bioRxiv preprint doi: https://doi.org/10.1101/2020.07.27.213165. this version posted July 28, 2020. The copyright holder for this preprint (which was not certified by peer review) is the author/funder. All rights reserved. No reuse allowed without permission.

Haloargentinum marplatensis gen. nov., sp. nov., a novel extremely halophilic bacterium isolated from salted-ripened anchovy (*Engraulis anchoita*)

Silvina Perez[1,2*], Margarita Gomila[3], Silvia Elena Murialdo[1,4], Irene Mabel Ameztoy[2], Narjol Gonzalez-Escalona[5], Elida Elvia Ramírez[2], María Isabel Yeannes[1,2]

[1]Dpto. de Ingeniería Química y en Alimentos, Facultad de Ingeniería, Universidad Nacional de Mar del Plata, 7600 Mar del Plata, Argentina

[2]Grupo de Investigación Preservación y Calidad de Alimentos (GIPCAL), Instituto de Ciencia y Tecnología de Alimentos y Ambiente (INCITAA), CONICET, Facultad de Ingeniería-Universidad Nacional de Mar del Plata, 7600 Mar del Plata, Argentina

[3]Microbiologia, Departament de Biologia, Universitat de les Illes Balears, 07122 Palma de Mallorca, Spain

[4]Grupo de Ingeniería Bioquímica (GIB), Instituto de Ciencia y Tecnología de Alimentos y Ambiente (INCITAA), CIC-PBA, Facultad de Ingeniería-Universidad Nacional de Mar del Plata, 7600 Mar del Plata, Argentina

[5]Division of Microbiology, Center for Food Safety and Applied Nutrition, Food and Drug Administration, College Park, MD, United States of America

***Corresponding author: silvinaperez@fi.mdp.edu.ar**

Keywords

Haloargentinum, *Firmicutes*, halophile, fermented fish

ABSTRACT

A facultative aerobic, Gram-negative, motile, non-endospore forming and extremely halophilic bacterium, strain 11aii^T, isolated from salted-ripened anchovy, was examined using a polyphasic approach to characterize and clarify its phylogenetic and taxonomic position. Sequences of the 16S rRNA gene revealed close relationships to species of the genera *Lentibacillus* and *Virgibacillus* (94.2% similarity). The organism grew optimally in the

bioRxiv preprint doi: https://doi.org/10.1101/2020.07.27.213165. this version posted July 28, 2020. The copyright holder for this preprint (which was not certified by peer review) is the author/funder. All rights reserved. No reuse allowed without permission.

32 presence of 20-35 % NaCl. The major fatty acids of strain 11aii^T were $C_{16:0}$ (42.1%) and
33 anteiso-$C15_{:0}$ (31.2%) and also presented iso-$C_{16:0}$ (11.0%), anteiso-$C_{17:0}$ (10.4%) and $C_{18:0}$
34 (5.2%). Based on data presented here, strain 11aii^T is considered to represent a novel genus
35 and species, for which the name *Haloargentinum marplatensis* gen. nov. sp. nov. is
36 proposed with the strain 11aii^T as type strain.

37

38

---

39 Introduction

40

41 Salting is an ancient method that has been applied for fish preservation. It can be followed by the
42 ripening stage consisting of chemical and physicochemical changes that modify the characteristics of
43 the muscle tissue and thus the sensory properties of the fish. These changes require months in the
44 presence of high salt content (NaCl). Salting and ripening of different pelagic species is a worldwide
45 common and traditional practice [1, 2]. Among this type of product, salted-ripened anchovy
46 (*Engraulis anchoita*) produced in Latin American countries can be mentioned. Due to the high NaCl
47 content and low water activity values that characterize this type of products, the microbiota is
48 mainly constituted by halophilic or halotolerant microbes. The role of microorganisms during the
49 ripening is under continuous investigation [1, 3–7]. Recent studies have reported the isolation of
50 many novel bacteria and archaea from salted and fermented seafood: *Lentibacillus jeotgali* Grbi^T [8],
51 *Halomonas shantousis* SWA25^T [9], *Halobacterium piscisalsi* HPC1-2^T [10], *Natrinema gari* HIS40-3^T
52 [11], *Haloterrigena jeotgali* A29^T [12], *Haloarcula salaria* HST01-2R^T and *Haloarcula tradensis* HST03^T
53 [13]. Here, we report the taxonomic characterization of an halophilic isolate which closest relatives
54 are members of the genera *Lentibacillus* and *Virgibacillus* belonged to the family *Bacillaceae* [8, 14,
55 15]. The genus *Lentibacillus* was defined by Yoon et al. [16], with the description of *Lentibacillus*
56 *salicampi* SF-20^T, a Gram-variable endospore-forming rod-shaped strain. Its last described species
57 corresponds to *Lentibacillus lipolyticus* SSKP1-9^T isolated from salted shrimp paste in Thailand [17].
58 The genus *Virgibacillus* was established by the reclassification of *Bacillus pantothenticus* CN3028^T
59 [18] as *Virgibacillus pantothenticus* [19], and the genus description was later emended by Heyrman
60 et al [20]. Members of this genus are Gram-positive or Gram-variable, endospore-forming, motile
61 rods [20, 21]. At the time of writing, *Lentibacillus* and *Virgibacillus* genera contained 17 and 36
62 validly named species, respectively, as reported on the LSPN website
63 (www.bacterio.net/lentibacillus.html and www.bacterio.net/virgibacillus.html). Notably, the
64 reported halophilic isolate here presented remarkable morphotype differences with the mentioned
65 genera. Based on the results of our taxonomic study and previous characterizations of the most
66 closely related genera, we consider that the halophilic strain should be included within a novel genus
67 and species for which the name *Haloargentinum marplatensis* gen. nov., sp. nov. is proposed.

68

69

---

bioRxiv preprint doi: https://doi.org/10.1101/2020.07.27.213165. this version posted July 28, 2020. The copyright holder for this preprint (which was not certified by peer review) is the author/funder. All rights reserved. No reuse allowed without permission.

70

71 ## Isolation and Ecology

72 Strain 11aii^T was isolated from beheaded and partially gutted salted-ripened anchovies collected
73 from a local factory (Mar del Plata, Argentina). Homogenates were prepared in saline broth (NaCl,
74 150 g/L; meat peptone, 3 g/L; meat extract, 3 g/L) in duplicate, followed by a subsequently
75 enrichment step performed by incubation at 35–37 °C for 60 min and successive serial dilutions were
76 carried out [22]. Homogenates (0.1 mL) were spread onto the growth media named Tryptone-salt-
77 yeast extract (TSL: NaCl, 200 g/L; $MgSO_4(7H_2O)$, 20 g/L; KCl, 5 g/L; $CaCl_2(6H_2O)$, 0.2 g/L; tryptone, 5
78 g/L; yeast extract, 4 g/L; agar-agar, 17 g/L) [23] in duplicate and incubated at 35–37 °C during 21
79 days. Colonies with different macroscopic characteristics (colour, size, shape and density) were re-
80 streaked on fresh agar plates and incubated at 35–37 °C until growth. Pure isolates were transferred
81 to TSL broth (NaCl, 200 g/L; $MgSO_4(7H_2O)$, 20 g/L; KCl, 5 g/L; $CaCl_2(6H_2O)$, 0.2 g/L; tryptone, 5 g/L;
82 yeast extract, 4 g/L) [23]. Colony stocks were kept at 4 °C for further analyses.

83

84

85

86 ## 16S RNA phylogeny

87 DNA was extracted and purified as described by D´Ippólito *et al.* [24] and Sheu *et al.* [25]. The
88 reaction mixture for PCR was performed with 5 µL of DNA template (cell-by-heat lysate), 2.5 µL of
89 buffer 1X, 1.5 µL of MgCl2 50 mM, 1.25 µL of dimethyl-sulphoxide, (DMSO), 1.25 µL dNTPs 10 mM,
90 0.8 µL of each primer (F43Eco 5'-CGGAATTCCAGGCCTAACACATGCAAGTC-3' and R1387Eco 5'-
91 CGGAATTCGGGCGGWGTGTACAAGGC-3'), 0.25 µL of Taq polimerase, to a final volume of 25 µL. PCR
92 reaction was executed by Biometra UNO-Thermoblock Thermal Cycler. Amplifications were carried
93 out using the following program: (94 °C 3 min) x 1; (94 °C 1 min, 55 °C 1 min, 72 °C 90 s) x 30, (72 °C
94 10 min) x 1. PCR products were purified by QIAquick PCR Purification kit (Qiagen, Alemania). PCR
95 products (10 µl each) were analyzed on 2 % TAE pre-cast agarose gels (Bio-Rad, Hercules, CA) and
96 run at 75 V for 1 h in 1X TAE with a molecular weight standard (100 bp ladder, Promega, WI, USA).
97 Amplification products were visualized by ethidium bromide staining (5 ug/ml). PCR product
98 consisted of a single band. PCR product was sequenced in both directions by MCLAB (South San
99 Francisco, CA, USA) employing primers 27F, 357F (5'-CTCCTACGGGAGGCAGCAG-3'), 518R (5'-
100 CGTATTACCGCGGCTGCTGG-3'), and 1492R sequenced by MCLAB company (www.mclab.com). DNA
101 sequences were assembled using Bioedit [26].

102 For 16S rDNA phylogenetic analysis, a BLAST analysis of the 11aii^T strain 16S rDNA sequence showed
103 that it matched 94 % to 16S rDNA sequences from strains *Lentibacillus* sp. KM1091 and *Lentibacillus*
104 *juripiscarius* strain P1-ASH. Ninety eight 16S rDNA partial sequences representing 90 highly related
105 bacterial taxa (publicly available at GenBank - Supplementary Table 1) and with a 92-99 % identical
106 to the 16S rDNA from strain 11aii^T were retrieved in order to re-construct the phylogenic
107 relationship of the strains. The sequences were aligned and an internal 16S rDNA fragment of 1190

bioRxiv preprint doi: https://doi.org/10.1101/2020.07.27.213165. this version posted July 28, 2020. The copyright holder for this preprint (which was not certified by peer review) is the author/funder. All rights reserved. No reuse allowed without permission.

108 bp was used for the phylogenetic study. The phylogeny was reconstructed using the maximum
109 likelihood method using Mega software v7 [27] and using the kimura-2 parameter model to estimate
110 the genetic distances [28]. The statistical support of the nodes in the ML tree was assessed by 500
111 bootstrap re-sampling.

112 Figure 1 shows the phylogenetic tree with the most of the Lineages compressed for visualization
113 purposes. The original phylogenetic tree with the highest log likelihood is shown in Supplementary
114 Figure 1. The 16S rRNA gene sequence of strain 11aii^{T} was closely related to species from the genera
115 *Lentibacillus* and *Virgibacillus* (94.2% similarity) by phylogentic analysis. It was closely related to
116 *Lentibacillus juripiscarius* (93.4%), *Lentibacillus jeotgali* (92.2%), *Virgibacillus flavescens* (92.0%) and
117 *Virgibacillus phasianinus* (91.7%).

118

119

120

121 Physiology and Chemotaxonomy

122 Morphological, physiological and biochemical characteristics were studied. The cell morphology was
123 carried out on the basis of the Gram staining (optic microscope) [29]. Focused on the capacity to
124 produce histamine by decarboxylation of its precursor (histidine), the histidine-decarboxylase test
125 was carried out. Cultures were inoculated on slanting surface of a solidified selective medium
126 (tryptone, 5 g/L; yeast extract, 5 g/L; L-histidine, 27 g/L; $CaCO_3$, 1 g/L; agar-agar, 20 g/L; bromocresol
127 purple, 0.06 g/L; pH 5.3), and incubated at 35–37°C during 10 days. Positive result was indicated by
128 the medium towards violet [30, 31]. Isolate with positive histidine-decarboxylase test was submitted
129 to a further characterization. Therefore, Ziehl-Neelsen staining was carried out and spore staining
130 was done by the Schaeffer and Fulton [32] technique. NaCl requirement was determined in the
131 above growth broth containing various NaCl concentrations (0–6 M). Inoculums were incubated at
132 35–37 °C and positive result was indicated by growing. Growth at different pH values (5.0 to 8.5,
133 with an interval of 0.5) was examined using TSL. Tests for catalase and cytochrome oxidase activities,
134 motility, nitrate reduction, urease reaction, lysine decarboxylase in lysine iron agar, histidine and
135 arginine dihydrolase by inoculation in basal broth with the respective amino acid, citrate utilization
136 on Simmons citrate agar (Britania) and the hydrolysis of gelatine and starch were performed as
137 described by MacFaddin [29]. The hydrolysis of Tween 80 was detected by screening for zones of
138 hydrolysis around colonies growing in a solid medium containing 1% v/v of this subtract [33].
139 Hydrogen sulphide (H_2S) production was tested by inoculation in TSI medium (Britania) which allows
140 the investigation of the production of H_2S and also the production of acid and gas from glucose,
141 lactose and sucrose. Indole formation was studied by inoculation and growing in peptone broth and
142 subsequently reaction with Kovacs's reagent. Acid production from carbohydrates was determined
143 in red phenol broths with 1% w/v of each subtract under study (galactose, sucrose, glucose, fructose,
144 lactose, maltose, sorbitol, mannitol, trehalose, xylose and arabinose). Oxidative/fermentative
145 metabolism of glucose was determined on OF basal medium [29]. Proteolytic and lipolytic activities
146 were determined by streaking pure culture in skim milk agar (yeast extract, 3 g/L; meat peptone, 5

bioRxiv preprint doi: https://doi.org/10.1101/2020.07.27.213165. this version posted July 28, 2020. The copyright holder for this preprint (which was not certified by peer review) is the author/funder. All rights reserved. No reuse allowed without permission.

147 g/L; agar, 15 g/L; milk, 10 mL/L) and in a solid medium containing 1% v/v of tributyrin, respectively.
148 Inoculated plates were incubated at 35–37 °C for 10 days. Clear zones around the streaks were
149 regarded as positive reactions [34]. All culture media used for biochemical tests were supplemented
150 with NaCl to a final concentration of 200 g/L, with K^+ (10 ppm) and Mg^{2+} (0.1 ppm) in order to
151 provide the specific nutrients needed by halophilic bacteria [23]. All analyses were carried out in
152 duplicate.

153 Based on colony macroscopic characteristics, two isolates were distinguished, namely, 11ai and
154 11aii^T. 11ai colonies were pale-pink pigmented, their cells were Gram-negative long-rods-shaped and
155 this isolate resulted negative for histidine-decarboxylase test. The 11aii^T colonies formed on agar
156 plates were circular (1–2 mm in diameter), smooth, translucent and salmon-reddish pigmented. This
157 isolate was Gram-negative and cells coccobacilli and disc-shaped (pleomorphic) were observed.
158 Regarding to the histidine-decarboxylase test, 11aii^T was positive, indicating that it could form
159 histamine. The presence of this biogenic amine is regulated because of in high concentrations
160 represents a potential food safety hazard [35, 36]. Therefore, 11aii^T was selected for further
161 investigations. Ziehl-Neelsen staining exhibited a negative result. Endospores was not observed. The
162 strain was able to grow at high NaCl concentrations, from 3.4 M (approximately 20 %) to 6 M
163 (approximately 35 %), and pH between 5.5 and 8. The strain was positive for oxidase and catalase.
164 Acid was not produced from sugars by red phenol broths, TSI or OF basal medium. Cells did not
165 hydrolyse gelatine or Tween 80 but they did starch. The strain was positive for nitrate reduction and
166 indole formation in the presence of tryptophan and but negative for urease reaction, citrate
167 utilization and hydrogen sulphide production. This isolate was positive for histidine decarboxylase
168 but negative for lysine decarboxylase and arginine dihydrolase. The strain was lipolytic and non-
169 proteolytic by the method of FIL IDF 73 [34], i.e. tributyrin was hydrolysed but casein was not.
170 Characteristics that distinguish isolate 11aii^T from recognized members of the genus *Lentibacillus*
171 and *Virgibacillus* are summarized in Table 1. The new strain can be differentiated from other closely
172 related species by several phenotypic properties, noting that it is Gram-negative, pleomorphic,
173 absent endospores and no sugars fermenter.

174 For cellular fatty acid analysis, strain was cultured on halophilic growth broth for a week at 35 °C and
175 the fatty acids were extracted as described by Bligh and Dyer [37] and the extract was dried under
176 nitrogen gas. The determination of Fatty acid methyl esters (FAME) profile was realized by gas
177 chromatography coupled to mass spectrometry (GC-MS) using 2% sulphuric acid–methanol (v/v) as
178 methylating reagent and methyl-nonadecanoate as internal standard [38]. The Thermo Scientific
179 TRACE 1300 Mainframe MS 230V gas chromatograph was used with the TG-5MS column (0.25 mm,
180 30 m, Thermo Scientific) coupled to the Thermo Scientific ISQ mass detector (single quadrupole)
181 with vacuum closing system. The GC-MS program consisted of programmed temperature vaporizer
182 (PTV) at 200°C, flow rate of 40.5 mL/min with split ratio 1/45 and oven temperature of 160 °C
183 maintained for 5 min, 5°C/min up to 300°C and maintained 5 min. The relative amount of each CFA
184 was expressed as percentage of the total fatty acids. The fatty acids of strain 11aii^T were $C_{16:0}$ (42.1%)
185 and anteiso-$C_{15:0}$ (31.2%) and also presented iso-$C_{16:0}$ (11.0%), anteiso-$C_{17:0}$ (10.4%) and $C_{18:0}$ (5.2%).
186 Comparison of CFA profile of the strain 11aii^T and closely related is indicated in Table 2. As in other
187 species of related genera, Anteiso-$C_{15:0}$, Iso-$C_{16:0}$ and Anteiso-$C_{17:0}$ represented an important
188 proportion of the cellular fatty acids. However, 11aii^T major fatty acid was $C_{16:0}$ differentiating from
189 the other species where it did not exceed 3%.

bioRxiv preprint doi: https://doi.org/10.1101/2020.07.27.213165. this version posted July 28, 2020. The copyright holder for this preprint (which was not certified by peer review) is the author/funder. All rights reserved. No reuse allowed without permission.

190 In conclusion, results of phenotypic, genotypic and phylogenetic studies presented in this study
191 demonstrate that strain 11aii^{T} represents a novel genus and species for which the name
192 *Haloargentinum marplatensis* gen. nov., sp. nov. is proposed as a new representative of the phylum
193 *Firmicutes*. Strain 11aii^{T} is the type strain of *Haloargentinum marplatensis*.

194

195

196 Protologue

197

198 **Description of *Haloargentinum* gen. nov.**

199 *Haloargentinum* (Ha.lo.ar.gen.ti.num. Gr. masc. n. hals, *halos*, salt; N.L. neut. adj. argentina,
200 pertaining to Argentina, where the bacteria was isolated; N.L. neut. n. *Haloargentinum*, salt (-
201 requiring) and Argentina). Cells are Gram-negative, coccobacilli/Disc-shaped or pleomorphic
202 bacteria, phylogenetically affiliated in the phylum *Firmicutes*. Aerobic. Oxidase- and catalase-
203 positive. Extremely halophilic, requiring at least 200 g salt / L for growth. Habitat: salted and ripened
204 anchovies. The type species is *Haloargentinum marplatensis*.

205 **Description of *Haloargentinum marplatensis* sp. nov.**

206 Cells are motile, Gram-negative coccobacilli/disc-shaped (pleomorphic) without endospores.
207 Colonies formed on agar plates are circular (1–2 mm in diameter), smooth, translucent and salmon-
208 reddish pigmented. Growth occurs in the presence of 20–35 % (w/v) NaCl and pH 5.5-8. The isolate
209 is positive for oxidase and catalase and negative for Ziehl-Neelsen staining. Acid is not produced
210 from carbohydrates (galactose, sucrose, glucose, fructose, lactose, maltose, sorbitol, mannitol,
211 trehalose, xylose and arabinose). Cells hydrolyse starch but no gelatine and Tween 80. Positive for
212 nitrate reduction and indole formation in the presence of tryptophan and negative for urease
213 reaction, citrate utilization and hydrogen sulphide production. Histidine decarboxylase is present
214 and lysine decarboxylase, arginine dihydrolase and phenylalanine deaminase are absent. Tributyrin
215 hydrolysis is produced but no milk proteolysis (casein hydrolysis). Major fatty acids are n-C16:0 and
216 anteiso-C15:0.

217 The type strain is 11aii^{T}, was isolated from salted-ripened anchovies, a traditional fermented food
218 elaborated in Argentina. The GenBank/EMBL/DDBJ accession number for the 16S rRNA gene
219 sequence of strain 11aii^{T} is MH010317.

220

221

---

222 AUTHOR STATEMENTS

223

bioRxiv preprint doi: https://doi.org/10.1101/2020.07.27.213165. this version posted July 28, 2020. The copyright holder for this preprint (which was not certified by peer review) is the author/funder. All rights reserved. No reuse allowed without permission.

224 **Authors and contributors**

225 Silvina Perez: Investigation, Visualization, Writing – original draft. Margarita Gomila: Visualization,
226 Writing – review & editing. Silvia Elena Murialdo: Funding acquisition, Supervision. Irene Mabel
227 Ameztoy: Investigation. Narjol Gonzalez-Escalona: Software, Resources. Elida Elvia Ramírez:
228 Investigation. María Isabel Yeannes: Conceptualization, Funding acquisition.

229

230 **Conflicts of interest**

231 The authors declare that there are no conflicts of interest.

232

233 **Funding information**

234 This work was financially supported by the Consejo Nacional de Investigaciones Científicas y Técnicas
235 (PIP 2013 N° 0403 and PIP 2016 N° 0437), Agencia Nacional de Promoción Científica y Tecnológica,
236 MINCyT (PICT 2015 N° 2855), Comisión de Investigaciones Científicas de la Pcia de Bs. As. (C.I.C.), and
237 Universidad Nacional de Mar del Plata (ING447/15).

238

239 **Acknowledgements**

240 Narjol Gonzalez Escalona was supported by the FDA Foods Program Intramural Funds. Silvina Perez,
241 Irene Mabel Ameztoy and María Isabel Yeannes were supported by Consejo Nacional de
242 Investigaciones Científicas y Técnicas (CONICET).

243

244

---

245 # REFERENCES

246 1. **Czerner M, Yeannes MI**. Bacterial contribution to salted anchovy (*Engraulis anchoita* Hubbs &
247 Marinni, 1935) ripening process. *J Aquat Food Prod Technol* 2014;23:102–114.

248 2. **Skåra T, Axelsson L, Stefánsson G, Ekstrand B, Hagen H**. Fermented and ripened fish
249 products in the northern European countries. *J Ethn Foods* 2015;2:18–24.

250 3. **Campello F**. Approche microbiologique de l'anchoitage. *Rev Trav Inst Pech Marit*
251 1985;47:217–226.

252 4. **Felix MM, Czerner M, Ramírez E, Yeannes MI**. Investigation of *Halococcus morrhuae* in
253 salted-ripened anchovy products. *Int Food Res J* 2016;23:2668–2674.

254 5. **Hernández-Herrero MM, Roig-Sagués AX, Rodriguéz-Jerez JJ, Mora-ventura MT**.
255 Halotolerant and halophilic histamine-forming bacteria isolated during the ripening of salted
256 anchovies (*Engraulis encrasicholus*). *J Food Prot* 1999;62:509–514.

257 6. **Villar M, de Ruiz Holgado AP, Sanchez JJ, Trucco RE, Oliver G**. Isolation and characterization

bioRxiv preprint doi: https://doi.org/10.1101/2020.07.27.213165. this version posted July 28, 2020. The copyright holder for this preprint (which was not certified by peer review) is the author/funder. All rights reserved. No reuse allowed without permission.

258 of *Pediococcus halophilus* from salted anchovies (*Engraulis anchoita*). *Appl Environ Microbiol*
259 1985;49:664–666.

260 7. **Pons-Sánchez-Cascado S, Bover-Cid S, Veciana-Nogués MT, Vidal-Carou MC**. Amino acid-
261 decarboxylase activity of bacteria isolated from ice-preserved anchovies. *Eur Food Res*
262 *Technol* 2005;220:312–315.

263 8. **Jung MJ, Roh SW, Kim MS, Bae JW**. *Lentibacillus jeotgali* sp. nov., a halophilic bacterium
264 isolated from traditional Korean fermented seafood. *Int J Syst Evol Microbiol* 2010;60:1017–
265 1022.

266 9. **Jiang W, Li C, Xu B, Dong X, Ma N, *et al.*** *Halomonas shantousis* sp. nov., a novel biogenic
267 amines degrading bacterium isolated from Chinese fermented fish sauce. *Antonie Van*
268 *Leeuwenhoek* 2014;106:1073–1080.

269 10. **Yachai M, Tanasupawat S, Itoh T, Benjakul S, Visessanguan W, *et al.*** *Halobacterium piscisalsi*
270 sp. nov., from fermented fish (pla-ra) in Thailand. *Int J Syst Evol Microbiol* 2008;58:2136–
271 2140.

272 11. **Tapingkae W, Tanasupawat S, Itoh T, Parkin KL, Benjakul S, *et al.*** *Natrinema gari* sp. nov., a
273 halophilic archaeon isolated from fish sauce in Thailand. *Int J Syst Evol Microbiol*
274 2008;58:2378–2383.

275 12. **Roh SW, Nam Y Do, Chang HW, Kim KH, Sung Y, *et al.*** *Haloterrigena jeotgali* sp. nov., an
276 extremely halophilic archaeon from salt-fermented food. *Int J Syst Evol Microbiol*
277 2009;59:2359–2363.

278 13. **Namwong S, Tanasupawat S, Kudo T, Itoh T**. *Haloarcula salaria* sp. nov. and *Haloarcula*
279 *tradensis* sp. nov., isolated from salt in Thai fish sauce. *Int J Syst Evol Microbiol* 2011;61:231–
280 236.

281 14. **Yoon JH, Oh TK, Park YH**. Transfer of *Bacillus halodenitrificans* Denariaz et al. 1989 to the
282 genus *Virgibacillus* as *Virgibacillus halodenitrificans* comb. nov. *Int J Syst Evol Microbiol*
283 2004;54:2163–2167.

284 15. **Tak EJ, Kim HS, Lee JY, Kang W, Sung H, *et al.*** *Virgibacillus phasianinus* sp. Nov., a halophilic
285 bacterium isolated from faeces of a Swinhoe's pheasant, *Lophura swinhoii*. *Int J Syst Evol*
286 *Microbiol* 2018;68:1190–1196.

287 16. **Yoon J-H, Kang KH, Park Y-H**. *Lentibacillus salicampi* gen. nov., sp. nov., a moderately
288 halophilic bacterium isolated from a salt field in Korea. *Int J Syst Evol Microbiol*
289 2002;52:2043–2048.

290 17. **Booncharoen A, Visessanguan W, Kuncharoen N, Yiamsombut S, Santiyanont P, *et al.***
291 *Lentibacillus lipolyticus* sp. nov., a moderately halophilic bacterium isolated from shrimp
292 paste (Ka-pi). DOI: 10.1099/ijsem.0.003658.

293 18. **Proom H, Knight BCJG**. *Bacillus pantothenticus* (n.sp.). *J Gen Microbiol* 1950;4:539–541.

294 19. **Heyndrickx M, Lebbe L, Kersters K, De Vos P, Forsyth G, *et al.*** *Virgibacillus*: A new genus to
295 accommodate *Bacillus pantothenticus* (Proom and Knight 1950). Emended description of
296 *Virgibacillus pantothenticus*. *Int J Syst Bacteriol* 1998;48:99–106.

297 20. **Heyrman J, Logan NA, Busse HJ, Balcaen A, Lebbe L, *et al.*** *Virgibacillus carmonensis* sp. nov.,
298 *Virgibacillus necropolis* sp. nov. and *Virgibacillus picturae* sp. nov., three novel species

bioRxiv preprint doi: https://doi.org/10.1101/2020.07.27.213165. this version posted July 28, 2020. The copyright holder for this preprint (which was not certified by peer review) is the author/funder. All rights reserved. No reuse allowed without permission.

299 isolated from deteriorated mural paintings, transfer of the species of the genus *Salibacillus* to
300 *Virgibacillus*, as *Virgibacillus*. *Int J Syst Evol Microbiol* 2003;53:501–511.

301 21. **Xu B, Hu B, Wang J, Lan Y, Zhu Y, *et al.*** *Virgibacillus indicus* sp. nov. and *Virgibacillus profundi*
302 sp. nov, two moderately halophilic bacteria isolated from marine sediment by using
303 microfluidic streak plates. *Int J Syst Evol Microbiol* 2018;68:2015–2023.

304 22. **ICMSF**. *Microorganismos de los alimentos. Técnicas de análisis microbiológico. Volume I.*
305 Zaragoza, España.: Acribia; 1983.

306 23. **IRAM**. Productos de la industria pesquera: Método para la determinación de
307 microorganismos halófilos extremos. IRAM 15.139.

308 24. **D´Ippólito S, de Castro RE, Herrera Seitz K**. Chemotactic responses to gas oil of *Halomonas*
309 spp. strains isolated from saline environments in Argentina. *Rev Argent Microbiol*
310 2011;43:107–110.

311 25. **Sheu DS, Wang YT, Lee CY**. Rapid detection of polyhydroxyalkanoate-accumulating bacteria
312 isolated from the environment by colony PCR. *Microbiology* 2000;146:2019–2025.

313 26. **Hall TA**. BioEdit: a user-friendly biological sequence alignment editor and analysis program
314 for Windows 95/98/NT. *Nucl Acids Symp Ser* 1999;41:95–98.

315 27. **Kumar S, Stecher G, Tamura K**. MEGA7: Molecular Evolutionary Genetics Analysis Version 7.0
316 for Bigger Datasets. *Mol Biol Evol* 2016;33:1870–1874.

317 28. **Kimura M**. A simple method for estimating evolutionary rates of base substitutions through
318 comparative studies of nucleotide sequences. *J Mol Evol* 1980;16:111–120.

319 29. **MacFaddin JF**. *Pruebas bioquímicas para la identificación de bacterias de importancia clínica*.
320 Buenos Aires: Médica Panamericana; 1980.

321 30. **Niven CF, Jeffrey MB, Corlett DA**. Differential plating medium for quantitative detection of
322 histamine-producing bacteria. *Appl Environ Microbiol* 1981;41:321–322.

323 31. **Ramirez EE, Ameztoy IM, Yeannes MI**. Bacterias halófilas como formadoras de histamina.
324 *Proc VI Congr Argentino Cienc y Tecnol Aliment 1er Encuentro Técnicos Aliment del Cono Sur*.

325 32. **Schaeffer AB, Fulton MD**. A simplified method of staining endospores. *Science (80-)*
326 1933;77:194.

327 33. **Sánchez-Porro C, Martín S, Mellado E, Ventosa A**. Diversity of moderately halophilic bacteria
328 producing extracellular hydrolytic enzymes. *J Appl Microbiol* 2003;94:295–300.

329 34. **FIL IDF 73**. Determinación de microorganismos lipolíticos de la manteca.

330 35. **Lehane L, Olley J**. Histamine fish poisoning revisited. *Int J Food Microbiol* 2000;58:1–37.

331 36. **Yeannes MI**. Histamina en productos pesqueros. In: Silvestre A (editor). *Toxicología de los*
332 *Alimentos*. Buenos Aires: Hemisferio Sur S.A.; 1996. pp. 30–45.

333 37. **Bligh EG, Dyer WJ**. A rapid method of total lipid extraction and purification. *J Biochem Physiol*
334 1959;37:911–917.

335 38. **Antolín EM, Delange DM, Canavaciolo VG**. Evaluation of five methods for derivatization and
336 GC determination of a mixture of very long chain fatty acids (C24:0-C36:0). *J Pharm Biomed*

bioRxiv preprint doi: https://doi.org/10.1101/2020.07.27.213165. this version posted July 28, 2020. The copyright holder for this preprint (which was not certified by peer review) is the author/funder. All rights reserved. No reuse allowed without permission.

337 *Anal* 2008;46:194–199.

338 39. **Namwong S, Tanasupawat S, Smitinont T, Visessanguan W, Kudo T, *et al.*** Isolation of
339 *Lentibacillus salicampi* strains and *Lentibacillus juripiscarius* sp. nov. from fish sauce in
340 Thailand. *Int J Syst Evol Microbiol* 2005;55:315–320.

341 40. **Senghor B, Seck EH, Khelaifia S, Bassène H, Sokhna C, *et al.*** Description of '*Bacillus*
342 *dakarensis*' sp. nov., '*Bacillus sinesaloumensis*' sp. nov., '*Gracilibacillus timonensis*' sp. nov.,
343 '*Halobacillus massiliensis*' sp. nov., '*Lentibacillus massiliensis*' sp. nov., '*Oceanobacillus*
344 *senegalensis*' sp. nov., '*Oceanobacillus timonensis*' sp. nov., '*Virgibacillus dakarensis*' sp. nov.
345 and '*Virgibacillus marseillensis*' sp. nov., nine halophilic new species isolated from human
346 stool. *New Microbes New Infect* 2017;17:45–51.

347 41. **Zhang DC, Schumann P, Wu J, França L, Neuner K, *et al.*** *Virgibacillus flavescens* sp. nov.,
348 isolated from marine sediment. *Int J Syst Evol Microbiol* 2016;66:1138–1143.

349 42. **Chen YG, Cui XL, Frítze D, Chai LH, Schumann P, *et al.*** *Virgibacillus kekensis* sp. nov., a
350 moderately halophilic bacterium isolated from a salt lake in China. *Int J Syst Evol Microbiol*
351 2008;58:647–653.

352 43. **Tanasupawat S, Chamroensaksri N, Kudo T, Itoh T**. Identification of moderately halophilic
353 bacteria from Thai fermented fish (pla-ra) and proposal of *Virgibacillus siamensis* sp. nov. *J*
354 *Gen Appl Microbiol* 2010;56:369–379.

355

356

357

358
FIGURES AND TABLES

359

360 Figure 1. Maximum likelihood phylogeny of strain 11aii^{T} with closest relatives using a 1190 bp
361 fragment of the 16S rDNA gene. The evolutionary history was inferred by using the Maximum
362 Likelihood method based on the Kimura 2-parameter model [28]. The tree shown has most of the
363 Lineages compressed for visualization purposes. Bootstrap support above 50% are shown above the
364 branches. In red fonts are the strains sequenced in this study. The tree is drawn to scale, with branch
365 lengths measured in the number of substitutions per site. Evolutionary analyses were conducted in
366 MEGA7 [27].

367

368 Supplementary figure 1. Phylogenetic tree with the highest log likelihood phylogeny of strain 11aiiT
369 with closest relatives using an 1190 bp fragment of the 16S rRNA gene. The evolutionary history was
370 inferred by using the Maximum Likelihood method based on the Kimura 2-parameter model [28].
371 Bootstrap support above 50% are shown above the branches. In red fonts are the strains sequenced
372 in this study. The tree is drawn to scale, with branch lengths measured in the number of
373 substitutions per site. Evolutionary analyses were conducted in MEGA7 [27].

bioRxiv preprint doi: https://doi.org/10.1101/2020.07.27.213165. this version posted July 28, 2020. The copyright holder for this preprint (which was not certified by peer review) is the author/funder. All rights reserved. No reuse allowed without permission.

Table 1. Differential phenotypic characteristics between strain *Haloargentinum marplatensis* gen. nov. sp. nov. 11aii[T] and species of the closely related *Lentibacillus* and *Virgibacillus* genera.

Strains: 1, 11aii[T] (data from the present study); 2, *Lentibacillus jeotgali* Grbi [8]; 3, *Lentibacillus juripiscarius* IS40-3[T] [39]; 4, *Lentibacillus massiliensis* Marseille-P3089[T] [40]; 5, *Virgibacillus flavescens* S1-20[T] [41]; 6, *Virgibacillus halodenitrificans* [14] ; 7, *Virgibacillus kekensis* YIM kkny16[T] [42]; 8, *Virgibacillus phasianinus* LM2416[T] [15]; 9, *Virgibacillus siamensis* MS3-4[T] [43]. Symbols: +, positive reaction; -, negative reaction; w, weakly positive; v, variable; ND, no data.

Characteristic	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**
Isolation source	Salted-ripened anchovies, Argentina	Fermented scallops, Korea	Fish sauce, Thailand	Salty stool, Senegal	Marine sediment, China	Marine solar saltern, Korea	Salt lake, China	Faeces of *Lophura swinhoii*, Korea	Fermented fish, Thailand
Pigmentation	Salmon-reddish	-	-	yellow	Light yellow	-	creamy grey	-	red color
Gram staining	-	+	+	+	v	V	+	+	+
Cell morphology	Pleomorphic	Rods	Rods	ND	Rods	Rods	Rods	Rods	Rods
Endospore forming	-	+	+	+	ND	+	+	ND	+
Motility	+	-	-	ND	+	+	+	+	+
NaCl range (% w/v)	20-35	3-20	3-30	0.5-20	0-20	2–25	0–25	0-20	1-20
pH range	5.5-8.0	6.0-8.0	5.0-9.0	ND	7.0-9.0	5.8-9.6	6.0-10.0	6.0-7.0	5.0-8.0
Growth at 35 °C	+	+	+	ND	-	+	+	-	+
Nitrate reduction	+	+	+	ND	-	+	+	+	-
Indole formation	+	ND	-	ND	-	-	-	ND	ND
Oxidase	+	-	+	+	+	+	+	-	+
Catalase	+	+	+	-	+	+	+	+	+
Urease reaction	-	-	-	ND	-	-	-	-	ND
Lysine decarboxylase	-	ND	ND	ND	-	-	ND	+	ND
Arginine dihydrolase	-	-	-	ND	-	-	ND	-	ND
Citrate utilization	-	ND	ND	ND	-	ND	ND	+	-
H_2S production	-	ND	-	ND	-	-	-	ND	-
Acid production from:									
Galactose	-	+	+	-	ND	+	-	+	-
Sucrose	-	-	+	W	ND	+	-	-	-
Glucose	-	+	+	+	ND	+	+	+	-
Fructose	-	+	+	+	ND	+	-	+	-
Lactose	-	-	v	-	ND	+	-	+	-
Maltose	-	+	+	-	ND	+	+	+	-
Sorbitol	-	ND	-	-	ND	+	-	ND	ND
Mannitol	-	+	v	-	ND	+	w	ND	-
Trehalose	-	-	+	-	ND	+	w	-	-
Xylose	-	-	-	+	ND	-	-	-	-
Arabinose	-	-	-	-	ND	-	-	-	-
Hydrolysis of									
Gelatin	-	ND	+	+	ND	ND	-	+	+
Starch	+	ND	-	-	ND	ND	+	+	-
Tween 80	-	-	-	+	ND	ND	-	-	-
Tributyrin	+	ND	ND	-	ND	ND	ND	ND	ND
Casein	-	-	+	+	ND	ND	-	+	+

Table 2. Comparison of fatty acid compositions between characteristics between strain *Haloargentinum marplatensis* gen. nov. sp. nov. 11aii[T] and species of the closely related *Lentibacillus* and *Virgibacillus* genera.

Strains: 1, 11aii[T] (data from the present study); 2, *Lentibacillus jeotgali* Grbi[T] [8]; 3, *Lentibacillus juripiscarius* IS40-3[T] [39]; 4, *Virgibacillus flavescens* S1-20[T] [41]; 5, *Virgibacillus halodenitrificans* [14]; 6, *Virgibacillus kekensis* YIM kkny16[T] [42]; 7, *Virgibacillus phasianinus* LM2416[T] [15]; 8, *Virgibacillus*

bioRxiv preprint doi: https://doi.org/10.1101/2020.07.27.213165. this version posted July 28, 2020. The copyright holder for this preprint (which was not certified by peer review) is the author/funder. All rights reserved. No reuse allowed without permission.

390 *siamensis* MS3-4[T] [43];. Data are percentages of the total fatty acids; components representing less
391 than 1.0% of the total are not shown.

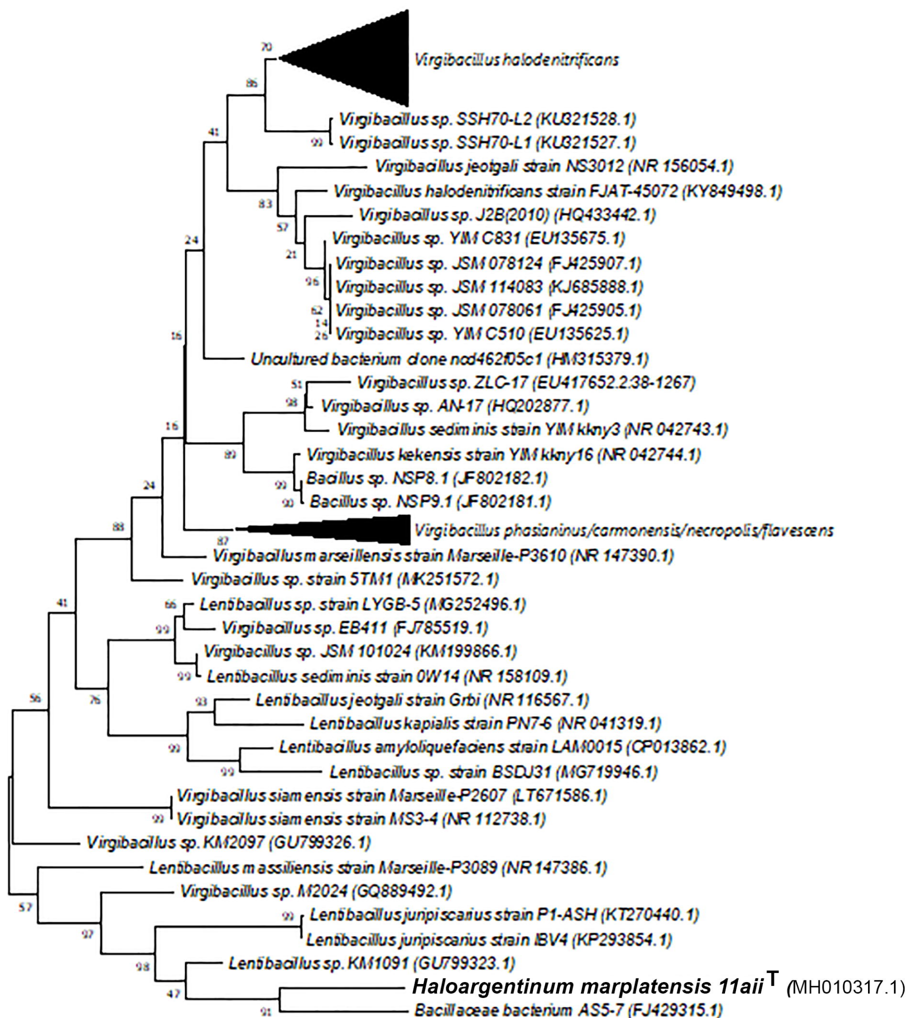
Fatty acid	1	2	3	4	5	6	7	8
Satured								
$C_{14:0}$					1.7			
$C_{16:0}$	42.1			1.2	2.7			1.5
$C_{18:0}$	5.2				2.1			
Unsatured								
$C_{16:1}\omega7c$ alcohol			2.6		8.1			
$C_{18:1}\omega9c$					1.1			
Branched								
Iso-$C_{14:0}$		5–13	1	18.2	13.3		3.1	3.9
Iso-$C_{15:0}$		3–18	4.4	2.1	2.6		5.3	11.3
Anteiso-$C_{15:0}$	31.2	38–54	61.9	30.3	50.4	54.1	73.0	55.8
Iso-$C_{16:0}$	11.0	13–30	4.5	36.4	6.1		5.2	6.6
Iso-$C_{17:0}$								1.5
Anteiso-$C_{17:0}$	10.4	13–18	20	9.8	7.0	32.0	9.7	17.7
Summed feature*								
4			2.5					

392 *Summed feature 4 comprises anteiso-C17:1 B and/or iso-C17:1 I

393

394

395



Virgibacillus halodenitrificans
Virgibacillus sp. SSH70-L2 (KU321528.1)
Virgibacillus sp. SSH70-L1 (KU321527.1)
Virgibacillus jeotgali strain NS3012 (NR 156054.1)
Virgibacillus halodenitrificans strain FJAT-45072 (KY849498.1)
Virgibacillus sp. J2B(2010) (HQ433442.1)
Virgibacillus sp. YIM C831 (EU135675.1)
Virgibacillus sp. JSM 078124 (FJ425907.1)
Virgibacillus sp. JSM 114083 (KJ685888.1)
Virgibacillus sp. JSM 078061 (FJ425905.1)
Virgibacillus sp. YIM C510 (EU135625.1)
Uncultured bacterium clone ncd462f05c1 (HM315379.1)
Virgibacillus sp. ZLC-17 (EU417652.2:38-1267)
Virgibacillus sp. AN-17 (HQ202877.1)
Virgibacillus sediminis strain YIM kkny3 (NR 042743.1)
Virgibacillus kekensis strain YIM kkny16 (NR 042744.1)
Bacillus sp. NSP8.1 (JF802182.1)
Bacillus sp. NSP9.1 (JF802181.1)
Virgibacillus phasianinus/carmonensis/necropolis/flavescens
Virgibacillus marseillensis strain Marseille-P3610 (NR 147390.1)
Virgibacillus sp. strain 5TM1 (MK251572.1)
Lentibacillus sp. strain LYGB-5 (MG252496.1)
Virgibacillus sp. EB411 (FJ785519.1)
Virgibacillus sp. JSM 101024 (KM199866.1)
Lentibacillus sediminis strain 0W14 (NR 158109.1)
Lentibacillus jeotgali strain Grbi (NR 116567.1)
Lentibacillus kapialis strain PN7-6 (NR 041319.1)
Lentibacillus amyloliquefaciens strain LAM0015 (CP013862.1)
Lentibacillus sp. strain BSDJ31 (MG719946.1)
Virgibacillus siamensis strain Marseille-P2607 (LT671586.1)
Virgibacillus siamensis strain MS3-4 (NR 112738.1)
Virgibacillus sp. KM2097 (GU799326.1)
Lentibacillus massiliensis strain Marseille-P3089 (NR 147386.1)
Virgibacillus sp. M2024 (GQ889492.1)
Lentibacillus juripiscarius strain P1-ASH (KT270440.1)
Lentibacillus juripiscarius strain IBV4 (KP293854.1)
Lentibacillus sp. KM1091 (GU799323.1)
Haloargentinum marplatensis 11aii T (MH010317.1)
Bacillaceae bacterium AS5-7 (FJ429315.1)
70
86
41
24
83
57
21
16
16
24
88
87
41
66
76
56
57
97
98
47