Hydromonas

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2. KEYWORDS: *Hydromonas duriensis*; freshwater; aerobe; chemo-organotroph;

3. ABSTRACT:

Rods with electron-dense intracellular inclusions, non-spore forming, Gram-negative. Aerobic, mesophilic, with the ability to grow between 15 and 30 ºC, pH 6-8 and a concentration of NaCl of up to 1% and no special growth requirements. Chemo-organotroph with limited metabolic versatility, being able to assimilate D-glucose, D-mannitol, and N-acetylglucosamine. Catalase- and cytochrome *c* oxidase positive. The respiratory quinone is ubiquinone 8 and major fatty acids are $C_{16:1}$ ω7c and $C_{14:0}$ 3-OH. Major polar lipids are phosphatidylethanolamine, phosphatidylglycerol, and diphosphatidylglycerol. Isolated from freshwater.

4. DEFINING PUBLICATION:

Hydromonas duriensis Vaz-Moreira, Narciso-da-Rocha, De Brandt, Vandamme, Silva Ferreira, Lobo-da-Cunha, Nunes, Manaia 2015, 4136^{VP}

5. ETYMOLOGY:

Hydromonas [Hy.dro.mo.nas. Gr. n. *hydro* water; L. fem. n. *monas* a unit, monad; N.L. fem. n. *Hydromonas* a unit (rod) from water].

6. GENERIC DEFINITION:

Rods with electron-dense intracellular inclusions, non-spore forming, Gram-negative. **Aerobic**, **mesophilic**, with the ability to grow between 15 and 30 ºC, pH 6-8 and a concentration of NaCl of up to 1% and no special growth requirements. Chemo-organotroph with **limited metabolic versatility**, being able to assimilate D-glucose, D-mannitol, and N-acetylglucosamine. Catalase- and **cytochrome *c* oxidase positive**. The respiratory quinone is **ubiquinone 8** and major fatty acids **$C_{16:1}$ ω7c** and **$C_{14:0}$ 3-OH**. Major polar lipids are phosphatidylethanolamine, phosphatidylglycerol, and diphosphatidylglycerol. Isolated from freshwater.

The DNA G+C content (mol %) is 47 ± 1 (HPLC).

Type species: ***Hydromonas duriensis*** Vaz-Moreira, Narciso-da-Rocha, De Brandt, Vandamme, Silva Ferreira, Lobo-da-Cunha, Nunes, Manaia 2015, 4136^{VP}

Number of species with validated names: 1.

7. FAMILY CLASSIFICATION:

Burkholderiaceae (fbm00181)

8. FURTHER DESCRIPTIVE INFORMATION:

8.1. Cell morphology:

A single species is described within the genus *Hydromonas*, *Hydromonas duriensis* (Vaz-Moreira et al., 2015). Cells are Gram-negative non-spore forming rods, with 0.7-2.2 µm long and 0.4±0.1 µm width. Electron-dense intracellular inclusions are observed by transmission electron microscopy (Fig. 1).

<Figure 1 near here>

8.2. Colonial and cultural characteristics:

On solid agar medium (modified Luria-Bertani agar), after 48-72 h of incubation at 30 ºC, the type strain of *Hydromonas duriensis* forms small (~1 mm diameter), convex, slightly dry beige-colored colonies.

8.3. Nutrition and growth conditions:

Limited catabolic versatility, with the ability to assimilate the carbon sources D-glucose, D-mannitol, and N-acetylglucosamine. Unable to assimilate L-arabinose, D-fructose, D-fucose, D-galactose, D-xylose, glycerol, potassium gluconate, potassium 2-ketogluconate, trisodium citrate, adipate, caprate, malate or phenylacetate.

Able to grow in the temperature range of 15 to 30 ºC, at pH of 6-8, and at NaCl concentrations up to 1% (w/v). Abundant growth is observed at 30 ºC, pH 8 and 0.1% (w/v) NaCl. Growth is not observed at 37 ºC, pH 9.0 and 3% NaCl.

8.4. Metabolism:

Aerobic. Nitrate is reduced to nitrite, which is not further reduced to nitrogen. Chemo-organotroph with limited metabolic versatility, being able to assimilate D-glucose, D-mannitol, and N-acetylglucosamine. Glucose is not fermented. Simmons citrate is not utilized. H_2S, indole, and acetoin are not produced. Aesculin is not hydrolyzed. The following enzymes are produced: beta-galactosidase, tryptophan deaminase, alkaline phosphatase, acid phosphatase, esterase (C4), esterase lipase (C8), leucine arylamidase, naphthol-AS-BI-phosphohydrolase, and alpha-glucosidase. Valine arylamidase production exhibits a weak positive reaction.

8.5. Chemotaxonomic characteristics:

The predominant fatty acids are $C_{16:1}$ ω7c, from summed feature 3 ($C_{16:1}$ ω7c and/or iso-$C_{15:0}$ 2-OH), and $C_{14:0}$ 3-OH, from summed feature 2 ($C_{12:0}$ aldehyde, an unknown fatty acid with equivalent chain-length value of 10.928, and $C_{14:0}$ 3-OH and/or iso-$C_{16:1}$ I). The major respiratory quinone is ubiquinone 8 and the polar lipids are phosphatidylethanolamine, phosphatidylglycerol, diphosphatidylglycerol, one unknown aminophospholipid, and three unknown aminolipids.

8.6. Ecology:

Hydromonas is associated with freshwater habitats.

Among others, it was co-isolated with *Sphingobium*, *Delftia*, *Vogesella*, *Aeromonas*, *Acinetobacter*, *Pseudomonas*, *Chryseobacterium*, *Flavobacterium*, *Enterobacter* and *Leclercia* species (Vaz-Moreira et al., 2017).

9. ENRICHMENT/ISOLATION PROCEDURES:

Hydromonas duriensis strain A2P5^T was isolated from a freshwater sample (river water) using the membrane filtration method and incubation on Pseudomonas Isolation Agar at 30 °C for 7 days (Vaz-Moreira et al., 2011, Vaz-Moreira et al., 2013). The culture was purified by sub-culturing on modified Luria–Bertani agar (mLA, per litre: 5 g tryptone, 2.5 g yeast extract, 1 g NaCl and 15 g agar) (Vaz-Moreira et al., 2011, Vaz-Moreira et al., 2013).

10. MAINTENANCE PROCEDURES:

Recommended *Hydromonas duriensis* strains maintenance is on modified Luria–Bertani agar (per liter: 5 g tryptone, 2.5 g yeast extract, 1 g NaCl and 15 g agar) for short periods or frozen at -80 ºC in nutritive broth with 15% (v/v) glycerol.

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

The closest related genera to *Hydromonas* are *Ephemeroptericola* (Kim et al., 2019), *Formosimonas* (Chen et al., 2017), *Ralstonia* (see gbm00941) and *Cupriavidus* (see gbm00936). Differential characteristics between the type species of these five genera are described in Table 1.

<Table 1 near here>

12. TAXONOMIC COMMENTS:

Based on the 16S rRNA gene sequence analysis, *Hydromonas duriensis* is a member of the family *Burkholderiaceae* with similarity values of 96.8% with *Ephemeroptericola cinctiostellae* F02^T, 96.2% with *Formosimonas limnophila* AHQ-12^T, and lower than 91% with *Ralstonia* spp. and *Cupriavidus* spp.

<Figure 2 near here>

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

1. ***Hydromonas duriensis*** Vaz-Moreira, Narciso-da-Rocha, De Brandt, Vandamme, Silva Ferreira, Lobo-da-Cunha, Nunes, Manaia 2015, 4136^{VP}

duriensis (du.ri.en.sis. L. neut. adj. *duriensis* inhabiting the Portuguese Douro region).

The characteristics are as described for the genus.

The DNA G+C content (mol %) is 47 ± 1 (HPLC).

Type strain: A2P5, LMG 28428, CCUG 66137

GenBank accession number (16S rRNA): LM653273

RELATED ARTICLES:

gbm00941

gbm00936

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182 **TABLES:**

183 Table 1. Differentiating characteristics between *Hydromonas duriensis* A2P5^T and type strains of the type species of the closest genera:

184 *Ephemeroptericola cinctiostellae* F02^T, *Formosimonas limnophila* AHQ-12^T, *Ralstonia pickettii* LMG 5942^T (see gbm00941) and *Cupriavidus necator*

185 LMG 8453^T (see gbm00936).

Characteristic	***Hydromonas duriensis* A2P5^T**	***Ephemeroptericola cinctiostellae* F02^T**	***Formosimonas limnophila* AHQ-12^T**	***Ralstonia pickettii* LMG 5942^T**	***Cupriavidus necator* LMG 8453^T**
Cellular morphology	rods	rods	rods	rods	coccoid rods
Growth at 37 ºC	-	-	+	+	+
Denitrification (N_2)	-	-	-	+	+
Acetoin production	-/+^{w}*	-	+	-	+^{w}
Aesculin hydrolysis	-	+	-	-	-
Assimilation of:					
L-Arabinose	-	n.a.	-	+	-
D-Fructose	-	n.a.	+	+	+
D-Fucose	-	n.a.	n.a.	+^{w}	-
D-Galactose	-	n.a.	n.a.	+	-
D-Glucose	+/-*	+	+	+	-

D-Xylose	-	n.a.	n.a.	+	-
Glycerol	-	n.a.	n.a.	+	-
D-Mannitol	+	n.a.	+	-	-
N-Acetylglucosamine	+/-*	+	+/-*	-	-
Potassium gluconate	-	n.a.	n.a.	+/-*	+/-*
Potassium 2-ketogluconate	-	n.a.	n.a.	+/-*	+/-*
Trisodium citrate	-	n.a.	-	+	+
Adipic acid	-	n.a.	+	$+^{w}$/+*	$+^{w}$
Capric acid	-	n.a.	-	+	+
Malic acid	-	+	-	+	+
Phenylacetic acid	-	n.a.	-	$+^{w}$/-*	+
Enzymes:					
α-Glucosidase	+/-*	$+^{w}$	+/-*	-	-
β-Glucosidase	-	+	-	-	-
β-Galactosidase	+	+	-	-	-
Alkaline phosphatase	+	-	$+^{w}$	+	+
Leucine arylamidase	+/-*	$+^{w}$	$+^{w}$	+	+

Tryptophan deaminase	+	n.a.	n.a.	+	-
Lipase (C14)	-	-	+	+	-
Major FAMES (>15%)[a]	$C_{14:0}$ 3-OH and/or iso-$C_{16:1}$ I; $C_{16:1}$ ω7c and/or iso $C_{15:0}$ 2-OH	$C_{16:1}$ ω7c and/or $C_{16:1}$ ω6c	iso-$C_{15:0}$ 3-OH $C_{16:1}$ ω7c and/or $C_{16:1}$ ω6c	$C_{16:0}$ $C_{16:1}$ ω7c and/or iso $C_{15:0}$ 2-OH	$C_{16:0}$ $C_{18:1}$ ω7c $C_{14:0}$ 3-OH and/or iso-$C_{16:1}$ I; $C_{16:1}$ ω7c and/or iso $C_{15:0}$ 2-OH
Polar lipids[a]	PE, PG, DPG, 3 minor AL, 1 APL	PE, PG, DPG, 3PL, 5AL, 1APL	PE, PG, DPG, 2APL, 3L	PE, PG, DPG, 1 minor APL	PE, PG, DPG
DNA G+C content (mol%)	47±1 (HPLC)	48.3 (genome)	50.4±1 (HPLC)	64 (NMF)	57±1 (Tm)
Source of isolation of the type strain	River water	Gut of the aquatic insect *Cincticostella levanidovae*	Lake freshwater	Human clinical specimen	Soil

[w], weakly positive; *variable result depending on the testing conditions (gbm00936, gbm00941, Vaz-Moreira et al., 2015, Kim et al., 2019, and Chen et al., 2017); n.a., not available.

[a], data from Vaz-Moreira et al. (2015) for *Hydromonas duriensis* A2P5^T, *Ralstonia pickettii* LMG 5942^T and *Cupriavidus necator* LMG 8453^T, and from Kim et al. (2019) for *Ephemeroptericola cinctiostellae* F02^T and *Formosimonas limnophila* AHQ-12^T.

190 PE, phosphatidylethanolamine; PG, phosphatidylglycerol; DPG, diphosphatidylglycerol; AL, unidentified aminolipid; APL, unidentified aminophospholipid;
191 PL, unidentified phospholipid; and L, unidentified lipid.

192 HPLC, High-Performance Liquid Chromatography; NMF (determined with the nitrocellulose membrane filter technique); Tm (determined by the thermal
193 melting point)

FIGURES:

Figure 1. Transmission electron micrographs of *Hydromonas duriensis* A2P5^T after 3 days of growth at 30 ºC on modified Luria-Bertani agar. A) cell morphology. B) detail of a cell with two unknown electron-dense bodies. Bars, 1 µm (A) and 0.5 µm (B). (copyright authorization pending)

Figure 2. Phylogenetic tree, based on 16S rRNA gene sequences, showing the position of the *Hydromonas* species in relation to the closest phylogenetic genera *Ephemeroptericola*, *Formosimonas*, *Ralstonia* and *Cupriavidus*. *Aquicella lusitana* SGT-39^T was used as outgroup. The dendrogram was generated by the Neighbor-Joining method. Bootstrap values, generated from 1000 re-samplings, are indicated at branch points. Bar, 1 substitution per 50 nucleotide positions.