

Oryzisolibacter

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2. KEYWORDS: *Oryzisolibacter propanilivorax*; rice paddy soil; aerobe; chemo-organotroph; propanil and 3,4-DCA degradation

3. ABSTRACT:

Rods $1.9 \pm 0.35 \mu\text{m}$ long and $0.6 \pm 0.04 \mu\text{m}$ wide. **Motile**. **Gram-negative**. Nonsporulating. **Facultative aerobe**. Nitrate is reduced to N_2 . Mesophilic, with the ability to grow between 15 and 37 °C, pH 6-9 and at a NaCl concentration up to 4%. **Chemo-organotroph**, being able to assimilate glycerol, potassium gluconate, adipic acid, malic acid, and (trisodium) citrate. Catalase- and cytochrome *c* oxidase positive. The respiratory quinone is **ubiquinone 8** and major fatty acids are $\text{C}_{18:1} \omega 7c$, $\text{C}_{16:0}$, and the summed feature 3 ($\text{C}_{16:1} \omega 7c$ / iso- $\text{C}_{15:0}$ 2-OH). Major polar lipids are phosphatidylethanolamine, phosphatidylglycerol, and diphosphatidylglycerol. Isolated from rice paddy soil through an enrichment culture in the presence of propanil and 3,4 –dichloroaniline; the type strain of the type species *Oryzisolibacter propanilivorax* is able to degrade both compounds.

4. DEFINING PUBLICATION:

Oryzisolibacter, Vaz-Moreira, Narciso-da-Rocha, Lopes, Carvalho, Lobo-da-Cunha, Whitman, Snauwaert, Vandamme, Manaia and Nunes, 2017, 3755^{VP}

5. ETYMOLOGY:

Oryzisolibacter [O.ry.zi.so.li.bac'ter. L. n. *oryza*, rice; L. n. *solum*, soil; N.L. masc. n. *bacter*, rod; N.L. masc. n. *Oryzisolibacter* rod from rice crop bulk soil].

6. GENERIC DEFINITION:

Rods $1.9 \pm 0.35 \mu\text{m}$ long and $0.6 \pm 0.04 \mu\text{m}$ wide. **Motile**. **Gram-negative**. Nonsporulating. **Facultative aerobe**. Nitrate is reduced into N_2 . Mesophilic, with the ability to grow between 15 and 37 °C, pH 6-9 and at a NaCl concentration up to 4%. **Chemo-organotroph**, being able to assimilate glycerol, potassium gluconate, adipic acid, malic acid, and (trisodium) citrate. Catalase- and cytochrome *c* oxidase positive. The respiratory quinone is **ubiquinone 8** and major fatty acids are $\text{C}_{18:1} \omega 7c$, $\text{C}_{16:0}$, and the summed feature 3 ($\text{C}_{16:1} \omega 7c$ / iso- $\text{C}_{15:0}$ 2-OH). Major polar lipids are phosphatidylethanolamine, phosphatidylglycerol, and diphosphatidylglycerol. Isolated from rice paddy soil through an enrichment culture in the presence of propanil and 3,4 -dichloroaniline; the type strain of the type species *Oryzisolibacter propanilivorax* is able to degrade both compounds.

The DNA G+C content (mol %) is 69.4 (HPLC) or 69.2 (genome analysis).

Type species: *Oryzisolibacter propanilivorax*, Vaz-Moreira, Narciso-da-Rocha, Lopes, Carvalho, Lobo-da-Cunha, Whitman, Snauwaert, Vandamme, Manaia and Nunes, 2017, 3755^{VP}

Number of species with validated names: 1.

69 **7. FAMILY CLASSIFICATION:**

70 *Comamonadaceae* (fbm00182)

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73 **8. FURTHER DESCRIPTIVE INFORMATION:**

74 **8.1. Cell morphology:**

75 A single species is described within the genus *Oryzisolibacter*, *Oryzisolibacter*
76 *propanilivorax* (Vaz-Moreira et al., 2017). Cells are Gram-negative, non-spore forming,
77 motile rods, $1.9 \pm 0.35 \mu\text{m}$ long and $0.6 \pm 0.04 \mu\text{m}$ wide.

78

79 **8.2. Colonial and cultural characteristics:**

80 On solid agar medium (Luria-Bertani agar), after 48 h of incubation at 30 °C, the type strain
81 of *Oryzisolibacter propanilivorax* forms circular (2 mm diameter), convex, light yellow-
82 colored colonies.

83

84 **8.3. Nutrition and growth conditions:**

85 The type strain of *Oryzisolibacter propanilivorax* is able to assimilate the carbon sources
86 glycerol, potassium gluconate, adipic acid, malic acid, and (trisodium) citrate. It is unable to
87 assimilate erythritol, D- or L-arabinose, D- or L-xylose, adonitol, methyl-beta-D-
88 xylopyranoside, D-galactose, D-fructose, D-mannose, L-sorbose, L-rhamnose, D-ribose
89 dulcitol, inositol, D-mannitol, D-sorbitol, methyl-alpha-D-mannopyranoside, methyl-alpha-
90 D-glucopyranoside, N-acetyl-glucosamine, amygdalin, arbutin, esculin, salicin, D-cellobiose,
91 D-maltose, D-lactose, D-melibiose, D-saccharose, D-trehalose, inulin, D-melezitose, D-

raffinose, amidon, glycogen, xylitol, gentiobiose, D-turanose, D-lyxose, D-tagatose, D- or L-fucose, D- or L-arabitol, potassium 5-ketogluconate, phenylacetic acid and capric acid. It is also not able to ferment glucose, mannose, inositol, sorbitol, rhamnose, sucrose, melibiose, amygdalin, or arabinose.

It is able to grow in the temperature range of 15 to 37 °C, at pH 6-9 and at a NaCl concentration up to 4%. Abundant growth is observed between 25 and 37 °C, pH 8 and 0.1-2% (w/v) NaCl. Growth is not observed at 40 °C, pH 10.0 or 5% NaCl.

8.4. Metabolism:

The type strain of *Oryzolibacter propanilivorax* is a facultative aerobe. Nitrate is reduced to nitrite, which is further reduced to nitrogen. Weak growth occurs under anaerobic conditions in the presence of nitrate. It is a chemo-organotroph, able to degrade the herbicide propanil and its transformation product 3,4-dichloroaniline (3,4-DCA) in resting cell assays performed in phosphate buffer with 0.25 mM of either of these compounds and a cell density corresponding to 2.5 mg L⁻¹ cells dry weight (Carvalho et al., 2010). Produces acetoin, tryptophan deaminase, esterase (C4), esterase lipase (C8), leucine and valine arylamidase, naphthol-AS-BI-phosphohydrolase, and weakly produces gelatinase, alkaline phosphatase, and cystine arylamidase. It is unable to grow in the presence of amoxicillin (25 mg), ticarcillin (75 mg), cephalothin (30 mg), ceftazidime (30 mg), meropenem (10 mg), colistin sulphate (50 mg), sulfamethoxazole/trimethoprim (23.75/1.25 mg), ciprofloxacin (5 mg), tetracycline (30 mg), gentamicin (10 mg) and streptomycin (10 mg).

8.5. Chemotaxonomic characteristics:

The predominant fatty acids are C_{18:1} ω 7c (12.6%), C_{16:0} (30.2%) and the summed feature 3 (C_{16:1} ω 7c / iso-C_{15:0} 2-OH) (40.0%). Other fatty acids include C_{10:0} 3-OH (7.2%) and C_{17:0} cyclo (3.6%). The major respiratory quinone is ubiquinone 8 and the major polar lipids are phosphatidylethanolamine, phosphatidylglycerol and diphosphatidylglycerol.

8.6. Genome:

The draft genome of the *Oryzolibacter propanilivorax* type strain EPL6^T is available under the DDBJ/EMBL/GenBank accession no. FNHP000000000.1 (Vaz-Moreira et al., 2017). It comprises 24 contigs in 24 scaffolds, with an N50 contig size of 341 147 bp, totalling 3.716 Mbp in size and is based on 1,452.5 Mbp of Illumina data with a mapped coverage of 391.1X. The draft genome sequence contains 3,302 candidate protein-coding genes and a G+C content of 69.2 mol%. According to the Rapid Annotation using Subsystem Technology (RAST) pipeline (<http://rast.theseed.org>) (Aziz et al., 2008, Overbeek et al., 2014, Brettin et al., 2015), among the candidate protein-genes, 57 are associated with the nitrogen metabolism, including nitrosative stress, nitrate and nitrite ammonification, ammonia assimilation, cyanate hydrolysis, and denitrification processes. Most of the candidate protein-genes are related with protein (183) and amino acid (342) metabolism, followed by those involved on the carbohydrates (190) and the lipid (124) metabolism, including fatty acids and isoprenoids. Although none of the protein-coding genes was annotated as involved in the anaerobic degradation of aromatic compounds, 22 genes were related with the metabolism of these compounds. Only four genes were annotated as involved on genetic mobility (phages, prophages, transposable elements or plasmids), but a high number of genes was annotated as related with stress response (58), mainly with oxidative stress response (23).

8.7. Ecology:

Oryzisolibacter is associated with agricultural soil habitats, namely rice paddy fields. Among others, *Oryzisolibacter propanilivorax* strain EPL6^T was co-isolated with *Pseudomonas aeruginosa*, *Brevundimonas vesicularis*, *Enterococcus faecium*, *Stenotrophomonas nitritireducens*, and *Rhodococcus erythropolis* (Vaz-Moreira et al., 2017).

9. ENRICHMENT/ISOLATION PROCEDURES:

Oryzisolibacter propanilivorax strain EPL6^T was isolated from an enrichment culture established from a mixture of Portuguese rice paddy soils from organic and conventional farming, where the herbicide propanil (0.5 mM) was used as the sole source of carbon and energy. The cultures were incubated at 30 °C and 120 rpm. When propanil was degraded, the culture was settled, decanted, and the solids (10%) were successively transferred into fresh culture medium using the same compound as carbon and energy source. After eliminating the soil residues through this process, the culture was centrifuged, and the pellet resuspended in fresh propanil-containing medium (0.45 mM). This process was repeated for 60 days. The resultant mixed culture was further enriched in a sequential batch reactor (SBR) for 130 days, at 28 °C, pH 7.2. During the first 76 days, the SBR operated with 0.5 mM propanil dump feeding (5 min), followed by 24-96 h of aeration, (>1 L min⁻¹), 1 h of settling and 0.25 h of decanting. After this period, the operation of the SBR changed to fed-batch, where per each 24 h-cycle the reactor was fed twice with 0.15 mM propanil, at the beginning and after 11 h of operation in each cycle. At days 15, 55, 63, 87, 89, 91, 107, 112, and 115, the reactor operated under batch conditions with concentrations of propanil varying between 0.075 and

0.59 mM. On days 123 and 129, propanil was substituted by 0.25 and 0.59 mM 3,4-DCA, respectively (Carvalho et al., 2010).

The cultivable bacteria present in this final enrichment culture were isolated by the spread plating method on Luria–Bertani agar. The culture of interest, strain EPL6^T, was purified by sub-culturing on the same medium (Carvalho et al., 2010).

10. MAINTENANCE PROCEDURES:

Recommended *Oryzisolibacter propanilivorax* maintenance is on Luria–Bertani agar for short periods or in nutritive broth with 15% (v/v) glycerol at -80 °C.

11. DIFFERENTIATION OF THE GENUS *ORYZISOLIBACTER* FROM OTHER GENERA:

The two closest related genera to *Oryzisolibacter* are *Alicyclophilus* (see gbm01825) and *Melaminivora* (see gbm01827). Differential characteristics between the type strains of the type species and of *M. jejuensis* are described in Table 1.

<Table 1 near here>

12. TAXONOMIC COMMENTS:

Based on the 16S rRNA gene sequence analysis, *Oryzisolibacter propanilivorax* is a member of the family *Comamonadaceae*. *Alicyclophilus denitrificans* and *Melaminivora alkalimesophila* are the nearest neighbour species, both sharing 96.8% 16S rRNA gene sequence identity with the type strain of *O. propanilivorax*. A slightly lower value (96.7%) is shared between the type strains of *M. jejuensis* and *O. propanilivorax*. Whole genome sequence comparative analyses confirmed that strain EPL6^T did not belong to any of the type species of these related genera (ANI values < 95%; (Goris et al., 2007)). However, all of these species share AAI values (76.5-80.3%) higher than the threshold proposed to include an organism in a given genus (60%) (Rodriguez-R and Konstantinidis, 2014), indicating relatively close taxonomic relatedness between genera within the *Comamonadaceae* family (Willems, 2014). In contrast, the analysis of the percentage of conserved proteins (POCP) supported the distinction of these three genera. POCP values shared by the type strains of these species (40%-45%) are lower than the 50% threshold value for genus delineation proposed by Qin et al. (2014). The analyses of differentiating phenotypic characteristics among *Alicyclophilus denitrificans* strain K601^T (=DSM 14773^T), *Melaminivora alkalimesophila* strain CY1^T (=DSM 26006^T), *M. jejuensis* strain KBB12^T and *O. propanilivorax* (Table 1), which allocation to distinct taxa was supported by the 16S rRNA gene sequence analyses, DNA:DNA, hybridization and/or other whole genome based parameters, suggests that nutritional and metabolic features may have a limited value for genus or species discrimination. Noticeably, more differentiating metabolic and nutritional features between the two type strains of the genus *Melaminivora* were observed than between *M. jejuensis* and *O. propanilivorax*. Also of note is the fact that *O. propanilivorax* was not included in the *M. jejuensis* description, possibly due to publication processing overlap.

13. LIST OF SPECIES OF THE GENUS *ORYZISOLIBACTER*:

1. *Oryzisolibacter propanilivorax* Vaz-Moreira, Narciso-da-Rocha, Lopes, Carvalho, Lobo-da-Cunha, Whitman, Snauwaert, Vandamme, Manaia and Nunes, 2017, 3755^{VP}

propanilivorax [pro.pa.ni.li.vo'rax. N.L. n. *propanilum*, the herbicide propanil; L. adj. *vorax* devouring, voracious; N.L. masc. adj. *propanilivorax* propanil-degrading].

In addition to the characteristics described for the genus, the cells are Gram-negative motile rods, able to grow between 15–37 °C, pH 6–9, and 0.1–4% (w/v) NaCl. Aesculin is not hydrolysed. Hydrogen sulfide or indole are not produced. Assimilates malic acid but not erythritol, D- or L-arabinose, D- or L-xylose, adonitol, methyl-beta-D-xylopyranoside, D-galactose, D-fructose, D-mannose, L-sorbose, L-rhamnose, dulcitol, inositol, D-mannitol, D-sorbitol, methyl-alpha-D-mannopyranoside, methyl-alpha-D-glucopyranoside, N-acetyl-glucosamine, amygdalin, arbutin, aesculin, salicin, cellobiose, maltose, lactose, melibiose, D-sucrose, trehalose, inulin, melezitose, raffinose, starch, glycogen, xylitol, gentiobiose, turanose, D-lyxose, D-tagatose, D- or L-fucose, D- or L-arabitol, potassium 5-ketogluconate, phenylacetic acid. Does not ferment glucose, mannose, inositol, sorbitol, rhamnose, sucrose, melibiose, amygdalin and arabinose. Produces acetoin, tryptophan deaminase, esterase (C4), esterase lipase (C8), leucine and valine arylamidase, naphthol-AS-BI-phosphohydrolase, and weakly produces gelatinase, alkaline phosphatase and cystine arylamidase, but not urease, arginine dihydrolase, lysine decarboxylase, ornithine decarboxylase, lipase (C14), trypsin, alpha-chymotrypsin, alpha-galactosidase, beta-galactosidase, beta-glucuronidase, alpha-glucosidase, beta-glucosidase, N-acetyl-beta-glucosaminidase, alpha-mannosidase or alpha-fucosidase. The type strain is able to degrade the herbicide propanil and its transformation product 3,4-dichloroaniline.

234 The DNA G+C content (mol %) is 69.4 (HPLC) or 69.2 (genome analysis).

235 Type strain: EPL6 (=LMG 28427 = CECT 8927)

236 GenBank accession number (16S rRNA): LM653274

237 GenBank accession number (genome): FNHP000000000.1

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293 **TABLES:**

294 Table 1. Differentiating characteristics between *Oryzisolibacter propanilivorax* EPL6^T and the type strains of the species with validated names of
 295 the closest genera.

Characteristic	<i>Oryzisolibacter</i> <i>propanilivorax</i> EPL6 ^T	<i>Alicyclophilus denitrificans</i> DSM 14773 ^T	<i>Melaminivora alkalimesophila</i> DSM 26006 ^T	<i>Melaminivora jejuensis</i> KBB12 ^T
Colonies colour	Light yellow	White	Translucent	Pale yellow
Cell morphology	Rods (0.6 µm x 1.9 µm)	Rods (0.6 µm x 1–2 µm)	Rods (0.7–0.9 µm x 2.0–3.0 µm)	Rods (0.5–0.7 µm x 2.0–3.5 µm)
Nitrite reduction	+	+	-	-
Growth at 50 °C	-	-	+	-
NaCl tolerance range (% , w/v)	0-4	n.a.	0–7	0-1
Citrate utilization	+	-	-	n.a.
Acetoin production	+	+	-	n.a.
Assimilation of				
Potassium gluconate	+	+	-	+
Adipic acid	+	+	-	+

Trisodium citrate	+	-	-	n.a.
Glycerol	+	+	-	n.a.
Enzymes				
Gelatinase	+	-	-	+
Acid phosphatase	-	+	+	+
Polar lipids	PE, PG, DPG	PE, PG, DPG	PE, PG, DPG, APL, PL	PE, PG, DPG, UL
DNA G+C content (mol%)	69.4	66	69.5	69.6
Source of the type strain and type of isolation (E, enrichment culture; I, direct isolation)	Rice field soil (E) with propanil and 3,4- dichloroaniline	Municipal sewage (E) with cyclohexanol as carbon source and nitrate as electron acceptor	Wastewater sludge of a melamine-producing factory (I)	Swinery waste (E) with Na ₂ S ₂ O ₃ as electron donor

296 Data from (Vaz-Moreira et al., 2017, Mechichi et al., 2003, Wang et al., 2014, Kim et al., 2018)

297 PE, phosphatidylethanolamine; PG, phosphatidylglycerol; DPG, diphosphatidylglycerol; APL, unidentified aminophospholipid; PL unidentified

298 phospholipid; UL, unidentified lipid. n.a., not available.