Transfer of the Nitrogen-Fixing Hydrogen Bacterium *Corynebacterium autotrophicum* Baumgarten et al. to *Xanthobacter* gen. nov.

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Thirty-five nitrogen-fixing, hydrogen-oxidizing bacteria, all members of *Corynebacterium autotrophicum* Baumgarten, Reh, and Schlegel 1974, and including the type strain of this species, were compared with 28 strains of coryneform bacteria by numerical taxonomy methods. The nitrogen-fixing hydrogen bacteria formed a well defined cluster; their similarity to the other strains tested was low, however. Furthermore, the chemotaxonomic characters of these strains excluded them from the coryneform bacteria. Therefore *C. autotrophicum* was reclassified, following the keys of *Bergey's Manual of Determinative Bacteriology* (8th ed.) as a species of the family *Azotobacteraceae*. Although taxonomically close to *Beijerinckia* and *Derrxia*, the nitrogen-fixing, hydrogen-oxidizing bacteria are regarded as representing a new genus, for which we propose the name *Xanthobacter*. *C. autotrophicum* Baumgarten, Reh, and Schlegel is transferred to the new genus as *Xanthobacter autotrophicus* (Baumgarten et al.) comb. nov.

From 1969 to 1974, several coryneform, yellow-pigmented, autotrophic, hydrogen-oxidizing bacteria were described (9, 10, 24, 28); they were placed in a new species—*Corynebacterium autotrophicum*—by Baumgarten et al. (3). All of these strains fixed nitrogen under microaerobic conditions (12, 30), and the physiology and efficiency of their nitrogen fixation have been studied (4).

After isolating many new strains of nitrogen-fixing hydrogen bacteria (30), we compared them with a number of well described coryneform bacteria by the method of numerical taxonomy. Since the nitrogen-fixing hydrogen bacteria formed a uniform cluster but had a very low similarity to the corynebacteria *sensu stricto* used in this investigation, a reclassification of *C. autotrophicum* appeared to be indicated. Additional biochemical properties of these organisms had to be determined before the organisms could be assigned to a gram-positive or a gram-negative taxon with confidence, for the evaluation of the Gram stain reaction, which differed from investigator to investigator, was the critical point in earlier descriptions of these organisms.

MATERIALS AND METHODS

Bacterial strains. The designations and sources of the bacterial strains used in this study are listed in Table 1. *C. autotrophicum* 7C SF is a slime-free mutant of strain 7C (1).

Growth media. The strains were maintained on nutrient broth medium (Difco) containing 0.2% succinate and 1.3% agar (Difco). For the taxonomic tests

the strains were grown on the following mineral medium: 1.95 g of KH_2PO_4 , 5.7 g of K_2HPO_4 , 3.0 g of $(\text{NH}_4)_2\text{SO}_4$, 0.2 g of $\text{MgSO}_4 \cdot 7 \text{H}_2\text{O}$, 0.04 g of $\text{CaCl}_2 \cdot 2 \text{H}_2\text{O}$, 0.003 g of FeCl_3 , 13 g of agar (Difco), 2 to 5 g of the carbon source, 1 ml of trace element solution (20), 10 ml of vitamin solution (21), and distilled water to 1,000 ml. For autotrophic growth the mineral medium of Schlegel et al. (22) was used; the cultures were incubated under an atmosphere of 10% O_2 , 10% CO_2 , and 80% H_2 . For growth on nitrogen-free medium, the N source in the mineral medium was omitted; the cultures were incubated under an atmosphere of 5% O_2 , 10% CO_2 , 10% H_2 , and 75% N_2 (nitrogen fixation under autotrophic growth conditions) or of 25% air and 75% N_2 in the presence of 0.5% sucrose or an appropriate carbon source (nitrogen fixation under heterotrophic growth conditions).

Staining methods. For the Gram stain, the modified method of Bartholomew (2) was used. Tetrazolium in vivo staining was performed by the method of Diena et al. (8). Volutin granules were stained for according to Albert and Laybourn's method as described in the *Manual of Microbiological Methods* (27).

Polyphosphate determination. Cells were grown on the mineral medium of Schlegel et al. (22) in which phosphate was gradually substituted by NaHCO_3 . No growth occurred in the original or modified medium of Neidhardt et al. (18). The polyphosphate content was then determined by the quantitative method of Kaltwasser and Schlegel (16).

Determination of poly- β -hydroxybutyric acid. Poly- β -hydroxybutyric acid was determined by the method described by Jüttner et al. (15).

Biochemical assays for numerical taxonomy. The characters used in the numerical taxonomy analysis are listed in Table 2. For determination of the

utilization of sole carbon sources, solid and liquid versions of the mineral medium described above were used. Sugars as carbon source were added in a concentration of 5 g/liter; alcohols, organic acids, and amino acids were added in a concentration of 2 g/liter. Tests for autotrophic growth with hydrogen as energy source and for growth in nitrogen-free medium were performed as described above. The other diagnostic characters used were determined by the methods given in the *Manual of Microbiological Methods* (27) and in

Laboratory Methods in Microbiology (13). The tyrosinase test was performed by the procedure of Yamada and Komagata (32). Deoxyribonuclease and urease productions were determined with the corresponding Difco media.

Computer analysis. For the calculation of similarities among the strains, the similarity coefficient S_j of Jaccard (25) was used. Clusters were then formed by single-linkage cluster analysis (26). The similarity data were then plotted as a dendrogram. The analysis

TABLE 1. *Bacterial strains used in this study*^a

Species	Strain	Source
<i>Arthrobacter atrocyaneus</i>	AJ1429	K. Komagata (Tokyo)
<i>A. simplex</i>	ATCC 6946	
<i>A. variabilis</i>	AJ 1434	K. Komagata
<i>A. tumescens</i>	ATCC 6947	K. Komagata
<i>Arthrobacter</i> sp.	9	G. Auling (Gottingen)
<i>Arthrobacter</i> sp.	RH 12	M. Reh (Gottingen)
<i>Arthrobacter</i> sp.	11 X	U. Eberhardt (Gottingen)
<i>Brevibacterium ammoniagenes</i>	ATCC 6871	K. Komagata
<i>B. ammoniagenes</i>	ATCC 6872	K. Komagata
<i>B. divaricatum</i>	AJ 1498	K. Komagata
<i>B. linens</i>	AJ 1473	K. Komagata
<i>B. lipolyticum</i>	AJ 1450	K. Komagata
<i>B. maris</i>	AJ 1480	K. Komagata
<i>B. stationis</i>	ATCC 14403	K. Komagata
<i>B. vitarumen</i>	ATCC 10234	K. Komagata
<i>Corynebacterium autotrophicum</i>	7C (DSM 432) (type strain)	DSM
<i>C. autotrophicum</i>	14g (DSM 431)	DSM
<i>C. autotrophicum</i>	RH 9, RH 10	M. Reh
<i>C. autotrophicum</i>	GZ 27, GZ 28, GZ 29, GZ30, GZ 37, GZ 38	G. Auling
<i>C. autotrophicum</i>	12/60/x, 19/-/x	U. Ebertardt
<i>C. autotrophicum</i>	OM 1	O. Meyer (Gottingen)
<i>C. autotrophicum</i>	JW 1, JW 3, JW 4, JW 5, JW 6, JW 7, JW 21, JW 22, JW 31, JW 32, JW 33, JW 34, JW 35, JW41, JW 42, JW 43, JW 44, JW 46, JW 47, JW 48, JW 49, JW 50	J. Wiegell (Gottingen)
<i>C. equi</i>	ATCC 6939	K. Komagata
<i>C. barkeri</i>	DSM 20145	DSM
<i>C. betae</i>	DSM 20141	DSM
<i>C. fascians</i>	DSM 20131	DSM
<i>C. flaccumfaciens</i>	DSM 20129	DSM
<i>C. flaccumfaciens</i> subsp. <i>aurantiacum</i>	DSM 20135	DSM
<i>C. herculis</i>	ATCC 13868	K. Komagata
<i>C. hoagii</i>	ATCC 7005	K. Komagata
<i>C. ilicis</i>	DSM 20138	DSM
<i>C. lilium</i>	DSM 20137	DSM
<i>C. manihot</i>	DSM 20158	DSM
<i>C. mediolanum</i>	DSM 20152	DSM
<i>C. poinsettiae</i>	DSM 20149	DSM
<i>C. rathayi</i>	DSM 20153	DSM
<i>C. xerosis</i>	DSM 20170	DSM
<i>Microbacterium flavum</i>	ATCC 13040	K. Komagata
<i>Mycobacterium flavum</i>	(301 = DSM 338)	J. Postgate (Brighton)
<i>Micrococcus glutamicus</i>	ATCC 13032	K. Komagata

^a Abbreviations: AJ, Central Research Laboratories, Ajinomoti Co., Japan; ATCC, American Type Culture Collection, Rockville, Md.; DSM, German Collection of Microorganisms, Germany. GZ, JW, OM, and RH are laboratory strain designations.

was performed with a UNIVAC 1108 computer of the Gesellschaft für Wissenschaftliche Datenverarbeitung, Göttingen, Federal Republic of Germany.

Identification of the carotenoid pigment. The carotenoid pigment (crude carotenoids and acetylated derivatives) of *C. autotrophicum* 14g and 7C and of *Mycobacterium flavum* 301 were compared by thin-layer chromatography and absorption spectroscopy as described by Nybraaten and Liaaen-Jensen (19).

RESULTS AND DISCUSSION

Numerical taxonomy. Thirty-five yellow coryneform nitrogen-fixing, autotrophic, hydrogen-oxidizing bacteria, all identified as strains of *C. autotrophicum* Baumgarten et al. (3), were compared by the methods of numerical taxonomy with 28 yellow or cream-colored strains of the genera *Arthrobacter*, *Brevibacterium*, *Corynebacterium*, and *Microbacterium*. The taxonomy of the reference strains was studied previously by Yamada and Komagata (32) and Schleifer and Kandler (23). Three arthrobacter-like autotrophic but not nitrogen-fixing strains were also included in the present study. One hundred-twenty (sometimes correlated) characters were used for the numerical taxonomy analysis; they were mainly those used by Yamada and Komagata (32) for grouping coryneform bacteria (Table 2).

The results of the single-linkage cluster analysis are given in Fig. 1.

The *C. autotrophicum* strains formed a distinct cluster above the level of 78% similarity; this cluster did not join any of the clusters formed by the other coryneform bacteria tested. Except for three strains (those of *Microbacterium flavum*, *C. rathayi*, and *C. manihot*), the reference strains linked before they joined the *C. autotrophicum* cluster. Linkage between the *C. autotrophicum* cluster and the other coryneform strains occurred at a similarity level of only 68%. The three autotrophic arthrobacter-like strains formed a distinct cluster but did not show any relationship to *C. autotrophicum*. Although this analysis did not contain any reference strain outside the coryneform bacteria, it was obvious that *C. autotrophicum* does not belong to *Corynebacterium* or to a genus related to *Corynebacterium*.

Determination of a hypothetical median organism. Table 2 shows the number of *C. autotrophicum* strains positive for the characters used in the numerical taxonomy analysis. The properties of a hypothetical median organism were determined by assuming a character to be positive for the organism if more than 17 of the 35 tested strains were positive. Three strains (JW 32, JW 33, and JW 42) had characters identical to those of the hypothetical me-

dian organism and therefore were most representative of *C. autotrophicum*. Four strains (JW 3, JW 7, JW 41, and JW 48) differed from the hypothetical median organism in only one character. These seven strains, together with four others with two characters different from those of the hypothetical median organism and one strain with three mismatches, formed a well-defined subcluster in the *C. autotrophicum* taxon (Fig. 1).

Strain 7C, selected as the type strain of *C. autotrophicum* by Baumgarten et al. (3), differed in eight characters from the hypothetical median organism and therefore is relatively atypical of the species. For further taxonomic studies, therefore, we recommend the use of strain JW 33 as a reference for the taxon in addition to the type strain, 7C.

Chemotaxonomic differences of *C. autotrophicum* from *Corynebacterium*. Besides the low overall similarity between *C. autotrophicum* and the coryneform reference strains, certain salient characters of *C. autotrophicum* precluded its membership in the genus *Corynebacterium*. None of the *C. autotrophicum* strains tested contained mycolic acids, which are typical of the coryneform bacteria (M. Goodfellow, personal communication; R. M. Kroppenstedt, personal communication). In all of the strains tested, *meso*-diaminopimelic acid was detected as diamino acid of the cell wall (O. Kandler and F. Fiedler, personal communication). The peptidoglycan was directly cross-linked by *m*-diaminopimelic acid (subgroup A1 [23]), as is typical of *Corynebacterium sensu stricto*, which is gram-positive, as well as of most gram-negative bacteria. However, besides peptidoglycan, *Corynebacteriaceae* contain arabinose and galactose as cell wall sugars; these were absent in the strains of *C. autotrophicum* tested (R. M. Kroppenstedt, personal communication). The fatty acid biosynthesis pathway of the nitrogen-fixing hydrogen bacteria so far examined is via *cis*-vaccenic acid, whereas that of the actinomycetes and the coryneform bacteria is via oleic acid (R. M. Kroppenstedt, personal communication).

Gram stain reaction and related characters. On the basis of the observed differences between *C. autotrophicum* and the genus *Corynebacterium*, we attempted to reclassify *C. autotrophicum* with the aid of the keys in *Bergey's Manual of Determinative Bacteriology* (6). One of the first characters used in the generic key was the Gram reaction of the cell wall. In the earlier descriptions of the nitrogen-fixing hydrogen bacteria, the statements on the Gram reaction were contradictory. Using Bartholomew's (2) modification of the Gram stain and a number of strains cultivated under various growth con-

TABLE 2. *Properties of 35 Corynebacterium autotrophicum isolates determined for numerical taxonomy analysis^a*

Property	No. of strains giving a positive reaction	Property	No. of strains giving a positive reaction
Growth on:		Gelatin liquefaction	0 (-)
Acetate	30 (+) ^a	Starch hydrolysis	0 (-)
Citrate	33 (+)	Casein hydrolysis	0 (-)
Lactate	34 (+)	Cellulose degradation	0 (-)
Formate	6 (-)	Tween hydrolysis	0 (-)
Oxalate	0 (-)	Growth on tellurite agar	15 (-)
Succinate	35 (+)	Growth with:	
		2.5% NaCl	6 (-)
Malate	35 (+)	5.0% NaCl	2 (-)
Fumarate	34 (+)	7.5% NaCl	0 (-)
Malonate	0 (-)	10.0% NaCl	0 (-)
Tartrate	0 (-)	Growth at:	
		25°C	35 (+)
Pyruvate	23 (+)	30°C	35 (+)
Mandelate	1 (-)	37°C	34 (+)
Benzoate	1 (-)	45°C	3 (-)
Gluconate	33 (+)	52°C	0 (-)
Glyoxylate	3 (-)	Colonial properties	
Glutarate	29 (+)	Circular	35 (+)
2-Oxoglutarate	31 (+)	Irregular	0 (-)
β -Hydroxybutyrate	0 (-)	Entire	35 (+)
Glutamate	35 (+)	Undulate	0 (-)
Alanine	2 (-)	Flat	0 (-)
Threonine	0 (-)	Convex	35 (+)
Valine	0 (-)	Umbonate	0 (-)
Leucine	1 (-)	Raised	2 (-)
Methionine	0 (-)	Translucent	0 (-)
Lysine	1 (-)	Opaque	35 (+)
Serine	0 (-)	Cream	0 (-)
Arginine	1 (-)	Yellow	35 (+)
Ornithine	0 (-)	Orange	0 (-)
α -Aminobutyrate	0 (-)	Pink	0 (-)
Histidine	0 (-)	Rough	0 (-)
Proline	1 (-)	Smooth	35 (+)
Cysteine	0 (-)	Filiform	35 (+)
Phenylalanine	0 (-)	Echinulate	0 (-)
Arabinose	0 (-)	Growth in liquid medium (test tubes):	
Xylose	0 (-)	Pellet	35 (+)
Ribose	0 (-)	Floppy	0 (-)
Rhamnose	0 (-)	Pellicular	0 (-)
Glucose	0 (-)	Ring	1 (-)
Fructose	28 (+)	Gram reaction	
Mannose	2 (-)	Positive	0 (-)
Galactose	0 (-)	Weakly positive	35 (+)
Trehalose	1 (-)	Negative	0 (-)
Sorbose	0 (-)	Metachromatic granules	35 (+)
Sucrose	24 (+)	Cell wall is acid fast	0 (-)
Lactose	1 (-)	Cell wall amino acid ^b	
Maltose	0 (-)	Ornithine	- (-)
Cellobiose	0 (-)	DL-Diaminopimelic acid	+ (+)
Glycerol	11 (-)	LL-Diaminopimelic acid	- (-)
Mannitol	1 (-)	Nitrate reduction	34 (+)
Sorbital	1 (-)	Voges-Proskauer reaction	0 (-)
Inositol	0 (-)	Methyl red reaction	0 (-)
Salicin	0 (-)	Indole production	0 (-)
Propanol	33 (+)	H ₂ S production (7 days)	0 (-)
Methanol	34 (+)	H ₂ S production (14 days)	0 (-)
Ethanol	34 (+)	Lecithine hydrolysis	0 (-)

TABLE 2.—Continued.

Property	No. of strains giving a positive reaction	Property	No. of strains giving a positive reaction
Autotrophic growth with H ₂ , O ₂ , and CO ₂	35 (+)	Tetrazolium chloride reduction	33 (+)
Nitrogen fixation	35 (+)	Litmus milk:	
		Alkaline	33 (+)
		Acid	0 (—)
		Clotting	0 (—)
		Clearing	0 (—)
		Production of:	
		Catalase	35 (+)
		Oxidase	35 (+)
		Urease	1 (—)
		Tyrosinase	0 (—)
		Phosphatase	31 (+)
		Deoxyribonuclease	0 (—)

^a Character of the hypothetical median organism is given in parentheses: +, more than 17 strains positive for the treated property; —, less than 18 strains positive for the tested property.

^b Only 12 strains were tested, all containing DL-diaminopimelic acid: 14g, 7C, GZ 29, GZ 38, 12/60/X, 9/-/x, OM 1, JW 21, JW34, JW43, and JW 44.

ditions, we found a positive reaction only in those regions of the cells containing refractile bodies; these bodies were visible by phase-contrast microscopy (30). Identical results were obtained using the tetrazolium staining method of Diena et al. (8). The refractile bodies, which apparently simulate gram-positive cell wall regions, were identified as polyphosphate granules by electron microscopy (29) as well as by volutin staining. Unfortunately, the polyphosphate content of the cells was high (15 mg of phosphate per g [dry weight]), and even at the minimum phosphate concentration in the growth medium (0.01 mM), the volutin granules did not disappear. By electron microscopy of thin sections of cells (29), it was shown that the cell envelope of *C. autotrophicum* has a thin multilayered structure, clearly resembling that of gram-negative cells. The peptidoglycan content of the cell wall of the *C. autotrophicum* strains (15 to 25%) was intermediate compared to that of gram-positive (30 to 70%) and gram-negative (10%) bacteria (23); teichoic acid and teichuronic acid, one or the other typically present in the cell walls of gram-positive bacteria, were absent (O. Kandler and F. Fiedler, personal communications). The cell envelopes of gram-negative bacteria contain lipopolysaccharides and lipoproteins. From strains 7C SF, 12/60/x, and JW 21, low but significant amounts of lipopolysaccharide could be isolated and analyzed (Wiegel and Mayer, manuscript in preparation). Furthermore, all strains tested contained ubiquinones (Q 10, Q 10 [H₂]) but no menaquinones (O. Thiele and M. Goodfellow, personal communications). These

properties indicate that *C. autotrophicum* is a gram-negative bacterium.

Cell morphology. Another salient characteristic in classifying bacteria—cell morphology—strongly favored inclusion of *C. autotrophicum* with the coryneform bacteria (3). The cell shape of *C. autotrophicum* was strongly influenced by growth conditions. Small rods (0.5 to 0.7 by 1.0 to 2.5 μ m) were formed on autotrophic as well as sugar-containing media, whereas irregularly branched cells arranged in palisades were produced on a succinate medium (30; Fig. 2). On media containing alcohols such as *n*-butanol, small coccoid cells as well as rods up to 10 μ m long were formed. Distinct resting stages (arthrospores or cysts), even in β -hydroxybutyrate- or butanol-containing media, were not found. The occurrence of a snapping type of cell division, as was assumed from light-microscope pictures of *C. autotrophicum* (3), was not confirmed by electron microscope studies. The illusion of snapping cell division may be due to copious amounts of slime or to outer cell wall layers holding the daughter cells together after cell division.

Reclassification of *C. autotrophicum*. According to the keys in *Bergey's Manual of Determinative Bacteriology* (6), the presently determined characters of *C. autotrophicum* lead one to the family *Azotobacteraceae*: gram-negative, aerobic to microaerophilic bacteria which do not produce endospores but which grow in nitrogen-free medium.

Table 3 shows the main diagnostic characters of the *Azotobacteraceae* and of *C. autotrophici-*

FIG. 1. Similarity dendrogram of strains used in the numerical taxonomic study.

cum. Within the *Azotobacteraceae*, *C. autotrophicum* is close to *Beijerinckia* and *Derxia*: small rods producing very tenacious extracellular slime or gum and conspicuous internal globular lipid bodies; slow growth; catalase may or may not be produced. The diagnostic difference between *Beijerinckia* and *C. autotrophicum* is the high guanine-plus-cytosine content (70%) of the latter, and between *C. autotrophicum* and *Derxia* it is the production of catalase by the

former. The characters of *C. autotrophicum* which clearly separate it from the four genera currently placed in the family *Azotobacteraceae* are its ability to grow autotrophically with hydrogen as energy source, its carbon utilization pattern, and its production of a water-insoluble, yellow, carotenoid pigment, zeaxanthin dirhamnoside (14, 19).

It is our opinion that *C. autotrophicum* belongs to a new genus of the family *Azotobacter-*

FIG. 2. Ultrathin section of *C. autotrophicum* 14g grown on succinate-containing medium. Glutaraldehyde-osmium tetroxide fixation, uranylacetate block staining, lead citrate post-staining; preparation by A. Walther-Mauruschat. Abbreviations: P, poly- β -hydroxybutyrate; PP, polyphosphate; Ps, small-type polyphosphate.

TABLE 3. Diagnostic characters useful within the family Azotobacteraceae^a

Character	<i>Corynebacterium autotrophicum</i>	<i>Azotobacter</i>	<i>Azomonas</i>	<i>Beijerinckia</i>	<i>Derxia</i>
G+C ^b content	69–70 mol%	63–66 mol%	53–59 mol%	55–59 mol%	70 mol%
Cyst formation	—	+	—	±	—
Storage material	PHB ^c	PHB	?	PHB	PHB
Refractile bodies	+	+	+	+	+
Slime production	+	+	±	+	+
Colony color	Yellow	Fluorescent	Fluorescent	Pink-brown	Yellow-brown
Pigments					
Water-soluble	—	+	+	+	+
Water-insoluble	+	+	—	—	—
Cell size (diam)	0.4–1 μ m	2 μ m	2 μ m	0.5–1.5 μ m	1 μ m
Pleomorphism	+	+	+	+	+
Motility	—	±	+	±	+
Catalase production	+	+	+	+	—
pH range of growth	5.8–9.0	5.5–8.5	4.5–9.0	3.0–10.0	5.5–9.0
Gram reaction	Variable	Negative to variable	Negative to variable	Negative	Negative

^a According to *Bergey's Manual of Determinative Bacteriology* (6).

^b G+C, Guanine plus cytosine

^c PHB, Poly- β -hydroxybutyrate.

aceae, for which we propose the name *Xanthobacter* (Xan.tho.bac'ter. Gr. adj. *xanthos* yellow; M.L. masc. n. *bacter* the equivalent of Gr. neut. n. *bacterion* rod, staff; M.L. masc. n. *Xanthobacter* yellow rod). The name of this organism thus becomes *Xanthobacter autotrophicus* (Baumgarten, Reh, and Schlegel 1974) comb. nov.

The description of *Xanthobacter* gen. nov. is as follows. Small, rod-shaped cells 0.4 to 1.0 by 0.8 to 6.0 μm ; branched, rather pleomorphic cells are produced on succinate-containing medium, and coccoid as well as cells up to 10 μm long are produced on media containing an alcohol as the sole carbon source. Refractile (polyphosphate) and lipid (poly- β -hydroxybutyrate) bodies are contained within the whole cell.

Nonmotile. Resting stages are unknown. Gram-negative type of cell wall, but the Gram reaction is variable; the cell wall contains peptidoglycan directly linked by *m*-diaminopimelic acid; lipopolysaccharides are present in the cell wall but teichoic and teichuronic acids are absent. Agar colonies are opaque and slimy; slime-free strains also exist. Colonies are yellow due to a water-insoluble carotenoid pigment, zeaxanthin dirhamnoside.

Grow as chemolithoautotrophs in mineral medium under an atmosphere of hydrogen, oxygen, and carbon dioxide (7:2:1 by volume) as well as chemoorganotrophically on methanol, ethanol, *n*-propanol, *n*-butanol, and many organic acids used as sole carbon sources; carbohydrate utilization is limited. Neither acid nor gas is produced from carbohydrates.

Atmospheric nitrogen is fixed in nitrogen-deficient media under reduced oxygen pressure with an efficiency of more than 10 and usually 20 mg of N per g of sucrose consumed.

Obligately aerobic. Catalase positive.

Optimal growth temperature: 25 to 30°C. pH range for optimal growth: 5.8 to 9.0.

Guanine-plus-cytosine content of the deoxyribonucleic acid: 69 to 70 mol%.

Isolated from soil, mud, and water.

Type species: *Xanthobacter autotrophicus* (Baumgarten, Reh, and Schlegel 1974) comb. nov.

The new taxonomic position of *C. autotrophicus*, as determined by the keys in *Bergey's Manual of Determinative Bacteriology* (6), does not suggest a systematic or evolutionary relationship to the other genera of the family *Azotobacteraceae*. The ribosomal ribonucleic acid homology might be taken as a criterion of the natural relationships between strains. The ribosomal ribonucleic acid properties of our strains were studied by J. DeLey (personal communication). They are far from those of *Azotobacter*

and *Derxia* and closest to, but still quite different from, those of *Beijerinckia*. The *Azotobacteraceae* themselves, however, are heterogeneous with respect to ribosomal ribonucleic acid homology. Therefore, the ribosomal ribonucleic acid property of *X. autotrophicus* is not considered as justification for excluding this species from this physiologically homogeneous family.

No taxonomic relationship of *X. autotrophicus* to members of *Xanthomonas*, *Flavobacterium*, *Chromobacterium*, *Cytophaga*, the *Methylobacteriaceae*, or other taxa of questionable taxonomic position was found with respect to such characteristic properties as irregular cell shape, colony pigmentation, slime production, guanine-plus-cytosine content, Gram variability, nitrogen fixation, autotrophic growth, methanol utilization, and limited pattern of carbohydrate utilization.

Relationship of *Mycobacterium flavum* 301 to *X. autotrophicus*. This study was not concerned with the taxonomy of *Mycobacterium flavum* 301, which was isolated as a nitrogen-fixing bacterium (11) and was later found to resemble the other nitrogen-fixing hydrogen bacteria (7; Zavarzin, personal communication). Nevertheless, there is no doubt that *Mycobacterium flavum* 301 does not belong to *Mycobacterium flavum* in Krasil'nikov's scheme (17) nor to *Microbacterium flavum* Orla-Jensen 1919 (5) but that it clearly resembles the members of *X. autotrophicus*: *Mycobacterium flavum* 301 does not contain arabinogalactan or any type of mycolic acid (R. M. Kroppenstedt, personal communication); it contains ubiquinone but no menaquinones (O. Thiele, personal communication); it has the same fatty acid pattern as *X. autotrophicus* (R. M. Kroppenstedt, personal communication); it has the same carotenoid pigment as *X. autotrophicus* 14g and 7C as determined by chromatography and by the absorption spectrum; its carbon utilization pattern is comparable to those of *X. autotrophicus* 14g and RH 9, which are also unable to utilize carbohydrates; it shows the same characteristic change in cell morphology caused by the addition of succinate to the medium; and the regulation of the leucine biosynthetic enzyme α -isopropylmalate synthase activity is similar to that of *Xanthobacter* but not to that of *Mycobacterium* and *Corynebacterium* (31). Strain 301 gives similar results as *X. autotrophicus* with the various staining procedures for cells and volutin granules, and the electron micrographs of thin sections show similar cell wall structures.

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