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1 **Reclassification of *Alteromonas fuliginea* (Romanenko *et al*. 1995) as**

2 ***Pseudoalteromonas fuliginea* comb. nov. and emended description**

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17 Running title: *Pseudoalteromonas fuliginea* comb. nov.

18 Content: New taxa (Proteobacteria)

Abstract

A new aerobic marine bacterium, strain S3431, was isolated from swab samples of an unidentified polychaete near Canal Concepción, Chile. This strain was thought to represent a new taxon within the *Pseudoalteromonas* genus. Although DNA-DNA reassociation values showed less than 70% genomic DNA relatedness to established *Pseudoalteromonas* type strains, it had 78% DNA-DNA homology with *Alteromonas fuliginea* DSM 15748 (= KMM 216) (Romanenko *et al.*, 1994). *A. fuliginea* has later been considered a heterotypic synonym of *Pseudoalteromonas citrea* (Ivanova *et al.*, 1998). Therefore we here studied the relatedness between strains S3431, *A. fuliginea* DSM 15748 and the type strain *P. citrea* LMG 12323^{T}. We found that physiological traits and genomic information are shared at a high level by strains S3431 and DSM 15748, but not between these and *P. citrea* LMG 12323^{T}. We found only approximately 20% DNA-DNA homology between the type strain of *P. citrea* LMG 12323^{T} and strains S3431 and DSM 15748. Based on the available phylogenetic and phenotypic data, reclassification of *Alteromonas fuliginea* DSM 15748 (Romanenko *et al.*, 1994) → *Pseudoalteromonas citrea* (Ivanova *et al.*, 1998) as *Pseudoalteromonas fuliginea* is proposed, and S3431 should be assigned to this new species. The name *Pseudoalteromonas fuliginea* is proposed and the type strain is KMM 216^{T} = DSM 15748^{T} = CIP 105339^{T}.

37 Bacteria of the genus *Pseudoalteromonas* within the *Gammaproteobacteria* are frequently
38 isolated from marine samples (Gram *et al.*, 2010). Several species produce bioactive
39 compounds, and often pigmentation co-occurs with production of bioactive metabolites
40 (Bowman, 2007;Egan *et al.*, 2002;Vynne *et al.*, 2011). Among the known biologically active
41 compounds produced by pigmented members of the genus are antifouling, anti-bacterial and
42 cytotoxic compounds, however, also non-pigmented species may be of biotechnological
43 interest due to the production of enzymes with novel hydrolytic activity (Bowman, 2007).
44 The so-called non-pigmented group of *Pseudoalteromonas* species contains several closely
45 related type strains, with 16S rRNA gene sequence identities of more than 99% in some cases
46 (Gauthier *et al.*, 1995).

47 During a research cruise, bacteria were collected from marine environments and screened for
48 antibacterial activity (Gram *et al.*, 2010). Of the isolated strains, more than one hundred were
49 identified by 16S rRNA gene sequence similarity as *Pseudoalteromonas* (Gram *et al.*,
50 2010;Vynne *et al.*, 2011). On February 3rd 2007, a swab sample was obtained from an
51 unidentified marine polychaete collected near Canal Concepción, Chile (-50.4498 ºN, -
52 74.8912 ºE). Dilutions of the swab sample were spread on Marine Agar 2216 and incubated
53 at 20ºC. Following incubation, strain S3431 was isolated due to a characteristic black
54 pigment and agarolytic ability.

55 We speculated that strain S3431 was a novel *Pseudoalteromonas* species and proceeded with
56 the experiments required for such analyses (Table S1) comparing to the closely related
57 *Pseudoalteromonas* type strains (Figure 1). During our work and reading, we came across a
58 potentially related bacterium named *Alteromonas fuliginea*, which also produces a black
59 pigment. The name was previously validly published (Romanenko *et al*. 1995, 879, following
60 effective publication by Romanenko *et al*., 1994), with *A. fuliginea* KMM 216 as a proposed
61 type strain out of four isolated strains (KMM 216, KMM 256, KMM 250 and KMM 504). In

62 1998, Ivanova *et al.* (Ivanova *et al.*, 1998) proposed rejection of the name
63 *Pseudoalteromonas fuliginea* (referring to *A. fuliginea*) suggesting it was a junior subjective
64 synonym (now called later heterotypic synonym) of *Pseudoalteromonas citrea* (Gauthier,
65 1977). Since then *A. fuliginea* has been considered a heterotypic synonym of *P. citrea*.

66 Here we present the phenotypic and genotypic differences and similarities between *P. citrea*
67 LMG 12323^T, *A. fuliginea* KMM 216 = DSM 15748, and our newly isolated
68 *Pseudoalteromonas* strain S3431.

69 Strains were routinely cultured for morphological and physiological characterization in
70 marine broth (MB) 2216 (Difco, cat. no. 279110) and on marine agar (MA) 2216 (Difco, cat.
71 no. 212185) at 25ºC. Marine minimal medium (MMM) (Östling *et al.*, 1991) with no carbon
72 source was used to inoculate Biolog assays (Biolog, cat. no. 1011). Temperature requirements
73 for growth were tested from 5º C to 42º C in 5º intervals and at 37º C and 42º C.
74 Requirements for Na^+-ions were tested on agar substrate containing 5.0 g $MgCl_2 \cdot 6H_2O$ l^{-1}, 2.0
75 g $MgSO_4 \cdot 7H_2O$ l^{-1}, 0.5 g $CaCl_2 \cdot 2H_2O$ l^{-1}, 1.0 g KCl l^{-1}, $FeSO_4 \cdot 7H_2O$ l^{-1}, 1.5 g yeast extract l^{-1},
76 2.5 g tryptone l^{-1}, 10 g agar l^{-1} and NaCl in concentrations of 0 to 15% w/v at 1% intervals
77 and 0.5% w/v. The pH range supporting growth was determined in broth containing 25 g
78 NaCl l^{-1}, 5.0 g $MgCl_2 \cdot 6H_2O$ l^{-1}, 2.0 g $MgSO_4 \cdot 7H_2O$ l^{-1}, 0.5 g $CaCl_2 \cdot 2H_2O$ l^{-1}, 1.0 g KCl l^{-1}, and
79 $FeSO_4 \cdot 7H_2O$ l^{-1}, 1.5 g yeast extract l^{-1}, and 2.5 g tryptone l^{-1} adjusted to pH 4, 5, 6, 7, 8, 9 and
80 10 and buffered according to desired pH. Since the pH of solutions with pH > 9.0 is not stable
81 due to acidification by CO_2, growth was evaluated after 3 days of incubation.

82 Catalase activity was tested using the 3% H_2O_2 method (Cowan, 1974). Oxidase activity was
83 tested by transfer of one colony onto a BBL DrySlide (BD, cat. no. 231746). A strain was
84 scored as oxidase positive if blue color developed within 20 seconds of application. Glucose
85 metabolism was tested by inoculation of colony mass into tubes containing Hugh & Leifson's

86 substrate (Hugh & Leifson, 1953) modified for marine microorganisms by adding 2.0% w/v
87 sea salts (Sigma, cat. no. S9883). The tubes were read after 2 days of incubation at 25ºC. API
88 20 NE strips supplemented with 2.5 ml sterile 8% sea salts per ampoule API AUX media
89 were used for comparative analysis of strain S3431^T and related type strains. Nutrient
90 assimilation by strain S3431 was tested in Biolog GN2 microtiterplates. Strains were grown
91 on MA overnight and colony mass was resuspended to OD_{600} = 1.0 in MMM supplemented
92 with 5 mM thioglycolate. 150 µl of this suspension was added to each well of the Biolog
93 GN2 plate. The plate was visually inspected for color change in wells after 10 days of
94 incubation at 25ºC.

95 Agarase activity was evaluated after 10 days of growth on MA at 25ºC. Caseinase activity
96 was tested on agar plates containing 100 g skim milk powder l^{-1} (Difco, cat. no. 232100), 30
97 g sea salts l^{-1} and 15 g agar l^{-1}. Chitinase production was assayed on agar plates containing 30
98 g sea salts and 0.2% w/v colloidal chitin (Weyland *et al.*, 1970). κ-carrageenase production
99 was tested on substrates containing MB solidified with 2% w/v κ-carrageenan (Sigma, cat.
100 no. 22048). Strains were further tested by spotting colony mass on substrate consisting of
101 28.75 ml buffer l^{-1} (85% phosphoric acid 0.08 M, boric acid 0.08 M, glacial acetic acid 0.08
102 M), 30 g Instant Ocean salts (Aquarium Systems, Inc., Sarrebourg, France) l^{-1} and 10 g agar l^{-}
103 ^{1}. The pH of the medium was adjusted to 6.0 and enzyme substrate was added to a
104 concentration of 0.1% w/v. Azurine-crosslinked (AZCL)-amylose, AZCL-curdlan, AZCL-
105 galactan, AZCL-rhamnogalacturon I, AZCL-xylose and AZCL-dextran were tested. Azo-
106 avicel was used as substrate to test production of endo-cellulases. Enzyme actitivity was
107 detected by the presence of a colored zone in the agar surrounding the colony. AZCL-
108 substrates and azo-avicel were purchased from Megazyme, Ireland. Hemolytic activity was
109 tested on Blood Agar Base (Oxoid, cat. no. CM55) with 5% v/v defibrinated calf blood.

110 Biomass for analysis of fatty acid composition, respiratory lipoquinones and polar lipids was
111 obtained from 48 hours old cultures incubated on MA at 25ºC. The biomass was lyophilized
112 prior to submission for analyses. Analyses of fatty acids, respiratory quinones and polar lipids
113 were carried out by the Identification Service and Dr. Brian J. Tindall, DSMZ, Braunschweig,
114 Germany (Table 2).

115 The nearly complete *Pseudoalteromonas* sp. S3431 16S-rRNA gene sequence was previously
116 obtained using standard PCR methods and the universal primers 27-f and 1492-r (Gram *et al.*,
117 2010) and deposited in GenBank under the accession number FJ457214. 16S rRNA gene
118 sequences of related type strains were obtained from GenBank, accession numbers are listed
119 in Figure 1. The sequences were aligned using the alignment tools in the CLC Main
120 Workbench (CLC Aarhus, Denmark version 7). Maximum Likelihood Phylogeny trees were
121 also generated using the CLC Main Workbench (CLC Aarhus, Denmark version 7) tools.
122 Neighbor Joining was the tree construction method used with the Jukes-Cantor nucleotide
123 distance measure. The design of the trees was finalized using MEGA 5 (Tamura *et al.*, 2011).
124 The topology of the tree was tested with 1000 bootstrap replications.

125 Determination of GC-mol% of genomic DNA and DNA-DNA hybridization values between
126 strain S3431, *P. citrea* LMG 12323^T and *A. fuliginea* DSM 15748 were carried out by the
127 DSMZ, Braunschweig, Germany or done *in silico* based on genome sequences (see below).
128 For DNA-DNA hybridization, cells were disrupted by using a French pressure cell (Thermo
129 Spectronic) and the DNA in the crude lysate was purified by chromatography on
130 hydroxyapatite (Cashion *et al.*, 1977). DNA-DNA hybridization was carried out as described
131 by (De Ley *et al.*, 1970) under the considerations of the modifications described by (HuS *et*
132 *al.*, 1983) using a model Cary 100 Bio UV/VIS-spectrophotometer equipped with a Peltier-
133 thermostatted 6x6 multicell changer and a temperature controller with *in situ* temperature
134 probe (Varian).

135 The genome of strain S3431 was sequenced by Beijing Genomic Institute and the genome of
136 *A. fuliginea* DSM 15748 by GATC using Illumina HiSeq Sequencing. In brief, genomic DNA
137 was extracted from each sample by using phenol:chloroform:isoamyl alcohol and then
138 precipitated with isopropanol. Samples were treated with RNase before quantification and
139 quality analysis using 1 % agarose gel electrophoresis, NanoDrop Spectrophotometer
140 (Saveen Werner, Sweden) and Qubit 2.0 Analyser (Invitrogen, United Kingdom). Libraries of
141 500 bp were used for 100 bp paired-end sequencing of genomes using the Illumina
142 sequencing technology on a HiSeq2000 with a minimum coverage of 100. We used the
143 Genome-to-Genome Distance Calculator by DSMZ (Auch *et al.*, 2010) to determine *in-silico*
144 DNA-DNA reassociation percent values (Table 3) and the Average Nucleotide Identity (ANI)
145 calculator to estimate the average nucleotide identity between two genomic datasets (Table 4)
146 (Goris *et al.*, 2007) using the newly sequenced genomes of *Pseudoalteromonas* sp. S3431
147 (JJNY01), *A. fuliginea* DSM 15748 (AF529062.1) and the publically available genome of *P.*
148 *citrea* LMG 12323^T (AHBZ02).

149 Strain S3431 and *A. fuliginea* DSM15748 differed phenotypically from *P. citrea* LMG
150 12323^T being able to grow at higher temperature and salinity (Table 1). Differences in
151 metabolism were observed between the strains: S3431 and *A. fuliginea* DSM 15748 were able
152 to utilize D-mannose, D-mannitol, D-maltose, adipic acid and malic acid, while *P. citrea*
153 LMG 12323 ^{T} was not. Furthermore, different enzymatic profiles were observed for S3431
154 and *A. fuliginea* DSM 15748 as compared to *P. citrea* LMG 12323 ^{T}. Strains S3431 and *A.*
155 *fuliginea* DSM 15748 had agarase, β-galactosidase and β-glucosidase activities, whilst *P.*
156 *citrea* LMG 12323 ^{T} had caseinase, chitinase and leucine arylimidase activities (Table 1).

157 The 16S rRNA gene sequences of *Pseudoalteromonas* type strains, S3431 and *A. fuliginea*
158 DSM 15748 were subjected to a maximum likelihood phylogenetic analysis. Within the
159 resulting phylogenetic tree S3431 and DSM 15748 strains were firmly placed within the so-

160 called non-pigmented group in contrast to the black pigment produced these strains (Figure
161 1). The low bootstrap values underline the difficulty of clearly resolving the species
162 relationship within this group of bacteria using only 16S rRNA gene sequences (Gauthier *et*
163 *al.*, 1995).

164 The DNA-DNA hybridization values of S3431 to *Pseudoalteromonas* type strains with >98%
165 16S rRNA identity were below the 70% recommended limit for delineating new species
166 (Table S1) (Wayne *et al.*, 1987). However, we found a 77.8% DNA-DNA reassociation with
167 *A. fuliginea* DSM 15748 (Table 3). Based on DNA-DNA hybridization studies and
168 physiological and morphological traits Ivanova and co-workers previously proposed that *A.*
169 *fuliginea* DSM 15748 should be placed in the species *P. citrea* (Gauthier, 1977), and the
170 name *A. fuliginea* (Romanenko *et al.*, 1994) should be rejected because it was a junior
171 subjective synonym (now called later heterotypic synonym) of *P. citrea* (Ivanova *et al.*,
172 1998). However, in our study, the DNA-DNA-hybridization between DSM 15748 and *P.*
173 *citrea* LMG 12323^T was only 19.6 % indicating that these two organisms do not belong to the
174 same species. Accordingly, a 77.8% DNA-DNA reassociation value between S3431 and
175 DSM 15748 cannot place S3431 within *P. citrea,* since DSM 15748 is not the type strain of
176 the species. The DNA-DNA hybridization value between S3431 and the type strain *P. citrea*
177 LMG 12323^T was 20.2% hence S3431 does not belong to the species *P. citrea.*

178 The analysis of whole-genome sequence data of the three strains also revealed several
179 differences and allowed the confirmation of the wet-lab DNA-DNA hybridizations (Table 3).
180 We found a very good correlation between wet-lab based analyses and the *in silico* data on
181 DNA-DNA hybridization, and confirmed that the S3431 and *A. fuliginea* DSM 15748 are the
182 same species but they cannot be classified as *P. citrea*. This conclusion is also reinforced
183 with the data from the ANI calculation, where both S3431 and DSM 15748 compare to *P.*

citrea at 78%, below the 95% threshold to be classified as the same species. ANI between S3431 and DSM 15748 is 98.5 %.

Both the pheno- and genotypic differences presented demonstrate that S3431 and *A. fuliginea* DSM15748 cannot be classified as *P. citrea*. Therefore we propose the reclassification of *A. fuliginea* Romanenko *et al*. 1995, 879^{VP} as *Pseudoalteromonas fuliginea* comb. nov. with the type KMM 216^{T} = DSM 15748^{T} = CIP 105339^{T}. We also provide an emended description of the species. Also strain S3431 (= LMG 26172 = NCIMB 14721) would be classified as *P. fuliginea*.

Description of *Pseudoalteromonas fuliginea* comb. nov.

Pseudoalteromonas fuliginea (fu.li.gi.ne'a. L. fem. adj. fuliginea – black-brown.).

Basonym: *Alteromonas fuliginea* Romanenko *et al*. 1995, 879

Cells are motile straight rods with polar flagella, 1.5 – 3.0 µm long and 0.5 – 0.8 µm wide. The cells are Gram-negative, non-spore forming, strictly aerobe and mainly occur as single cells. When grown on MA 2216 at 25º C, the strain forms raised circular black to dark brown colonies, which appear smooth and shiny, with 3 – 5 mm in diameter. A melanin-like brown pigment diffusing into the agar is produced. Growth occurs at 5 to 30º C, with no growth at 35º C, and within pH 6 to 9, the optimal pH being 7.5 to 8.5. Substrate NaCl content from 1-9% w/v supports growth. The strain utilizes D-glucose, D-mannose, D-galactose, maltose, sucrose, melibiose, lactose, succinate, D-gluconate, D-mannitol, sorbitol, citrate, xylose, trehalose, acetate, L-arginine, α-cyclodextrin, dextrin, glycogen, tween 40, tween 80, D-cellobiose, gentiobiose, lactulose, D-raffinose, acetic acid, D-galacturonic acid, α-ketoglutaric acid, propionic acid, succinic acid, L-alaninamide, L-alanine, L-alanyl-glycine,

207 L-asparagine, L-glutamic acid, glycyl-L-glutamic acid, L-proline, L-serine, L-threonine,
208 inosine, uridine, α-D-glucose-6-phosphate and D-glucose-6-phosphate as sole carbon sources.
209 It does not grow on D-fructose, sorbitol, *N*-acetylglucosamine or pyruvate. Reduction of
210 nitrate to nitrite is variable. The strain produces amylase and hydrolyzes agar, κ-carrageenan
211 and casein, but it is not hemolytic. The primary cellular fatty acids are summed feature 3
212 ($C_{16:1}$ ω7c/$C_{16:1}$ ω6c) and $C_{16:0}$. The sole respiratory quinone was Q8, and the polar lipids
213 were phosphatidylglycerol, phosphatidylethanolamine, an aminophospholipid, an aminolipid
214 and two glycolipids.

215 The type strain is KMM 216^T (= DSM15748 ^{T} = CIP105339 ^{T}), isolated from the homogenate
216 of purple sea squirt *Halocynthia aurantium* inhabiting the coastal waters of Peter the Great
217 Bay in the Sea of Japan. The DNA G+C mol content of the type strain ranges from 41.5 to
218 43.8%.

219

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229

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291

292

Table 1. Phenotypic, physiological and biochemical characteristics of strain S3431, DSM 15748 and LMG 12323. All tests were performed in the present study.

Strains: S3431; *P. citrea* LMG 12323^T; *A. fuliginea* DSM 15748. +, positive test; - negative test; w, weak positive test; o, oxidative glucose metabolism in marine Hugh & Leifson substrate.

Characteristic	**S3431**	**LMG 12323^T**	**DSM 15748**
Glucose metabolism	o	-	-
Growth at temp (ºC)	5-30	5-25	5-30
% NaCl required (w/v)	0.5-9	2-5	1-9
Growth at pH	6-9	6-9	6-9
Reduction of NO_3 to NO_2	+	-	-
Genome size (Mb)	4.20	5.32	4.77
GC-mol %	38.9	41.1	38.9
Utilization of:			
L-arabinose	-	+	w
D-mannose	+	-	+
D-mannitol	+	-	+
D-maltose	+	-	+
Potassium gluconate	+	-	-
Adipic acid	+	-	w
Malic acid	+	-	+
Enzyme activity:			
Agarase	+	-	+
Caseinase	-	+	-
Carrageenase	+	-	-
Chitinase	-	+	-
Urease	-	-	+
Leucine arylimidase	-	+	-
β-galactosidase	+	-	+
β-glucosidase	+	-	+

All the strains were oxidase and catalase positive, utilized D-glucose and exhibited protease, esterase (C 4), esterase lipase (C 8), lipase (C 14), valine arylimidase and acid phosphatase activities.

All the strains were negative for indole production, arginine dihydrolase, cystine arylimidase, trypsin, α-galactosidase and α-glucosidase activities, as well as for the utilization of N-acetyl-glucosamine, capric acid, trisodium citrate and phenylacetic acid.

Table 2. Cellular fatty acid profiles of strain S3431, DSM 15748 and LMG 12323. All data was generated in this study.

Strains: S3431; *P. citrea* LMG 12323^T; *A. fuliginea* DSM 15748. Values given are percentages of total fatty acids. -, not detected; tr, trace amounts (< 1%). Naming table: MIDI Sherlock TSBA6 6.10.

Fatty acids	**S3431**	**LMG 12323**	**DSM 15748**
10:0 3OH	1.1	5.3	tr
12:0	1.3	tr	1.3
11:0 3OH	tr	tr	tr
12:0 3OH	5.5	3.6	6.9
14:0	tr	tr	tr
Summed feature 1 (15:1 iso H/13:0 3OH)	tr	-	tr
15:1 ω8c	2.1	-	tr
16:1 ω9c	tr	-	tr
Summed feature 3 (16:1 ω7c/16:1 ω6c)	37.1	39.5	39.1
16:0	21.5	21.8	19.1
17:1 ω8c	7.99	tr	6.6
17:1 ω6c	tr	-	tr
17:0	6.5	tr	2.8
18:1 ω9c	tr	-	1.2
Summed feature 8 (18:1 ω7c/18:1 ω6c)	8.4	23.4	10.5
18:0	1.6	1.6	2.4
Summed feature 7	-	-	-
20:0	-	-	-

Table 3. Genomic DNA-DNA hybridization (DDH) percentages determined using *in silico* (diagonal up) and wet-lab approaches (diagonal down).

Strains: S3431; *P. citrea* LMG 12323^T; *A. fuliginea* DSM 15748. ND, not determined.

		***In silico* DDH (% ± SD)**		
		S3431	**LMG 12323**	**DSM 15748**
Wet-lab DDH (%)	**S3431**		19.9 ± 2.3	85.9 ± 2.4
	LMG 12323	20.2		19.6 ± 2.3
	DSM 15748	77.8	ND	

Table 4. Average Nucleotide Identity (ANI) percentages determined using *in silico* approaches.

Strains: S3431; *P. citrea* LMG 12323^T; *A. fuliginea* DSM 15748.

	S3431	**LMG 12323**	**DSM 15748**
S3431	100	78.3 ± 5.7	98.6 ± 2.1
LMG 12323		100	78.2 ± 5.6
DSM 15748			100

Figure 1. Maximum likelihood phylogenetic tree based on 16S rRNA gene sequences of *Pseudoalteromonas* and *Alteromonas* type strains. Strain S3431 appeared in the non-pigmented clade, near *A. fuliginea* DSM15748, *P. translucida* DSM 14402^T, *P. antarctica* CECT4664^T, *P. nigrifaciens* NCIMB 8614^T, and *P. haloplanktis* DSM6060^T. Scale bar: 0,01 substitutions per nucleotide site. Bootstrap support is indicated at nodes (1000 replications). GenBank accession numbers are indicated. *Salinispora arenicola* ATCC BAA-917 was used to root the tree (not included in figure), GenBank accession number NR_042725.

Figure 1

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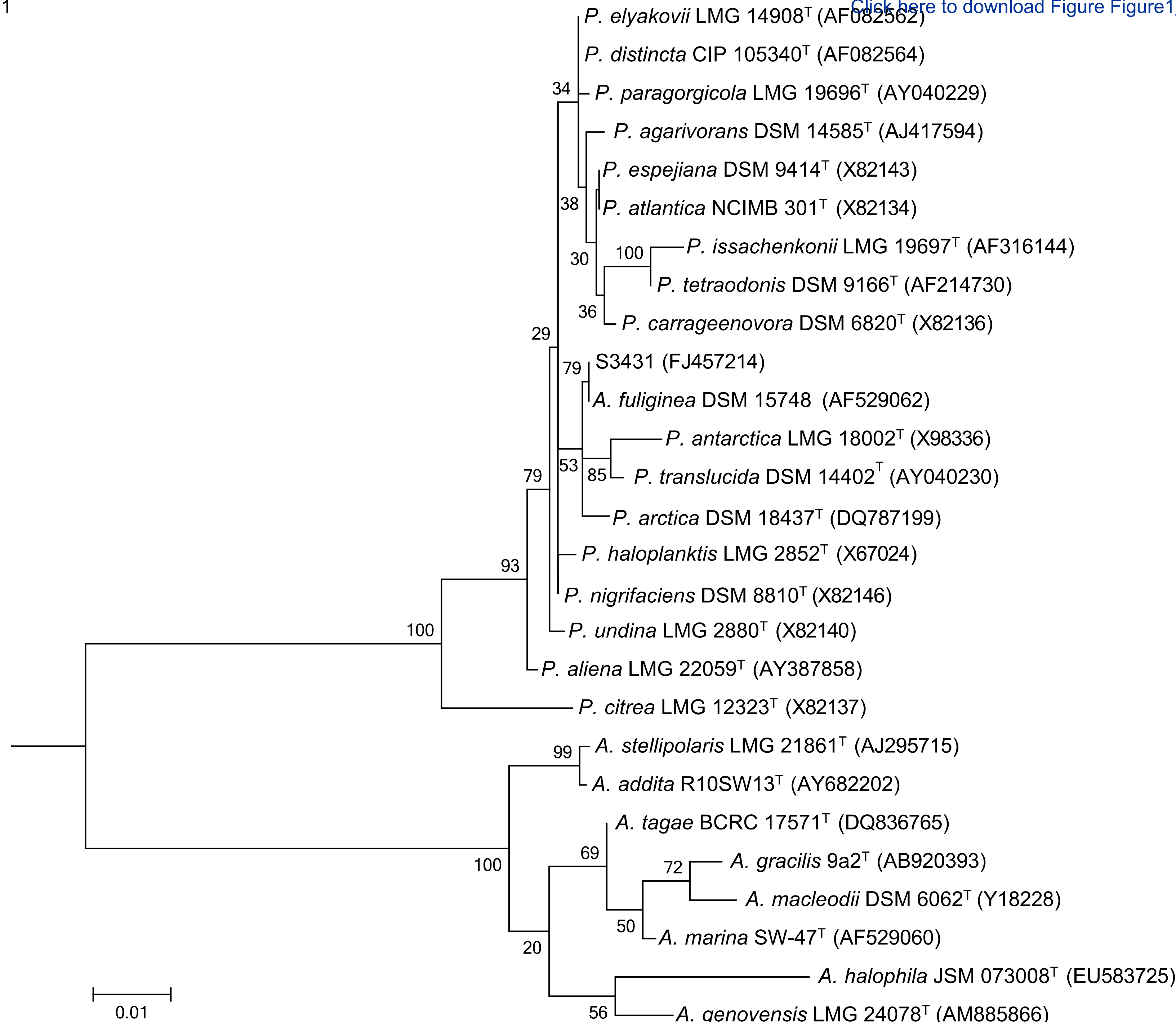

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Reclassification of *Alteromonas fuliginea* (Romanenko *et al.* 1995) as *Pseudoalteromonas fuliginea* comb. nov. and emended description

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Running title: *Pseudoalteromonas fuliginea* comb. nov.

Content: New taxa (Proteobacteria)

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Table S1. Phenotypic, physiological and biochemical characteristics of strain S3431 and related strains of the genus *Pseudoalteromonas*. All data were generated in this study.

Strains: 1, S3431; 2, *P. agarivorans* DSM 14585^T; 3, *P. aliena* LMG 22059^T; 4, *P. antarctica* LMG 18002^T; 5, *P. arctica* DSM 18437^T; 6, *P. atlantica* NCIMB 301^T; 7, *P. carrageenovora* DSM 6820^T; 8, *P. citrea* LMG 12323^T; 9, *A. fuliginea* DSM 15748; 10, *P. distincta* CIP 105340^T; 11, *P. elyakovii* LMG 14908^T; 12, *P. espejiana* DSM 9414^T; 13, *P. haloplanktis* LMG 2852^T; 14, *P. issachenkonii* LMG 19697^T; 15, *P. nigrifaciens* DSM 8810^T; 16, *P. paragorgicola* LMG 19696^T; 17, *P. tetraodonis* DSM 9166^T; 18, *P. translucida* DSM 14402^T; 19, *P. undina* LMG 2880^T. +, positive test; - negative test; w, weak positive test; o, oxidative glucose metabolism in marine Hugh & Leifson substrate; ND, not determined.

Characteristic	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19
Oxidase	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Catalase	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Glucose metabolism	o	o	-	-	o	o	o	-	-	-	o	o	-	-	-	o	o	-	o
Growth at temperature (ºC)	5-30	5-37	5-30	5-30	5-30	5-37	5-30	5-25	5-30	10-30	5-30	5-30	5-30	5-37	5-30	5-30	5-35	5-30	5-35
% NaCl required for growth (w/v)	0.5-9	0-11	0.5-9	0.5-10	0-10	0.5-12	0.5-10	2-5	1-9	0.5-9	0.5-11	0.5-12	0.5-11	0-12	0.5-9	0.5-10	0.5-11	0.5-10	0.5-11
Growth at pH	6-9	6-9	6-9	6-9	6-9	6-9	6-9	6-9	6-9	6-9	6-9	6-9	6-9	6-9	6-9	6-9	6-9	6-9	6-9
Reduction of nitrates to nitrites	+	-	-	-	-	-	-	-	-	+	-	-	-	-	-	-	-	-	-
Indole production	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Arginine DiHydrolase	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
% DNA-DNA reassociation to S3431	-	35.8	40.1	41.0	41.0	22.8	11.7	20.2	77.8	44.9	52.5	55.7	22.9	40.6	11.6	54.0	14.5	6.2	21.7
% DDH estimate (GLM-based)	100	-	-	-	39.0±2.5	-	-	19.9±2.3	85.9±2.5	-	-	-	20.8±2.3	-	-	-	-	-	22.2±2.
Utilization of:																			
D-glucose	+	+	+	+	+	+	+	+	+	+	+	+	w	+	+	+	+	+	+
L-arabinose	-	-	w	-	-	-	-	+	w	+	-	-	-	-	-	-	-	-	+
D-mannose	+	+	-	+	+	+	+	-	+	+	+	-	-	-	-	+	-	+	-
D-mannitol	+	+	+	+	+	+	+	-	+	+	+	+	-	-	+	-	-	+	-
N-acetyl-glucosamine	-	+	+	+	-	-	-	-	-	+	-	-	-	+	+	-	+	+	+

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D-maltose	+	+	+	+	+	+	+	-	+	+	+	+	+	+	+	+	+	+	+
Potassium gluconate	+	+	-	+	+	-	-	-	-	-	-	-	-	-	+	+	-	+	-
Capric acid	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Adipic acid	+	+	+	+	-	-	-	-	w	+	+	-	-	+	+	-	+	+	-
Malic acid	+	+	+	+	+	-	+	-	+	+	+	-	-	+	+	+	+	+	+
Trisodium citrate	-	-	-	+	-	-	+	-	-	-	+	+	-	-	+	-	-	+	-
Phenylacetic acid	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	-
Enzyme activity:																			
Agarase	+	+	-	-	-	+	-	-	+	-	-	-	-	-	-	-	-	-	-
Protease	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Caseinase	-	+	+	+	+	+	+	+	-	+	+	+	+	+	-	w	+	-	+
Carrageenase	+	-	-	-	-	-	+	-	-	-	-	-	-	-	-	-	-	-	-
Chitinase	-	-	+	-	-	-	-	+	-	-	-	-	-	+	-	-	-	-	+
Urease	-	-	-	+		-	-	-	+	-	-	-	-	-	+	-	-	+	-
Esterase (C 4)	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Esterase Lipase (C 8)	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Lipase (C 14)	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Leucine arylimidase	-	-	-	-	-	-	-	+	-	-	-	-	-	-	-	-	-	-	-
Valine arylimidase	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Cystine arylimidase	-	-	-	-	-	-	+	-	-	-	-	-	-	-	-	-	-	-	-
Trypsin	-	-	-	-	-	+	+	-	-	-	-	-	-	-	-	-	-	-	-
Acid phosphatase	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
α-galactosidase	-	-	-	-	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-
β-galactosidase	+	+	-	-	+	+	+	-	+	-	+	+	-	-	+	+	-	-	-
α-glucosidase	-	-	-	-	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-
β-glucosidase	+	+	-	-	+	+	+	-	+	+	+	+	-	+	-	+	+	-	+

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