Thauera selenatis gen. nov., sp. nov., a Member of the Beta Subclass of *Proteobacteria* with a Novel Type of Anaerobic Respiration

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A recently isolated, selenate-respiring microorganism (strain AX^T [T = type strain]) was classified by using a polyphasic approach in which both genotypic and phenotypic characteristics were determined. Strain AX^T is a motile, gram-negative, rod-shaped organism with a single polar flagellum. On the basis of phenotypic characteristics, this organism can be classified as a *Pseudomonas* sp. However, a comparison of the 16S rRNA sequence of strain AX^T with the sequences of other organisms indicated that strain AX^T is most similar to members of the beta subclass (level of similarity, 86.8%) rather than to members of the gamma subclass (level of similarity, 80.2%) of the *Proteobacteria*. The presence of the specific polyamine 2-hydroxyputrescine and the presence of a ubiquinone with eight isoprenoid units in the side chain (ubiquinone Q-8) excluded strain AX^T from the authentic genus *Pseudomonas* and allowed placement in the beta subclass of the *Proteobacteria*. Within the beta subclass, strain AX^T is related to *Iodobacter fluvatile*. The phylogenetic distance (level of similarity, less than 90%), as well as a lack of common phenotypic characteristics between these organisms, prevents classification of strain AX^T as a member of the genus *Iodobacter*. In addition, strain AX^T possesses a unique mechanism for anaerobic respiration, which allows it to utilize selenate as an electron acceptor without interference by nitrate. Therefore, we propose that strain AX^T should be the first member of a new genus and species, *Thauera selenatis*.

In the past, gram-negative, aerobic, rod-shaped bacteria with polar flagella have frequently been assigned to the genus *Pseudomonas*. The genetic heterogeneity of this genus became apparent when levels of rRNA cistron similarity were determined, which revealed the presence of several clusters of related organisms (28). These results were later confirmed by the results of the extensive DNA-DNA and DNA-rRNA hybridization studies of De Vos et al. (11–14) and by 16S rRNA cataloging results (41, 43), which revealed that members of the genus *Pseudomonas* branch off within the alpha, beta, and gamma subclasses of the *Proteobacteria* (34). Only the members of the *Pseudomonas fluorescens* branch in the gamma subclass represent the authentic pseudomonads. As pointed out by Willems et al. (36), all of the other *Pseudomonas* species are considered to be misclassified. Chemotaxonomy provides a rapid alternative approach for excluding organisms from or including organisms in the authentic pseudomonad taxon on the basis of the results of fatty acid, ubiquinone, and polyamine analyses (1, 2, 8–10, 27). Below, those organisms that have the genus name *Pseudomonas*, but are not part of the phylogenetic nucleus of the true pseudomonad taxon (see above) are indicated by enclosing the genus name in single quotation marks (e.g., '*Pseudomonas*' *cepacia*).

In a polyphasic approach to bacterial systematics, genotypic and phenotypic properties are combined to delineate taxa, and it was not until recently that several species of the obviously heterogeneous taxon *Pseudomonas* were either

placed in other genera (e.g., the genera *Xanthomonas* [35] and *Comamonas* [13]) or elevated to genus rank (e.g., the genera *Sphingomonas* [44], *Hydrogenophaga* [36], and *Acidovorax* [38]) by using this approach.

The same polyphasic strategy was used in this study to classify an organism that was tentatively and incorrectly classified as a *Pseudomonas* strain (22). Only one strain of this organism has been isolated, and this strain has the unique characteristic of reducing selenate to selenite by anaerobic respiration.

MATERIALS AND METHODS

Media and growth conditions. The media which were used (defined minimal medium and minimal medium containing 0.4% yeast extract) have been described previously (22). Strain AX^T (T = type strain) was grown as described previously (22). This organism has been deposited with the American Type Culture Collection as strain ATCC 55363^T.

Bacterial strains tested for anaerobic growth with selenate or nitrate. Strains were obtained from the American Type Culture Collection (ATCC) and from the Culture Collection of the Department of Microbiology, University of Queensland, and were tested for the ability to grow by using selenate or nitrate for respiration in anaerobic ATCC media (16) containing 5 or 10 mM selenate or 10 mM nitrate (22). The following bacterial strains were obtained from the ATCC: *Comamonas terrigena* ATCC 8461 (tested in ATCC medium 386 containing glucose [final concentration, 2 g/liter]), *Janthinobacterium lividum* ATCC 12473 (tested in ATCC medium 3 containing glucose [final concentration, 2 g/liter]), *Hydrogenophaga flava* ATCC 33667 (tested in ATCC medium 1246 containing gluconate [final concentration, 2 g/liter]), *Chromobacterium violaceum* ATCC 12472

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(tested in ATCC medium 3 containing acetate [final concentration, 1.4 g/liter]), *Iodobacter fluviatilis* ATCC 33051 (tested in ATCC medium 3 containing acetate [final concentration, 1.4 g/liter]), '*Pseudomonas*' *cepacia* ATCC 25416 (tested in ATCC medium 3 containing acetate [final concentration, 1.4 g/liter]), *Alcaligenes faecalis* ATCC 8750 (tested in ATCC medium 3 containing gluconate [final concentration, 2 g/liter]), and *Alcaligenes xylosoxydans* subsp. *xylosoxydans* ATCC 27061 (tested in ATCC medium 3 containing gluconate [final concentration, 2 g/liter]). The bacterial strains obtained from the Culture Collection of the Department of Microbiology, University of Queensland, were '*Pseudomonas*' *mixta* UQM 1762 (= ATCC 49108) (tested in ATCC medium 416 containing sucrose [final concentration, 20 g/liter]), *Acidovorax facilis* UQM 1918 (= ATCC 11228) (tested in ATCC medium 72 containing glucose [final concentration, 2 g/liter]), *Variovorax paradoxus* UQM 1905 (= ATCC 17713) (tested in ATCC medium 3 containing acetate [final concentration, 1.4 g/liter]), and *Derxia gumosa* UQM 2144 (= ATCC 15994) (tested in ATCC medium 165 containing glucose [final concentration, 2 g/liter]).

Phenotypic characterization. The presence of oxidase, catalase, arginine dihydrolase, and lysine decarboxylase and the ability to hydrolyze starch and gelatin were determined as described by Smibert and Krieg (31). The presence of a diffusible, fluorescent pigment was determined by using King media A and B (28). The denitrification test was carried out in anaerobic minimal medium containing 0.4% yeast extract, 20 mM nitrate, 20 mM acetate, and a Durham tube; growth and gas production were recorded within 48 h after inoculation (31). The nature of the gas produced during denitrification was determined by using the method of Jennehan et al. (18). Strain AX^T was grown for 48 h in an anaerobic chamber (Coy Laboratory Products, Inc., Ann Arbor, Mich.) on minimal medium containing 2% agar, 20 mM acetate, 40 mM nitrate, and 0.0001% resazurin. The production of N₂O was detected by the formation of a pink color around the colonies, which was due to oxidation of the resazurin by N₂O; N₂ production does not cause the oxidation of resazurin.

The ability of strain AX^T to use various carbon sources was tested on aerobic minimal medium containing 2% agar as described below. All substrates were prepared as 2 M solutions or as saturated solutions (0.18 to 1.4 M) and were sterilized by filtration through sterile Acro Disc filters (pore size, 0.2 µm; diameter, 25 mm; Gelman Sciences). Sterile Whatman no. 1 filter discs (diameter, 7 mm) were saturated with each carbon source and were dropped onto a lawn of strain AX^T spread on minimal agar medium containing no carbon source. The substrate diffused into the agar, and the cells grew at all concentrations up to the highest concentration that they could tolerate. Three discs were placed on each plate at equal distances from each other. To prepare the lawn of strain AX^T, the organism was grown overnight aerobically in minimal medium broth supplemented with 10 mM acetate. The culture was harvested by centrifugation at 8000 × g for 30 min and washed once. The cells were suspended in 0.1 volume of minimal medium, and a 0.1-ml portion was spread onto each minimal medium agar plate. The plates were incubated at 28°C and were examined each day by using a dissecting microscope during an incubation period of at least 7 days. On these plates the organisms formed haloes around the discs and growth was classified as good, moderate, or poor on the basis of the width and thickness of these haloes.

Growth on H₂-O₂-CO₂ was determined in 10-ml portions

of minimal medium broth in closed 20-ml serum bottles; 10 ml of H₂ and 10 ml of air were added to the bottle after inoculation, and CO₂ was supplied by the HCO₃⁻ present in the medium (22). Growth on methanol, isobutyrate, and propionate was tested in 10-ml portions of minimal medium broth in closed roll tubes under a 100% N₂ atmosphere (22). The following concentrations were used: methanol, 1 and 3 mM; isobutyrate, 5 mM; propionate, 5 mM.

The agar method described above, which was used to test the range of substrates that could support growth of strain AX^T, was used for the following reasons: (i) with certain substrates, cells tended to lyse after growth ceased, and therefore, it was desirable not to do the tests in broth cultures; and (ii) neither the optimum concentrations nor the inhibitory concentrations of the substrates were known, and therefore, use of a concentration gradient permitted the organism to grow at the concentrations that best suited it.

DNA base composition. DNA was extracted and the melting temperature was determined by using the method of Nelson et al. (25), with some modifications. Strain AX^T was grown aerobically on a shaker in 100 ml of minimal medium containing 0.4% yeast extract, 20 mM nitrate, and 20 mM acetate. After 24 h of incubation at 28°C, 1 g (wet weight) of cells was collected by centrifugation at 10,000 × g for 15 min. DNAs isolated from *Escherichia coli* and *Pseudomonas putida* were used as controls; both organisms were grown in nutrient broth (Difco) at 37°C, and after 24 h of incubation, 2 g (wet weight) of cells was harvested by centrifugation at 10,000 × g for 15 min. All of the cells were broken by using a French press (16,000 lb/in²; Aminco Chance, Silver Spring, Md.). DNA from each resulting cell extract was isolated, and the melting temperature was determined and used to calculate the moles percent G+C content as described by Nelson et al. (25).

Scanning electron microscopy. Strain AX^T was grown at 28°C for 6 to 12 h in minimal medium supplemented with 10 mM acetate and 10 mM nitrate. The cells either were filtered onto Nuclepore filters (pore size, 0.4 µm) and fixed in Sorenson buffer (17) containing 2.5% glutaraldehyde or were fixed by adding 2.5% glutaraldehyde to the medium and then filtered onto a Nuclepore filter. The fixed and filtered cells were washed in Sorenson buffer, dehydrated three times in a series consisting of increasing ethanol concentrations in water (50, 75, 95, and 100% [vol/vol] ethanol), critical point dried, mounted on a stub, gold coated in a sputter coater, and then viewed in the first stage of a model ISI DS130 scanning electron microscope (Topcon, Pleasanton, Calif.).

Phylogenetic analysis. All of the steps involved in sequence analysis of 16S ribosomal DNA, including isolation of genomic DNA, amplification of 16S ribosomal DNA, purification of polymerase chain reaction products, and subsequent sequence analysis of these products have been described in detail previously (29). A partial sequence consisting of 1,346 nucleotides was aligned with 57 homologous sequences from members of the alpha, beta, and gamma subclasses of the *Proteobacteria* (5a, 15, 24, 26). Positions 1 through 31 and 1376 through 1542 (*E. coli* nomenclature [42]) were omitted from the analysis. In order to include restricted information for about 150 nucleotides of the 16S rRNAs from additional members of the beta subclass of the *Proteobacteria* (6, 9), all of the sequences of the reference organisms were shortened to the same length in an additional comparison. Equally weighted (Hamming distances) and percent similarities between each pair of sequences were calculated. The phylogenetic analysis was carried out by using the neighborliness method (30). The

TABLE 1. Percent similarity between one 1,358-nucleotide-long stretch of the 16S ribosomal DNA sequences of strain AX^T and other members of the beta subclass of the *Proteobacteria* (8) (above the diagonal) and equally weighted (Hamming) distances between the 16S ribosomal DNA sequences (below the diagonal)

Organism	Strain AX ^T	<i>Iodobacter fluviatilis</i>	<i>Chromobacterium violaceum</i>	<i>Vitreoscilla stercoraria</i>	<i>Neisseria gonorrhoeae</i>	<i>Kingella denitrificans</i>	<i>Kingella kingae</i>	<i>Eikenella corrodens</i>	<i>Comamonas testosteroni</i>	' <i>Pseudomonas</i> ' <i>cepacia</i>	<i>Alcaligenes xylosoxydans</i>	<i>Alcaligenes faecalis</i>	<i>Escherichia coli</i>	<i>Pseudomonas aeruginosa</i>
Strain AX ^T		88.7	87.7	86.3	85.4	87.6	85.6	87.0	85.1	87.6	86.2	85.5	79.1	83.6
<i>Iodobacter fluviatilis</i>	154		90.3	88.0	86.7	88.1	87.2	88.1	87.9	88.7	89.2	87.9	80.7	83.7
<i>Chromobacterium violaceum</i>	167	132		89.3	88.1	91.2	89.5	91.5	86.5	88.2	88.4	86.8	80.0	82.6
<i>Vitreoscilla stercoraria</i>	186	163	145		91.4	90.6	90.1	91.8	85.2	86.5	86.5	86.8	81.9	82.0
<i>Neisseria gonorrhoeae</i>	198	181	162	117		91.9	91.5	91.7	82.8	85.8	86.2	85.3	80.7	81.2
<i>Kingella denitrificans</i>	168	161	119	128	110		93.9	95.8	85.0	86.9	86.7	87.3	81.5	82.8
<i>Kingella kingae</i>	196	174	143	135	116	83		93.3	83.9	86.5	85.8	85.9	79.7	80.9
<i>Eikenella corrodens</i>	177	162	116	112	113	58	92		84.8	86.7	87.0	87.4	80.3	82.0
<i>Comamonas testosteroni</i>	203	164	183	201	234	205	219	207		87.3	87.7	85.6	80.1	81.7
' <i>Pseudomonas</i> ' <i>cepacia</i>	169	154	160	183	193	178	184	180	172		89.2	88.5	79.2	82.3
<i>Alcaligenes xylosoxydans</i>	187	146	158	184	188	181	193	177	167	147		92.4	79.5	81.6
<i>Alcaligenes faecalis</i>	197	164	179	179	200	173	192	171	196	156	103		80.2	81.1
<i>Escherichia coli</i>	284	262	272	246	262	251	276	267	270	283	278	269		82.5
<i>Pseudomonas aeruginosa</i>	223	221	236	244	255	233	260	244	249	240	250	256	237	

algorithm was implemented as part of the program package SAGE (Technoma GmbH, Heidelberg, Germany).

Chemotaxonomic analysis. Quinones were extracted and analyzed as described by Auling et al. (3). Extraction, analysis, and quantification of polyamines were carried out as described by Busse and Auling (10).

Nucleotide sequence accession number. The rRNA sequence of strain AX^T has been deposited in the EMBL data bank under accession number X68491.

RESULTS AND DISCUSSION

Phylogeny. The average similarity values for the 16S rRNA sequences of strain AX^T and representatives of the alpha, beta, and gamma subclasses of the *Proteobacteria* range from 73.0 to 76.8% (average, 75.4%), 85.1 to 88.7% (average, 86.8%), and 77.5 to 82.2% (average, 80.2%), respectively. Consequently, strain AX^T can be considered a member of the beta subclass (Table 1). This placement is supported by the findings that the polyamines 2-hydroxyputrescine and putrescine, as well as ubiquinone Q-8, are present in strain AX^T cells (Table 2). 2-Hydroxyputrescine is the specific polyamine of the beta subclass of the *Proteobacteria* (10). Ubiquinone Q-8 is the major quinone compound in members of the beta subclass of the *Proteobacteria* (9), whereas ubiquinone Q-9 is characteristic of the authentic pseudomonads (8).

A phylogenetic analysis was carried out between strain AX^T and members of the beta subclass of the *Proteobacteria* for which 16S rRNA and DNA data are listed in the Ribosome RNA Database Project, as well as '*Pseudomonas*' *mixta*, *Pseudomonas chitinolytica*, *Pseudomonas pickettii*, *Pseudomonas solanacearum*, *Pseudomonas andropogonis*, *Pseudomonas caryophylli*, and *Pseudomonas gladioli* (unpublished data). The phylogenetic distance between strain AX^T and each of these organisms, including representatives of the genera *Rhodocyclus*, *Alcaligenes*, *Chromobacterium*, *Iodobacter*, *Spirillum*, *Neisseria*, *Eikenella*, *Kingella*, *Simonsiella*, and *Vitreoscilla*, nonauthentic pseudomonads, and ammonium-oxidizing organisms, was less than 90%. Strain

TABLE 2. Biochemical and physiological characteristics of *T. selenatis*^a

Characteristic	<i>T. selenatis</i>
Quinone.....	Ubiquinone Q-8
Polyamines ^b	2-Hydroxyputrescine, putrescine
Catalase.....	+
Oxidase.....	+
Arginine dehydrolase.....	-
Lysine decarboxylase.....	-
H ₂ Autotrophy.....	+
Denitrification to N ₂ O.....	+
Selenate respiration.....	+
Poly-β-hydroxybutyrate accumulation.....	+
N ₂ fixation.....	-
Hydrolysis of:	
Gelatin.....	-
Starch.....	-
Growth factors required ^c	+

^a In carbon source utilization tests performed on minimal medium containing 2% agar (see text), good growth occurred with the following carbon sources: acetate, alanine, arginine, aspartate, benzoate, D-amino-N-butyrate, 3-hydroxybutyrate, isobutyrate, citrate, fructose, glutamate, lactate, lactose, proline, propionate, pyruvate, serine, succinate, sucrose, Tween 40, Tween 80, and H₂-O₂-CO₂. Moderate growth occurred with *p*-anisate, asparagine, ferulate, fucose, hydroxyproline, lactulose, leucine, maltose, raffinose, phenylacetate, veratrate, and glucose. Poor growth occurred with *cis*-aconitate, *p*-aminobenzoate, arabinose, erythritol, formate, galactose, D-galacturonate, gentiobiose, DL-α-glycerophosphate, histidine, hydroxycinnamate, *m*-inositol, mannose, 3-phenylpropanoate, phenoxyacetate, phenylalanine, rhamnose, sorbitol, trehalose, turanose, and vanillin. And no growth occurred with L-alanylglycine, *m*-anisate, catechol, ethyl vanillin, α-Ketoglutarate, glycerol, *o*-methoxyphenoxyacetate, and methanol.

^b The concentrations of the polyamines were as follows: 2-hydroxyputrescine, 33.1 μmol/mg (dry weight) of cells; putrescine, 68.9 μmol/mg (dry weight) of cells.

^c The vitamins added to support growth were folic acid, riboflavin, thiamine hydrochloride, pantothenate, niacinamide, vitamin B₁₂, biotin, pyridoxine hydrochloride, and *p*-aminobenzoate (22).

TABLE 3. Characteristics that differentiate *Thauera selenatis* gen. nov., sp. nov., from taxa that have similar DNA base compositions and belong to the beta subclass of the *Proteobacteria*

Characteristic	' <i>Pseudomonas</i> ' <i>mixta</i> ^a	<i>Pseudomonas solanacearum</i> rRNA branch ^b	<i>Comamonas</i> ^c	<i>Acidovorax</i> ^c	<i>Hydrogenophaga</i> ^c	<i>Variovorax</i> ^c
Cell morphology	Rods	Rods	Rods or spirilla	Rods	Rods	Rods
Flagellation						
Polar	+	+	—	+	+	—
Lateral	+	—	—	—	—	—
Bipolar tufts	—	—	+	—	—	—
Peritrichous	—	—	—	—	—	+
Yellow nondiffusible pigment	—	—	—	—	+	+
Starch hydrolysis	+	D ⁱ	—	—	—	—
Occurrence ^f	S	S, FW, CS, IP	S, FW, CS, IP	S, FW, CS	S, FW	S, FW
Growth on nutrient agar	—	+	+	+	+	+
Tolerance to 1.5% NaCl	—	+	+	d	+	+
Chemolithic growth on H ₂	—	—	—	d	+	d
Nitrogen fixation	—	—	—	—	—	—
Denitrification	—	D	—	D	+ ^m	— ^m
Selenate respiration ^m	—	ND ⁿ	—	—	—	—
Oxidative metabolism	+	+	+	+	+	+
Utilization of:						
D-Glucose	+	+	—	+	+	+
Lactose	d	—	—	—	—	—
Inulin	+	—	—	—	—	—
Acetate	—	+	+	+	+	+
Propionate	—	+	+	d	d	d
Isobutyrate	—	+ ^o	+	d	—	d
meso-Inositol	—	+ ^p	d	—	+	d
Sorbitol	—	+ ^p	—	d	+	+
DL-4-Aminobutyrate	—	+	d	+	—	+
β-Alanine	—	+	d	+	—	+
L-Proline	—	+	+	+	+	+
G+C content (mol%)	67–70	64–70	60–69	62–66	67–69	65–69

^a Data from reference 5.^b Data from reference 28.^c Data from references 28 and 36 through 40.^d Data from reference 4.^e Data from references 32 and 33.^f Data from reference 20 (including data for *Aquaspirillum anulus*, *Aquaspirillum delicatus*, *Aquaspirillum giesbergeri*, *Aquaspirillum gracile*, *Aquaspirillum metamorphum*, *Aquaspirillum psychrophilum*, and *Aquaspirillum sinosum*).^g Data from references 21 and 23.^h Data from references 7 and 19.ⁱ +, positive; —, negative; D, variable among the species of a genus; d, 11 to 89% of the strains are positive; (w), weak.^j Produces violacein.^k Colonies are brownish on minimal agar medium containing 0.4% yeast extract.^l Abbreviations: S, soil; FW, fresh water; CS, clinical samples; IP, infected plants.^m Results for the type species only.ⁿ ND, not determined (pathogens not available from the ATCC in accordance with a U.S. Department of Agriculture decision).^o Isobutyrate was not utilized by *Pseudomonas caryophylli*.^p meso-Inositol and sorbitol were not utilized by *Pseudomonas mallei*.

AX^T exhibits the highest degree of relatedness with *I. fluviatilis* ATCC 33051 (88.7%). This relationship is confirmed by the position of the new isolate in the phylogenetic tree (Fig. 1). The phylogenetic distance between strain AX^T and *I. fluviatilis* (level of similarity, less than 90%) and the lack of common phenotypic characteristics (Table 3) indicate that these two organisms are not members of the same genus. The branching order of the reference strains is almost identical to the topology of the tree published previously (15).

Although strain AX^T is *Pseudomonas*-like in morphology and many phenotypic characteristics, no close genomic relatedness exists between this strain and the true pseudomonads (i.e., organisms belonging to the gamma subclass) or even between this strain and '*Pseudomonas*' *cepacia* (a member of the beta subclass and not a true pseudomonad). Also, the results of an analysis of a short stretch of the 16S rRNAs from 29 members of the beta subclass, as well as *Pseudomonas aeruginosa*, '*Pseudo-*

monas' *mixta*, a number of *Pseudomonas* isolates (9), and members of the genus *Hydrogenophaga* (formerly members of the genus *Pseudomonas*) (6), support the conclusion that strain AX^T should not be considered a member of one of the several subgroups of nonauthentic *Pseudomonas* species (data not shown).

Growth characteristics. Strain AX^T grew in minimal medium only when it was supplemented with vitamins (Table 2). This organism can grow aerobically or anaerobically with nitrate or selenate as the terminal electron acceptor (22). The generation time for growth in minimal medium broth for all electron acceptors was about 170 min.

Colonies on minimal medium containing 2% agar after 5 days of aerobic growth at 28°C were small (diameter, 0.75 mm), round with entire edges, and convex. The colonies were light brown and adhered to the agar. Colonies grown aerobically on agar supplemented with 0.4% yeast extract were 1.1 mm in diameter after 3 days of incubation.

TABLE 3—Continued

<i>Xylophilus</i> ^c	<i>Derxia</i> ^d	<i>Janthinobacterium</i> ^e	<i>Aquaspirillum</i> ^f	<i>Chromobacterium violaceum</i> ^e	<i>I. fluviatile</i> ^g	<i>Alcaligenes faecalis</i> and <i>Alcaligenes xylosoxydans</i> ^h	<i>T. selenatis</i>
Rods	Rods	Rods	Helices (clockwise)	Rods	Rods	Rods and cocci	Rods
+	+	+	—	+	+	—	+
—	—	+	—	+	+	—	—
—	—	—	+	—	—	—	—
—	—	—	—	—	—	+	—
+	—	—	—	—	—	—	+ ^k
d ⁱ	—	—	—	—(w) ⁱ	—	—	—
IP	S	S, FW	FW	S, FW	FW	S, FW, CS	FW
—	—	+	—	+	+	+	—
—	—	+	—	+	+	—	+
—	+	—	—	—	—	—	+
—	+	—	—	—	—	—	—
—	— ^m	d	—	— ^m	— ^m	+	+
+	+	—	—	—	—	—	+
+	+	+	+	D	—	+	+
+	+	+	D	—	—	D	+
—	—	—	—	—	—	—	+
—	—	d	d	d	—	+	+
—	—	—	—	—	—	+	+
—	—	—	—	—	—	D	+
—	—	+	—	—	—	—	+ (w)
—	+	+	—	—	—	—	+ (w)
—	—	—	—	+	—	D	+
—	—	—	—	d	—	D	+
—	—	—	—	—	—	+	+
68–69	69–72	61–67	D 57–65	65–68	50–52	56–70	66

The organism was able to grow on many different carbon sources and also chemolithotrophically with H₂-CO₂-O₂ (air) (Table 3). When strain AX^T was grown with acetate, poly-β-hydroxybutyrate was accumulated (Table 2) (22).

Morphological characteristics. The cells were gram negative and rod shaped (1.4 by 0.56 μm) with rounded ends (Fig. 2). The cells were motile by means of a single polar flagellum (Fig. 2).

Biochemical and physiological characteristics. The bio-

chemical and physiological characteristics of strain AX^T are shown in Tables 2 and 3.

Taxonomic position. The polyphasic characterization of strain AX^T showed that this organism could not be assigned to any currently recognized genus on the basis of phenotypic characteristics and the results of a phylogenetic analysis of 16S rRNA sequences. In addition, this organism possesses a unique mechanism for anaerobic respiration, because it utilizes selenate (SeO₄²⁻; Se^{VI}) as the terminal electron acceptor.

Strain AX^T is the only member of a single line of descent within the beta subclass of the *Proteobacteria* and can be differentiated phenotypically from taxa with similar DNA base compositions (Table 3), including its closest phylogenetic relatives, *I. fluviatile* and *Chromobacterium violaceum* (Fig. 1 and Table 3). None of the organisms tested that belonged to the beta subclass of the *Proteobacteria* was able to grow anaerobically by using SeO₄²⁻ as the terminal electron acceptor (Table 3).

Consequently, we propose that strain AX^T should be the first member of a new genus and new species, *Thauera selenatis*.

Description of the genus *Thauera* gen. nov. *Thauera* (Thau'e.ra. N. L. fem. n. *Thauera*, in honor of R. Thauer). Gram-negative, rod-shaped cells are 1.4 μm long by 0.56 μm wide, have rounded ends, and contain poly-β-hydroxybutyrate inclusions. Cells are motile, and each cell has a single polar flagellum. Oxidative metabolism. Contains ubiquinone Q-8. Grows aerobically in defined media containing acetate as the carbon source and electron donor. Under anaerobic conditions exhibits anaerobic respiration with selenate or

FIG. 1. Phylogenetic position of strain AX^T among members of the beta subclass of the *Proteobacteria*. Bar = 20 nucleotide exchanges.

FIG. 2. Scanning electron micrograph of strain AX^T grown anaerobically in minimal medium supplemented with 10 mM acetate and 10 mM nitrate. Bar = 1 μ m.

nitrate as the electron acceptor. Nitrate is reduced to N₂O. Selenate is reduced to selenite. Acetate is metabolized to CO₂. Growth is also supported by the carbon and energy sources alanine, arginine, aspartate, benzoate, D-amino-*N*-butyrate, 3-hydroxybutyrate, isobutyrate, citrate, fructose, glutamate, lactate, lactose, proline, propionate, pyruvate, serine, succinate, and sucrose. Less vigorous growth occurs with glucose. No growth occurs on nutrient agar (Difco). Vitamins are required for growth in defined media. Colonies are small, round with entire edges, convex, adherent, and light brown. Optimal growth occurs at 25 to 30°C. The optimal pH for growth with nitrate is pH 8, and the optimal pH for growth with selenate is pH 7. Grows autotrophically with hydrogen, carbon dioxide, and air. Unable to fix dinitrogen. Catalase and oxidase positive. Arginine dihydrolase is absent. The major polyamines are putrescine and 2-hydroxyputrescine. Phylogenetically, the genus is a member of the beta subclass of the *Proteobacteria*. The base composition of the DNA of the type strain of the type species is 66 mol% G+C (as determined by the thermal denaturation method). The type species is *Thauera selenatis*.

Description of *Thauera selenatis* sp. nov. *Thauera selenatis* (se.le.na'tis. N.L. chem. term *selenas*, selenate; N.L. gen. n. *selenatis*, of selenate, because the organism reduces selenate to selenite). In addition to the characteristics given above in the genus description, *T. selenatis* has the characteristics described below. Colonies on defined medium containing acetate are 0.75 mm in diameter after 5 days of aerobic growth at 28°C. On agar supplemented with 0.4% yeast extract colonies are 1.1 mm in diameter after 3 days. Growth occurs at 4°C but not at 39°C. Does not hydrolyze gelatin or starch. The DNA base composition of the type

strain is 66 mol% G+C (as determined by the thermal denaturation method). Other characteristics are shown in Tables 2 and 3.

The type strain is strain AX (= ATCC 55363), which was isolated from selenium-contaminated drainage water in the San Joaquin Valley of California.

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