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Genus *Paludifilum*

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1 **Genus *Paludifilum***

2

3 **Defining publication:** *Frikha-Dammak, Fardeau, Cayol, Ben Fguira-Fourati, Najeh,*
4 *Ollivier and Maale 2016, 5371* ^{VP}

5

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13 **Etymology:**

14 Pa.lu.di.fi'lum L. n. *palus*, -dis a marsh; L.neut. n. *filum* a thread; N.L. neut. n. *Paludifilum* a
15 thread from a marsh).

16

17 **Abstract**

18 The genus *Paludifilum* at the time of writing, comprises one validly published species,
19 *Paludifilum halophilum*, which was isolated from superficial sediment of a solar saltern in
20 Sfax, Tunisia. The species of the genus is thermotolerant, halophilic, aerobic and
21 chemoorganotrophic. Aerial and substrate mycelia are long and well-developed with long
22 chains containing fluorescent and circular spores. Phylogenetic analyses using 16S rRNA
23 gene sequences placed the species as representative of *Thermoactinomycetaceae* family.

24

25 **Keywords:** moderate thermophile, halophile, aerobe

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27

28 **Description:**

29 Aerial and substrate mycelia are long and well-developed with long chains containing
30 fluorescent and circular spores. Cells present a typical **Gram-positive**-type cell wall structure.

31 **Mesophilic, halophile and thermotolerant, aerobic**, and fermentative growth. The major
32 cellular fatty acids are anteiso-C15: 0, iso-C15: 0 and iso-C17: 0. Yeast extract is required for
33 growth.

34 *DNA G+C content (mol %): 51.2*

35

36 *Type species: Paludifilum halophilum* Frikha-Dammak, Fardeau, Cayol, Ben Fguira-
37 *Fourati, Najeh, Ollivier and Maale 2016, 5371* ^{VP}

38

39 Number of species with validated names: 1.

40

41 **Family classification:**

42 *Thermoactinomycetaceae*

43 Further descriptive information

44 The genus *Paludifilum*, at the time of writing, is *comprised* of one validly named species, *P.*
45 *halophilum*, which was isolated from the superficial sediment of noncrystallizer M1 pond of
46 Sfax solar saltern in Tunisia (Frikha-Dammak et al., 2016). The occurrence of new genera of
47 halophilic thermoactinomycetes has been reported in the hypersaline environments of distant
48 geographical sites such as the Algerian salt lake (Addou et al., 2012) and the soil of
49 hypersaline wetland in the north of Iran (Zarparvar et al., 2014). The saltern of Sfax can thus
50 be considered as an additional geographical site harboring new members of halophilic
51 thermoactinomycetes (Frikha-Dammak et al., 2017). Moreover, this is the first report
52 revealing that these microbes could have an antimicrobial potential. It was shown for
53 *Paludifilum halophilum*, that the observed antimicrobial activity is most likely explained by
54 the production of Gramicidin S and four cyclic dipeptides identified as cyclo(L-4-OH-Pro-L-
55 Leu), cyclo(L-Tyr-L-Pro), cyclo(L-Phe-L-Pro), and cyclo(L-Leu-L-Pro). The potent
56 inhibitory effect of these compounds covered the growth of the plant pathogen *A. tumefaciens*
57 and the human pathogens *S. aureus*, *S. enterica*, *E. coli*, and *P. aeruginosa*. This is the first
58 time that the bioactivity of cyclic antimicrobial peptides from halophilic thermoactinomycete
59 against agriculturally and medically important bacteria is reported.

60

61 Properties of the species of the genus are described in Table 1. Cells stain Gram-positive,
62 The substrate and aerial mycelia were long and well developed on all tested media with long
63 chains containing fluorescent and circular spores. Colonies, 4.0–8.0 mm in diameter, are
64 circular and irregular with wrinkles between the centre and the edge of the colony. The strain
65 forms yellow aerial mycelium on Bennett's medium.

66

67 **Growth conditions.** The temperature range for growth is 30–55°C, with an optimum between
68 40 and 45°C. The pH range for growth is 5-11, with an optimum near 8.0 and 9.0. The salinity
69 range for growth is from 5–20% NaCl (w/v) with an optimum at 10%.

70 Glucose, sucrose, maltose, xylose, mannose, fructose, arabinose, inositol, starch and sorbitol
71 are used as sole carbon sources for growth, but not mannitol. Acids and acetoin were not
72 produced from D-glucose. Proline, lysine, glycine and ornithine can be used as sole sources of
73 nitrogen, but not leucine, arginine, glutamine, tryptophan or phenylalanine. The strain did not
74 reduce nitrate and nitrite. Casein, starch and gelatin were hydrolysed, while H₂S and indole
75 were not produced. The strain was resistant to lysozyme and phenol. Casein, gelatin and
76 starch are hydrolysed, but not lipids, cellulose, tyrosine, xanthine, hypoxanthine and DNA.
77 (Table 1).

78

79 Enrichment and isolation procedures

80

81 *Paludifilum halophilum* was isolated by suspending 1 g collected superficial sediment in 9.0
82 ml saline water (150 g/l). After vigorous shaking by using vortex mixer, a 0.1 ml aliquot of
83 each serial dilution (10⁻¹-10⁻⁵) was taken and spread over the surface of *Streptomyces* isolation
84 agar medium. After incubation for 3 weeks at 37°C under aerobic conditions, colonies were
85 picked and streaked several times on the same isolation agar medium until deemed to be
86 axenic (Frikha-Dammak et al, 2016).

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88 Maintenances procedures

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90 Stock cultures can be maintained in culture medium described by Frikha-Dammak et al.
91 (2016). Liquid cultures retain viability for several months after storage at -80°C in the basal
92 medium containing 20% glycerol (v/v).

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94 Phylogenetic and taxonomic comments

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96

97 Phylogenetic analyses using 16S rRNA gene sequences indicated that *Paludifilum halophilum*
98 belongs to an independent phylogenetic lineage of the family *Thermoactinomycetaceae* that is
99 distinct from all other reference genera within the family. *P. halophilum* shows a gene

100 sequence similarity of 94.5% with *Kroppenstedtia eburnea* (von Jan et al., 2011) and 94.6%
101 with *Melghirimyces algeriensis* (Addou et al., 2012). (Figure 1).

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104 ***List of species of the genus Paludifilum***

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107 1. ***Paludifilum halophilum*** Frikha-Dammak, Fardeau, Cayol, Ben Fguira-Fourati, Najeh,
108 Ollivier and Maale 2016, 5371 ^{VP}

109 ha.lo'phi.lum. Gr. n. *hals*, *halos* salt; Gr. adj. *philos* loving; N.L. neut. adj. *halophilum* salt
110 loving

111 Thermotolerant, halophilic, aerobic bacterium. Aerial mycelia were long and well developed
112 on all tested media with long chains containing fluorescent and circular spores. Colonies, 4.0–
113 8.0 mm in diameter, are circular and irregular with wrinkles between the centre and the edge
114 of the colony. Gram-staining is positive, catalase-and oxidase positive. The temperature range
115 for growth is 30-55°C, with optimum growth at 40-45°C. The optimum pH for growth is 8-9.
116 The salinity range for growth is 50-200 g/l (optimum 100 g/l). D-Glucose, D-xylose, sucrose,
117 maltose, D-mannose, D-inositol and D-sorbitol are assimilated as sole carbon sources. Acids
118 and acetoin are not produced from D-glucose. L-Proline, L-lysine and L-ornithine are used as
119 carbon and nitrogen sources. Nitrate and nitrite are not reduced. The major cellular fatty acids
120 are anteiso-C15:0, iso -C15:0 and iso-C17:0. The major mena-quinone is MK-7 and MK-8 is
121 also detected. In addition of the four major known polar lipids, five minor unknown lipids
122 were detected and identified as two phospholipids, one phosphoaminolipid, one lipid and one
123 glycolipid. Isolated from the superficial sediment of noncrystallizer M1 pond of Sfax solar
124 saltern in Tunisia.

125 *DNA G+C content (mol %):* 51.2

126 *Type strain:* SMBg3 (DSM 102817, CCUG 68698).

127 *EMBL/GenBank accession number (16S rRNA gene):* KP229518

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157

158 **FIGURE CAPTIONS**

159

160 **Figure 1** Phylogenetic tree based on 16S rRNA gene sequences showing the relationship of
161 *Paludifilum halophilum* with the type strains of related genus of the family
162 *Thermoactinomycetaceae*. The consensus tree was constructed with mega 6 software (Tamura
163 *et al.*, 2013) using the Neighbor-Joining, Maximum-Parsimony and Maximum-Likelihood
164 methods. There were a total of 1364 positions in the final dataset. GenBank accession
165 numbers are given in parentheses. *Alicyclobacillus acidoterrestris* DSM 3922^T (GenBank
166 accession no. AJ133631) was used as the outgroup. Bar, 2 substitutions per nucleotide
167 position.

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Table 1. Descriptive characteristics of the species of the genus *Paludifilum*, and the type strains of two phylogenetically related species of the family *Thermoactinomycetaceae*: *Kroppenstedtia eburnea* (von Jan et al., 2011) and *Melghirimyces algeriensis* (Addou et al., 2012); PE, phosphatidylethanolamine; PG, phosphatidylglycerol; PS, phosphatidylserine; DPG, diphosphatidylglycerol; PME, phosphatidylmethylethanolamine; UL, unknown lipid; UPL, unknown phospholipid; UPN, unknown phosphoaminolipid; UGL, unknown glycolipid.

Characteristics	<i>Paludifilum halophilum</i>	<i>Kroppenstedtia eburnea</i>	<i>Melghirimyces algeriensis</i>
Morphology	pale yellow aerial mycelium	white aerial mycelium	yellow aerial mycelium
Optimum temperature (°C)	40-45	45	40-55
Optimum pH	8-9	5-8.5	6-8
Optimum NaCl (%)	10	ND	10
G + C content (mol%)	51.2	54.6	52.6
Main substrates used	glucose, xylose, sucrose, maltose, mannose, inositol, sorbitol	sucrose, i-inositol, mannitol, rhamnose	Glycerol, maltose, cellobiose, rhamnose, sucrose, myo-inositol, fructose, ribose, mannose, glucose, raffinose and amino-acids
Polar lipids	PE, PG, DPG, PME, UPN, 2UPLs, UGL, UL	DPG, PE, PG	. PG, DPG, PE, PME, 5UPLs, UL

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182 **Table 2** Main cellular fatty acids of *Paludifilum halophilum* and the type strains of two
 183 phylogenetically related species of the family *Thermoactinomycetaceae*: *Kroppenstedtia*
 184 *eburnea* (von Jan et al., 2011) and *Melghirimyces algeriensis* (Addou et al., 2012).

Fatty acids	<i>Paludifilum halophilum</i>		<i>Melghirimyces algeriensis</i>
iso-C14 : 0	1.7	3.9	8.5
iso-C15 : 0	24.3	73.3	59.1
Anteiso-C15 : 0	29.2	13.1	18.2
Iso-C16 : 0	7.3	4.5	1.8
C16 : 0	5.4	ND	4.6
Iso-C17 : 0	17.2	ND	6.7
Anteiso-C17 : 0	8.9	ND	1.5