1 **_Streptomyces iconiensis_ sp. nov. and _Streptomyces smyrnaeus_ sp. nov., two halotolerant**
2 **actinomycetes isolated from Tuz (Salt) Lake and Camalti Saltern in Turkey**

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14 **Subject category**: New Taxa: Actinobacteria

15

16 **Running title:** *Streptomyces iconiensis s* sp. nov. and *Streptomyces smyrnaeus* sp. nov.

17

18 The GenBank accession number for the 16S rRNA gene sequence of ***Streptomyces iconiensis***
19 BNT558^T(= KCTC 29198^T = DSM 42109^T) is **KC959223** and ***Streptomyces smyrnaeus***
20 SM3501^T(= KCTC 29214^T = DSM 42105^T) is **KF006349**.

21

22 **Keywords:** *Streptomycetaceae*, *Streptomyces iconiensis*, *Streptomyces smyrnaeus*, Polyphasic
23 taxonomy

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26

27 **Abstract**

28 The taxonomic positions of two novel actinomycetes, designated BNT558^T and SM3501^T,
29 were established by using a polyphasic approach. The organisms had chemical and
30 morphological features that were consistent with their classification in the genus
31 *Streptomyces*. The whole-cell hydrolysates of the two strains contained LL-diaminopimelic
32 acid as the diagnostic diamino acid. The predominant menaquinones were MK-9(H_6) and
33 MK-9(H_8) for strain BNT558^T and MK-9(H_8) and MK-9(H_6) for strain SM3501^T. Major fatty
34 acids of the strains were *anteiso*-$C_{15:0}$, *anteiso*-$C_{17:0}$ and *iso*-$C_{16:0}$. The polar lipid profiles of
35 strain BNT558^T contained diphosphatidylglycerol, phosphatidylethanolamine,
36 phosphatidylinositol, one unidentified glycolipid and one unidentified aminophospholipid,
37 while that of strain SM3501^T consisted of diphosphatidylglycerol, phosphatidylglycerol,
38 phosphatidylinositol, phoshphatidylethanolamine and three unidentified atypical aminolipids,
39 one unidentified aminolipid and two unidentified glycolipids. The G+C contents of the
40 genomic DNAs were 70.2 and 69.6 mol % for strains BNT558^T and SM3501^T, respectively.
41 16S rRNA gene sequence data supported the classification of the isolates in the genus
42 *Streptomyces* and showed that they formed two distinct branches within the genus
43 *Streptomyces*. Based on the almost complete 16S rRNA gene sequences isolate BNT558^T was
44 most closely related to *Streptomyces albiaxialis* NRRL B-24327^T and isolate SM3501^T was
45 most closely related to *Streptomyces cacaoi* subsp. *cacaoi* NBRC 13860^T. DNA-DNA
46 relatedness between each of the isolates and its closest phylogenetic neighbours showed that
47 they belonged to distinct species. The two isolates were readily distinguished from one
48 another and from the type strains of the other species classified in the genus *Streptomyces*
49 based on a combination of phenotypic and genotypic properties. Based on the genotypic and
50 phenotypic evidence, strains BNT558^T and SM3501^T belong to two novel species in the genus
51 *Streptomyces* for which the names *Streptomyces iconiensis* sp. nov. (type strain BNT558^T =

52 KCTC 29198^T = DSM 42109^T) and *Streptomyces smyrnaeus s*p. nov. (type strain SM3501^T =
53 KCTC 29214^T = DSM 42105^T) are proposed, respectively.

54 Saline lakes and man-made marine salterns are interesting model systems for microbiologist
55 to work on microbial diversity and ecosystem functions in extreme environments. Recent
56 studies have shown that such environments are highly productive and have been a valuable
57 source of novel microorganisms (Anton *et al*., 2002; Yoon *et al*., 2002; Li *et al*., 2004; Tang
58 *et al*., 2010 & 2011; Wang *et al*., 2011; Yang *et al*., 2012; Tatar *et al*., 2013). While new
59 advances have been slow, there is great interest in the use of halophilic or halotolerant
60 microorganisms to degrade organic pollutants and to produce biopolymers, biosurfactants and
61 compatible solutes (Marhuenda-Egea & Bonete, 2002; Le Bornge *et al*., 2008). In this
62 context, members of the genus *Streptomyces* remain an unique source of natural products,
63 including clinically significant antibiotics, antimetabolities and antitumour agents (Watve *et*
64 *al*., 2001; Igarashi *et al*., 2005; Fiedler *et al*., 2005; Shiomi *et al*., 2005; Olano *et al*., 2009;
65 Kim *et al*., 2012a). Another unique feature of the genus is the large number of species it
66 contains; at the time of writing the genus *Streptomyces* contained over 600 species with
67 validly published names (Euzéby, 2012; http://www.bacterio.cict.fr/s/streptomycesa.html).
68 The present investigation was designed to establish the taxonomic positions of two novel
69 *Streptomyces* strains, designated BNT558^T and SM3501^T, that were isolated from Tuz (Salt)
70 Lake and Camalti Saltern, in Turkey. A polyphasic taxonomic study based on a combination
71 of genotypic and phenotypic procedures showed that isolates BNT558^T and SM3501^T should
72 be recognized as two novel species of the genus *Streptomyce*s, *Streptomyces iconiensis* sp.
73 nov. and *Streptomyces smyrnaeus* sp. nov., respectively.
74 Strain BNT558^T was isolated from soil samples collected from Tuz (Salt) Lake, Konya,
75 Turkey, by using Modified Bennett's agar (Jones, 1949), supplemented with 5 % (w/v) NaCl
76 while strain SM3501^T was isolated by using SM3 medium (Tan *et al*., 2006) supplemented

77 with filter sterilized cycloheximide (50 µg ml^{-1}) and 15 % (w/v) NaCl from a sediment sample
78 collected from the first pool of Camalti Saltern, Izmir, Turkey. The isolation plates were
79 incubated at 28˚C for 21 days. The strains $BNT558^T$ and $SM3501^T$ were maintained on
80 modified Bennett's agar slopes and on starch-mineral salt agar supplemented with 10 % (w/v)
81 NaCl (DSMZ medium 1240), respectively, at room temperature and in 20 % (v/v) glycerol
82 solution at -20 °C.

83 Genomic DNA extraction, PCR-mediated amplification of the 16S rRNA gene and
84 purification of the PCR products were carried out following Chun & Goodfellow (1995). The
85 almost complete 16S rRNA gene sequences of strains $BNT558^T$ and $SM3501^T$ were
86 determined using an ABI PRISM 3730 XL automatic sequencer. The resultant 16S rRNA
87 gene sequences were aligned with corresponding sequences of representative type strains of
88 the genus *Streptomyces* (retrieved from EzTaxon-e server; Kim *et al*., 2012b) by using
89 CLUSTAL W in MEGA version 5.0 (Tamura *et al*., 2011). The sequence similarities were
90 calculated using PHYDIT program version 3.1. Phylogenetic analysis was carried out by
91 using three tree-making algorithms: neighbour-joining (Saitou & Nei, 1987), maximum-
92 likelihood (Felsenstein, 1981) and maximum parsimony (Fitch, 1971) with MEGA version
93 5.0 (Tamura *et al*., 2011). Evolutionary distances were calculated using model of Jukes &
94 Cantor (1969). Topologies of the resultant trees were evaluated by bootstrap analyses
95 (Felsenstein, 1985) based on 1000 resamplings.

96 Almost complete 16S rRNA gene sequences of strains $SM3501^T$ (1494 bp) and $BNT558^T$
97 (1473 bp), were determined. These sequences were analysed by preliminary comparison with
98 sequences in the GenBank database and the results indicated that the two isolates were most
99 closely related to members of the genus *Streptomyces*. It is apparent from Fig. 1 that strain
100 $BNT558^T$ and $SM3501^T$ separated to two different subclades and shared 97.14 % 16S rRNA
101 similarity, which corresponds to 42 nt differences at 1471 locations. This relationship was

102 supported by all the tree-making algorithms used in this study (Supplementary Fig. S1 and
103 S2). The 16S rRNA gene sequence similarity between strain BNT558^T and the most closely
104 related type strains *Streptomyces albiaxialis* NRRL B-24327^T, *Streptomyces daliensis* YIM
105 31724^T, *Streptomyces sclerotialus* DSM 43032^T, *Streptomyces rimosus* subsp. *rimosus* ATCC
106 10970^T, *Streptomyces ramulosus* NRRL B-2714^T, *Streptomyces olivaceiscleroticus* DSM
107 40595^T, and *Streptomyces niger* NBRC 13362^T were 98.91 % (16 nt differences at 1471),
108 98.37 % (24 nt differences at 1471), 98.15 % (27 nt differences at 1461), 98.10 % (28 nt
109 differences at 1471), 98.02 % (29 nt differences at 1466), 98.02 % (29 nt differences at 1465)
110 and 98.02 % (29 nt differences at 1464), respectively. Sequence similarities with all other type
111 strains of the genus *Streptomyces* were below 98.0 %.

112 Strain SM3501^T was most closely related to *Streptomyces cacaoi* subsp. *cacaoi* NBRC
113 12748^T. The two strains share 98.43 % 16S rRNA sequence similarity, which corresponds to
114 23 nt differences at 1464. High sequence similarity values were also shown with the type
115 strains of *Streptomyces qinglanensis* 172205^T (98.37 %; 24 nt differences at 1472),
116 *Streptomyces flocculus* NBRC 13041^T (97.95 %; 30 nt differences at 1463), *Streptomyces*
117 *rangoonensis* LMG 20295^T (97.82 %; 32 nt differences at 1465), *Streptomyces gibsonii*
118 NBRC 15415^T (97.81 %; 32 nt differences at 1464), *Streptomyces almquistii* NRBC 13015^T
119 (97.81 %; 32 nt differences at 1459), *Streptomyces albus* subsp. *albus* NRRL B-2365^T (97.76
120 %; 33 nt differences at 1472), *Streptomyces hygroscopicus* subsp. *hygroscopicus* NRRL
121 2387^T (97.54 %; 36 nt differences at 1463) and *Streptomyces sporocinerus* NBRC 100766^T
122 (97.54 %; 36 nt differences at 1463). Sequence similarities with the type strains of all other
123 species with validly published names of the genus *Streptomyces* were lower.

124

125 DNA-DNA relatedness values were determined between strain BNT558^T and *S. albiaxialis*
126 DSM 41799^T, and between strain SM3501^T and the type strains of *S. cacaoi* subsp. *cacaoi*

127 KCTC 9758^{T}, *S. qinglanensis* DSM 42035^{T} and *S. flocculus* DSM 40327^{T}. DNA was isolated
128 using a French pressure cell (Thermo Spectronic) and was purified by chromatography on
129 hydroxyapatite as described by Cashion *et al*. (1977). DNA-DNA hybridization was carried
130 out as described by De Ley *et al.* (1970) under consideration of the modifications described
131 by Huss *et al*. (1983) using a model Cary 100 Bio UV/VIS-spectrophotometer equipped with
132 a Peltier-thermostatted 6X6 multicell changer and a temperature controller with *in situ*
133 temperature probe (Varian).

134 The taxonomic integrity of the test strains were supported by DNA-DNA relatedness data.
135 Strain BNT558^{T} showed DNA relatedness values 18.4 % ± 1.2 % to *S. albiaxialis* DSM
136 41799^{T}, while strain SM3501^{T} showed DNA relatedness values 32.8 % ± 6.3 % to *S. cacaoi*
137 subsp. *cacaoi* KCTC 9758^{T}, 46.2 % ± 2.7 % to *S. qinglanensis* DSM 42035^{T} and 47.7 % ± 7.4
138 % to *S. flocculus* DSM 40327^{T} (based on the average of duplicate determinations in 2X SSC
139 and 10 % formamide at 70 °C), below the recommended criterion of 80 % for species
140 delineation of the genus *Streptomyces* (Labeda, 1992). Further DDH experiments can safely
141 be omitted, because the risk to miss a DDH value above the species discrimination threshold
142 of 80 % is neglectable (below 0.03 % for strain SM3501^{T} and below 0.25 % for strain
143 BNT558^{T}, even when the more strict 70 % DDH threshold proposed by Wayne et al. (1987)
144 would be considered (Meier-Kolthoff et al. 2013).

145 Biomass for chemotaxonomic studies were prepared by growing strains BNT558^{T} and
146 SM3501^{T} in Bennett’s broth at 160 rpm for 18 days at 28 ºC; cells were harvested by
147 centrifugation, washed in distilled water, re-centrifuged and freeze-dried. Whole cell amino
148 acids and sugars were prepared according to Lechevalier & Lechevalier (1970) and analysed
149 by thin layer chromatography (Staneck & Roberts, 1974). Polar lipids were extracted and
150 analyzed by the method of Minnikin *et al*. (1984) as modified by Kroppenstedt & Goodfellow
151 (2006). The isoprenoid quinones were extracted and purified using the method of Collins *et*

152 *al*. (1977) and analysed by HPLC (Kroppenstedt, 1982). For extraction and analysis of
153 cellular fatty acids, physiological age of each strain was standardized by consistently choosing
154 the last quadrant streaked on Bennett's agar plates incubated at 28 ºC for 4 days. Analysis was
155 conducted using the Microbial Identification System (MIDI) Sherlock software version 4.5
156 (method TSBA40, TSBA6 database) as described by Sasser (1990). FAME peaks were
157 analysed using soft ware version TSBA 5.0. The DNA G+C content of strains BNT558^T and
158 SM3501^T were determined following the procedure developed by Gonzalez & Saiz-Jimenez
159 (2005).

160 The cell wall of strains BNT558^T and SM3501^T contained LL-diaminopimelic acid. Whole-
161 cell hydrolysates consisted of fucose, mannose, ribose and traces of galactose and glucose for
162 strain BNT558^T, galactose, glucose, ribose and a trace of mannose for strain SM3501^T. The
163 polar lipid profile of strain BNT558^T consisted of diphosphatidylglycerol,
164 phosphatidylethanolamine, phosphatidylinositol, one unidentified glycolipid and one
165 unidentified aminophospholipid. Whereas strain SM3501^T contained diphosphatidylglycerol,
166 phosphatidylglycerol, phosphatidylinositol, phoshphatidylethanolamine and three unidentified
167 atypical aminolipids, one unidentified aminolipid and two unidentified glycolipids (Supp. Fig.
168 S3-4). The major menaquinones of the strain BNT558^T were MK-9(H_6) (54.0 %) and MK-
169 9(H_8) (27.0 %). Minor amounts of MK-9(H_2) (2.0 %), MK-9(H_4) (5.0 %), MK-10(H_4) (1.0
170 %), MK-10(H_6) (1.0 %), MK-10(H_8) (1.0 %), and some minor unidentifed components were
171 also detected. Strain SM3501^T exhibited MK-9(H_8) (51.0 %) and MK-9(H_6) (32.0 %) as the
172 predominant menaquinones. Minor amounts of MK-9(H_4) (1.0 %), MK-10(H_2) (2.0 %), MK-
173 10(H_4) (1.0 %), MK-10(H_6) (3.0 %), MK-10(H_8) (4.0 %) and in addition some minor
174 unidentified components were also detected. The major cellular fatty acids were *anteiso*-$C_{15:0}$
175 (46.52 %), *anteiso*-$C_{17:0}$ (18.18 %), *iso*-$C_{16:0}$ (13.31 %) and *iso*-$C_{15:0}$ (10.0 %) for strain
176 BNT558^T, and *anteiso*-$C_{15:0}$ (37.27 %), *anteiso*-$C_{17:0}$ (32.28 %) and *iso*-$C_{16:0}$ (17.31 %) for

177 strain SM3501^T (Supplementary Table S1). The G+C content of the DNA was 70.2 and 69.6

178 mol % for strains BNT558^T and SM3501^T, respectively.

179 Cultural characteristics were investigated on media from the International *Streptomyces*

180 Project (ISP) agar (Shirling & Gottlieb, 1966), modified Bennett's agar, Czapek's agar and

181 tryptic soy agar (TSA; Difco). The degree of growth, aerial mycelium and pigmentation were

182 recorded after 14 days of incubation at 28 °C. National Bureau of Standards (NBS) Colour

183 Name Charts (Kelly, 1964) was used for determining colour designation and names. Colony

184 morphology and micromorphological properties of strains BNT558^T and SM3501^T were

185 determined by examined gold coated dehydrated specimens of 30-days cultures from

186 modified Bennett's agar and DSMZ medium 1240, respectively, using a JEOL JSM 6060

187 scanning electron microscope. Growth at different temperatures (4, 10, 28, 37, 45, 50 and 55

188 ºC), and pH 4.0–12.0 (at intervals of 1.0 pH unit), and in the presence of NaCl (0–10, 15, 20,

189 30 %; w/v) was determined on yeast extract-malt extract agar (ISP 2) (Shirling & Gottlieb,

190 1966). Established methods were used to determine whether the strains degraded Tween 40

191 and 80 (Nash & Krent, 1991); the remaining degradation tests were examined using methods

192 described by Williams *et al*. (1983). Carbon source utilization was tested using carbon source

193 utilization (ISP 9) medium (Shirling & Gottlieb, 1966) supplemented with a final

194 concentration of 1 % of the tested carbon sources. Nitrogen source utilization was examined

195 using the basal medium recommended by Williams *et al.* (1983) supplemented with a final

196 concentration of 0.1 % of the tested nitrogen sources. Antimicrobial activity of strains

197 BNT558^T and SM3501^T to inhibit the growth Gram (+) bacteria, such as *Bacillus subtilis*

198 NRRL B-209, *Bacillus cereus* NRRL B-3711, *Bacillus licheniformis* NRRL B-1001, *Bacillus*

199 *pumilus* NRRL-BD 142, *Liysteria monocytogenes* ATCC 19117, *Micrococcus luteus* NRRL

200 B-1018, *Staphylococcus aureus* ATCC 29213 and *Staphylococcus aureus* NRRL B-767, and

201 Gram (-) bacteria, such as *Citrobacter freundii* NRRL B-2643, *Escherichia coli* ATCC 25922,

202 *Escherichia coli* MC4100, *Enterobacter aerogenes* NRRL B-427 and *Pseudomonas*
203 *aeruginosa* NRRL B-2679 or fungi, such as *Aspergillus niger, Aspergillus parasiticus* NRRL-
204 465^T and *Candida utilis* NRRL Y-900 were observed using an agar well method described by
205 Zamanian *et al.*, (2005). The type strains *S. albiaxialis* DSM 41799^T, *S. ferralitis* DSM
206 41836^T, *S. cacaoi* subsp. *cacaoi* KCTC 9758^T, *S. qinglanensis* DSM 42035^T, *S. flocculus*
207 DSM 40327^T and *S. rangoonensis* DSM 40452^T were included for comparison in all tests.
208
209 BNT558^T and SM3501^T had morphological proporties consistent with their classification in
210 the genus *Streptomyces*. Strain BNT558^T formed a brownish gray substrate mycelium and
211 dark grayish brown aerial hyphae which differentiated into open loops of warty-surfaced
212 spore on modified Bennett's agar while strain SM3501^T formed a branched white substrate
213 mycelium and white aerial hyphae which differentiated into spiral chains of smooth-surfaced
214 spores on DSMZ medium 1240, (Fig. 2 and 3). The organism grew well on ISP 2, 3, 4, 5, 6
215 and 7, and modified Bennett's agar. Strain BNT558^T produced strong reddish-brown
216 pigments on ISP 2, grayish-brown ones on ISP 3, strong brown ones on ISP 4 and dark red
217 pigments on modified Bennett's agar, whereas strain SM3501^T produced moderate yellowish-
218 brown pigments on ISP 3 medium. BNT558^T and SM3501^T did not produce any melanoid
219 pigments on ISP 6 and ISP 7 medium. The physiological and biochemical properties are given
220 in Table 1 and the species description.
221 The genotypic and phenotypic data presented here show that the two isolates can be
222 distinguished from one another and from the type strains of species previously classified in
223 the genus *Streptomyces*. Therefore, it is concluded that strains BNT558^T and SM3501^T
224 represent two novel species within the genus *Streptomyces*, for which the names *Streptomyces*
225 *iconiensis* sp. nov., *Streptomyces smyrnaeus s*p. nov. are proposed.
226

227 **Description of *Streptomyces iconiensis* sp. nov.**

228 ***Streptomyces iconiensis*** (i.co.ni.en'sis. L. masc. adj. iconiensis pertaining to Iconium, the
229 present day Konya, from where the type strain was isolated).

230 Aerobic, Gram stain-positive, non-motile, non-acid-alcohol-fast actinomycete which forms a
231 brownish gray substrate mycelium and dark grayish brown aerial hyphae which differentiated
232 into open loops of warty-surfaced spores. Growth occurs at pH 5.0-12.0, and at 28-45 ºC, but
233 not at pH 4.0 and at temperatures of 4, 10, 50 and 55 ºC. Arbutin, allantoin and urea are
234 hydrolysed. Nitrate reduction is negative. Adenine, starch, Tweens 40 and 80 are degraded but
235 casein and xanthine are not. Utilizes adonitol, D-arabinose, L-arabinose, D-cellobiose, D-
236 fructose, D-galactose, D-mannitol, D-mannose, lactose, maltose, sucrose, dextrin and xylose
237 as sole carbon sources, but not D-sorbitol, dextran, inulin, L-sorbose, L-glutamic acid, xylitol
238 and succinic acid. Utilizes glycine, L-alanine, L-arginine, L-methionine, L-serine as sole
239 nitrogen sources, but not alpha-*iso*-leucine, L-phenylalanin, L-histidine, L-threonine, L-proline,
240 L-hydroxyproline, L-cysteine, L-valine. Antimicrobial activity is shown against *Aspergillus*
241 *parasiticus* NRRL-465, *Candida utilis* NRRL Y-900, *Escherichia coli* MC4100*, Bacillus*
242 *subtilis* NRRL B-209, *Bacillus pumilus* NRRL-BD 142, *Staphylococcus aureus* ATCC 29213,
243 *Staphylococcus aureus* NRRL B-767 The predominant menaquinones were MK-9(H_6) and
244 MK-9(H_8). Major fatty acids were *anteiso*-$C_{15:0}$, *anteiso*-$C_{17:0}$ and *iso*-$C_{16:0}$. The polar lipid
245 profile contains diphosphatidylglycerol, phosphatidylethanolamine, phosphatidylinositol, one
246 unidentified glycolipid and one unidentified aminophospholipid. The G+C content of the
247 genomic DNA was 70.2 mol %. The type strain, BNT558^T (= KCTC 29198^T = DSM 42109^T)
248 was isolated from a soil sample collected from Tuz (Salt) Lake, Konya, Turkey.

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252 **Description of *Streptomyces smyrnaeus* sp. nov.**

253 ***Streptomyces smyrnaeus*** (smyr.nae'us. L. masc. adj. *smyrnaeus* pertaining to Smyrna, the
254 present-day Izmir, from where the type strain was isolated)

255 Aerobic, Gram stain-positive, non-motile, non-acid-alcohol-fast actinomycete which forms a
256 branched white substrate mycelium and white aerial hyphae which differentiated into spiral
257 chains of smooth-surfaced spores. Growth occurs at pH 4.0-12.0, and at 28-45 ºC, but not at
258 temperatures of 4, 10, 50 and 55 ºC. Arbutin, allantoin are hydrolysed, but not urea. Nitrate
259 reduction is negative. Adenine, starch, Tweens 40 and 80 are degraded but casein and
260 xanthine are not. Utilizes adonitol, L-arabinose, D-cellobiose, D-fructose, D-galactose, D-
261 sorbitol, D-mannitol, D-mannose, lactose, maltose, sucrose, dextrin, inulin, xylitol, xylose and
262 succinic acid as sole carbon sources, but not D-arabinose, dextran, L-sorbose and L-glutamic
263 acid. Utilizes alpha-*iso*-leucine, glycine, L-alanine, L-arginine, L-hydroxyproline, L-threonine,
264 L-proline, L-serine, L-valine as sole nitrogen sources, but not L-phenylalanin, L-methionine,
265 L-histidine, L-cysteine. Antimicrobial activity is shown against *Aspergillus parasiticus*
266 NRRL-465, *Candida utilis* NRRL Y-900, *Bacillus subtilis* NRRL B-209, *Bacillus cereus*
267 NRRL B-3711, *Bacillus pumilus* NRRL-BD 142. The predominant menaquinones were MK-
268 9(H_8) and MK-9(H_6). Major fatty acids of the strains were *anteiso*-$C_{15:0}$, *anteiso*-$C_{17:0}$ and *iso*-
269 $C_{16:0}$. The polar lipid profile contains diphosphatidylglycerol, phosphatidylglycerol,
270 phosphatidylinositol, phoshphatidylethanolamine and three unidentified atypical aminolipids,
271 one unidentified aminolipid and two unidentified glycolipids, The G+C content of the
272 genomic DNA was 69.6 %. The type strain, SM3501^T(= KCTC 29214^T = DSM 42105^T) was
273 isolated from a sediment sample collected from first pool of Camalti Saltern, Izmir, Turkey.

274

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Table 1. Phenotypic properties of strains BNT558^{T}, SM3501^{T} and the most related type species. Strains: **1,** BNT558; **2,** *Streptomyces albiaxialis* DSM 41799^{T}; **3,** *Streptomyces ferralitis* DSM 41836^{T}; **4,** SM3501; **5,** *Streptomyces cacaoi* subsp. *cacaoi* KCTC 9758^{T}; **6,** *Streptomyces qinglanensis* DSM 42035^{T}; **7,** *Streptomyces flocculus* DSM 40327^{T}; **8,** *Streptomyces rangoonensis* DSM 40452^{T}.Strains were positive for arbutin hydrolysis, ability of growth at D-fructose, D-galactose, D-mannose, D-mannitol, dextrin, lactose, maltose, xylose as sole carbon sources 1.0 % (w/v), L-alanine and L-arginine as sole nitrogen sources 0.1 % (w/v), growth at pH:6.0, pH:8.0, pH:10, temperatures 28 °C, 37°C, 45°C and 0-5 % NaCl concentrations. But negative for ability of growth at dextran, L-sorbose, L-glutamic acid, growth at 4°C, 10°C, 55°C and 20 %, 30 % NaCl concentrations . All data were obtain in this study.

	1	**2**	**3**	**4**	**5**	**6**	**7**	**8**
Biochemical Tests								
Allantoin Hydrolysis	+	+	+	+	+	-	+	+
Nitrate Reduction	-	-	-	-	-	-	-	+
Urea Hydrolysis	+	-	-	-	-	+	+	-
pH tolerence								
4.0	-	+	-	+	+	-	+	+
5.0	+	+	+	+	+	-	+	+
11.0	+	+	-	+	+	+	+	+
12.0	+	+	-	+	+	+	+	+
Temperature								
50°C	-	-	-	-	-	-	+	+
NaCl (%)								
8.0	+	+	-	+	+	+	+	+
9.0	+	+	-	+	+	+	+	+
10.0	+	+	-	+	+	+	+	+
15.0	-	-	-	+	+	+	-	-
Degradation								
Adenine (0.5%)	+	+	-	+	+	+	-	-
Casein (1.0 %)	-	-	+	-	-	-	-	-
Starch (1.0 %)	+	+	+	+	+	+	-	-
Tween 40 (1.0 %)	+	+	-	+	+	+	+	+
Tween 80 (1.0 %)	+	+	-	+	+	+	+	+
Use of sole C sources 1.0 % (w/v)								
Adonitol	+	+	-	+	+	+	+	+
D-arabinose	+	+	-	-	+	+	-	-
L-arabinose	+	-	-	+	+	+	-	-
D-cellobiose	+	+	-	+	+	+	+	+
D-sorbitol	-	-	-	+	-	-	-	-
Inulin	-	+	-	+	+	+	-	+
Succinic acid (0.1 %)	-	+	-	+	-	+	+	+
Sucrose (Saccharose)	+	+	-	+	+	+	+	+
Xylitol	-	-	-	+	-	-	-	-
Use of sole N sources 0.1 % (w/v)								
Alpha-isoleucine	-	+	+	+	+	+	+	+
Glycine	+	+	+	+	+	-	+	+
L-cysteine	-	+	+	-	+	-	+	+
L-histidine	-	+	+	-	+	-	+	+
L-hydroxyproline	-	+	+	+	-	-	-	+
L-methionine	+	+	+	-	-	-	-	-
L-phenylalanine	-	+	+	-	-	-	-	+
L-proline	-	+	+	+	+	-	+	+
L-serine	+	+	+	+	+	-	+	+
L-threonine	-	+	+	+	+	+	+	+
L-valine	-	+	+	+	+	-	+	+

430 **Legends for Figures**

431 **Fig. 1.** Neighbour-joining tree (Saitou & Nei, 1987) based on almost complete 16 rRNA gene
432 sequences showing the position of strains BNT558^T and SM3501^T amongst their phylogenetic
433 neighbours. *Actinomadura glomerata* IMSNU 22179^T (AJ293704) was used as an out group.
434 Numbers at the nodes indicate the levels of bootstrap support (%); only values ≥ 50% are
435 shown. GenBank accession numbers are given in parentheses. Bar, 0.01 substitutions per site.

436

437 **Fig. 2.** Scanning electron micrograph of strain BNT558^T grow on modified Bennett's agar at
438 28°C for 21 days.

439

440 **Fig. 3.** Scanning electron micrograph of strain SM3501^T grow on starch-mineral salt agar
441 supplemented with 10 % (w/v) NaCl (DSMZ medium 1240) at 28°C for 21 days.

442

443 **Supplementary Fig. S1.** Maximum parsimony tree based on almost complete 16 rRNA gene
444 sequences showing the position of strains BNT558^T and SM3501^T amongst their phylogenetic
445 neighbours.

446 **Supplementary Fig. S2.** Maximum likelihood tree based on almost complete 16 rRNA gene
447 sequences showing the position of strains BNT558^T and SM3501^T amongst their phylogenetic
448 neighbours.

449

450 **Supplementary Fig. S3.** Molybdophosporic acid stained two-dimensional TLC of polar
451 lipids from strain BNT558^T.

452

453 DPG: Diphosphatidylglycerol, PE: Phosphatidylethanolamine, PI: Phosphatidylinositol, GL:
454 Glycolipid and PN: Aminophospholipid

455 **Supplementary Fig. S4.** Molybdophosporic acid stained two-dimensional TLC of polar
456 lipids from strain SM3501^T.
457
458 DPG: Diphosphatidylglycerol, PE: Phosphatidylethanolamine, PG: Phosphatidylglycerol and
459 AL, atyp: Aminolipid, AL: Aminolipid and GL: Glycolipid.
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461 **Supplementary Table S1.** Fatty acids profiles of strains BNT558^T and SM3501^T and closely
462 related type species.
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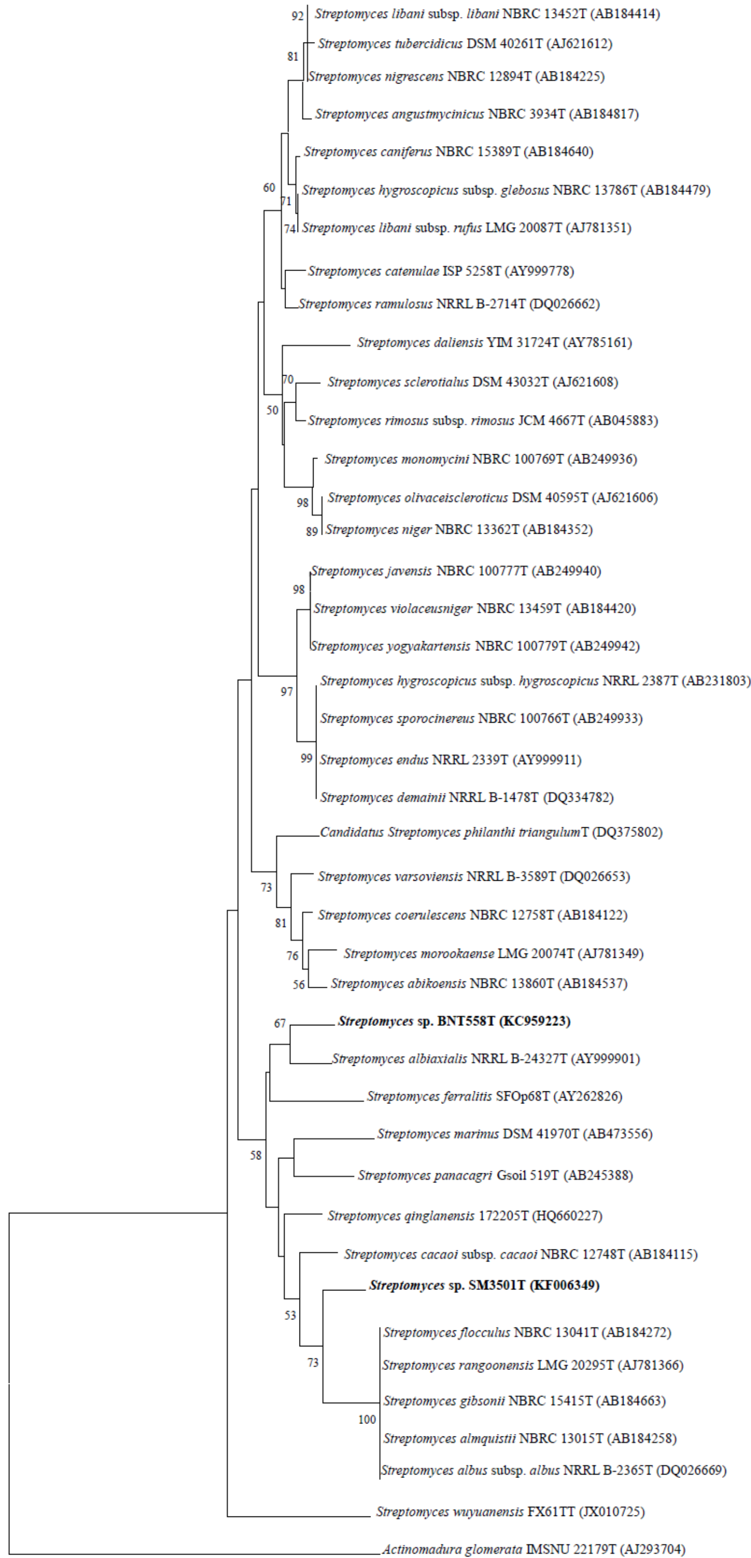

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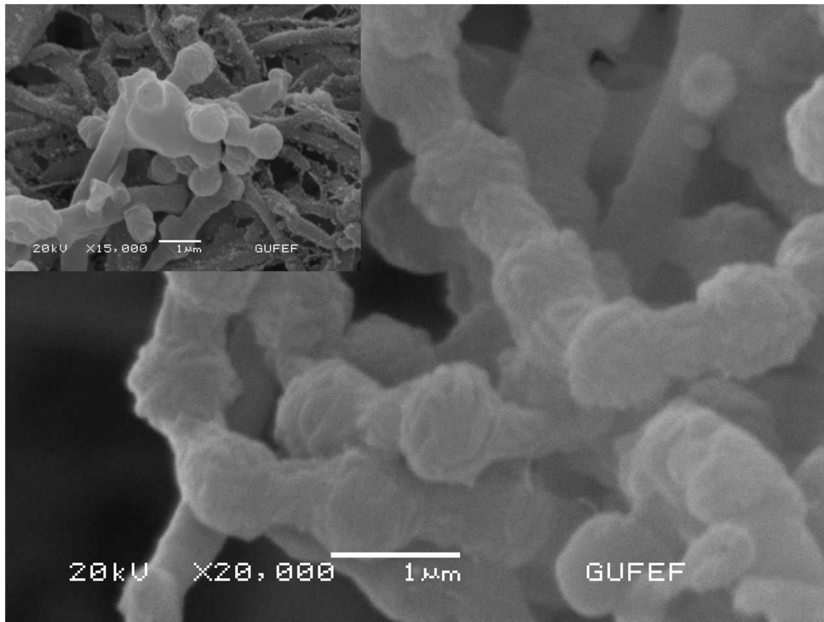

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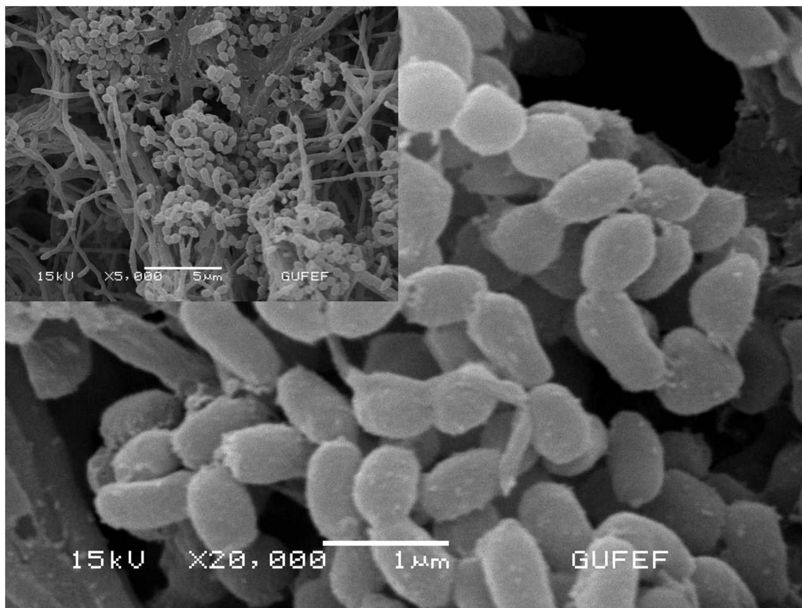

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