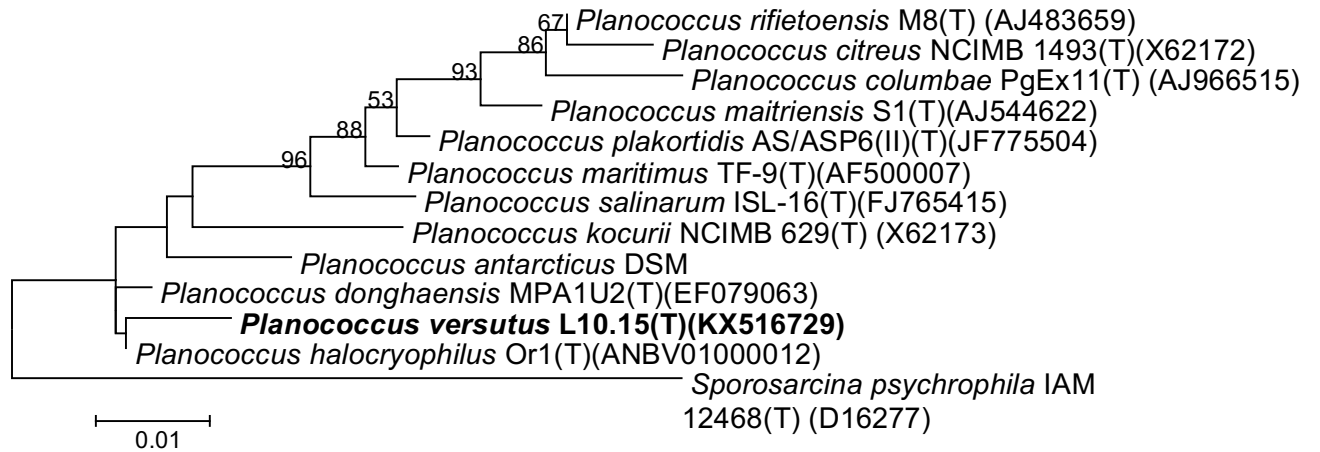
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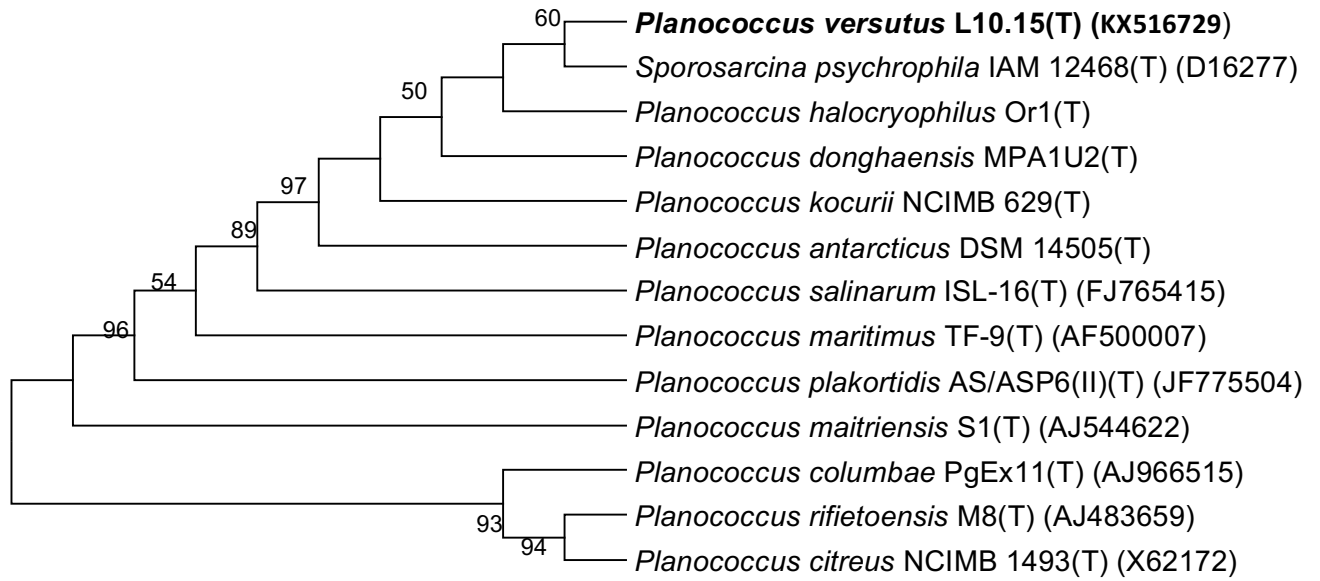
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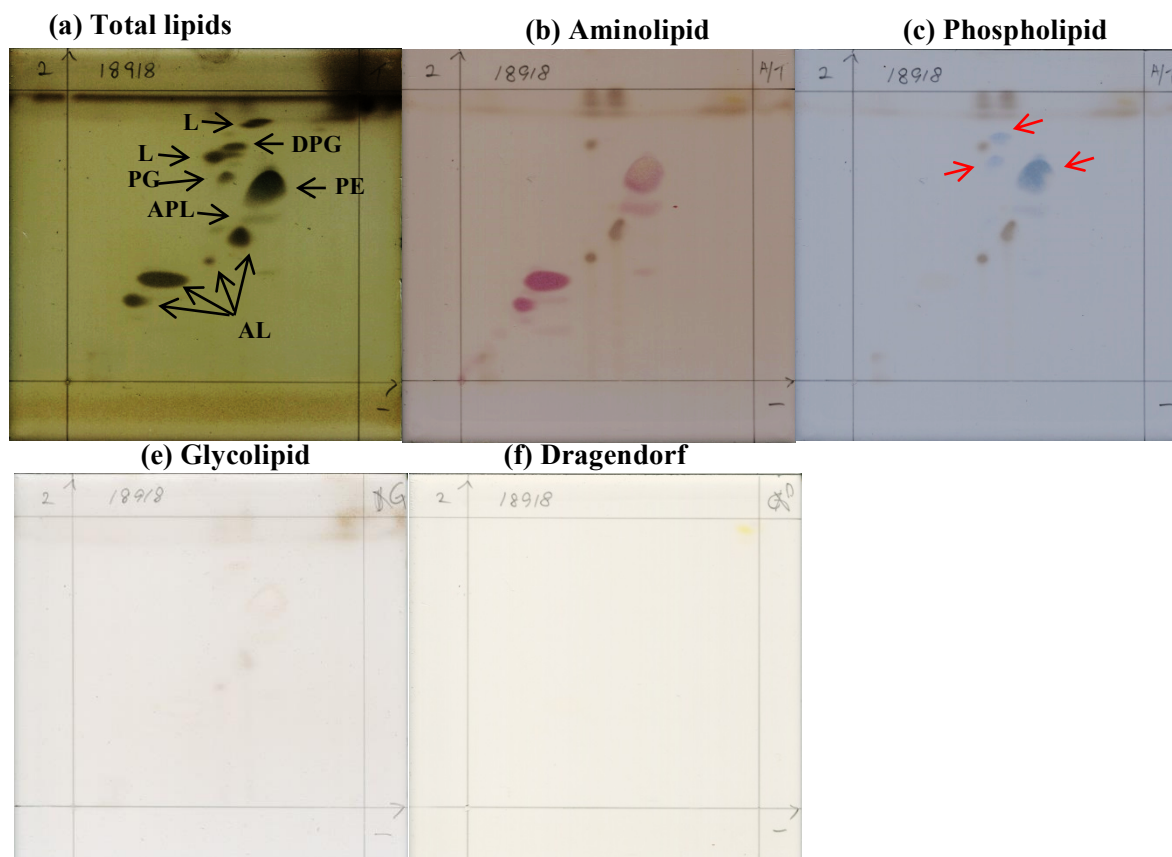




**Supplementary Fig. S1.** Phylogenetic tree constructed by maximum-likelihood analysis based on 16S rDNA sequences, depicting the phylogenetic relationship of strain L10.15<sup>T</sup> with closely related type species of the genus *Planococcus*. Scale bar represents evolutionary distance as 0.01 change per nucleotide position. Bootstrap values (%) > 50 % from 1,000 replicates are shown.



**Supplementary Figure S2.** 16S rRNA phylogeny constructed using maximum parsimony phylogeny showing the taxonomic position of strain L10.15<sup>T</sup> against 12 closest relatives retrieved from EzTaxon database. Bootstrap values (expressed as percentages of 100 replicates) greater than 50 % are shown at the branch points. *Sporosarcina psychrophila* IAM 12468<sup>T</sup> was used as an outgroup.



**Supplementary Fig. S3.** TLC chromatograms of polar lipid distribution of *P. versutus* strain L10.15<sup>T</sup> visualized with 5 % ethanolic molybdato-phosphoric acid for total lipids (a); ninhydrin (Sigma) for amino lipids (b); molybdenum blue (Sigma) for phospholipids (c);  $\alpha$ -naphthol/sulphuric acid reagent for glycolipids (d) and Dragendorff reagent for choline-containing lipids (e). The polar lipid profile consisted of a mixture of phosphatidylethanolamine (PE), phosphatidylglycerol (PG), diphosphatidylglycerol (DPG), an unidentified aminophospholipid (APL), two unidentified Lipids (L) and four unidentified aminolipids (AL).

**Supplementary Table S1.** ANI-MUMmer analysis and the aligned percentage in [#] of

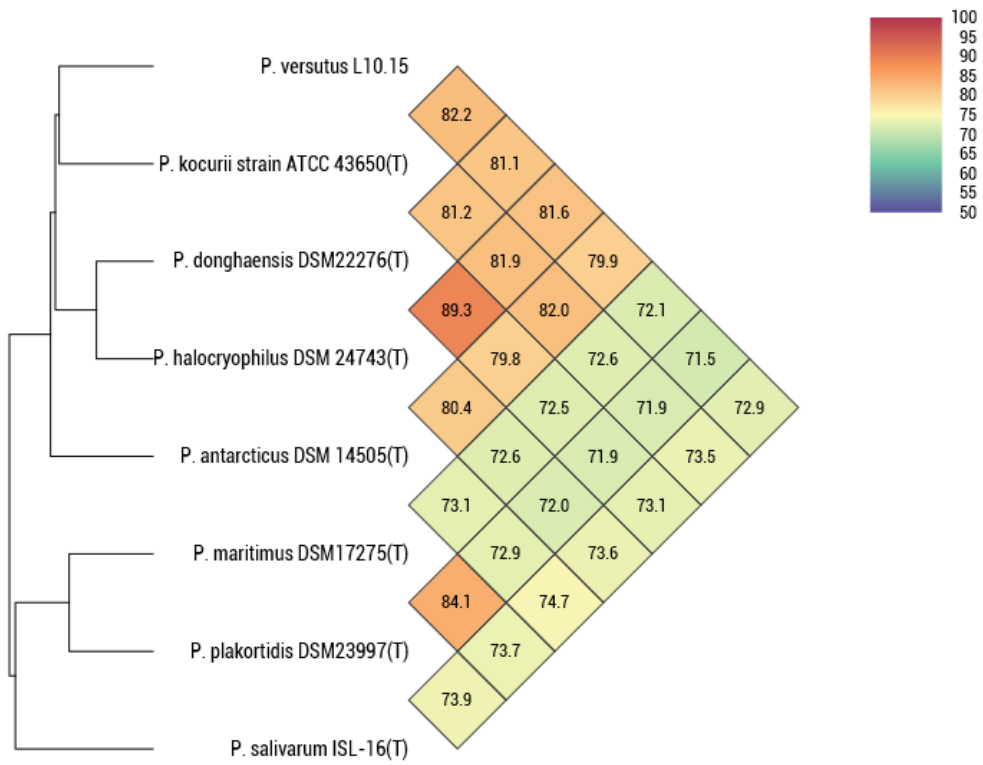
*P. versutus* L10.15<sup>T</sup> and closest type species based on JSpeciesWS.

#ANIm and aligned percentage	<i>P. salivarium</i> ISL-16 <sup>T</sup>	<i>P. versutus</i> L10.15 <sup>T</sup>	<i>P. donghaensis</i> JH1 <sup>T</sup>	<i>P. halocryphilus</i> Or1 <sup>T</sup>	<i>P. antarcticus</i> CMS 26or <sup>T</sup>	<i>P. plakortidis</i> AS/ASP6 (II) <sup>T</sup>	<i>P. maritimus</i> TF-9 <sup>T</sup>	<i>P. kocurii</i> ATCC 43650 <sup>T</sup>
<i>P. salivarium</i> ISL-16 <sup>T</sup>	*	88.2 [7.0]	89.3 [7.8]	88.8 [8.0]	86.0 [11.3]	86.2 [8.8]	87.8 [7.7]	88.9 [7.7]
<i>P. versutus</i> L10.15 <sup>T</sup>	88.2 [2.4]	*	84.8 [41.5]	84.8 [46.6]	84.4 [39.5]	85.0 [4.9]	85.0 [5.5]	86.1 [46.6]
<i>P. donghaensis</i> JH1 <sup>T</sup>	88.6 [3.0]	84.8 [43.1]	*	89.6 [86.2]	84.0 [38.5]	85.5 [5.8]	85.5 [6.4]	85.2 [46.5]
<i>P. halocryphilus</i> Or1 <sup>T</sup>	88.4 [2.9]	84.8 [46.3]	89.6 [83.3]	*	84.1 [41.5]	85.1 [5.7]	84.4 [6.6]	85.6 [48.7]
<i>P. antarcticus</i> CMS 26or <sup>T</sup>	86.0 [3.7]	84.3 [35.0]	84.0 [33.0]	84.2 [37.1]	*	84.1 [5.8]	84.7 [6.1]	84.9 [46.0]
<i>P. plakortidis</i> AS/ASP6 (II) <sup>T</sup>	86.2 [3.1]	85.0 [5.0]	85.2 [5.5]	85.1 [5.8]	84.1 [6.7]	*	85.8 [69.4]	85.4 [5.5]
<i>P. maritimus</i> TF-9 <sup>T</sup>	87.4 [2.7]	84.6 [5.6]	85.5 [6.1]	84.4 [6.7]	84.7 [6.9]	85.8 [69.1]	*	84.8 [6.8]
<i>P. kocurii</i> ATCC 43650 <sup>T</sup>	88.9 [2.5]	86.1 [45.0]	85.2 [43.3]	85.6 [47.4]	84.9 [49.8]	85.4 [5.2]	84.8 [6.4]	*

**Supplementary Table S2.** ANI-Blast analysis and the aligned percentage in [#] of *P.*

*versutus* L10.15<sup>T</sup> and closest type species based on JSpeciesWS.

#ANiB and aligned percentage	<i>P. salivarium</i> ISL-16 <sup>T</sup>	<i>P. versutus</i> L1015 <sup>T</sup>	<i>P. donghaensis</i> JH1 <sup>T</sup>	<i>P. halocryphilus</i> Or1 <sup>T</sup>	<i>P. antarcticus</i> CMS 26or <sup>T</sup>	<i>P. plakortidis</i> AS/ASP6 (II) <sup>T</sup>	<i>P. maritimus</i> TF-9 <sup>T</sup>	<i>P. kocurii</i> ATCC 43650 <sup>T</sup>
<i>P. salivarium</i> ISL-16 <sup>T</sup>	*	73.2 [52.9]	73.4 [58.2]	73.4 [58.4]	74.7 [61.2]	74.2 [56.8]	73.8 [56.1]	73.6 [55.4]
<i>P. versutus</i> L1015 <sup>T</sup>	73.0 [16.7]	*	80.8 [68.9]	81.2 [71.4]	79.8 [70.2]	71.3 [52.8]	72.0 [53.7]	81.8 [72.2]
<i>P. donghaensis</i> JH1 <sup>T</sup>	73.4 [19.4]	80.8 [70.5]	*	89.0 [85.3]	79.6 [72.2]	71.7 [57.3]	72.3 [58.8]	81.2 [72.3]
<i>P. halocryphilus</i> Or1 <sup>T</sup>	73.6 [18.2]	81.2 [70.8]	88.9 [82.6]	*	80.2 [72.4]	71.6 [57.3]	72.2 [58.7]	81.7 [73.0]
<i>P. antarcticus</i> CMS 26or <sup>T</sup>	74.8 [17.9]	79.6 [63.0]	79.5 [63.3]	80.1 [65.0]	*	72.6 [50.5]	72.7 [51.9]	81.5 [65.1]
<i>P. plakortidis</i> AS/ASP6 (II) <sup>T</sup>	73.7 [19.3]	71.1 [54.7]	71.4 [58.8]	71.5 [59.6]	72.5 [58.8]	*	83.6 [80.6]	71.6 [57.0]
<i>P. maritimus</i> TF-9 <sup>T</sup>	73.7 [18.2]	72.0 [54.9]	72.2 [58.8]	72.3 [60.0]	72.8 [59.3]	83.7 [79.5]	*	72.5 [57.4]
<i>P. kocurii</i> ATCC 43650 <sup>T</sup>	73.3 [17.1]	81.7 [69.9]	80.9 [69.0]	81.6 [71.4]	81.5 [70.2]	71.6 [52.9]	72.3 [54.3]	*



**Supplementary Fig. S4.** OrthoANI analysis of *P. versutus* L10.15<sup>T</sup> and closest type species.