Hydromonas duriensis gen. nov., sp. nov., isolated from freshwater

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An aerobic, Gram-stain-negative rod, designated strain A2P5T, was isolated from the Douro river, in Porto, Portugal. Cells were catalase- and oxidase-positive. Growth occurred at 15–30 °C, at pH 6–8 and in the presence of 1 % (w/v) NaCl. The major respiratory quinone was Q8, the genomic DNA had a G+C content of 47 ± 1 mol%, and phosphatidylethanolamine, phosphatidylglycerol and diphosphatidylglycerol were amongst the major polar lipids. On the basis of 16S rRNA gene sequence analysis, strain A2P5T was observed to be a member of the family Burkholderiaceae, but could not be identified as a member of any validly named genus. The low levels of 16S rRNA gene sequence similarity to other recognized taxa (< 91 %), together with the comparative analysis of phenotypic and chemotaxonomic characteristics, supported the proposal of a novel species of a new genus within the family Burkholderiaceae. The name Hydromonas duriensis is proposed. The type strain of Hydromonas duriensis is A2P5T (=LMG 28428T=CCUG 66137T).

A bacterial strain, designated A2P5T, was isolated from freshwater of the river Douro in northern Portugal, during a study on the bacterial diversity of drinking water before and after treatment (Vaz-Moreira et al., 2011, 2013). Based on 16S rRNA gene sequence analysis, the isolate was identified as a member of the family Burkholderiaceae. Highest 16S rRNA gene sequence similarity values were approximately 90 % with members of the genera Cupriavidus and Ralstonia. Members of these genera comprise bacteria occurring in both environmental and clinical settings, including opportunistic human and plant pathogens, and potential biodegraders of recalcitrant xenobiotics (Coenye et al., 1999; Chen et al., 2001; Cuadrado et al., 2010). To test the hypothesis that strain A2P5T represents a member of a new genus, the strain was compared with the type strains of the type species of the closest genera, Cupriavidus necator LMG 8453T and Ralstonia pickettii LMG 5942T.

Strain A2P5T was isolated from river Douro surface water on Pseudomonas Isolation Agar medium, after 72 h of incubation at 30 °C. Strain A2P5T was found in the water sample at a density of approximately 100 c.f.u. ml−1 and was co-isolated with members of taxa such as Acinetobacter junii, Pseudomonas simiae, Aeromonas veronii or Chryseobacterium sp. Strain A2P5T was purified by subculturing on modified Luria–Bertani agar (mLA, per litre: 5 g tryptone, 2.5 g yeast extract, 1 g NaCl and 15 g agar), on which, after 48–72 h of incubation, formed small, convex and beige-coloured colonies. The culture was preserved frozen at −80 °C in nutritive broth with 15 % (v/v) glycerol. Phenotypic and chemotaxonomic assays were performed simultaneously for strain A2P5T and Ralstonia pickettii LMG 5942T and Cupriavidus necator LMG 8453T. Unless stated otherwise, bacteria were cultivated on mLA, and incubated at 30 °C. Colony and cell morphology, Gram staining, cytochrome c oxidase and catalase tests were analysed based on the methodologies of Murray et al. (1994) and Smibert & Krieg (1994). Cell morphology was observed by transmission electron microscopy, as

The GenBank/EMBL/DDBJ accession number for the 16S rRNA gene sequence of isolate A2P5T is LM653273.
described previously (Vaz-Moreira et al., 2012). Briefly, bacteria were fixed in 2.5 % glutaraldehyde and 4 % formaldehyde in cacodylate buffer (0.1 M, pH 7.2), washed in the same buffer, and post-fixed overnight in 2 % OsO4 buffered with cacodylate. After brief washing, bacteria were treated with 1 % uranyl acetate, dehydrated in increasing concentrations of ethanol and embedded in Epon. Ultrathin sections were stained with uranyl acetate and lead citrate and observed in a JEOL 100CXII transmission electron microscope (60 kV). Cell size was determined based on optical microscopy, with the software ImageJ 1.48v. The pH range for growth was examined in modified Luria–Bertani broth (per litre: 5 g tryptone, 2.5 g yeast extract and 1 NaCl) containing 12 mM MES (Sigma) to adjust the pH to 5.0, 6.0 and 7.0, 12 mM TAPS (Sigma) to adjust the pH to 8.0, and 12 mM CAPS (Sigma) to adjust the pH to 9.0 or 10.0. NaCl tolerance and temperature range for growth were assayed, respectively, in culture medium supplemented with 0.1, 1, 2 and 3 % (w/v) NaCl or incubated at 10, 15, 18, 25, 30 and 37 °C. Biochemical and nutritional tests were performed by using the commercial kits API 20E, API 20NE, API ZYM and API 50CH (bioMérieux) following the manufacturer’s instructions. The API 50CH strips were inoculated with the bioMérieux AUX medium. Growth under anaerobic conditions was tested on mLA incubated in an anaerobic chamber. The ability to reduce nitrate was tested in modified Luria–Bertani broth supplemented with 0.25 % (w/v) KNO3, under aerobic and anaerobic conditions.

After 48–72 h of incubation on mLA, strain A2P5T produced beige-coloured, small and convex colonies. Cells were rods ranging from 0.7 to 2.2 μm in length, with observable electron-dense bodies (Fig. 1). In modified Luria–Bertani broth, optimal growth occurred at 30 °C, at pH 8 and with 0.1 % (w/v) NaCl. No growth was observed at 37 °C, in the presence of 3 % NaCl or at pH 9.0. Of the 54 carbon sources tested, only D-glucose, D-mannitol and N-acetylglucosamine were used as single sources of carbon (Table 1).

The nucleotide sequence of the 16S rRNA gene was determined after PCR amplification of total DNA extracted using primers 27F and 1492R as described previously (Ferreira da Silva et al., 2007). The 16S rRNA gene sequence was compared with others available in the EzTaxon database (Kim et al., 2012). Phylogenetic analysis was conducted using the MEGA6 software (Tamura et al., 2013). Sequence similarity was estimated based on the model of maximum composite likelihood and the dendrogram was created with the neighbour-joining statistical method. The maximum-likelihood method was used to assess tree stability. Non-homologous and ambiguous nucleotide positions were excluded from the calculations and a total of 1248 nt positions were included in the analysis. This analysis showed that strain A2P5T belongs to the family Burkholderiaceae, with the genera Cupriavidus and Ralstonia as closest neighbours, with sequence similarities of 90.3 and 90.6 % with the type strains of the type species Cupriavidus necator and Ralstonia pickettii, respectively (Fig. 2).

Sequence similarity values with the type strains of the type species Polynucleobacter necessarius subsp. necessarius and Oxalobacter formigenes were 87.9 and 88.9 %, respectively.

Fatty acid methyl esters were extracted and analysed using 48 h cultures on trypticase soy broth agar, incubated at 28 °C, as described by Vandamme et al. (1992), and separated and identified by using the Sherlock Microbial Identification System (version 3.1; MIDI). For strain A2P5T, these analyses were complemented by GC-MS identification of the major fatty acid methyl ester components, using the conditions described by Manaia & Moore (2002), using a Varian 3800 gas chromatograph coupled with Varian Saturn 2000 ion trap GC-MS workstation software, version 6.9.1.

Fig. 1. Transmission electron micrographs of cells of strain A2P5T. (a) Cells after growth for 3 days at 30 °C on mLA, showing cell morphology. (b) Detail of a cell with two unknown electron-dense bodies. Bars, 1 μm (a), 0.5 μm (b).
The fatty acid methyl ester profile of strain A2P5T was characterized by the predominance of fatty acids identified as summed feature 3 (C16:1ω7c and/or iso-C15:0 2-OH), in which C16:1ω7c predominated, and summed feature 2 (C12:0 aldehyde, an unknown fatty acid with equivalent chain-length value of 10.928, and C14:0 3-OH and/or iso-C16:1 I), in which C14:0 3-OH predominated (Table 2). The polar lipid profile was composed of phosphatidylethanolamine, phosphatidylglycerol, diphosphatidylglycerol, one unknown aminophospholipid and three unknown aminolipids (Fig. 3). The respiratory quinone was ubiquinone 8. The DNA G+C content was 47 ± 1 mol%. These chemotaxonomic characteristics do not exclude the affiliation of strain A2P5T to the family Burkholderiaceae (Yabuuchi et al., 2005).

Confirming the 16S rRNA gene sequence comparative analysis, which suggested that strain A2P5T could represent a member of a new genus, phenotypic and chemotaxonomic characterization supported its differentiation from the type strains of the type species of related genera. In particular, in contrast to Cupriavidus necator LMG 8453T and Ralstonia pickettii LMG 5942T, strain A2P5T was able to assimilate D-mannitol and N-acetylglucosamine, exhibited β-galactosidase and α-glucosidase activity, and was unable to utilize citrate, to assimilate D-fructose, potassium gluconate, potassium 2-ketogluconate, trisodium citrate or some organic acids and to reduce nitrite (Table 1). The polar lipid profile of strain A2P5T was distinct from that of Cupriavidus necator LMG 8453T and Ralstonia pickettii LMG 5942T, in particular due the presence of three unknown aminolipids and one aminophospholipid (Fig. 3). Also the fatty acid profile allowed the differentiation of strain A2P5T from its closest neighbours, showing a higher percentage of the fatty acids summed feature 2 (C12:0 aldehyde, and C14:0 3-OH and/or iso-C16:1 I) and C12:0 and a lower percentage of C16:0 and C18:1ω7c (Table 2). These differentiating characteristics and the unique phylogenetic position (Fig. 2) suggest that strain A2P5T should be most appropriately allocated to a novel species of a new genus, for which the name Hydromonas duriensis gen nov., sp. nov. is proposed.

**Description of Hydromonas gen. nov.**

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].

Cells are non-spore-forming, Gram-stain-negative rods. Catalase- and cytochrome c oxidase-positive. Mesophilic. Chemo-organotrophic with aerobic respiratory metabolism. Poor metabolic versatility, although sugars or derivatives thereof can be used as carbon sources. The respiratory quinone is ubiquinone 8 and the DNA G+C content is 47 mol%. Major fatty acids are summed feature 3 (C16:1ω7c and/or iso-C15:0 2-OH), in which C16:1ω7c predominated, and summed feature 2 (C12:0 aldehyde, and C14:0 3-OH and/or iso-C16:1 I), with C16:1ω7c and C14:0 3-OH predominating.
respectively, in each of those categories. The polar lipids comprise phosphatidylethanolamine, phosphatidylglycerol, diphosphatidylglycerol and minor unidentified amino(phospho)lipids. Phylogenetically, belongs to the family Burkholderiaceae. The type species is Hydromonas duriensis.

**Description of *Hydromonas duriensis* sp. nov.**

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

Displays the following characteristics in addition to those given for the genus. Colonies are beige-coloured, small...
(~ 1 mm in diameter), convex and slightly dry on mLA after 48–72 h of incubation. Cells are 0.7–2.2 μm in length and 0.4 ± 0.1 μm in width, with poor growth under anaerobic conditions in the presence of nitrate. Good growth occurs on mLA, at 15–30 °C, at pH 6–8 and in the presence of up to 1 % (w/v) NaCl [optima at about 30 °C, pH 8 and 0.1 % (w/v) NaCl]. Reduces nitrate to nitrite, but does not reduce nitrite to nitrogen. Simmons citrate is not utilized. Aesculin is not hydrolysed. H2S, indole and acetoin are not produced. Glucose is not fermented, and none of the API 20E carbon sources leads to acid production under aerobic conditions. Produces β-galactosidase, tryptophan deaminase, alkaline phosphatase, acid phosphatase, esterase (C4), esterase lipase (C8), leucine arylamidase, naphthol-AS-Bl-phosphohydrolase and α-glucosidase, but not the other enzymes present in the API ZYM, 20E or 20NE systems. Presents a weakly positive reaction for valine arylamidase activity. Assimilates D-glucose, D-mannitol and N-acetylglucosamine, but not the other carbon sources available in the API 50CH or API 20NE systems.

The type strain, A2P5T (LMG 28428T = CCUG 66137T), was isolated from freshwater of the river Douro. The DNA G+C content of the type strain is 47 ± 1 mol%.

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**Table 2.** Fatty acid composition of strain A2P5T and the type strains of the type species of the closest related genera

<table>
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<tr>
<th>Fatty acid</th>
<th>ECL</th>
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<th>2</th>
<th>3</th>
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<td>C10:0</td>
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<td>ND</td>
<td>ND</td>
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<tr>
<td>iso-C11:0</td>
<td>10.61</td>
<td>5.2</td>
<td>ND</td>
<td>ND</td>
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<tr>
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<td>12.00</td>
<td>7.1</td>
<td>TR</td>
<td>ND</td>
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<tr>
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<td>1.2</td>
<td>ND</td>
<td>ND</td>
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<tr>
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</tr>
<tr>
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<tr>
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<td>5.1</td>
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<tr>
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<td>1.3</td>
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<tr>
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<tr>
<td>SF3 †</td>
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<td>15.82</td>
<td>32.7</td>
<td>31.7</td>
<td>28.2</td>
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*SF2, Summed feature 2 (C12:0 aldehyde, an unknown fatty acid with ECL of 10.928, and C14:0 3-OH and/or iso-C16:1 I). These two components have distinct ECLs. For that reason they appear in two different rows in the table. For strain A2P5T, SF2 is composed of about two times more C14:0 3-OH than iso-C16:1 I.
†SF3, Summed feature 3 (C16:1ω7c and/or iso-C15:0 2-OH). For strain A2P5T, SF3 includes about four times more C16:1ω7c than iso-C15:0 2-OH.

**Fig. 3.** Polar lipid patterns of strain A2P5T and its closest neighbours *Ralstonia pickettii* LMG 5942T and *Cupriavidus necator* LMG 8453T. PE, Phosphatidylethanolamine; PG, phosphatidylglycerol; DPG, diphosphatidylglycerol; AL, unidentified aminolipid; APL, unidentified aminophospholipid.
References


