***Acidisphaera rubrifaciens* gen. nov., sp. nov., an aerobic bacteriochlorophyll-containing bacterium isolated from acidic environments**

Akira Hiraishi,¹ Yukiko Matsuzawa,¹ Toshio Kanbe² and Norio Wakao³

Author for correspondence: Akira Hiraishi. Tel: +81 532 446913. Fax: +81 532 446929.
e-mail: hiraishi@eco.tut.ac.jp

¹ Department of Ecological Engineering, Toyohashi University of Technology, Toyohashi 441-8580, Japan

² Laboratory of Medical Mycology, Nagoya University School of Medicine, Nagoya 466-8550, Japan

³ Department of Bioscience and Technology, Iwate University, Morioka 020-8550, Japan

Four strains of aerobic, mesophilic, acidophilic bacteria that produced bacteriochlorophyll (BChl) *a* were isolated from acidic hot springs and mine drainage. The characteristics of the four isolates were almost identical. The isolates were strictly aerobic and chemo-organotrophic. They were Gram-negative, non-motile cocci and coccobacilli, formed salmon-pink colonies on solidified media and produced BChl *a* and carotenoids only under aerobic growth conditions. The cells also produced small amounts of zinc-substituted BChl *a* when grown in the presence of 1 mM zinc sulfate. Anaerobic growth in the light was not found, but aerobic growth was stimulated by continuous incandescent illumination. The isolates grew in a pH range of 3.5–6.0, with pH optima of 4.5–5.0. A phylogenetic analysis based on 16S rDNA sequences showed that the isolates clustered in the major acidophilic group of the class *Proteobacteria*, which includes species of the genera *Acidiphilium* and *Rhodopila*. The anaerobic phototrophic bacterium *Rhodopila globiformis* was the closest relative to the new isolates (95% level of sequence similarity). The G+C content of the genomic DNA of the isolates was 69.1–69.8 mol%. On the basis of these results, it was concluded that the four isolates should be classified into a new genus and a new species, for which the name *Acidisphaera rubrifaciens* is proposed. The type strain is strain HS-AP3^T (= JCM 10600^T).

Keywords: *Acidisphaera rubrifaciens*, acidophilic bacterium, aerobic BChl-containing bacterium, phylogeny, zinc-bacteriochlorophyll

INTRODUCTION

Obligately aerobic bacteria that have bacteriochlorophylls (BChls) and photochemical activity are categorized into a group of aerobic phototrophic or aerobic BChl-containing bacteria. Most of these bacteria have been classified as members of new genera of the class *Proteobacteria*, including *Erythrobacter*, *Erythromicrobium*, *Porphyrobacter*, *Roseobacter*, *Roseococcus* and *Sandaracinobacter* (for reviews, see Shimada, 1995; Yurkov & Beatty, 1998). Recently, several new genera including *Craurococcus*, *Paracraurococcus* (Saitoh *et al.*, 1998), *Roseovarius*

(Labrenz *et al.*, 1999), *Rubrimonas* (Suzuki *et al.*, 1999), *Roseateles* (Suyama *et al.*, 1999), *Roseivivax* (Suzuki *et al.*, 1999) and ‘*Citromicrobium*’ (Yurkov *et al.*, 1999) have been described as aerobic BChl-containing bacteria. While most of these genera consist of neutrophilic species, the genus *Acidiphilium* includes species of aerobic phototrophic acidophiles that inhabit strongly acidic mineral environments such as mine drainage (Kishimoto *et al.*, 1995). Interestingly, the main photopigment of *Acidiphilium* species is bacteriochlorophyll *a* chelated with zinc (Zn-BChl *a*) in place of magnesium as the central metal (Wakao *et al.*, 1996; Hiraishi *et al.*, 1998). Zn-BChl *a* is more stable than Mg-BChl *a* under acidic conditions (Wakao *et al.*, 1996; Kobayashi *et al.*, 1998). Thus, it is of major concern to determine whether the occurrence of Zn-BChl *a* in aerobic BChl-containing bacteria is related to the acidic environments of their natural habitats.

Abbreviations: BChl, bacteriochlorophyll; Mg-BChl, magnesium-chelated BChl; Zn-BChl, zinc-chelated BChl.

The DDBJ accession numbers for the 16S rDNA sequences determined in this study are D86512 and D86513.

During surveys of acidophilic phototrophic bacteria in various acidic environments, we isolated several strains of pink- to red-pigmented aerobic acidophiles that produced Mg-BChl *a* but not Zn-BChl *a*. Aerobic acidophilic bacteria that contain Mg-BChl *a* as the main photopigment have not been reported. This article, therefore, describes the taxonomic characteristics of the new aerobic Mg-BChl-producing acidophiles and their phylogenetic relationship to other acidophilic phototrophs. Here we propose to classify the new isolates as a new genus and species with the name *Acidisphaera rubrificiens*.

METHODS

Bacterial strains. Four strains designated HS-AP3^T (super-script T = type strain), HS-AP4, HS-AP16 and YO-704 were studied. Strains HS-AP3^T and HS-AP4 were isolated from surface water and sediment from an acidic hot spring, respectively, in Hakone, Kanagawa Prefecture, Japan. The pH of the water at the sampling site was 3.2 and the temperature was 32 °C. Strain HS-AP16 was isolated from sediment in an acidic hot spring stream (pH 2.8, 35 °C) in Unzen, Nagasaki Prefecture, Japan. Strain YO-704 was isolated from acidic mine drainage (pH 2.5, 22 °C) in Iwate Prefecture, Japan. *Acidiphilium cryptum* ATCC 33463^T, *Acidiphilium rubrum* ATCC 35905^T and *Rhodospila globiformis* DSM 161^T were used as the reference organisms. The strains with ATCC and DSM numbers were obtained from the American Type Culture Collection, Manassas, VA, USA, and from the DSMZ (Deutsche Sammlung von Mikroorganismen und Zellkulturen), Braunschweig, Germany.

Growth media and cultivation. Unless otherwise noted, GNYS medium (pH 4.5) was used for cultivation of the new isolates. This medium consisted of a mineral base (RM2; Hiraishi & Kitamura, 1984), 0.3 % sodium gluconate as the sole carbon source and 0.1 % yeast extract as the growth factor. Gluconate and other components of the medium were autoclaved separately and combined before use. The medium contained 0.8 % gellan gum (Wako Pure Chemicals) when used as a solidified medium (Kishimoto & Tano, 1987). For cultivation of *Acidiphilium* species, GYS medium (Hiraishi *et al.*, 1998) was used. All aerobic bacteria tested were grown aerobically on a reciprocal shaker at 30 °C in darkness or under incandescent illumination at 2000 lx. *R. globiformis* was grown anaerobically in the light with 10 mM fructose and 10 mM gluconate as carbon sources, as described by Pfennig (1974). For chemical and genetic tests, cells were harvested by centrifugation from a culture at the late-exponential phase of growth, washed twice with sterile 50 mM phosphate buffer (pH 6.8) and pelleted. The cell pellets were used immediately for analysis or stored at –20 °C until analysed.

Microscopic studies. General cell morphology was studied under an Olympus phase-contrast microscope. Gram staining was performed with a Gram stain reagent kit (Nakarai Tesque). Electron microscopy of the negatively stained cells and thin sections was performed with a JEOL electron microscope as described previously (Hiraishi *et al.*, 1991, 1995b).

Photopigment analysis. A portion of washed cells was resuspended in 50 mM phosphate buffer (pH 7.0), sonicated for 3 min and centrifuged to remove unbroken cells and

large debris. Absorption spectra of the resultant cell extracts were recorded with a Shimadzu BioSpec 1600 spectrophotometer. BChl *a* was extracted from washed cells with acetone:methanol (7:2, v/v) and its concentration was estimated spectrophotometrically by using the absorption coefficient 75 mM^{–1} cm^{–1} at 770 nm (Clayton, 1963). Also, lipid extracts were analysed using silica-gel TLC and spectrochromatography as described previously (Hiraishi *et al.*, 1998). Mg-BChl *a*, Zn-BChl *a* and spirilloxanthin purified from *A. rubrum* ATCC 35905^T were used as the standard photopigments.

Quinone analysis. Quinones were extracted, fractionated and analysed by spectrochromatography as described previously (Hiraishi *et al.*, 1996).

Physiological and biochemical tests. Precultures grown in GNYS medium in darkness were used for all phenotypic tests. Effects of pH on growth were studied with GNYS medium modified by replacing half of the amount of gluconate with D-xylose. The pH of the medium was adjusted by adding 1 N NaOH or H₂SO₄. Carbon nutrition tests were performed with mineral medium RM2 supplemented with 0.05 % yeast extract as the basal medium, to which a neutralized, filter-sterilized carbon substrate was added. The concentration of carbon substrates used was 0.2 % unless otherwise noted. The test tubes were incubated for 2 weeks before the final reading and growth was estimated turbidimetrically by comparing the optical density, at 660 nm (OD₆₆₀), of the test tubes with that of the control tube without an added carbon source. The optical density in the test tubes was measured directly with a Pharmacia Novaspec spectrophotometer. All other physiological and biochemical tests were performed as described previously (Wakao *et al.*, 1994), GNYS medium being used as the basal medium (pH 5.5) in most cases.

DNA base composition and DNA–DNA hybridization. Genomic DNA was extracted and purified by the method of Marmur (1961). The DNA base composition (G + C content) was determined using the HPLC method (Katayama *et al.*, 1984; Hiraishi *et al.*, 1991). DNA–DNA hybridization studies were performed using the quantitative dot-blot hybridization method with biotin labelling and colorimetric detection as reported previously (Hiraishi *et al.*, 1991).

16S rDNA sequencing and phylogenetic analysis. The 16S rDNA fragments that corresponded to positions 8–1543 of *Escherichia coli* 16S rRNA (Brosius *et al.*, 1978) were amplified directly from cell lysates by the PCR as described previously (Hiraishi *et al.*, 1994). PCR products were purified by the PEG precipitation method (Hiraishi *et al.*, 1995a), sequenced directly with a SequiTherm Long-Read Cycle sequencing kit (Epicentre Technologies) and fluorescent primers and analysed with a Pharmacia DNA sequencer. PCR products were also subcloned with a SureClone Ligation kit (Pharmacia), according to the manufacturer's instructions, then sequenced. Sequence data were compiled with the GENETYX-MAC program (Software Development). Multiple alignment of sequence, calculation of the corrected evolutionary distance based on Kimura's two-parameter model (Kimura, 1980) and construction of a phylogenetic tree by the neighbour-joining method (Saitou & Nei, 1987) were performed with the CLUSTAL W program (Thompson *et al.*, 1994). Branching patterns of trees were evaluated by bootstrapping with 1000 resamplings (Felsenstein, 1985). Alignment positions with gaps and unidentified bases were excluded for the calculations. The tree was illustrated by using the TREEVIEW program (Page, 1997).

RESULTS

Enrichment and isolation

Test tubes containing either GNYS or GYS medium were inoculated with hot-spring water, hot-spring sediment or mine-drainage samples and incubated aerobically on a reciprocal shaker at 30 °C in darkness. While all GYS tubes gave negative results for pigmented growth, some GNYS tubes gave a pink colour after 1–4 weeks incubation. Subsequent transfers on to solidified media of the same composition resulted in the appearance of tiny pink or salmon-pink colonies, some of which were then purified after repeated streak culturing. Five pigmented isolates thus obtained were designated HS-AP3^T, HS-AP4, HS-AP16 and YO-704 (for their sources, see Methods).

Morphology and ultrastructure

Cells of the four isolates were cocci and coccobacilli measuring 0.7–0.9 µm in width and 1.0–1.6 µm in length (Fig. 1). They occurred singly or in pairs and reproduced by binary fission. Motile cells were not observed at any growth stage. Transmission electron microscopy revealed that negatively stained cells of the isolates had no flagella. The Gram reaction was negative.

Ultrathin-section electron microscopy indicated that cells of strain HS-AP3^T had the ultrastructure typical

Fig. 1. (a) Phase-contrast micrographs (bar, 10 µm) and (b) electron micrographs of negatively stained cells (bar, 1 µm), showing general cell morphology of strain HS-AP3^T.

Fig. 2. Thin-section electron micrographs of strain HS-AP3^T. Bar, 0.5 µm.

Fig. 3. Absorption spectrum of a sonicated cell extract from strain HS-AP3^T.

of the Gram-negative membrane (Fig. 2). We could not find any type of intracytoplasmic membrane systems in these cells. The cells frequently contained one or two electron-dense granules which might be polyphosphate granules.

Photopigment production

Spectroscopic experiments showed that the ultrasonic cell extracts of the isolates grown in darkness had absorption maxima at 474–476, 502–503, 545 (shoulder), 590 (shoulder), 801 and 873–874 nm (Fig. 3). Also, the acetone:methanol extracts had absorption maxima at 386, 466–467 (shoulder), 494–495, 525–526 and 770 nm (data not shown). These spectroscopic data indicate the presence of Mg-BChl *a* and carotenoids. The very low absorption peak at around 800 nm compared to the peak at 873–874 nm suggests

Fig. 4. Time-course of aerobic growth of strain HS-AP3^T in GNYS medium in the dark (filled circles) and in the light (open circles).

that the isolates contain the photosynthetic reaction centre and core light-harvesting complexes, but lack peripheral antenna complexes.

HPLC analyses of the acetone:methanol extracts demonstrated the presence of Mg-BChl *a* and spirilloxanthin as the main component of BChls and carotenoids, respectively. When the cells were grown in the presence of 1 mM zinc sulfate, an additional photopigment, Zn-BChl *a*, occurred in trace amounts (1–2% of the peak area of Mg-BChl *a*) (data not shown). The isolates also contained a considerable amount of a polar carotenoid component which hardly migrated on silica-gel TLC with benzene:acetone (1:1, v/v) as the developing solvent. The chemical structure of this carotenoid is possibly new and will be reported elsewhere.

Like the situation in many other species of aerobic BChl-containing bacteria (Shimada, 1995; Yurkov & Beatty, 1998), aerobic and dark conditions were most favourable for BChl production by the isolates. The BChl *a* content of the isolates ranged from 35 to 90 nmol (g dry wt cells)⁻¹. BChl production was repressed completely by continuous illumination.

Effects of light and pH on growth

The new isolates grew well under aerobic conditions with shaking but never exhibited anaerobic growth even if they were exposed to light. They showed a doubling time of 11–13 h when grown in GNYS medium in darkness. Continuous incandescent illumination stimulated aerobic growth of the isolates significantly, provided that the precultures were grown in darkness. The growth yield was 1.5- to 1.8-fold higher in illuminated cultures than in dark cultures (Fig. 4). For example, illuminated and dark cultures after 7 d incubation had an OD₆₆₀ of 1.05 and 0.70, respectively. Light-stimulated aerobic growth has been reported in some aerobic BChl-containing bacteria

Fig. 5. Effect of pH on growth of strain HS-AP3^T.

Table 1. Carbon-source utilization by the isolates

Symbols: ++, good growth ($0.5 > OD_{660}$); +, moderate growth ($OD_{660} = 0.1-0.5$); (+), poor growth ($OD_{660} = 0.05-0.1$); +/-, little, or ambiguous, growth; -, no growth. No strains grew with inositol, lactose, maltose, sucrose, butanol, methanol, propanol, acetate (0.05%), benzoate (0.05%), butyrate (0.05%), citrate, formate, propionate, alanine, asparagine, aspartate, glutamate or leucine.

Substrate	Growth response of:			
	HS-AP3 ^T	HS-AP4	HS-AP16	YO-704
Sugar/sugar alcohol				
L-Arabinose	+/-	(+)	(+)	+/-
D-Fructose	+/-	(+)	+/-	+/-
D-Galactose	+	++	(+)	+
D-Glucose	(+)	(+)	(+)	(+)
Glycerol	+	+	+	+
D-Mannitol	(+)	(+)	+/-	(+)
D-Mannose	-	+/-	+/-	-
D-Sorbitol	+	+	(+)	+
D-Xylose	+	+	+	+
Alcohol				
Ethanol	+	+	+	+
Organic acid				
Fumarate	+	+	+	+
Gluconate	++	++	++	++
Lactate	+	++	+	++
Malate	++	++	++	++
Pyruvate	++	++	++	++
Succinate	+	+	+	+
Amino acids and others				
Casamino acids	+/-	+/-	+/-	+/-
Peptone	+/-	+/-	+/-	+/-
Yeast extract	(+)	(+)	(+)	(+)

(Harashima *et al.*, 1987; Yurkov & Van Gernerden, 1993). Illumination also had positive effects on the viability of the isolates under starvation conditions, as is the case in other aerobic BChl-producing bacteria (Shiba, 1984).

Fig. 6. Distance-matrix tree showing phylogenetic affiliations of the new isolate HS-AP3^T, other aerobic and anaerobic photosynthetic bacteria and their non-photosynthetic relatives of the α -subclass of *Proteobacteria*, based on 16S rDNA sequences. *Roseateles depolymerans* (β -subclass) was used as a member of outgroups to root the tree. Bootstrap values obtained with 1000 bootstrap resamplings are given at branching points of interest. Anaerobic phototrophs are asterisked and non-photosynthetic relatives are shown in small letters. The DDBJ/EMBL/GenBank accession numbers of the 16S rDNA sequences used are shown in parentheses. Bar, 5 nucleotide substitutions per 100 nucleotides.

Table 2. DNA base composition of, and DNA–DNA relatedness between, the new isolates and related organisms

Test organism	DNA G + C content (mol %)	Hybridization (%) to labelled DNA from:	
		HS-AP3 ^T	<i>R. globiformis</i> DSM 161 ^T
Isolate			
HS-AP3 ^T	69.3	100	7
HS-AP4	69.1	83	4
HS-AP16	69.8	81	9
YO-704	69.6	93	6
<i>Rhodopila globiformis</i> DSM 161 ^T	65.1*	6	100
<i>Acidisphaera cryptum</i> ATCC 33463 ^T	67.1†	2	2
<i>Acidisphaera rubrum</i> ATCC 35905 ^T	63.6†	2	2

* Cited from Pfennig (1974).

† Cited from Wakao *et al.* (1994).

The four isolates grew in a pH range of 3.5 to 6.0 and failed to grow at pH 3.0 and 6.5. As an example, the growth response of strain HS-AP3^T to different pH conditions is shown in Fig. 5. The pH optimum was 4.5–5.0. Thus, the isolates were obligately acidophilic.

Physiological and biochemical characteristics

The new isolates were aerobic chemo-organotrophic acidophiles that had a respiratory-type of metabolism, with oxygen as the terminal electron acceptor. Anaerobic growth with nitrate was not found. Nitrate was not reduced to nitrite. Neither sulfide, elemental sulfur, thiosulfate nor Fe²⁺ served as the electron donor for

chemolithotrophic growth. The optimum temperature for growth was 30–35 °C. Growth occurred in the presence of 0–1 % NaCl and was inhibited by 3 % NaCl. Catalase and cytochrome oxidase were produced. Hydrolysis of starch, Tween 80 or casein was negative.

The results of carbon nutrition tests are shown in Table 1. All isolates grew well with gluconate, lactate, malate and pyruvate. Moderate growth was observed with xylose, galactose, glycerol, sorbitol, ethanol and some intermediates of the tricarboxylic acid cycle. Little or no growth occurred with lower fatty acids, citrate, benzoate, amino acids, peptone and Casamino acids.

Quinone composition

HPLC experiments indicated that ubiquinone-10 accounted for 98–99% of the total quinone contents of the new isolates. The remainder detected was ubiquinone-9. No other quinone ring groups, such as menaquinones or rhodoquinones, were detected.

Phylogenetic analyses

The phylogenetic positions of the new isolates were evaluated by studying the 16S rDNA sequence information. We determined nearly full-length 16S rDNA sequences from strains HS-AP3^T and YO-704 and *R. globiformis* DSM 161^T. Partial 16S rDNA sequences (approx. 700 bases) of strains HS-AP4 and HS-AP16 were also determined. The 16S rDNA sequences of the new isolates were identical in the compared regions. A neighbour-joining phylogenetic tree was constructed on the basis of the distance-matrix data of the test strains and several reference bacteria (Fig. 6). The new isolates clustered in a major acidophilic group of the *Proteobacteria* containing species of the genera *Acetobacter*, *Acidicella*, *Acidiphilium* and *Rhodopila*. The 16S rDNA sequences of the isolates were most similar to the sequence of *R. globiformis* at a level of sequence similarity of 95.0% (corrected distance = 0.0455).

The results of DNA base ratio determination and DNA–DNA pairing studies are shown in Table 2. The DNA base ratios of the new isolates ranged from 69.1 to 69.7 mol% and levels of DNA–DNA hybridization greater than 81% were found among them. These results, together with phenotypic data and 16S rDNA sequence information, indicate that the new isolates form a genetically coherent group at the species level.

DISCUSSION

The discovery of Zn-BChl *a* in the acidophilic bacteria of the genus *Acidiphilium* (Wakao *et al.*, 1996; Hiraishi *et al.*, 1998) has not only provided a new insight into bacterial photosynthesis but has also raised the question as to whether this pigment is essential for photosynthesis in such acidophilic bacteria as those capable of growing at pH 4.0 and below. Since the BChl dimers, called the special pair in the photosynthetic reaction centre, are located near the periplasmic side of the membrane, they may be affected by external pH. This may be a plausible reason why *Acidiphilium* species contain Zn-BChl *a* rather than Mg-BChl *a* as the main photopigment. The former pigment is much more stable than the latter under acidic conditions (Wako *et al.*, 1996; Kobayashi *et al.*, 1998). The above-noted question motivated us to survey phototrophic acidophiles other than *Acidiphilium* species in strongly acidic environments. As reported here, our surveys have resulted in the isolation of the aerobic phototrophic acidophiles that are able to grow in a pH range of 3.5–6.0, with pH optima at 4.5–5.0. However, unlike *Acidiphilium* species, the new

isolates do not produce Zn-BChl *a* but instead have Mg-BChl *a* as the major component. This finding not only indicates that there are major phenotypic differences between the new acidophilic isolates and members of the genus *Acidiphilium* but also suggests that the occurrence of Zn-BChl *a* is not always essential for photosynthesis in obligately acidophilic bacteria. However, since the isolates are not so strongly acidophilic as *Acidiphilium* species, the relationship between Zn-BChl-involved bacterial photosynthesis and acidic environments is still a major subject of study.

The phylogenetic analysis based on the 16S rDNA sequence information has demonstrated that the new isolates form a major cluster with members of some genera of aerobic and anaerobic phototrophic bacteria including *Acidiphilium*, *Craurococcus*, *Paracraurococcus*, *Roseococcus* and *Rhodopila*. Morphologically, our isolates are similar to species of the genera of aerobic phototrophic bacteria, *Craurococcus*, *Paracraurococcus* and *Roseococcus*. However, these established genera of aerobic BChl-containing bacteria are not acidophilic (Yurkov *et al.*, 1994; Saitoh *et al.*, 1998). In this respect, and in terms of some other phenotypic traits, our isolates are clearly distinguishable from the previously known genera showing coccoid forms (Table 3). The nearest phylogenetic relative to the new isolates is the anaerobic phototrophic bacterium *R. globiformis*. The isolates are similar to *R. globiformis* in terms of cell morphology and the preference for growth under acidic conditions. However, *R. globiformis* grows best under anaerobic/light conditions, whereas our isolates never grow anaerobically in the light. Also, there are major differences between the isolates and *R. globiformis* in chemotaxonomic characteristics, including quinone profiles and carotenoid composition.

Therefore, although the new isolates have a relatively close phylogenetic relationship to *R. globiformis*, it is our conclusion that the isolates should be classified into a new genus separate from the genus *Rhodopila* as well as from all established genera of the aerobic BChl-containing bacteria. We propose the name *Acidisphaera rubrifaciens* gen. nov., sp. nov. for the new isolates. The differential characteristics of *Acidisphaera* and phylogenetically related genera of phototrophic bacteria are shown in Table 3.

Description of *Acidisphaera* gen. nov.

Acidisphaera (A.ci.di.sphae'ra. M.L. n. *acidum* an acid; M.L. fem. n. *sphaera* a sphere; M.L. fem. n. *Acidisphaera* acid-requiring coccoid micro-organism).

Cells are non-motile cocci and coccobacilli. Non-spore-forming and unencapsulated. Gram-negative. Obligately aerobic chemo-organotroph and facultative photo-organotroph. Light stimulates aerobic growth and viability. Does not grow anaerobically in the light. Produces bacteriochlorophyll *a* and carotenoids. Mesophilic and obligately acidophilic. Grows between pH 3.5 and pH 6.0. No growth occurs in the presence

Table 3. Differential characteristics of *Acidisphaera* gen. nov. and phylogenetically related genera of phototrophic bacteria

Information on *Acidiphilium* is based on Kishimoto *et al.* (1995) and Hiraishi *et al.* (1998), information on *Craurococcus* and *Paracraurococcus* is based on Saitoh *et al.* (1998), information on *Roseococcus* is based on Yurkov *et al.* (1994) and information on *Rhodopila* is based on Pfennig (1974), Imhoff & Bias-Imhoff (1985) and this study.

Characteristic	<i>Acidisphaera</i> gen. nov.	<i>Acidiphilium</i>	<i>Craurococcus</i>	<i>Paracraurococcus</i>	<i>Roseococcus</i>	<i>Rhodopila</i>
Cell shape	Cocci, short rods	Rods	Cocci	Cocci	Cocci	Cocci
Cell diameter	0.7–0.9	0.5–0.7	0.8–2.0	0.8–1.5	0.9–1.3	1.6–1.8
Motility	—	+	—	—	+	+
Colour of colonies	Salmon-pink	Pink, red	Pink	Red	Pink	Purple-red
Zn-BChl as main pigment	—	+	—*	—*	—*	—*
Near IR peak for BChl <i>a</i>	874	864	872	856	855	865
Anaerobic phototrophy	—	—	—	—	—	+
Optimum pH for growth	4.5–5.0	3.0–3.5	7.5	6.6–6.8		4.8–5.0
Growth at pH 7	—	—	+	+		—
Quinone(s)	Q-10	Q-10	Q-10	Q-10	Q-10	Q-9(10), MK-9(10), RQ-9(10)
DNA G + C content (mol %)	69.1–69.8	62.9–68.3	70.5	70–71	70.4	66.3

* A. Hiraishi (unpublished data).

of 3 % NaCl. Catalase- and oxidase-positive. Grows with a number of simple organic compounds as electron donors and carbon sources. Growth on complex media containing peptone is poor. Neither sulfide, elemental sulfur, thiosulfate nor Fe²⁺ serve as the electron donor for chemolithotrophic growth. Ubiquinone-10 is present as the major respiratory quinone. The G + C content of the genomic DNA ranges from 69.1 to 69.8 mol %. 16S rDNA sequence information places the genus in the α -subclass of the *Proteobacteria*, with *Rhodopila* as its phylogenetic neighbour. The type species is *Acidisphaera rubrifaciens*.

Description of *Acidisphaera rubrifaciens* sp. nov.

Acidisphaera rubrifaciens (rub.ri.fa'ci.ens. L. adj. *ruber* red; L. v. *facio* to make; M.L. part. adj. *rubrifaciens* red-producing).

Cells are Gram-negative non-motile cocci or coccobacilli measuring 0.7–1.0 μ m wide and 0.8–1.2 μ m long. Any type of intracytoplasmic membrane system is absent. Polyphosphate granules are present. Colonies on solidified media are circular, smooth, convex, salmon-pink to red and reach 2 mm in diameter after 1 week incubation. Cell membranes have absorption maxima at 474–476, 502–503, 545, 590, 801 and 873–874 nm. Acetone:methanol extracts from cells have absorption maxima at 386, 466–467, 494–495, 525–526 and 770 nm. Spirilloxanthin and an unknown polar pigment are the major carotenoids. The temperature range for growth is 20–40 °C (optimum 30–35 °C). The pH optimum for growth is 4.5–5.0. Nitrate is not reduced to nitrite. Hydrolysis of starch,

casein or Tween 80 is absent. Gluconate, lactate, malate and pyruvate are good carbon sources. Other carbon sources that support growth are galactose, glucose, D-xylose, glycerol, mannitol, sorbitol, fumarate, malate, succinate and yeast extract. Little or no growth occurs with L-arabinose, fructose, mannose, lactose, sucrose, inositol, butanol, ethanol, methanol, propanol, formate, acetate, propionate, butyrate, citrate, benzoate, Casamino acids and peptone. The G + C content of the genomic DNA ranges from 69.1 to 69.8 mol %. The habitats are strongly acidic mineral environments, including acidic hot springs and pyritic mine drainage. The type strain is strain HS-AP3^T, which has been deposited with the Japan Collection of Microorganisms, RIKEN, Wako, Japan, as JCM 10600^T.

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