

Genus Paludifilum

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1	Genus Paludifilum
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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 <i>Thermoactinomycetaceae</i> family.
24	
25	Keywords: moderate thermophile, halophile, aerobe
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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

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

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Number of species with validated names: 1.

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Family classification:

- 42 Thermoactinomycetaceae
- 43 Further descriptive information
- 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
- 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
- against agriculturally and medically important bacteria is reported.

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- Properties of the species of the genus are described in Table 1. Cells stain Gram-positive,
- The substrate and aerial mycelia were long and well developed on all tested media with long
- 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.

- 67 **Growth conditions**. The temperature range for growth is 30–55°C, with an optimum between
- 40 and 45°C. The pH range for growth is 5-11, with an optimum near 8.0 and 9.0. The salinity
- 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
- 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
- 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
- starch are hydrolysed, but not lipids, cellulose, tyrosine, xanthine, hypoxanthine and DNA.
- 77 (Table 1).

Enrichment and isolation procedures

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- 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
- 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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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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Phylogenetic and taxonomic comments

- 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

sequence similarity of 94.5% with Kroppenstedtia eburnea (von Jan et al., 2011) and 94.6% 100 101 with Melghirimyces algeriensis (Addou et al., 2012). (Figure 1). 102 103 104 List of species of the genus Paludifilum 105 106 107 1. Paludifilum halophilum Frikha-Dammak, Fardeau, Cayol, Ben Fguira-Fourati, Najeh, Ollivier and Maale 2016, 5371 VP 108 109 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 128

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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
- et al., 2013) using the Neighbor-Joining, Maximum-Parsimony and Maximum-Likelihood
- methods. There were a total of 1364 positions in the final dataset. GenBank accession
- numbers are given in parentheses. *Alicyclobacillus acidoterrestris* DSM 3922^T (GenBank
- accession no. AJ133631) was used as the outgroup. Bar, 2 substitutions per nucleotide
- position.

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. 2).

1	7	7

Characteristics	Paludifilum	Kroppenstedtia	Melghirimyces
	halophilum	eburnea	algeriensis
Morphology	pale yellow aerial	white aerial	yellow aerial
	mycelium	mycelium	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, i-inositol,	Glycerol, maltose,
	sucrose, maltose,	mannitol	cellobiose, rham-
	mannose, inositol,	rhamnose	nose, sucrose,
	sorbitol		myo-inositol,
			fructose, ribose,
			mannose, glu-
			cose, raffinose and
			amino-acids
Polar lipids	PE, PG, DPG,	DPG, PE, PG	. PG, DPG, PE,
	PME, UPN,		PME,
	2UPLs, UGL, UL		5UPLs, UL

Table 2 Main cellular fatty acids of *Paludifilum halophilum* 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).

Fatty acids	Paludifilum		Melghirimyces
	halophilum		algeriensis
		1	-
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
		T	I
C16:0	5.4	ND	4.6
L C17 0	17.0	ND	
Iso-C17:0	17.2	ND	6.7
Antoine C17 : 0	9.0	ND	1.5
Anteiso-C17: 0	8.9	ND	1.5