JOURNAL OF BACTERIOLOGY, Aug. 1969, p. 597-602
Vol. 99, No. 2

Printed in U.S.A.

Rhodopseudomonas acidophila, sp. n., a New Species of the Budding Purple Nonsulfur Bacteria

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Received for publication 24 May 1969

A succinate-mineral salts medium of *p*H 5.2 provided selective enrichment conditions for *Rhodomicrobium vannielii* and for a new species belonging to the *Athiorhodaceae*, described herein as *Rhodopseudomonas acidophila*. Seven strains of the new species have been isolated from different sources in the United States and Germany. The cells are rod-shaped or ovoid, 1.0 to 1.3 μm wide and 2 to 5 μm long, and motile by means of polar flagella. Multiplication occurs by budding. The photopigments consist of bacteriochlorophyll *a* and carotenoids of the spirilloxanthin series, together with new carotenoids. All strains can grow either under anaerobic conditions in the light or under microaerophilic to aerobic conditions in the dark. No growth factors are required. The range of simple organic substrates photoassimilated resembles that characteristic of *Rhodomicrobium*. Good photolithotrophic growth is possible at the expense of molecular hydrogen; thiosulfate and sulfide are not utilized.

In his monograph on the purple nonsulfur bacteria, van Niel (4) showed that the use of different substrates provides a convenient means for the selective enrichment of different members of the *Athiorhodaceae*. He noted that extensive experiments on the effect of *p*H on the enrichment of these organisms had not been performed. His enrichment experiments were usually conducted at a *p*H between 7 and 8, and yeast extract was added to the medium. The *p*H range for growth of the resulting pure cultures obtained by such methods is generally between *p*H 6 and 8, the *p*H optimum being somewhat above 7.

We examined the effect of low *p*H values (5.1) on the enrichment of the *Athiorhodaceae*, with succinate as a carbon source. From such enrichments, seven closely similar strains which grow best at *p*H 5.5 and 6 were isolated. The new strains differ from all known species of the *Athiorhodaceae* and are described here as a new species of the genus *Rhodopseudomonas*: *R. acidophila* sp. n.

MATERIALS AND METHODS

Sources of strains. The first strain of the new species (strain 7050) was isolated by enrichment from a water sample from Crystal Lake, Urbana, Ill. Many water and mud samples from different localities have been studied subsequently in order to find out how common and widespread the new species is. The additional six strains described in this paper were isolated from the following natural sources: strain 7150, Lake Monroe near Bloomington, Ind.; strain 7250, cypress swamp, Okeefenokee State Park, Ga.; strain 77550, mud vulcano (*p*H: 4.5), Yellowstone National Park, Wyo.; strain 7750, farm pond near Athens, Ga.; strain 2751, forest pond near Grünenplan, Germany; strain 3251, sphagnum peat bog near Kolshorn/Hanover, Germany.

Media and conditions of cultivation. Unless otherwise indicated, the following mineral base was used for all media: KH_2PO_4, 1 g; NH_4Cl, 0.5 g; $MgSO_4 \cdot 7H_2O$, 0.4 g; NaCl, 0.4 g; $CaCl_2 \cdot 2H_2O$, 0.05 g; trace element solution of Pfennig and Lippert (3), 10 ml; water to 1,000 ml. For enrichment cultures, agar-shake cultures and stock cultures, 1.5 g of disodium succinate per liter was added as the carbon source. In solid media, the agar concentration was 1%. The *p*H of the enrichment was adjusted to give 5.1 to 5.2 after autoclaving. Screw-cap bottles (50 ml) were used as culture vessels. Pure cultures were obtained by repeated application of the agar-shake culture method, with the succinate medium used at a *p*H of 5.6. Tests for the utilization of simple organic substrates (sodium salts were used in case of organic acids) were performed by adding the substrates from sterile stock solutions (final concentration, 0.1% w/v) to the autoclaved mineral medium; tests were carried out in triplicate at a *p*H of 5.8 to 6.0. Growth was estimated from measurements of the optical density at 650 nm in 1-cm cuvettes by using a Unicam Spectrophotometer SP700, final readings being made after incubation for 2 weeks. Tests for photolithotrophic growth with molecular hydrogen and carbon

dioxide were performed in cotton-plugged Erlenmeyer flasks placed in desiccators containing a mixture of 80% H_2 and 20% CO_2. If not otherwise stated, the incubation temperature was about 25 C, and the light intensity was 50 to 100 ft-c from a 100 w tungsten lamp. Tests for ability to grow in the dark under aerobic or microaerophilic conditions were performed in several different ways, by using the mineral medium (*p*H 5.8) with 0.1% pyruvate or lactate as the carbon source. Cultures were prepared in 100 ml of liquid medium in 300-ml Erlenmeyer flasks and incubated without agitation; and agar-shake cultures (1% agar) were prepared with 10 ml of medium per tube, control tubes being sealed with sterile Vaspar to exclude air. The ability to grow on agar slants was also determined.

The absorption spectra of intact cells were measured on 3.5-ml samples of cell suspensions of freshly grown cultures in which 5 g of sucrose had been dissolved, in order to reduce light scattering. They were determined with a Unicam Spectrophotometer SP700.

Deoxyribonucleic acid (DNA) base composition. The moles per cent guanine plus cytosine of the DNA of all strains was determined by Manley Mandel, Houston, Texas, by the caesium chloride density gradient method (5).

RESULTS

Enrichment and isolation. The members of the *Athiorhodaceae* which developed in anaerobic illuminated enrichment cultures in a succinate-mineral medium with an initial of *p*H 5.1 to 5.2 depended primarily on the natural sample used as inoculum. In three cases, *R. acidophila* was the only phototrophic bacterium which developed in the enrichments (strains 7050, 7150, and 3251). Other enrichment cultures yielded *Rhodomicrobium vannielii*, either alone or together with *R. acidophila* (strain 7250). The low *p*H-succinate mineral medium evidently provides excellent enrichment conditions for *R. vannielii*, an organism for which a specific enrichment method has not so far been available. A large number of enrichments yielded, after several weeks of incubation, one or two of the well-known *Rhodopseudomonas* species (*R. palustris*, *R. capsulata*, *or R. gelatinosa*). These species all require growth factors, and developed only as members of the mixed population of the enrichment culture. Strains 7550, 7750, and 2751 of *R. acidophila* developed in enrichment cultures together with *R. palustris* or *R. capsulata*; by repeated transfer to fresh enrichment media, it was possible in every case to obtain *R. acidophila* as the predominant member of the population.

Morphology. As a result of their large size and characteristic shape, individual cells of *R. acidophila* can be readily recognized among the cells of other phototrophs in enrichment cultures. The cells are ovoid or slightly curved rods, 1 to 1.3 μm wide and 2 to 4 μm long, and in some strains are as much as 7 μm long (*see* Table 1 and Fig. 1). A tendency to form somewhat irregular and swollen cells is apparent in all strains. The formation of rosette-like clusters, typical for *R. palustris*, has been observed only in strains 7050, 7250, and 2751. All strains show polar cell growth and multiply by budding (Fig. 2). In this respect, the new species is similar to *R. palustris* (6). In contrast to *Rhodomicrobium*, the strains of *R. acidophila* do not form a tube or filament between mother cell and bud; the bud is sessile on the mother cell and separates by constriction when the bud reaches the size of the mother cell. After separation, both mother and daughter cell again bud at the newly formed poles (*see* Fig. 2).

All strains are motile by means of polar flagella and are gram-negative. The photosynthetic membrane system has been studied in thin sections by electron microscopy. As in *R. palustris* and *Rhodomicrobium*, the membrane system consists of parallel lamellae which underlie and are possibly continuous with the cytoplasmic membrane [strains 7150, 7250, 7550, 7750, 2751, and 3251 (G. Cohen-Bazire, *personal communication*); strain 7050 (D. S. Hoare, *personal communication*)].

Physiological and biochemical characteristics. *R. vannielii* is the only purple nonsulfur bacterium so far described which does not require growth factors. All enrichment cultures in which *R. acidophila* was detected microscopically yielded pure cultures of this organism which grew well in succinate mineral medium. *R. acidophila* is accordingly a second species of the *Athiorhodaceae* which does not require organic growth factors. The addition of Casamino Acids or yeast extract to the succinate mineral medium does not increase the growth rate of *R. acidophila*, and may even inhibit growth at higher concentrations. The shortest doubling times measured with strain 7050 using the succinate-mineral medium in stationary bottle cultures were between 3.5 and 5.0 hr.

All *R. acidophila* strains are capable of good photolithotrophic growth with molecular hydrogen and carbon dioxide (80:20, v/v). Thiosulfate is not utilized, and hydrogen sulfide strongly inhibits growth.

The results of tests for phototrophic growth of *R. acidophila* strains on single organic carbon sources are summarized in Table 2. The species is characterized by the inability to utilize amino acids, sugars, higher fatty acids, cyclohexane carboxylate, and benzoate for growth.

Three different methods were used to test for the ability of *R. acidophila* strains to grow in the dark under aerobic or microaerophilic conditions. The results indicate (*see* Table 1) that all strains are able to grow in the dark if oxygen is present as

TABLE 1. *Characteristic features of Rhodopseudomonas acidophila strains compared with Rhodopseudomonas palustris and Rhodomicrobium vannielii*

Organism and strain	Color of dim-light-grown anaerobic cultures	pH-range for growth on succinate	Optimal temp for growth	Size of cells		Growth in the dark under aerobic or microaerophilic conditions (lactate or pyruvate carbon source)			Moles % guanine plus cytosine in DNA
				Width	Length	In stationary liquid cultures	In agar-shake cultures: distance below surface	On slants	
			C	*μm*	*μm*		*mm*		
R. acidophila strain									
7050	Purple-red	4.9–7.1	30	1.1–1.3	2–5	+	0–2	+	65.3
7150	Orange-brown	5.2–7.2	25–28	1.1–1.3	2–7	+	2	−	62.2
7250	Orange-red	5.1–6.2	25–28	1.0	2.3–6.5	+	7	−	65.3
7550	Orange-brown	4.8–7.0	30	1.0	1.8–4.0	+	0–2	+	66.8
7750	Orange-brown	4.8–7.0	30	1.0	1.8–4.0	+	0–2	+	64.3
2751	Orange-brown	5.3–7.0	25–28	1.0–1.3	2–5	+	2	−	62.8
3251	Purple-red	5.0–7.0	25–28	1.3	2.5–6.5	+	3–4	−	62.2
R. palustris strain 1850	Brownish-red	6.0–8.5	30–37	0.6–0.8	1.2–2.0	+	0–2	+	64.8
R. vannielii strain 7255	Orange-brown	5.2–7.5	30	1.0–1.2	2.0–2.8	+	0–2	−	63.8

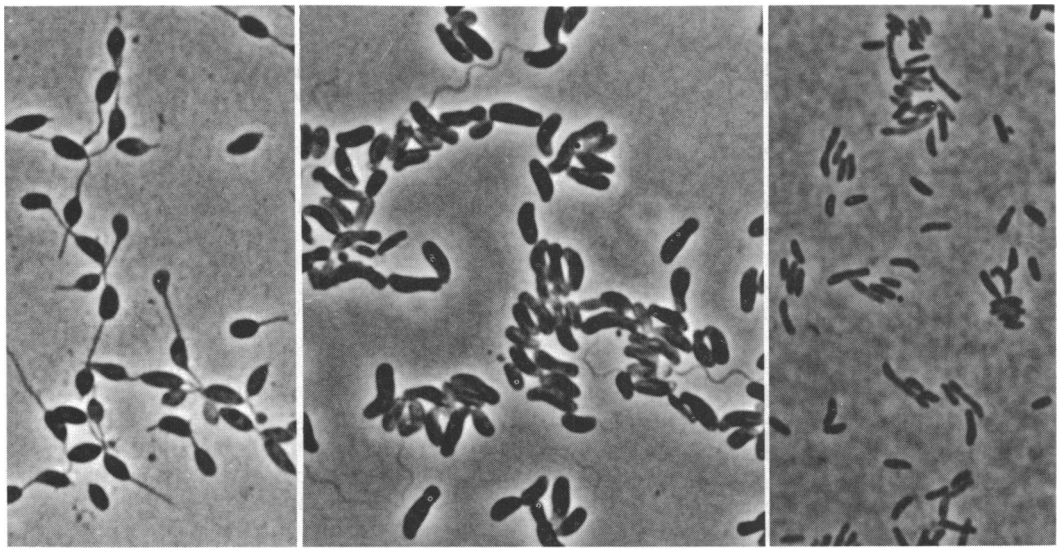

FIG. 1. *Cell form of Rhodopseudomonas acidophila compared with Rhodomicrobium vannielii and Rhodo pseudomonas palustris. Center: R. acidophila, type strain 7050; some detached flagella are seen. Left: Rhodomicrobium vannielii strain 7255. Right: R. palustris strain 1850. All strains succinate-grown. Phase contrast, ×1,830.*

an electron acceptor. The strains differ in their tolerance with respect to air. Only strains 7050, 7550, and 7750 develop on the surface of slants at full atmospheric oxygen tension, whereas the remaining four strains require the lower oxygen concentrations provided by stationary liquid cultures or agar-shake cultures.

Poly-β-hydroxybutyrate has been identified as a storage product in acetate-grown cells of strain 7050 (D. S. Hoare, *personal communication*).

Photopigments. Absorption spectra of intact cells of all strains of *R. acidophila* show the maxima characteristic of bacteriochlorophyll *a*. A typical spectrum is given in Fig. 3. The carotenoid pigments are currently being studied by K. Schmidt, Göttingen, Germany (*personal com-*

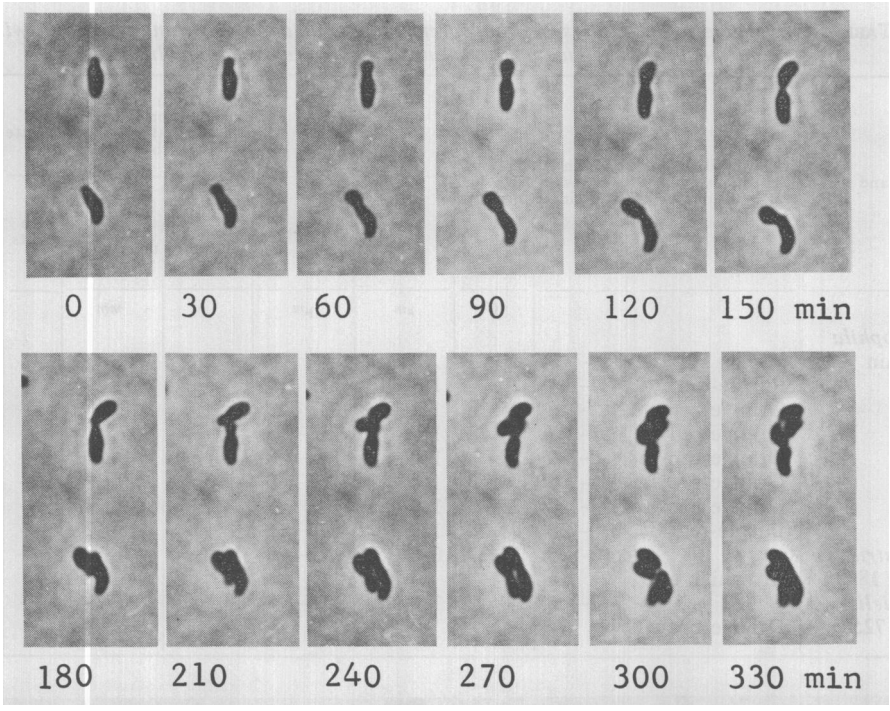

FIG. 2. *Rhodopseudomonas acidophila strain 7050. Agar slide culture; pictures of the same cells taken at 30-min intervals to show the outgrowth of the sessile buds, the division by constriction, and the further outgrowth at the poles of the former division. Phase contrast, × 1,500. Pictures by Heather M. Johnston.*

munication). Preliminary results indicate that all strains contain small amounts of carotenoids of the normal spirilloxanthin series, in addition to four carotenoids of unknown structure which have not been found in other purple bacteria. These latter carotenoids appear to be a particular characteristic of the new species.

DISCUSSION

The use of an acid succinate-mineral medium (*p*H 5.2) for the enrichment of purple nonsulfur bacteria provides a simple method for the selective isolation of the new species *R. acidophila* and of *R. vannielii*. Both these species share a relatively low *p*H optimum for growth (approximately *p*H 6.0 for *Rhodomicrobium* as shown by studies with six strains) and do not require growth factors. They utilize similar ranges of simple organic substrates for phototrophic growth. Both species can grow in the dark under aerobic or microaerophilic conditions. (This is true for the six strains of *Rhodomicrobium* that we have examined.) However, the growth rate of *Rhodomicrobium* is higher in the presence of amino acids or complex organic nutrients, whereas that of *R. acidophila* is not affected or even inhibited.

On the basis of its reproduction by the formation of a bud at the tip of a filament extending from the mother cell, *R. vannielii* (1, 2) has been excluded from the *Athiorhodaceae* and placed in the order *Hyphomicrobiales* in the seventh edition of *Bergey's Manual of Determinative Bacteriology*. However, Whittenbury and McLee (6) recently showed that *Rhodopseudomonas palustris* and *R. viridis* both multiply by budding; the bud forms at the end of a tube which develops at the pole of the mother cell that does not bear the flagellum. These three budding species of purple nonsulfur bacteria have in common a photosynthetic membrane system which consists of parallel lamellae underlying and continuous with the cytoplasmic membrane; the membrane system is formed de novo in each developing bud. Whittenbury and McLee (6) discussed the possibility that this particular type of membrane system is correlated with reproduction by budding.

R. acidophila also multiplies by budding, and all strains have a membrane system which is similar to that of the other budding species (G. Cohen-Bazire and D. S. Hoare, *personal communication*). Within the genus *Rhodopseudomonas*, *R. acidophila* is the species with the largest cells; they are almost twice as large as the morphologically similar cells of *R. palustris*. *R. acidophila* also

TABLE 2. *Utilization of single organic substrates and electron donors by Rhodopseudomonas acidophila strains, Rhodopseudomonas palustris strain 1850, and Rhodomicrobium vannielii strain 7255*

Carbon source and electron donor	Utilization[a]								
	R. acidophila strains							*R. palustris*[b]	*R. vannielii*
	7050	7150	7250	7550	7750	2751	3251		
Formate	0	0	0	0	0	0	0	2	0
Acetate	2	3	1	3	2	1	2	3	3
Propionate	1	2	1	3	2	2	2	3	1
Butyrate	1	1	1	1	1	0	1	3	1
Valerate	2	1	2	2	1	1	1	3	1
Caproate	1	0	0	0	0	1	0	2	1
Caprylate	0	0	0	0	0	0	0	2	1
Pelargonate	0	0	0	i	i	0	0	0	0
Glycolate	0	0	0	0	0	1	0	1	0
Pyruvate	3	3	3	3	3	3	3	3	3
Lactate	3	3	3	3	3	3	3	3	3
Citrate	0	0	0	1	1	0	0	0	0
Malate	3	3	3	3	3	3	3	3	3
Fumarate	3	3	3	3	2	3	3	3	3
Succinate	3	3	3	3	3	3	3	3	3
Tartrate	1	0	0	0	0	1	0	0	0
Malonate	0	0	0	0	0	1	3	2	3
Benzoate	0	0	0	0	0	0	0	3	0
Cyclohexane-carbonxylate	0	0	0	0	0	0	0	3	0
Methanol	0	0	1	1	1	1	1	0	0
Ethyl alcohol	1	1	1	1	1	2	1	1	1
Glycerol	0	0	0	1	1	1	1	2	0
Glucose	0	0	0	0	1	1	0	0	0
Fructose	0	0	0	0	0	0	0	0	0
Mannitol	0	0	0	0	0	0	0	0	0
Asparaginate	1	0	0	0	0	0	0	0	0
Glutamate	0	0	0	0	0	0	0	1	0
Arginine	0	0	0	0	0	0	0	0	0
Casamino Acids	0	1	0	1	1	1	1	1	1
Yeast extract	1	1	0	1	1	2	1	1	1
Bicarbonate plus thiosulfate	0	0	0	0	0	0	0	2	0
Bicarbonate plus sulfide	i	i	i	i	i	i	i	i	i
Bicarbonate plus molecular hydrogen	3	3	3	3	3	3	3	2	3

[a] Optical density (OD) at 650 nm; 1 = up to 0.1 OD; 2 = 0.2 to 0.5 OD; 3 = 0.6 to 0.9 and higher OD; i = growth completely inhibited; 0 = OD as in the control without added substrate.

[b] In the presence of 0.02% yeast extract.

differs from *R. palustris* in the following respects. The buds are sessile and not formed at the end of a tube or filament; it contains characteristic new carotenoid components; and growth factors are not required. In addition, the following substrates which are photoassimilated by *R. palustris* cannot be utilized by *R. acidophila*: amino acids, cyclohexanecarboxylate, benzoate, and thiosulfate plus bicarbonate (*see* Table 2). On the basis of their particular characteristics and the above-mentioned differences in comparison to *R. palustris* and *R. vannielii*, it appears proper to recognize the seven strains as a new species of the genus *Rhodopseudomonas*.

Rhodopseudomonas acidophila n. sp.

a.ci.do'phi.la. L. adj. *acidus* sour; M. L. noun *acidum* an acid; Gr. adj. *philus* loving; M. L. adj. *acidophila* acid-loving.

Morphology: Cells rod-shaped to elongated ovoid, slightly curved, 1.0 to 1.3 μm wide and 2.0 to 5.0 μm long, some strains up to 7 μm long; multiplication by budding; no tube or filament between mother cell and bud. Motile by means of polar flagella. Gram-negative. Photosynthetic membrane system: parallel lamellae underlying and possibly continuous with the cytoplasmic membrane.

Culture: Photolithotrophic (with molecular

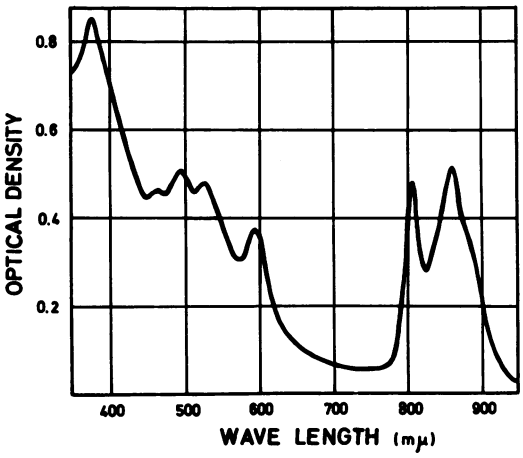

FIG. 3. *In vivo absorption spectrum of Rhodopseudomonas acidophila strain 7050. Cells suspended in 60% (w/w) sucrose solution.*

hydrogen) and facultatively photoorganotrophic, growing either anaerobically in the light or aerobically in the dark; some strains are microaerophilic. Optimum *p*H 5.8. Optimum temperature 25 to 30 C. Color of anaerobic cultures dependent on light intensity and carbon source: purple red (dim to regular light intensity) to orange-brown (bright light). Aerobically grown cells colorless to pink. No growth factors required; growth rate not increased in the presence of yeast extract or complex organic nutrients. Under certain conditions, the cells form clusters reminiscent of *R. palustris*. In media lacking Ca ions the cells are immotile.

Photolithotrophic growth with H_2 and CO_2. Photoassimilation of lactate, pyruvate, succinate, fumarate, malate, ethyl alcohol, acetate, propionate, butyrate, valerate. No growth in mineral media with sulfide, thiosulfate, formate, caprylate, pelargonate, glycerol, glutamate, and other amino acids, benzoate, sugars, and sugar alcohols.

Pigments: Absorption spectra of living cell suspensions show the maxima of bacteriochlorophyll *a*-containing organisms (375, 590, 805, 855 nm and a shoulder at about 890 nm), together with carotenoid maxima at 460, 490, and 525 nm. In addition to carotenoids of the normal spirilloxanthin series, carotenoids which differ from those hitherto known of phototrophic bacteria are present. Hydrogenase and catalase activity present.

Storage material: Poly-β-hydroxybutyrate.

DNA base composition (from determinations of buoyant density): 62.2 to 66.8 moles per cent guanine plus cytosine. The value for the type strains is 65.3.

Habitat: Mud and water exposed to light, particularly acidic habitats and pools in peat bogs.

Type: Strain 7050 (Crystal Lake). Type cultures are deposited with the American Type Culture Collection (ATCC 25092) and the culture collection (strain 7050) of the Institut für Mikrobiologie der Universität Göttingen, Germany.

ACKNOWLEDGMENTS

I thank Roger Y. Stanier for stylistic improvements of the English text. I am indebted to Germaine Cohen-Bazire, Berkeley, Calif., and Derek S. Hoare, Austin, Tex., for the information on the fine structure and to Manley Mandel, Houston, Tex., for the determinations of the DNA base ratios.

I gratefully acknowledge the cooperation of Heather M. Johnston for making agar slide cultures and serial pictures of growing cells. Hans Trüper kindly provided a number of water samples from the United States. Dorothee Pecksen is thanked for reliable technical assistance.

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