Arhodomonas aquaeolei gen. nov., sp. nov., an Aerobic, Halophilic Bacterium Isolated from a Subterranean Brine

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Arhodomonas aquaeolei gen. nov., sp. nov., isolated from a petroleum reservoir production fluid, is described. The single isolate was an obligately halophilic, aerobic, gram-negative, oval rod-shaped bacterium that was actively motile by means of a single polar flagellum. It was catalase and oxidase positive. The isolate had a specific requirement for NaCl; growth occurred at NaCl concentrations between 6 and 20%, and optimal growth occurred in the presence of 15% NaCl. This species metabolized primarily organic acids and required biotin for growth. The name *Arhodomonas* is proposed for the new genus, which was placed in the gamma subclass of the *Proteobacteria* on the basis of the results of a 16S rRNA sequence analysis. Although *A. aquaeolei* is most closely related to purple sulfur bacteria (the genera *Ectothiorhodospira* and *Chromatium*), it is not a phototrophic microorganism, which is consistent with its isolation from a subterranean environment. The major components of its cellular fatty acids were C_{16:0}, C_{18:1}, C_{19:0}, C_{16:1}, and C_{18:0} acids. The DNA base composition of the type strain is 67 mol% G+C. The type and only strain is strain HA-1 (= ATCC 49307).

Halophiles are microorganisms which require high concentrations of NaCl for optimal growth and utilize various active processes of haloadaptation to cope with the osmotic stress of concentrated salt solutions (35, 45). Halophiles have been isolated from various natural and man-made saline environments, including salterns (5), hypersaline lakes (10), saline soils (32), seawater (27), and petroleum reservoir production fluids (1, 6). Halophiles can be classified as halotolerant, moderately halophilic, or extremely halophilic, according to the concentration of NaCl required for optimal growth (20). Moderately halophilic microorganisms grow optimally at NaCl concentrations between 0.5 and 2.5 M, and this group includes a taxonomically and physiologically diverse array of bacteria.

An obligately halophilic, gram-negative, heterotrophic, aerobic, rod-shaped organism was isolated from an oil field brine. The phenotypic and phylogenetic characteristics of this organism were significantly different from the characteristics of all previously described halophiles and justify its designation as a new genus and new species. We describe the new genus *Arhodomonas* for this non-red-cell organism related to the purple sulfur bacteria. The type species is *Arhodomonas aquaeolei*, which was isolated from an oil field brine. The type strain is strain HA-1 (= ATCC 49307).

(A portion of this work has appeared previously [2]).

MATERIALS AND METHODS

Bacterial isolation. An obligately halophilic, aerobic, rod-shaped eubacterium was isolated by Woody Jenkins from a petroleum reservoir production fluid (native produced water) obtained from a producing well in the Southeast Vassar Vertz Sand Unit in Payne County, Okla. (19). Produced brine samples were streaked onto solid medium containing 8.5 g of plate count broth (Difco Laboratories, Detroit, Mich.) per liter, 2% agar, and 10% NaCl and incubated

aerobically at 37°C. A single isolate, designated strain HA-1^T (T = type strain), was recovered from these aerobic plates.

Media and cultivation methods. The basal medium contained the following components (per liter): 150 g of NaCl, 1.0 g of NH₄Cl, 0.1 g of KCl, 0.1 g of KH₂PO₄, 0.2 g of MgSO₄ · 7H₂O, 0.04 g of CaCl₂ · 2H₂O, 10 g of TES buffer [*N*-tris(hydroxymethyl)methyl-2-aminoethanesulfonic acid], 5 ml of a trace metal solution (39), and 10 ml of a vitamin solution (39). The pH of the medium was 7.0 to 7.3. A complex medium contained the following components (per liter): 150 g of NaCl, 17 g of plate count broth (Difco), and 2 g of KNO₃. The pH was adjusted to 7.2. Solid media were prepared by adding 20 g of purified agar (BBL, Cockeysville, Md.) per liter. Incubation was at 37°C. Anaerobic growth was tested in media prepared and inoculated by a strictly anaerobic technique (3, 4) in aluminum-sealed anaerobic culture tubes (catalog no. 2048-18150; Bellco Glass, Inc., Vineland, N.J.).

Nutritional and growth characteristics. The temperature range for growth was determined on a solid complex medium incubated at 5 to 45°C; growth was scored visually. The pH range for growth was determined in complex broth medium whose final pH was adjusted to 5.0 to 9.0 with HCl or KOH; growth was scored visually.

For the nutritional tests, a filter-sterilized substrate was added to the basal medium at a concentration of 0.1%. Carbohydrates were used at a concentration of 0.2% (wt/vol). When amino acids were tested as sole carbon, nitrogen, and energy sources, NH₄Cl and KNO₃ were omitted from the basal medium. Growth was scored visually with reference to a negative control tube containing basal medium with no added organic compound. Positive results were scored after at least three consecutive transfers in the same medium. The nitrogen requirement was tested in basal medium containing acetate from which NH₄Cl and KNO₃ were omitted. The compounds tested were NH₄Cl, KNO₃, tryptone, and L-amino acids.

To determine vitamin requirements, basal medium was prepared as described above except that the vitamin solution

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was not added. Individual vitamins were added from filter-sterilized solutions.

Phototrophic growth medium contained the following components (per liter): 150 g of NaCl, 2 g of KNO₃, 0.5 g of NH₄Cl, 0.3 g of KCl, 0.3 g of KH₂PO₄, 0.5 g of MgSO₄ · 7H₂O, 0.2 g of CaCl₂ · 2H₂O, 1 g of yeast extract, 10 g of TES buffer, 5 ml of a trace metal solution (39), and 20 ml of a vitamin solution (39). After autoclaving, the medium was supplemented with 1.5 g of NaHCO₃ per liter; the NaHCO₃ was added from a freshly prepared, CO₂-saturated, filter-sterilized solution. Sodium sulfide and sodium thiosulfate were added from freshly prepared filter-sterilized solutions to final concentrations of 1 and 5 mM, respectively, to test for photoautotrophic growth with sulfide or thiosulfate as the electron donor. The final pH values of the media were 7.0 to 7.3. Photoheterotrophic growth was tested in medium containing 2 g of sodium acetate per liter. Phototrophic media were prepared aerobically and anaerobically. Cultures were incubated at 37°C with and without incandescent light. Growth was scored visually and compared with the growth of a positive control culture grown aerobically in medium containing acetate.

Ion specificity determination. Other salts were substituted for NaCl at concentrations of 1.5, 2.0, and 2.6 M in basal medium containing 12 mM acetate to examine the isolate's requirement for NaCl. KCl, LiCl, MgCl₂ · 2H₂O, and NH₄Cl were used to test for a specific Na⁺ requirement. A Cl⁻ requirement was examined by using NaBr, NaNO₃, NaSO₄, and Na₂S₂O₃. Growth was scored visually and compared with the growth of a positive control in medium containing NaCl.

Phenotypic characterization. The isolate was tested for general phenotypic characteristics by using previously described procedures (34, 37, 43). Unless otherwise indicated, the tests were carried out in media containing 15% NaCl at pH 7.0 to 7.3 and the test preparations were incubated at 37°C.

Antibiotic susceptibility was tested by spreading bacterial suspensions on plates containing complex medium and applying the following antibiotic discs (Difco): ampicillin (10 µg), carbenicillin (100 µg), chloramphenicol (30 µg), erythromycin (15 µg), gentamicin (10 µg), neomycin (30 µg), penicillin G (10 U), streptomycin (10 µg), tetracycline (30 µg), and vancomycin (30 µg). Zones of inhibition were measured, and susceptibility was determined by comparison with standard inhibition zones (Difco).

Microscopy. Cellular morphology was determined by phase-contrast microscopy and electron microscopy. Micrographs were prepared from log-phase cells grown on a complex medium. For transmission electron microscopy, cells were spread onto carbon-coated Formvar grids, fixed with 1% glutaraldehyde in 15% NaCl, and negatively stained with 1% phosphotungstic acid (pH 7). Micrographs were taken with a Zeiss model EM-10 transmission electron microscope.

Cell membrane fatty acid analysis. A cell membrane fatty acid analysis was performed by Microcheck, Inc., Northfield, Vt. Whole-cell fatty acids were analyzed as fatty acid methyl esters by gas chromatography with a MIDI microbial identification system (MIDI, Inc., Newark, Del.). Fatty acid methyl ester extracts were prepared and chromatographed by using previously described methods (24, 25, 36). The cells used for analysis were harvested from a culture grown for 72 h at 37°C on tryptic soy broth agar (Difco) supplemented with 15% NaCl and 0.2% KNO₃.

Determination of DNA base composition. The DNA was

extracted and purified by the method of Marmur (22). The guanine-plus-cytosine (G+C) content of the DNA was determined from the midpoint value of the thermal denaturation profile (23) obtained with a Beckman model DU-8B spectrophotometer. The G+C content of reference DNA from *Escherichia coli* b strain ATCC 11303 is 51 mol%.

16S rRNA sequence analysis. *Arhodomonas aquaeolei* HA-1^T (= ATCC 49307^T) was grown on complex medium as described above. The 16S rRNAs from *Arhodomonas aquaeolei*, *Ectothiorhodospira halophila* SL1 (= DSM 244), *Ectothiorhodospira halochloris* ATCC 35916, *Chromatium vinosum* ATCC 17899, *Pseudomonas aeruginosa* ATCC 25330, and *Oceanospirillum linum* ATCC 11336 were sequenced by using methods described previously (21, 30). The sequences for *Proteus vulgaris*, *Escherichia coli*, and *Agrobacterium tumefaciens* DSM 30150 have been published previously (7, 26, 49). Sequences were analyzed by a distance matrix analysis, using a program for fitting trees to distance data adapted by workers in the laboratory of Carl R. Woese (8, 17, 30).

Nucleotide sequence accession numbers. The 16S rRNA sequences of *Arhodomonas aquaeolei*, *Ectothiorhodospira halophila* SL1, *Ectothiorhodospira halochloris* ATCC 35916, *Chromatium vinosum* ATCC 17899, *Pseudomonas aeruginosa* ATCC 25330, and *O. linum* ATCC 11336 determined in this study were deposited in the GenBank data base under accession numbers M26631, M59152, M26630, M26629, M34133, and M22365, respectively.

RESULTS AND DISCUSSION

In this paper we describe the isolation and characterization of a new type of moderately halophilic eubacterium which has phenotypic and phylogenetic characteristics different from the characteristics of the moderately halophilic gram-negative rods previously described.

Cellular and colonial morphology. The isolate is a gram-negative rod-shaped eubacterium that is 2 by 0.8 µm. It is characteristically found singly or in pairs. Cells are actively motile by means of a single polar flagellum (Fig. 1).

Colonies on complex or defined media containing 2.6 M NaCl are circular, convex, smooth with entire margins, 0.5 mm in diameter, and nonpigmented.

Cultural and physiological characterization. *Arhodomonas aquaeolei* was an obligate halophile that grew in medium containing 1.0 to 3.4 M NaCl. Optimal growth occurred in medium containing about 2.5 M NaCl. Strain HA-1^T had a specific requirement for NaCl. Salts of K⁺, Mg²⁺, Li⁺, or NH₄⁺ did not support growth. The chloride anion could not be replaced by Br⁻, NO₃⁻, SO₄²⁻, or S₂O₃²⁻. Growth was not stimulated by increased levels of Mg²⁺, Ca²⁺, or K⁺. The salt requirement of this organism reflects the conditions in the natural environment from which it was isolated (6, 19).

Nitrogen could be supplied as ammonium, nitrate, or tryptone. Glutamic acid and glutamine could serve as sole carbon, nitrogen, and energy sources.

Growth was aerobic. Catalase and oxidase activities were produced. The optimum growth temperature was 37°C. The temperature range for growth was 20 to 45°C. The pH range for growth was 6.0 to 8.0, with an optimum pH of 6.5 to 7.5. Strain HA-1^T primarily metabolized organic acids (Table 1). The biotin requirement of strain HA-1^T was determined in basal medium by performing at least six consecutive transfers in medium containing only biotin. Growth was stimulated by yeast extract. Biochemical characteristics are listed below in the description of the species.

FIG. 1. Transmission electron micrograph of *Arhodomonas aquaeolei*.TABLE 1. Utilization of organic carbon sources by *Arhodomonas aquaeolei* HA-1^T

Compound ^a	Utilization by <i>Arhodomonas aquaeolei</i>
Acetate	+ ^b
Butyrate	+
Isobutyrate	-
DL-β-Hydroxybutyrate	+
γ-Amino- <i>n</i> -butyrate	+
γ-Hydroxybutyrate	-
Citrate	-
Crotonate	+
Ethanol	+
Formate	-
D-Fructose	-
Fumarate	+
D-Galactose	-
D-Gluconate	+
D-Glucose	-
L-Glutamic acid ^c	+
L-Glutamine ^c	+
Glycerol	+
Lactate	+
D-Malate	-
D-Mannitol	-
Methanol	-
Propionate	+
Pyruvate	+
Starch	-
Succinate	+
Sucrose	-
Valerate	+
D-Xylose	+

^a When supplied as the sole source of carbon and energy.^b +, positive; -, negative.^c When supplied as the sole source of carbon, nitrogen, and energy.

Since the results of the phylogenetic analysis (see below) suggests that there is a relationship between *Arhodomonas aquaeolei* and the anoxic phototrophic purple sulfur bacterial genera *Ectothiorhodospira* and *Chromatium*, attempts were made to culture *Arhodomonas aquaeolei* phototrophically (15, 31). No difference in the growth of *Arhodomonas aquaeolei* was observed in aerobic phototrophic growth test medium incubated with illumination compared with cultures grown in the dark. No growth of *Arhodomonas aquaeolei* was observed in anaerobic phototrophic growth test medium when sulfide (1 mM) and thiosulfate (5 mM) were tested as photosynthetic electron donors. Phototrophic growth test medium (without added NaCl) supported growth of *Chromatium vinosum* ATCC 17899 and environmental enrichment cultures of anoxic phototrophic *Chromatium* species. Although *Arhodomonas aquaeolei* is most closely related to purple sulfur bacteria (the genera *Ectothiorhodospira* and *Chromatium*), it is not a phototrophic microorganism, which is consistent with its isolation from a subterranean environment.

Like *Arhodomonas aquaeolei*, a specific requirement for sodium exists in all *Ectothiorhodospira* species (16); however, growth of *Ectothiorhodospira mobilis* occurs in the absence of added chloride (14). Although all *Ectothiorhodospira* species grow well under anaerobic conditions in the light, *Ectothiorhodospira mobilis* and *Ectothiorhodospira shaposhnikovii* can grow microaerobically in the dark (15). Both *Arhodomonas aquaeolei* and *Ectothiorhodospira* species utilize a small number of simple organic compounds, such as certain fatty acids (15).

DNA base composition. The DNA base composition of *Arhodomonas aquaeolei* was 67.0 mol% G+C.

Phylogeny. A matrix of sequence dissimilarity values for *Arhodomonas aquaeolei* and selected reference organisms belonging to the gamma subclass of the *Proteobacteria* is shown in Table 2. The results of the 16S rRNA sequence

TABLE 2. Dissimilarity matrix derived from comparisons of 16S rRNA sequences of various species in the gamma subclass of the *Proteobacteria*

Organism	% Dissimilarity to ^a :							
	<i>Arhodomonas aquaeolei</i>	<i>Ectothiorhodospira halochloris</i>	<i>Ectothiorhodospira halophila</i>	<i>Chromatium vinosum</i>	<i>Pseudomonas aeruginosa</i>	<i>O. linum</i>	<i>Escherichia coli</i>	<i>Proteus vulgaris</i>
<i>Ectothiorhodospira halochloris</i>	9.5							
<i>Ectothiorhodospira halophila</i>	8.0	3.6						
<i>Chromatium vinosum</i>	10.0	12.1	11.6					
<i>Pseudomonas aeruginosa</i>	13.2	13.1	13.0	12.5				
<i>Oceanospirillum linum</i>	12.5	14.2	13.6	13.1	9.9			
<i>Escherichia coli</i>	16.5	17.8	18.4	14.8	14.7	14.2		
<i>Proteus vulgaris</i>	17.9	17.5	18.2	16.6	15.9	13.9	6.3	
<i>Agrobacterium tumefaciens</i> ^b	19.3	18.2	18.1	19.0	21.9	20.4	24.4	23.8

^a Percentages of dissimilarity were estimated from the number of changes per 100 nucleotides. The sequences used were obtained from the Ribosomal Database Project collection (28, 29). The strain designations and GenBank accession numbers are given in the text.

^b Member of the alpha subclass of the *Proteobacteria*.

analysis (Fig. 2) show that *Arhodomonas aquaeolei* represents a deeply branching lineage in the gamma subclass of the *Proteobacteria* of the eubacterial kingdom (38, 47, 48). *Agrobacterium tumefaciens* was included as an outgroup reference organism and is a species in the alpha subclass of the *Proteobacteria* (38, 47, 49). The results of the 16S rRNA sequence analysis indicate that the genus *Arhodomonas* is a separate genus specifically related to the genus *Ectothiorhodospira* (15). *Arhodomonas aquaeolei* does not grow phototrophically. The 16S rRNA of *Arhodomonas aquaeolei* has structural features that link it strongly to the gamma subclass and separate it from members of the alpha, beta, and delta subclasses. The 16S rRNA of *Arhodomonas aquaeolei* has structural similarities with the 16S rRNAs of the purple sulfur bacteria. The loop starting at position 420 (*Escherichia coli* numbering) is UGCG in *Arhodomonas aquaeolei* and species of the genus *Ectothiorhodospira*, a composition not

found in other members of the gamma subclass except *Chromatium* species. The base C is added in the loop covering position 1361 in *Arhodomonas aquaeolei* and members of the genus *Ectothiorhodospira*; this feature is not found in other species of the gamma subclass of the *Proteobacteria*.

The taxonomy of moderately halophilic, gram-negative, aerobic or facultatively anaerobic, rod-shaped eubacteria has been studied in some detail (41, 44). The following species have been validly published: *Vibrio costicola* (12), *Chromohalobacter marismortui* (42), *Deleya halophila* (34), *Deleya salina* (40), *Halomonas elongata* (46), *Halomonas halmophila* (9), *Mesophilobacter marinus* (27), and *Volcaniella eurihalina* (33). The family *Halomonadaceae*, which includes members of the genera *Halomonas* and *Deleya*, constitutes an individual subline of descent within the gamma subclass of the *Proteobacteria* as determined by 16S rRNA cataloging (11). The phylogenetic position of *Vibrio costicola* within the family *Vibrionaceae*, as determined by 16S rRNA sequence analysis, has recently been reported (18). The phylogenetic positions of *Chromohalobacter marismortui*, *Mesophilobacter marinus*, and *Volcaniella eurihalina* based on 16S rRNA analysis data have not been reported.

Recently, a new species of halophilic eubacteria belonging to the gamma subclass of the *Proteobacteria*, *Marinobacter hydrocarbonoclasticus*, was described (13). The genera *Marinobacter* and *Arhodomonas* are not related, as indicated by the G+C content of *Marinobacter hydrocarbonoclasticus* (52.7 mol%) and 16S rRNA sequence analysis data (13). Although *Arhodomonas aquaeolei* and *Marinobacter hydrocarbonoclasticus* have similar phenotypic characteristics, they can be differentiated by the following tests: optimal NaCl concentration, lecithinase activity, and tetracycline and streptomycin susceptibility.

Arhodomonas aquaeolei can be easily differentiated from *Mesophilobacter marinus* since *Mesophilobacter marinus* is a nonmotile coccobacillus, grows on glucose and sucrose, and has a G+C content of 44.0 to 46.9 mol% (27). Useful characteristics for distinguishing *Arhodomonas aquaeolei* from other moderately halophilic, aerobic, heterotrophic, gram-negative, rod-shaped bacteria are shown in Table 3.

Since there are significant phenotypic and phylogenetic differences between our isolate (strain HA-1^T) and the previously described moderately halophilic bacteria, we propose a new genus, *Arhodomonas*, and a new species, *Arhodomonas aquaeolei*, for this organism.

FIG. 2. Phylogeny of *Arhodomonas aquaeolei* as determined by a 16S rRNA sequence analysis. The total horizontal distance between two species indicates the level of difference between their sequences. Bar = 5% difference. Genus names are given in the text.

TABLE 3. Differential characteristics for *Arhodomonas aquaeolei* HA-1^T (= ATCC 49307^T) and other aerobic, moderately halophilic, gram-negative, rod-shaped eubacteria^a

Strain	Optimum salt concn (%)	Oxygen relationship	Motility	Oxidase production	Nitrate reduction	Growth on ^b :	
						Glucose	Sucrose
<i>Arhodomonas aquaeolei</i> ATCC 49307 ^T	15 ^c	Aerobe	+	+	+	—	—
<i>Chromohalobacter marismortui</i> ATCC 17056 ^T	10 ^d	Aerobe	+	—	—	+	+
<i>Deleya halophila</i> CCM 3662 ^T	7.5 ^d	Aerobe	+	+	+	+	+
<i>Deleya salina</i> ATCC 49509 ^T	5 ^d	Aerobe	—	+	+	—	—
<i>Halomonas elongata</i> ATCC 33173 ^T	3.5–8 ^c	Facultative anaerobe	+	—	+	+	+
<i>Halomonas halmophila</i> CCM 2833 ^T	10 ^c	Aerobe	+	+	—	+	+
<i>Vibrio costicola</i> NCMB 701 ^T	10 ^c	Facultative anaerobe	+	+	—	+	+
<i>Volcaniella eurihalina</i> ATCC 49336 ^T	7.5 ^d	Aerobe	—	—	+	—	+

^a Data from references 9, 12, 33, 34, 40, 42, and 46 and from this study.^b When glucose and sucrose were supplied as sole sources of carbon and energy.^c Optimum NaCl concentration.^d Optimum total-salts concentration.

Description of *Arhodomonas* gen. nov. *Arhodomonas* (A' rho.do.mo.nas. Gr.pref. a, not; Gr. adj. rhodos, red; Gr. n. monas, a unit; M.L. fem. n., *Arhodomonas*, a non-red cell).

Gram-negative, short, straight, rod-shaped cells occurring singly or in pairs. Motile by means of a single polar flagellum. Not spore forming and not encapsulated.

Respiratory metabolism. Catalase and oxidase positive. Nitrate is reduced. Nitrite is not reduced. Nonphototrophic.

Phylogenetically, the genus *Arhodomonas* represents a deeply branching lineage in the gamma subclass of the *Proteobacteria* (as determined by 16S rRNA sequence analysis). The G+C content of the DNA of the type strain of the type species is 67 mol% (as determined by the thermal denaturation method).

The type species is *Arhodomonas aquaeolei*.

Description of *Arhodomonas aquaeolei* sp. nov. *Arhodomonas aquaeolei* (a.quae.o'le.i. L. n. aqua, water; L. n. oleum, oil; M.L. gen. n. *aquaeolei*, from water of oil, isolated from an oil field brine).

Growth in liquid cultures is uniformly turbid with a pellicle forming after 72 h of incubation.

Gram-negative, short, straight, rod-shaped cells that are 0.8 to 1.0 µm in diameter and 2.0 to 2.5 µm long and occur singly or in pairs. Motile by means of a single polar flagellum. Not spore forming and not encapsulated. Colonies on complex medium containing 2.6 M NaCl are circular with entire margins, convex, and smooth; they are 0.5 mm in diameter after 72 h and nonpigmented.

The optimal NaCl concentration is 15%; grows in the presence of NaCl concentrations between 6 to 20%. No growth occurs in the absence of NaCl. Growth occurs at 20 to 45°C and pH 6 to 8; optimal growth occurs at 37°C and pH 7.

Aerobic. Respiratory metabolism with O₂ as the terminal electron acceptor. Catalase and oxidase positive. Nitrate is reduced. Nitrite is not reduced.

Tween 20 and Tween 80 are hydrolyzed. Casein, DNA, esculin, gelatin, starch, and tyrosine are not hydrolyzed. Urease is produced. Negative for arginine, lysine, and ornithine decarboxylase activities. The following tests are negative: Simmons citrate, methyl red, Voges-Proskauer, indole, lecithinase, H₂S production, phenylalanine deaminase, and phosphatase. Cells are susceptible to ampicillin (10 µg), carbenicillin (100 µg), chloramphenicol (30 µg), erythromycin (15 µg), penicillin G (10 U), and tetracycline (30 µg). Cells are resistant to gentamicin (10 µg), neomycin (30 µg), streptomycin (10 µg), and vancomycin (30 µg).

The following compounds are utilized as sole carbon and energy sources: acetate, butyrate, crotonate, ethanol, fumarate, gluconate, glycerol, lactate, propionate, pyruvate, succinate, valerate, isovalerate, xylose, yeast extract, and Casamino Acids. Acids are not produced from these compounds.

The following compounds are not utilized as sole carbon and energy sources: adonitol, allantoin, arabinose, benzoate, betaine, butanol, cellobiose, citrate, esculin, formate, fructose, fucose, galactose, glucose, glucosamine, glycolate, heptanoate, hexanoate, hippurate, inulin, inositol, isopropanol, lactose, malate, maleate, malonate, maltose, mannitol, mannose, melezitose, methanol, propanol, oxamate, raffinose, rhamnose, ribose, saccharose, salicin, sorbose, starch, sucrose, tartarate, trehalose, turanose, and xylitol.

Glutamic acid and glutamine are used as sole carbon, nitrogen, and energy sources. The other common amino acids do not support growth.

The major components of the cellular fatty acids are C_{16:0} acid (21.74%), C_{18:1} acid (21.74%), C_{19:0} acid (12.72%), C_{16:1} acid (12.55%), and C_{18:0} acid (11.20%).

The G+C content of the DNA is 67 mol% (as determined by the thermal denaturation method).

The type and only strain is *Arhodomonas aquaeolei* HA-1, which was isolated from a petroleum reservoir production fluid; it has been deposited in the American Type Culture Collection as strain ATCC 49307.

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