Halomonas smyrnensis sp. nov., a moderately halophilic, exopolysaccharide-producing bacterium

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Four Gram-negative, moderately halophilic, exopolysaccharide-producing strains, designated AAD6T, AAD4, AAD17 and AAD21, were isolated from Camalti Saltern Area, a wildlife reserve in Sasali, Izmir province located in the Aegean Region of Turkey. The isolates grew at an optimum NaCl concentration of 10 % (w/v). The major cellular fatty acids were C16:0, C18:1\(^\text{\textasciitilde}7\)c, C16:1\(^\text{\textasciitilde}7\)c and C12:0 3OH, respectively and the predominant lipoquinone was ubiquinone Q-9. The G+C content of the genomic DNA of strains AAD6T, AAD4, AAD17 and AAD21 was 63.0, 63.3, 62.8 and 62.6 mol%, respectively. Comparative 16S rRNA gene sequence studies showed that the isolates belonged to the genus Halomonas. The DNA–DNA hybridization mean values between the representative strain AAD6T and the closely related species Halomonas salina DSM 5928T, Halomonas halophila DSM 4770T, Halomonas maura DSM 13445T, Halomonas organivorans DSM 16226T, Halomonas elongata DSM 2581T, Halomonas koreensis JCM 12237T and Halomonas nitroreducens LMG 24185, were 40.8, 39.6, 24.2, 23.3, 12.6, 14.5 and 12.2 %, respectively. Based on these data the strains represent a novel species of the genus Halomonas for which the name Halomonas smyrnensis sp. nov. is proposed. The type strain is AAD6T (=DSM 21644T =JCM 15723T).

The genus Halomonas belonging to the family Halomonadaceae within the class Gammaproteobacteria, comprised 77 species with validly published names at time of writing among which 7 have been reclassified into other genera (http://www.bacterio.cict.fr/h/halomonas.html). Most of these species were widely distributed in saline habitats such as salt lakes, marine environments, saltern areas, as well as saline sand and soils. Species of the genus Halomonas were originally described as Gram-negative, rod-shaped, aerobic, chemorganotrophic, halophilic bacteria (Vreeland et al., 1980; Franzmann et al., 1988). During the last three decades, many species isolated from saline environments (Franzmann et al., 1987; James et al., 1990; Mormile et al., 1999; Duckworth et al., 2000; Quillaguamán et al., 2004), solar salterns (Bouchotroch et al., 2001; Lim et al., 2004; Martinez-Checa et al., 2005; Lee et al., 2005) and saline sands or soils (Romano et al., 2006; Martinez-Cánovas et al., 2004; García et al., 2004) have been assigned to the genus Halomonas; however a number of them have been reclassified and their nomenclature has been changed because of their phylogenetic heterogeneity (Arahal et al., 2001, 2002; Mellado et al., 1995; Ventosa et al., 1998).

The aim of this work was to determine the exact taxonomic position of strains AAD6T, AAD4, AAD17 and AAD21, isolated from Camalti Saltern Area, in Sasah, Izmir, Turkey, based on their phenotypic, phylogenetic and genomic characteristics.

Several pond soil samples from the Aegean Region of Turkey, with salt contents in the range 30–50 % and pH values of 6.5–7.5, were serially diluted with 0.9 % (w/v)
saline solution and spread on medium A containing (w/v) 0.5% yeast extract, 0.3% sodium citrate, 2% MgSO₄·7H₂O, 0.2% KCl, 25% NaCl and 1% agar (Grant et al., 2001). The pH of the medium was adjusted to 6.9 and plates were incubated at 37°C. After one week of incubation a number of yellow–cream colonies had developed. They were purified using the repeated serial dilution technique followed by restreaking on solid enrichment medium A and the purity of the isolates was examined based on cell shape under a microscope and colony homogeneity on the plates. Four isolates, designated AAD6ᵀ, AAD4, AAD17 and AAD21 and belonging to the same genus based on 16S RNA gene sequence analysis, were studied for phenotypic characteristics. Subculturing was performed on same medium for 24 h at 37°C and bacterial isolates were maintained as glycerol stocks at −70°C for further studies.

Procedures for phenotypic characterization followed Mata et al. (2002) and Arahal et al. (2007). Cellular morphology and motility were determined by phase-contrast microscopy (Zeiss) and colony morphology was determined with a Leica M8 stereomicroscope using cultures grown on media A agar plates for 24 h at the optimal temperature. The NaCl requirement for growth and salt tolerance were studied in same medium supplemented with 0, 5, 10, 15, 20, 25 and 30% (w/v) NaCl (Arahal et al. 1996). Unless stated otherwise, the growth media contained 10% NaCl (w/v). Growth was tested at different temperatures (5, 10, 15, 20, 25, 30, 37, 40, 45, 50, 55, 60, 65°C). The pH tolerance of strains was tested at 37°C at different pH values by using buffered medium A (50 mM MES, HEPES, TAPS and CAPSO) over the pH values 5.0, 5.5, 6.0, 6.5, 7.5, 8.0, 8.5 and 9.0. To study the utilization of single carbon sources, the isolates were grown statically using medium B containing (g L⁻¹): 7 K₂HPO₄, 2 KH₂PO₄, 0.1 MgSO₄·7H₂O, 1 (NH₄)₂SO₄, 0.5 peptone, 100 NaCl and 10 carbon sources, at 37°C, pH 7.0. Exopolysaccharide production was observed on medium B after cultivation of the strains for 3 days as previously reported (Poli et al., 2009a). To study the requirement of Mg²⁺, SO₄²⁻ and K⁺ for the growth, the isolates were grown statically using medium B with the following modifications: for Mg requirement, medium B without 0.1 g L⁻¹ MgSO₄·7H₂O; for SO₄ requirement, medium B without 0.1 g L⁻¹ MgSO₄·7H₂O and 1 g L⁻¹ (NH₄)₂SO₄; but containing 0.1 g L⁻¹ MgCl₂; for K requirement, medium B where 7 g L⁻¹ K₂HPO₄ and 2 g L⁻¹ KH₂PO₄ were replaced with 7 g L⁻¹ Na₂HPO₄ and 2 g L⁻¹ NaH₂PO₄. When amino acids were tested as sole carbon, nitrogen and energy sources, medium B was used without (NH₄)₂SO₄. Antibiotic susceptibility tests were performed using the disc diffusion method on agar (Benola et al., 1984). Polyhydroxyalkanoate (PHA) production was determined as previously described (Romano et al., 2006). Oxidase activity was determined by observing the oxidation of tetramethyl-p-phenylenediamine, and catalase activity was determined by assessing bubble production in a 3% (v/v) hydrogen peroxide solution. For nitrate and nitrite reduction, liquid growth medium A containing 0.1% (w/v) KNO₃ or 0.001% (w/v) NaNO₃, respectively, were used. The hydrolysis of casein, gelatin, ascuscin, Tweens 20 and 80, DNA, starch and urea; the consumption of glucose either by oxidation or fermentation; and respiration on nitrate and nitrite were tested as described by Mata et al. (2002). Acid production was determined in liquid medium A according to Arahal et al. (1996). Indole production was detected in medium containing 1% (w/v) peptone, 10% (w/v) NaCl at pH 7.2 plus 1% (w/v) tryptophan using Kovacs’ reagent. For phenylalanine deamination and tyrosine decomposition test, solidified growth medium A containing 0.2% (w/v) Dl-phenylalanine or 0.05% (w/v) L-tyrosine, respectively, was used. Hydrolysis of hippurate was tested in standard growth medium A containing 1% (w/v) sodium hippurate. Lysine and arginine decarboxylases were performed according to Falkow (1958) and to Goldschmidt & Lockhart (1971), respectively. The methyl red and Voges–Proskauer tests were performed according to Clark & Lubs (1915) and Barritt (1936), respectively. Anaerobic growth test was performed as described by Poli et al. (2009a). Motility and other routine microbiological tests were performed as previously described (Poli et al., 2007). Other additional phenotypic characters of strain AAD6ᵀ were tested with GN2 MicroPlates (Biolog) according to Mata et al. (2002).

Strains AAD6ᵀ, AAD4, AAD17 and AAD21, the predominant micro-organisms in the enrichment media A at 37°C, were rod-shaped and formed cream–yellowish, circular and slightly irregular colonies. All four strains were able to produce exopolysaccharide, although strain AAD6ᵀ produced a higher yield of exopolysaccharide. All strains were positive for oxidation of glucose. None of the isolates grew anaerobically and produce nitrite and gas from nitrate. All strains were oxidase-negative, catalase-positive and produced acid from glucose, sucrose and mannose. The isolates did not hydrolyse gelatin or urea and did not produce PHA. All strains utilized mannose and maltose as a sole carbon and energy source, and alanine and serine as a sole carbon, nitrogen and energy source. Strains were negative in tests for methyl red reaction, Voges–Proskauer reaction, lysine and arginine decarboxylase activities, fermentation of D-glucose and respiration on nitrate or nitrite. Only strain AAD6ᵀ was weakly positive for ONPG activity. All strains required Mg²⁺ for growth but not SO₄²⁻ or K⁺. Other characteristics that differentiated the isolates are reported in Table 1. Cells of the representative strain AAD6ᵀ were non-motile. The type strain grew at salt concentration in the range of 3–25% (w/v) NaCl (optimum, 10%). No growth was observed in the absence of NaCl or above 25% (w/v) NaCl. Growth was observed at temperatures between 5 and 40°C (optimum, 37°C). Strain AAD6ᵀ grew at pH values between 5.5 and 8.5 (optimum, pH 7.0). Additional phenotypic characters of the type strain AAD6ᵀ tested by Biolog GN2 microplate, are given in Table S1 (available in IJSEM Online). Previous studies revealed that strain AAD6ᵀ produced high levels of an exopolysaccharide (EPS). Sugar analysis, methylation studies and NMR spectra of the EPS indicated the
repeating unit of this polysaccharide was composed of beta
(2,6)-D-fructofuranosyl residues (Poli et al., 2009a), a
unique case of a Halomonas species described as a levan
producer.

The DNA G+C content (mol%) and DNA–DNA hybrid-
ization analysis were performed as previously described
(Poli et al., 2009b). Genomic DNA was isolated using
DNAzol (Molecular Research Centre, Inc., USA) according
to the manufacturer's instructions. PCR amplification of
the 16S rRNA gene was performed with universal primers
27f and 1525r. An amplicon of approximately 1.5 kb was
purified by the EZ-10 Spin Column PCR purification kit
(Bio Basic Inc., Canada) according to manufacturer's
instructions. DNA sequencing on eight strands namely 27f,
343r, 357f, 514f, 907r, 1100r, 1392r, 1492r and 1525r were
performed by the dideoxy chain-termination method with
a Beckman Coulter CEQ 8800 DNA sequencer according to
manufacturer's protocol.

The DNA G+C content of the genomic DNA of strains
AAD6T, AAD4, AAD17 and AAD21 was 63.0, 63.3, 62.8
and 62.6 mol%, respectively. A partial 16S rRNA gene
sequence of strain AAD21 (GenBank accession no.
HM352832, 1361 nt) and nearly full-length 16S rRNA
gene sequences of strains AAD6T (DQ131909, 1445 nt),
AAD4 (GU397426, 1488 nt) and AAD17 (GU397431,
1459 nt) were determined and the phylogenetic analysis
revealed that all four isolates belonged to the genus
Halomonas. The DNA–DNA hybridization values between
strain AAD6T and strains AAD17, AAD21 and AAD4 were
89 %, 96 % and 93 %, respectively, indicated that they were
members of the same species.

The following reference strains Halomonas salina DSM
5928T, Halomonas halophila DSM 4770T, Halomonas maura
DSM 13445T, Halomonas organivorans DSM 16226T and
Halomonas elongata DSM 2581T, were obtained from
Deutsche Sammlung von Mikroorganismen und Zellkulturen
(DSMZ), Braunschweig, Germany and were grown in the
media suggested by the authors (Bouchotroch et al., 2001;
Garcia et al., 2004; Quesada et al., 1984; Valderrama et al.,
1991; Vreeland et al., 1980). Halomonas nitroreducens
LMG 24185T and Halomonas koreensis JCM 12237T, were obtained
from the Belgian Co-ordinated Collections of Micro-
organisms (BCCM-LMG) and from the Japan Collection
of Microorganisms (JCM), respectively, and were grown in
the media suggested by the authors (Gonzalez-Domenech
et al., 2008; Lim et al., 2004).

Sequences of related taxa were obtained from GenBank/
EMBL/DDBJ databases. The values for pairwise 16S rRNA
gene sequence similarity among the closest species were
determined using the EzTaxon-e server (http://eztaxon-e.
ezbiocloud.net/; Kim et al., 2012). Phylogenetic trees were
constructed by using MEGA software version 5 (Tamura

<table>
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<th>Characteristics</th>
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<th>AAD4</th>
<th>AAD17</th>
<th>AAD21</th>
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<td>Cream</td>
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<td>+</td>
<td>–</td>
</tr>
<tr>
<td>G + C content (mol%)</td>
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<td>62.6</td>
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<td>16S rRNA gene sequence similarity to strain AAD6T (%)</td>
<td>100</td>
<td>99.7</td>
<td>99.6</td>
<td>99.8</td>
</tr>
</tbody>
</table>

*These carbon sources did not support the growth of strain AAD6T.

Table 1. Differential phenotypic characteristics of the isolates from Çamaltı Saltern Area, in Sasalı, Izmir, Turkey

+ , Positive; –, negative; w, weak positive.
Moreover, strain AAD6T differed from anaerobes. Strain AAD6T was oxidase-negative while these two latter in that they were unique facultative nitroreducens were not. In addition, strain AAD6T differed from H. koreensis H. elongata and H. nitroreducens. Other phenotypic characteristics that distinguish strain AAD6T from the type strains of closely related species of the genus Halomonas are shown in Table 2.

Comparative 16S rRNA gene sequence analyses between the representative strain AAD6T and the closely related species showed that the strain AAD6T was related to Halomonas salina F8-11T (99.4 % 16S rRNA gene sequence similarity), Halomonas halophila CCM 3662T (99.4 %), Halomonas maura S-31T (98.1 %), Halomonas organivorans G-16.1T (98.0 %), Halomonas koreensis SS20T (97.4 %), Halomonas elongata DSM 2581T (97.2 %) and Halomonas nitroreducens 11S T (97.1 %). The phylogenetic tree constructed using the neighbour-joining method showed that strain AAD6T was a member of genus Halomonas and formed a clade with H. salina F8-11T and H. halophila CCM 3662T with a bootstrap value of 99 % (Fig. 1). The maximum-parsimony and the maximum-likelihood phylogenetic trees showed essentially the same position for strain AAD6T (Figs S1 and S2). The DNA–DNA hybridization mean values between strain AAD6T and H. salina DSM 5928T, H. halophila DSM 4770T, H. maura DSM 13445T, H. organivorans DSM 16226T, H. elongata DSM 2581T, H. koreensis JCM 12237T and H. nitroreducens DSM 21845T, were 41 %, 40 %, 24 %, 23 %, 13 %, 14 % and 12 %, respectively. These values are below the 70 % threshold values generally accepted for species delineation (Christensen et al., 2001; Rossello-Mora & Amann, 2001), and they were lower than the recommended threshold value accepted for defining a novel species (Murray et al., 1990; Tindall et al., 2010; Wayne et al., 1987), hence supporting the distinct position of strain AAD6T within the genus Halomonas. Strain AAD6T differed from H. halophila, H. organivorans, H. elongata and H. koreensis in that the latter did not produce EPS; it differed from H. salina, H. organivorans, H. maura H. elongata and H. nitroreducens in the hydrolysis of tyrosine and only from these two latter in that they were unique facultative anaerobes. Strain AAD6T was oxidase-negative while H. salina, H. halophila, H. maura, H. nitroreducens and H. koreensis were not. In addition, strain AAD6T differed from H. salina in the absence of phenylalanine deaminase activity. Regarding respiration on nitrate or nitrite, strain AAD6T differed from H. maura, H. elongata, H. nitroreducens and H. koreensis. l-histidine did not support the growth of strain AAD6T unlike H. organivorans, H. maura, H. elongata, H. nitroreducens and H. koreensis. Moreover, strain AAD6T differed from H. salina, H. maura, H. halophila, H. elongata, H. nitroreducens and H. koreensis for the reduction of nitrate and only from H. elongata and H. nitroreducens for nitrite reduction. Strain AAD6T differed from all closely related species of the genus Halomonas in that it was able to hydrolyse starch and casein. In addition, strain AAD6T hydrolysed Tween 80 unlike most of the closest relatives except for H. organivorans. Strain AAD6T produced acid from glucose unlike H. salina, H. maura and H. nitroreducens, and it produced acid from mannose unlike H. salina, H. maura, H. elongata, H. nitroreducens and H. koreensis. Other phenotypic characteristics that distinguish strain AAD6T from the type strains of closely related species of the genus Halomonas are shown in Table 2.

Polar lipids were obtained from 3.0 g of freeze-dried cells grown in aerobic conditions on TSB supplemented with 5 % (w/v) NaCl and harvested at stationary growth phase. Polar lipids were extracted with CHCl3/Methanol/H2O (65:25:4, by vol.). The lipid extract was analysed by thin layer chromatography (TLC) on silica gel (0.25 mm, F254, Merck) eluted in the first dimension with CHCl3/Methanol/H2O (65:25:4, by vol.) and in the second dimension with CHCl3/Methanol/Acetic acid/H2O (80:12:15:4, by vol.). All polar lipids were detected by spraying the plates with 0.1 % (w/v) Ce(SO4)2 in 1 M H2SO4 or with 3 % (w/v) methanolic solution of molybdophosphoric acid followed by heating at 100 °C for 5 min. Phospholipids and aminolipids were detected by spraying with the Dittmer-Lester and the ninhydrin reagents, respectively, and glycolipids were visualized with α-naphthol (Nicolaus et al., 2001). Polar lipids were also identified by 1H-NMR spectra. Quinones were analysed by LC/MS on a reverse-phase RP-18 Lichrospher (250×4 mm) column eluted with n-hexane/ethylacetate (99:1, by vol.) with a flow rate of 1.0 ml min⁻¹ and identified by ESI/MS and 1H-NMR spectra (Nicolaus et al., 2001). NMR spectra, recorded at the NMR Service of Institute of Biomolecular Chemistry of CNR (Pozzuoli, Italy), were acquired on a Bruker DPX-300 operating at 300 MHz, using a dual probe. Fatty acid methyl esters (FAMEs) were obtained from complex lipids by acid methanolysis and analysed using a Hewlett Packard 5890A gas chromatograph fitted with a FID detector, as previously reported (Nicolaus et al., 2001).

The total lipid content in strain AAD6T ranged between 8 % and 10 % of the total dry weight of cells grown at 37 °C in standard conditions and harvested in the stationary phase of growth. Four major phospholipids were found: 1,2 diacylglycerol-3-phosphorylethanolamine, phosphoglycerol, diphosphoglycerate and a glycocephospholipid [2-(α-D-glucopyranosylxyloxy)-3-hydroxy-propyl]-phosphatidylglycerol recently described in five members of Halomonas rRNA group1 (Giordano et al., 2007) (Fig. S3).

Chromatographic analysis of lipoquinones revealed the presence of two UV-absorbing bands. The 1H-NMR spectrum showed the presence of ubiquinone signals as in recognized species of the genus Halomonas. The LC/MS analysis gave two molecular peaks: the predominant respiratory quinone was identified as Q9. Q8 was also detected (less than 10 %).

The FAME composition of AAD6T, characterized by the abundance of unsaturated chains, was C16:0 (35 %),
**Fig. 1.** Neighbour-joining phylogenetic tree based on 16S rRNA gene sequences, showing the position of the novel isolate with respect to other related species. Bootstrap values (expressed as percentages of 1000 replications) >50% are shown at branch points. Bar, 0.01 substitutions per nucleotide position.

**Table 2.** Differential characteristics of strain AAD6\(^T\) and its closest phylogenetic relatives

<table>
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<td>–</td>
<td>+</td>
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<td>Colony pigmentation</td>
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<td>Temperature range (°C)</td>
<td>5.0–40</td>
<td>4.0–45</td>
<td>4.0–45</td>
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<td>15–45</td>
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<td>10–40</td>
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<td>pH range</td>
<td>5.5–8.5</td>
<td>5.0–10</td>
<td>5.0–10</td>
<td>6.0–10</td>
<td>6.0–9.0</td>
<td>5.0–9.0</td>
<td>7.0–9.0</td>
<td>5.5–10</td>
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<td>Acid production from:</td>
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<tr>
<td>D-Glucose</td>
<td>+</td>
<td>–</td>
<td>+</td>
<td>+</td>
<td>–</td>
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<td>–</td>
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</tr>
<tr>
<td>Mannose</td>
<td>+</td>
<td>–</td>
<td>+</td>
<td>+</td>
<td>–</td>
<td>–</td>
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<tr>
<td>Hydrolysis of:</td>
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</tr>
<tr>
<td>Aesculin</td>
<td>+</td>
<td>–</td>
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<td>+</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>+</td>
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<tr>
<td>Casein</td>
<td>+</td>
<td>–</td>
<td>–</td>
<td>–</td>
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<tr>
<td>Gelatin</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>+</td>
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<td>DNA</td>
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<td>–</td>
<td>–</td>
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<td>–</td>
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<td>–</td>
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<tr>
<td>Tween 20</td>
<td>–</td>
<td>+</td>
<td>–</td>
<td>+</td>
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<td>–</td>
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<tr>
<td>Tween 80</td>
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<td>–</td>
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<td>Urea</td>
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<td>–</td>
<td>+</td>
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<td>Growth on:</td>
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<tr>
<td>L-Arabinose</td>
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<td>+</td>
</tr>
<tr>
<td>d-Xylose</td>
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<td>+</td>
<td>–</td>
<td>–</td>
<td>+</td>
<td>+</td>
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<tr>
<td>Lactose</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>+</td>
<td>+</td>
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<tr>
<td>Sucrose</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>–</td>
<td>–</td>
<td>+</td>
<td>–</td>
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</tr>
<tr>
<td>L-Histidine</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
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<tr>
<td>G+C content (mol%)</td>
<td>15.0</td>
<td>60.4–64.2</td>
<td>66.7</td>
<td>61.0–62.9</td>
<td>62.2–64.1</td>
<td>60.5</td>
<td>65.3</td>
<td>70.0</td>
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</table>
C₁₈:₁₀₁₀₇C (22.6%), C₁₇:₁₀₁₀₇C (21.4%) and C₁₂: 3OH (10.7%), C₁₉:₀ cyclo ω₈c (5.6%), C₁₂:₀ (1.3 %) and trace amount of C₁₀:₀. This composition was similar to, and corresponded well with, those of members of the genus *Halomonas* (Bouchotroch et al., 2001; Lim et al., 2004; Yoon et al., 2002; Lee et al., 2005; Wang et al., 2007, 2008).

On the basis of physiological, biochemical and phylogenetic properties, strain AAD⁶ᵀ represents a novel species within the genus *Halomonas*, for which the name *Halomonas smyrnensis* sp. nov. is proposed.

**Description of Halomonas smyrnensis** sp. nov.

*Halomonas smyrnensis* (smyr.nen’is, N.L. fem. adj. smyr-nensis of or belonging to the classical town Smyrna, today Izmir, where this species was isolated).

Cells are Gram-negative, facultatively aerobic, and rod-shaped (1.8–2.2 × 1.4–1.8 μm). Oxidase-negative and catalase-positive. Colonies on complex agar medium are 1–2 mm in diameter, cream-yellowish, circular and slightly irregular after 2 days. No growth is observed in the absence of NaCl. Grows at an optimum NaCl concentration of 10 % (w/v), and at an optimum pH and temperature of 7.0 and 37 °C, respectively. Negative for indole formation, H₂S and PHA production. Hydrolyses starch and aesculin, but not gelatin, Tween 20, hippurate, phenylalanine, DNA or urea. Does not reduce nitrate and nitrite. Negative result in tests for methyl red reaction, Voges–Proskauer reaction, lysine and arginine decarboxylase activities, fermentation of D-glucose and respiration on nitrate or nitrite. Requires Mg²⁺ for growth but not SO₄²⁻ or K⁺. Utilizes D-glucose, sucrose, maltose, D-fructose, D-galactose and D-mannose as sole carbon sources, producing acid only from glucose, sucrose and mannose. Does not utilize raffinose, L-rhamnose, acetate or citrate. Oxidizes glucose and utilizes alanine and serine as sole carbon, nitrogen and energy sources but unable to use histidine. Cells produce an exopolysaccharide; the physical and chemical properties of this polymer produced by strain AAD⁶ᵀ are described elsewhere (Poli et al., 2009a). For all strains, the predominant ubiquinone is Q-9 and the predominant polar lipids are 1,2-diacylglycerol-3-phosphorylthanolamine, phosphoglycerol, diphosphoglycerate and (2-(2-D-glucopyranosyloxy)-3-hydroxy-propyl)-phosphatidyl diacylglycerol. Major fatty acids are C₁₆:₁₀, C₁₈:₁₀₁₀₇C, C₁₆:₁₀₁₀₇C and C₁₂:₀ 3OH, C₁₉:₀ cyclo ω₈c, C₁₂:₀ and trace amount of C₁₀:₀. Strain AAD⁶ᵀ is susceptible to (amount per disc): ampicillin (9 U), bacitracin (10 U), novobiocin (5 U), amikacin (30 U), tetracycline (30 U), aztreonam (30 U), cefepime (10 U), cefaperazone (75 U), but not to amoxicillin (25 μg), cefoxitin (30 μg), penicillin (10 U), cefotaxime (30 μg), cefuroxime (30 μg), ticarcillin (30 μg), isoxacinoline (30 μg), cefotaxime (30 μg), ampicillin (30 μg) and cefotaxime (30 μg).

The type strain, AAD⁶ᵀ (=DSM 21644ᵀ =JCM 15723ᵀ), was isolated from saltern area from Izmir province, Aegean Region of Turkey. The DNA G+C content of the type strain AAD⁶ᵀ is 63 mol%. Three additional strains of the species are AAD4, AAD17 and AAD21, with DNA G+C contents of 63.3, 62.8 and 62.6 mol%, respectively.

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