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Ectothiorhodospira mobilis Pelsh, a Photosynthetic Sulfur Bacterium Depositing Sulfur Outside the Cells[1]

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From salt flats on the Galapagos Islands, two strains of a red photosynthetic bacterium were isolated and identified as *Ectothiorhodospira mobilis*, an organism first described by Pelsh in 1937. The cells are curved in a short spiral, 0.7 to 1.0 μ wide and 2.0 to 4.8 μ long. They are motile by a polar tuft of flagella. Cells contain several large stacks of lamellar membranes, carrying the pigments bacteriochlorophyll *a* and carotenoids of the spirillo xanthin series. Cell division occurs by binary fission, not budding. The organism is strictly anaerobic and obligately photosynthetic. Its ability to grow well with sulfide, sulfur, thiosulfate, or sulfite as photosynthetic H donors puts it taxonomically in the *Thiorhodaceae*. During growth with sulfide, elementary sulfur is deposited outside the cells in the medium and disappears during further growth. A limited number of organic carbon compounds can be utilized as hydrogen donors in place of inorganic sulfur compounds. Under these conditions, sulfate can serve as the sulfur source. The enzymes catalase and hydrogenase are present. The newly isolated strains require vitamin B_{12}. They also require a salinity of 2 to 3% NaCl, but they are not extreme halophiles. The organism is not identical with any of the species listed in *Bergey's Manual*.

In 1931, C. B. van Niel (45) grouped the pure cultures of the *Thiorhodaceae* that he had isolated into three morphological types: the rod-shaped *Chromatium* type, the spherical *Thiocystis* type, and the small *Pseudomonas* type. In contrast to the first two types, the third, when growing with sulfide as the photosynthetic hydrogen donor, did not store elementary sulfur globules inside the cells but deposited them outside.

Pelsh (27) named the first species of van Niel's *Pseudomonas* type *Ectothiorhodospira mobile*. His work was not treated in *Bergey's Manual* (6th and 7th editions) or in *Index Bergeyana*, nor was it discussed by Scardovi (37), Osnitskaya (26), Kondratieva (17), or Yang Hui-Fang (52), who later described additional representatives of van Niel's *Pseudomonas* type as *Rhodopseudomonas vannielii*, *Rhodopseudomonas issatchenkoi*, and organisms resembling *Rhodopseudomonas palustris*.

Following the rules of the International Code of Nomenclature of Bacteria (Intern. J. Systematic Bacteriol. **16**:459-490, 1966), Pelsh's description of *Ectothiorhodospira mobile* is valid and legitimate. The correct form of the specific name is *mobilis*, since the generic name ending with *-spira*, is female. Pelsh (27) also proposed a subfamily, the *Ectothiorhodaceae*, to contain purple bacteria of van Niel's *Pseudomonas* type. The equivalent taxon for purple sulfur bacteria with intracellular sulfur storage, *Endothiorhodaceae*, had previously been proposed by Baas Becking (2). Pelsh's original strain was lost before much information concerning its properties had been obtained. Chesnokov and Saposhnikov (6) found that, in van Niel's (45) medium supplemented with 2% NaCl and 1% sodium phosphate, sulfide could be replaced by sulfite, thiosulfate, or elementary sulfur, with optimal *p*H values of 7.4, 7.5, and 8.5, respectively. They further demonstrated that a considerable number of organic carbon compounds could be metabolized by the organism (7). Saposhnikov (35) also showed that elementary sulfur could be replaced by elementary selenium, which was oxidized to selenate during photosynthetic growth at *p*H 8 to 9. Finally, Pelsh's strain was used in a study on the effect of the redox potential of the medium on quantum yields (36). The optimal *r*H range

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found was 12 to 16; i.e., the E_h was -100 to $+100$ mv.

In enrichments for photosynthetic sulfur bacteria from mud of estuarine salt flats at Academy Bay on the Galapagos Island of Santa Cruz, spirilloid photosynthetic bacteria developed in Pfennig's medium (29) containing sulfide as the sole photosynthetic hydrogen donor. Two strains of the organism were isolated and identified as *Ectothiorhodospira mobilis* Pelsh. Since Pelsh's original isolate is no longer available (19), it was the aim of this study to redescribe *E. mobilis*, and to differentiate it from other members of the *Thiorhodaceae* and *Athiorhodaceae*.

MATERIALS AND METHODS

Samples. The mud samples were collected during cruise no. 15 of the U.S. Research Vessel *Anton Bruun*, April 1966, from an estuarine salt flat at Academy Bay on Isla Santa Cruz (Indefatigable Island), Galapagos Islands (Archipelago de Colon). The samples had salinities of 25 to 29% (total salts, w/v), contained large amounts of decaying algal material, and had a definite hydrogen sulfide odor.

Organisms. For comparison with the isolated strains 8112 and 8113, *Rhodopseudomonas palustris* strains 17000, 17002 (ATCC), *Rhodospirillum rubrum* strain 6461 (H. W. Jannasch), and *Chromatium vinosum* strain 8214 (isolated from Juniper Point Pond, Woods Hole, Mass.) were used.

Media. Cells were photolithotrophically grown in Pfennig's (29) medium containing vitamin B_{12} (Cyanocobalamine, Merck & Co., Inc., Rahway, N.J.). For sulfide-free photoorgantrophic growth, the following modification of Pfennig's medium was used: $MgCl_2 \cdot 6H_2O$ was replaced by $MgSO_4$; NH_4Cl, by 0.1% ammonium acetate; and $Na_2S \cdot 9H_2O$, by 0.1% sodium ascorbate. In comparative growth experiments with organic acids as hydrogen donors (Table 1), NH_4Cl was omitted, and the acids were added in the form of ammonium salts. All media for *E. mobilis* were prepared with 30 g of NaCl per liter. If not otherwise indicated, the incubation temperature was 25 C, and the light intensity was 500 lux.

For all media, the trace element solution of Pfennig and Lippert (32) was used. The purity of the cultures was checked microscopically and by inoculation into *Desulfovibrio* medium (1) and Difco AC medium.

Staining. The Gram stain was performed as Hucker's modification (16); poly-β-hydroxybutyrate in the cells was stained with Sudan Black B (38), and polysaccharides were stained with Lugol's reagent (16).

Catalase. Sulfide-grown cells were harvested by centrifugation and taken up in 3% H_2O_2. Development of oxygen bubbles was considered as a positive catalase reaction.

Hydrogenase. A 50-ml amount of a sulfide-grown cell suspension was harvested by centrifugation, washed twice, and taken up in 10 ml of the following solution (per 1,000 ml of distilled water): $CaCl_2 \cdot 2H_2O$, 0.7 g; trace element solution (32), 16.7 ml; $MgCl_2 \cdot 6H_2O$, 0.33 g; NH_4Cl, 0.33 g; KCl, 0.33 g; KH_2PO_4, 0.33 g; NaCl, 30.0 g; *p*H 7.0. A 2-ml amount of this suspension was used per Thunberg tube. The bulb contained 1.0 ml of methylene blue solution (5 mg/100 ml of distilled water). The Thunberg tubes were twice evacuated and filled with H_2; control tubes were filled with N_2. After tipping of the bulb contents into the cell suspension, one set of tubes was incubated for 30 min at 25 C and a light intensity of 1,000 lux, and a parallel set was incubated in the dark. Decolorization of the methylene blue was taken as a positive hydrogenase reaction. Nitrite formation from nitrate was tested with Griess-Ilosvay reagent (40). All chemicals used were of analytical grade.

RESULTS

Enrichment and isolation. E. mobilis developed in daylight-illuminated enrichments in Pfennig's (29) medium supplied with 3% NaCl. While two *Chromatium* species were isolated also from these enrichments (Trüper and Jannasch, Arch. Mikrobiol. *in press*), no development of green photosynthetic sulfur bacteria was evident. Several attempts to enrich photosynthetic bacteria in media with the salinity of the samples used for inoculation were unsuccessful. Two strains of *E. mobilis*, 8112 and 8113, originating from different enrichments, were obtained in pure culture through repeated agar shake dilution series (29).

Morphology. In Pfennig's medium plus 3% NaCl, cells of *E. mobilis* are short spirilla or (directly after division) vibrios (Fig. 1A). The cell diameter is 0.7 to 1.0 μ; the length of young cells is 2.0 to 2.6 μ and that of full spirals is 3.6 to 4.8 μ. The average width of the spiral is 1.4 μ. These measurements agree well with those of Pelsh's original description, namely, 0.6 to 0.8 μ by 3.0 to 6.0 μ. In aged cultures, irregular cell shapes and even branching sometimes occur. The cells are motile by means of a polar tuft of flagella (Fig. 2). The Gram reaction is negative.

In cultures grown with sulfide, globules of elementary sulfur are deposited outside the cells in the medium (Fig. 1A). Although parts of the cells appear to be more dense than the rest of the cell in the phase-contrast microscope, inclusions of elementary sulfur were never seen.

The lens-shaped colonies in agar shake cultures are surrounded by a yellowish-white halo of sulfur globules during the early stages of growth. The cells divide by binary fission (Fig. 3), never by budding as reported for *Rhodopseudomonas palustris* (Fig. 1B) and *R. viridis* (51).

Thin sections of *E. mobilis* show a type of ultrastructure which, though described for several nonsulfur purple bacteria (10, 11, 13), has been previously unrecorded in photosynthetic sulfur bacteria. The cells contain several large stacks of lamellar membranes, which differ in number, size,

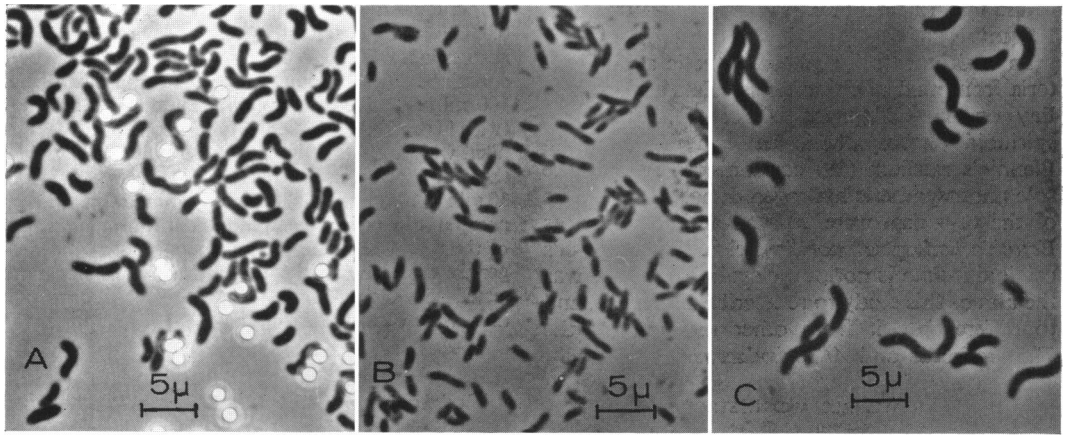

FIG. 1. *Cell morphology of Ectothiorhodospira mobilis compared with Rhodopseudomonas palustris and Rhodospirillum rubrum. (A) E. mobilis strain 8112, sulfide-grown. (B) Rhodopseudomonas palustris strain ATCC 17002, malate-grown. (C) Rhodospirillum rubrum strain 6461, malate-grown.*

and arrangement from those described for *Athiorhodaceae*. The lamellar membranes of *E. mobilis* are most probably the carriers of the photosynthetic pigments. Detailed studies on the ultrastructure of this organism are in progress. The recently described photosynthetic extreme halophile of Raymond and Sistrom (33) apparently possesses similar membrane stacks.

Photosynthetic pigments. Although single cells under the microscope do not show a discernible color, clusters of cells appear red. Cell suspensions have a red, sometimes brownish-red, color. Photoautotrophically grown cultures appear more red, and photoheterotrophically grown cultures have a more brownish cast. Young photoautotrophic culture suspensions are brownish and turn to red with further development. The disc-shaped colonies in agar shake cultures are dark cherry red.

The absorption spectrum of *E. mobilis* (Fig. 4) resembles that of *R. palustris*. The absorption maxima at 375, 590, 865 mμ, as well as the shoulder at 800 mμ, indicate the presence of bacteriochlorophyll *a*. From the shape of the absorption curve between 435 and 630 mμ, it may be concluded that the carotenoids of the organism belong to the spirillo xanthin series (39), and, within this series, to subgroup B, with rhodopin as the major carotenoid. In photoheterotrophically grown cells, the absorption spectrum is slightly different: a broad bacteriochlorophyll maximum at 830 mμ appears instead of that at 865 mμ with the 800-mμ shoulder in autotrophic cells. In the carotenoid area of the curve, in addition to the maximum at 490 mμ a new maximum appears at 520 mμ, where autotrophically grown cells show only a slight shoulder. A detailed study on the carotenoids of *E. mobilis* is in progress.

Physiology. The isolated strains of *E. mobilis* grew well under strictly anaerobic conditions in Pfennig's medium with bicarbonate as the sole carbon source and sulfide as the photosynthetic hydrogen donor. No growth was obtained in the dark or under aerobic conditions. The optimal *p*H for *E. mobilis* in Pfennig's medium is between 7.6 and 8.0, which is in the same range as the *p*H used by Pelsh (27) with van Niel's (45) medium, as well as with the optimal *p*H of Kondratieva's sulfide-utilizing *Rhodopseudomonas* sp. (44). For *Thiorhodaceae* with intracellular sulfur storage in Pfennig's medium, the optimal *p*H is 7.0 to 7.5 (30).

Like several other *Thiorhodaceae* (H. H. Thiele, Thesis, Univ. of Göttingen, Göttingen, Germany, 1966), *E. mobilis* may also be grown in media free from reduced sulfur compounds, as long as a suitable photosynthetic hydrogen donor (e.g., acetate) and sulfate as a sulfur source are provided. The organism is capable of assimilatory sulfate reduction. Also, in media with organic carbon compounds as hydrogen donors, no growth occurs in the dark or under aerobic conditions. Attempts to train the organism to grow aerobically in the dark by gradually lowering the light intensity and increasing the amounts of air in the bottles were unsuccessful.

E. mobilis does not lose its ability to utilize sulfide as the photosynthetic hydrogen donor when cultivated through several transfers under photoheterotrophic conditions.

Table 1 shows the utilization of photosynthetic

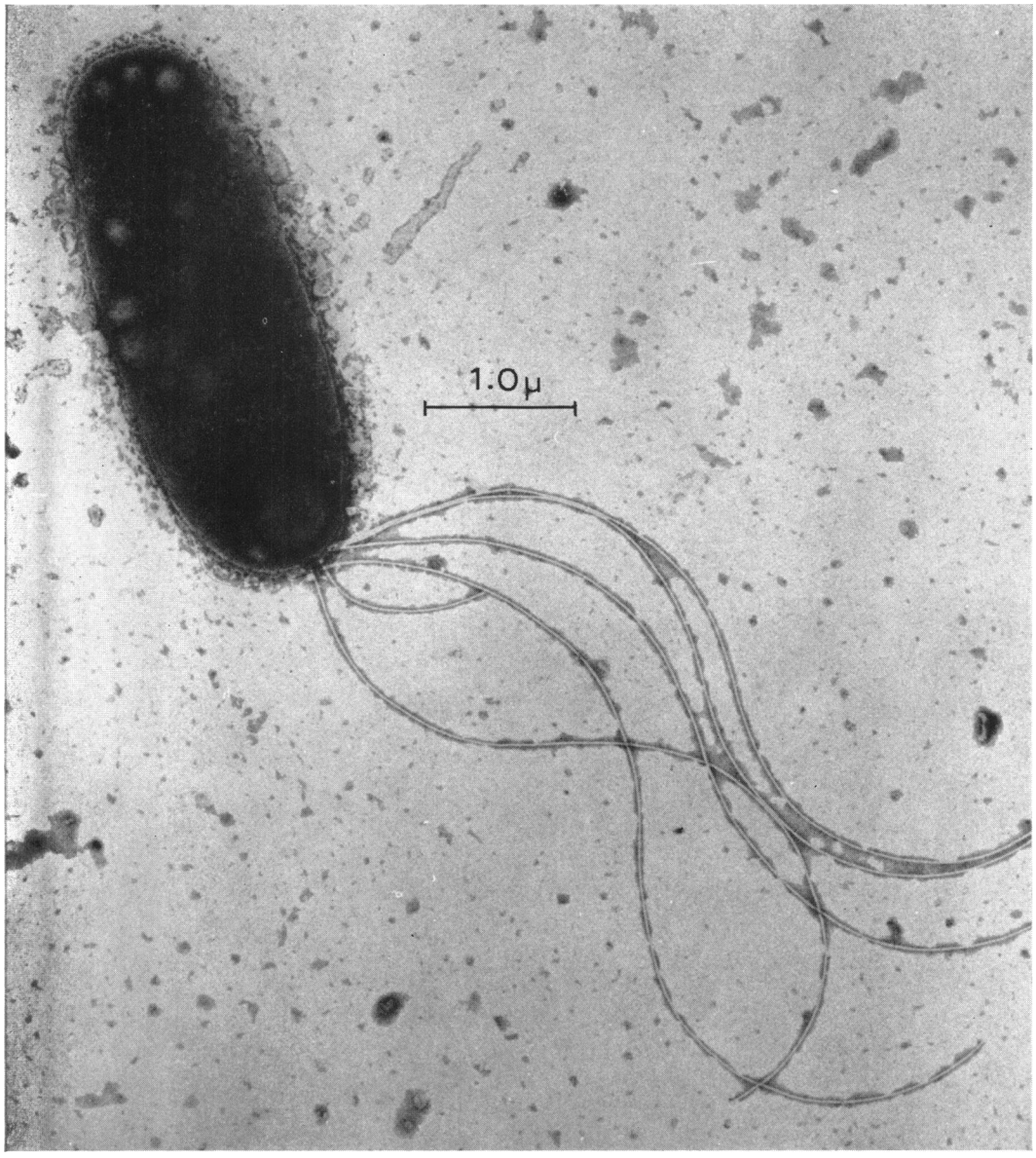

FIG. 2. *Flagellar tuft of Ectothiorhodospira mobilis, strain 8113; young cell. Negative stain (15). Courtesy of S. C. Holt, University of Massachusetts, Amherst.*

hydrogen donors in the presence of sulfide. The two strains differ with respect to utilization of glucose, butyrate, propionate, and lactate. Such differences should probably be considered minor strain differences; they occur in other species of both the *Thiorhodaceae* and the *Athiorhodaceae*. For comparison, data obtained with two *Chromatium vinosum* strains (D and 8214) are also listed.

Acetate-grown cells of *E. mobilis* store poly-β-hydroxybutyrate and malate-grown cells store polysaccharides, as shown by staining methods. The organism possesses catalase and hydrogenase. Since sulfate is utilized as a sulfur source, photoautotrophic growth with molecular hydrogen as the hydrogen donor is possible.

As nitrogen sources, ammonium salts are readily utilized. Nitrate is neither reduced to

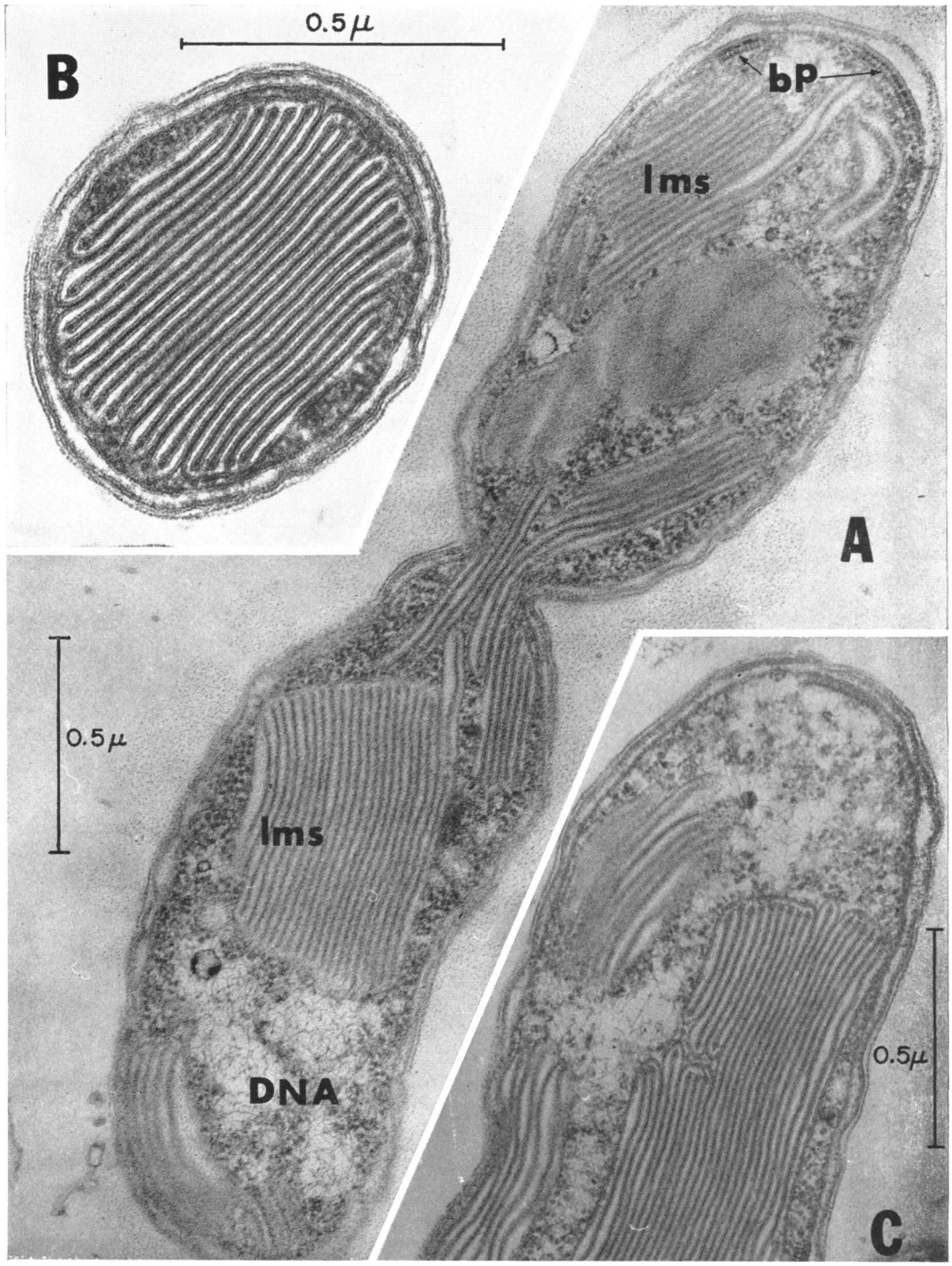

FIG. 3. *Ultrastructure of Ectothiorhodospira mobilis strain 8112. (A) Longitudinal section of a dividing cell. (B) Cross section. (C) Longitudinal section. Lamellar membrane stacks, lms; basal membrane of the flagellar tuft, bm; nucleoplasm, DNA. Fixation (34). Courtesy of J. B. Waterbury and S. W. Watson, Woods Hole Oceanographic Institution, Woods Hole, Mass.*

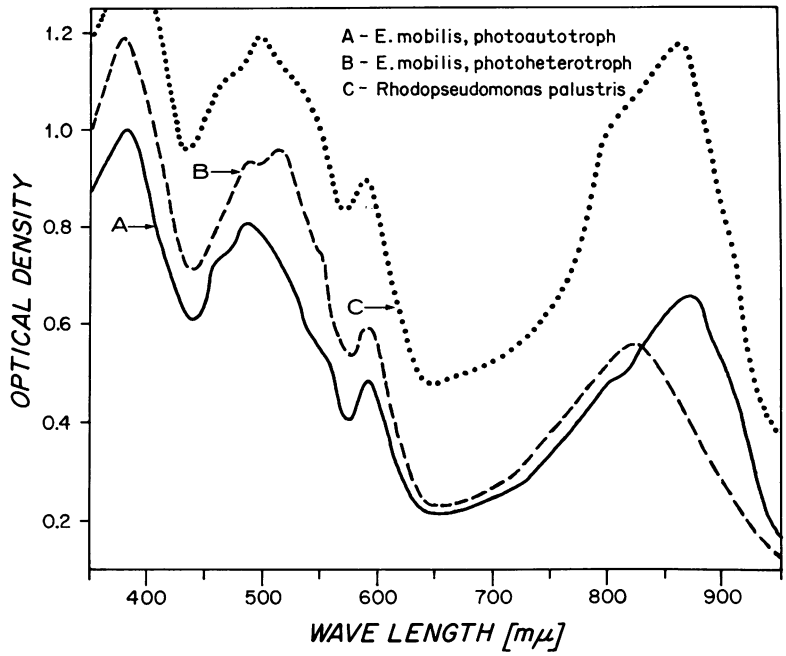

FIG. 4. *In vivo light absorption spectra of Ectothiorhodospira mobilis strain 8112, and Rhodopseudomonas palustris strain ATCC 17000. Measurements on glass fiber filters (43).*

nitrite nor utilized as a nitrogen source. The fixation of molecular nitrogen has not so far been convincingly demonstrated. Both strains of *E. mobilis* require vitamin B_{12}, since transfers into vitamin B_{12}-free medium failed to grow.

E. mobilis may be considered a moderate halophile. By measuring growth yields (turbidity at 650 mμ) at low salinities, it was found that with 2% NaCl the growth was 90% of that with 3% NaCl; the cells still maintained a vibrioid to spirilloid shape. With 1% NaCl, growth was only 46% of that with 3% NaCl; the majority of the cells had irregular forms. In a medium without NaCl, no growth occurred; the cells became immotile, clumped, and died.

Ecology. Pelsh's original strain of *E. mobilis* had been isolated from a layer of purple sulfur bacteria on the gypsum mud crust in the eastern reservoir of lake Sakskoe on the Crimea Peninsula. The salinity of this environment is given as 12 to 20° Be, i.e., 12.5 to 22% NaCl (27). However, the strain was not an extreme halophile. The newly isolated strains 8112 and 8113 were obtained from a salt bed on the Galapagos Islands with salinities of 25 to 29% NaCl. Strongly saline environments appear to be the favored natural habitat of *E. mobilis*. Baas Becking (2) observed a spirillum in mud of Owens Lake, Calif., that apparently deposited sulfur extracellularly; and Butlin and Postgate (5) reported "many spiral bodies" in the $CaSO_4$-saturated saline lake of Ain-ez-Zauia, Cyrenaica, Libya. It seems quite possible that these authors observed *Ectothiorhodospira* species. Enrichment cultures designed for isolation of *E. mobilis* should be prepared in media with 3% NaCl and a *p*H of 7.6 to 8.2, and they should be inoculated from saline aquatic environments. The extreme halophilic photosynthetic bacterium recently isolated by Raymond and Sistrom (33) from highly saline lakes in Oregon seems to be closely related to *E. mobilis*.

Relationships between E. mobilis and other photosynthetic bacteria. Table 2 shows the characteristic properties of several photosynthetic bacteria that have similarities with *E. mobilis*. The data for Table 2 were collected from *Bergey's Manual* and from references given in the Literature Cited section (6, 17–20, 22, 26, 27, 30, 31, 33, 37, 40, 44, 47, 50, 52).

As Fig. 1 shows, the cell shape of *E. mobilis* is more like that of *R. rubrum* than that of *R. palustris*, although its ultrastructure and pigments suggest a possible affinity to the latter organism. The lamellar membrane systems of *E. mobilis* show similarities with those of *R. palustris*, *R. viridis*, and *Rhodomicrobium vannielii*, as well as *Rhodospirillum fulvum*, *R. molischianum*, and *R. photometricum*. However, they are not organized as one extended system of closely packed lamellae parallel to the cell wall and appressed to the cell

TABLE 1. *Utilization of photosynthetic hydrogen donors by Ectothiorhodospira mobilis and Chromatium vinosum*[a]

Additions	Per cent	*E. mobilis*		*C. vinosum*	
		Strain 8112	Strain 8113	Strain 8214	Strain D[b]
Sulfide	0.05	Control	Control	Control	Control
Thiosulfate	0.1	+	++	++	+
Sulfur	0.1	+	+	−	+
Sulfite	0.05	+	+	+	+
Methanol	0.1	(i)	−	−	−
Ethyl alcohol	0.1	−	−	−	−
Glycerol	0.1	−	−	−	−
Glucose	0.1	+	(i)	−	−
Fructose	0.1	+	++	+	−
Ascorbate	0.1	−	−	−	−
Casamino Acids	0.1	(+)	(+)	(+)	+
Pyruvate	0.1	++	++	++	++
Acetate	0.1	++	++	++	++
Butyrate	0.05	−	++	−	−
Propionate	0.02	+	i	+	+
Lactate	0.05	i	+	+	−
Benzoate	0.05	−	−	−	−
Formate	0.02	−	−	−	+
Malate	0.1	+	++	++	++
Succinate	0.1	(+)	+	++	++
Citrate	0.1	ii	ii	−	−

[a] Growth was compared as optical density readings of cell suspensions at 650 mμ, the sulfide control figuring as 1.0; −, growth like control; (+), growth slightly better than control (1.1 to 1.3); +, growth enhanced (1.4 to 1.9); ++, strong enhancement of growth (>2.0); (i), growth slightly inhibited (0.9 to 0.7); i, growth inhibited (0.6 to 0.2); ii, growth completely inhibited. Incubation: 5 days at 500 lux, 25 C.

[b] Thiele, Thesis, Univ. of Göttingen.

membrane, as in *R. palustris* (10), *R. viridis* (13), and *R. vannielii* (3); nor are they numerous and positioned at distinct angles to the cell membrane, as in *R. molischianum* (11) or *R. fulvum* (10). In relation to the size of the cell, the lamellar membrane stacks of *E. mobilis* appear larger than those in *Rhodospirillum* spp. They are not positioned at distinct angles but appear appressed to the cell membrane. The arrangement of the lamellar membrane stacks in the extreme halophilic photosynthetic bacterium of Raymond and Sistrom (33) is almost identical to that in *E. mobilis*.

E. mobilis possesses a polar tuft of flagella (Fig. 2) emerging from a specialized basal membrane structure (Fig. 3A), as reported for *Spirillum serpens* (23), *R. rubrum* (8), and *R. fulvum* (9). The extreme halophile of Raymond and Sistrom (33) possesses only one thick flagellum. The mode of cell division suggests a closer relationship of *E. mobilis* to *Rhodospirillum* species than to *Rhodopseudomonas palustris*, *Rhodopseudomonas viridis*, and *Rhodomicrobium vannielii*, which divide by budding (51) rather than by binary fission.

Physiologically, *E. mobilis* fits into the family of the *Thiorhodaceae*, as demonstrated by the utilization of sulfide, sulfur, sulfite, and thiosulfate as photosynthetic hydrogen donors. Among the *Athiorhodaceae*, *Rhodopseudomonas palustris* is able to utilize thiosulfate, but no other reduced sulfur compounds. The utilization of fructose is quite common in the small *Chromatium*, *Thiocystis*, and *Thiocapsa* species, as is the nonutilization of ethyl alcohol, citrate, and benzoate (42; Thiele, Thesis, Univ. of Göttingen, 1966; Trüper, *unpublished data*). Being strictly anaerobic, *E. mobilis* differs from the species described by Scardovi (37), Osnitskaya (26), Kondratieva (17), and Yang Hui-Fang (52), and it appears to be more closely related to the *Endothiorhodaceae* and the strictly anaerobic brown *Rhodospirillum* species. *E. mobilis* cannot be identified with the poorly described *Thiospirillum rufum* (*Bergey's Manual*), since *Thiospirillum*, by definition, stores intracellular sulfur globules. Although several

TABLE 2. *Characteristic features of Ectothorhodospira mobilis as compared with the so far described Ectothiorhodaceae and several Athiorhodaceae*[a]

Feature	*Ectothiorhodaceae*							*Athiorhodaceae*				
	E. mobilis strains 8112, 8113	*E. mobile* (ref. 27)	*Rhodopseudomonas issatchenkoi* (ref. 26)	*Rhodopseudomonas vannielii* (ref. 37)	*Rhodopseudomonas* sp. (ref. 17)	*Rhodopseudomonas* sp. (ref. 52)	Extreme halophile "SL1" (ref. 33)	*Rhodopseudomonas palustris*	*Rhodospirillum fulvum*	*Rhodospirillum molischianum*	*Rhodospirillum photometricum*	*Rhodospi illum rubrum*
Morphology												
Curved/spiral	+	+	−	−	−	−	+	−	+	+	+	+
Budding	−	−	n	n	n	n	−	+	−	−	−	−
Polar flagella	+	+	+	+	+	+	+	+	+	+	+	+
Lamellar membranes	+	n	n	n	n	n	+	+	+	+	+	−
General physiology												
Salt requiring	+	+	−	−	−	−	+	−	−	−	−	−
Vitamin requiring	B_{12}	−	−	−	−	−	n	pAB	pAB	n	n	bio
Facultative aerobe	−	n	+	+	+	+	−	+	−	−	−	+
Assimilatory SO_4^{2-} reduction	+	n	+	+	+	+	n	+	+	+	+	+
Significant H-donors utilized												
Sulfide	+	+	+	+	+	+	+	−	−	−	−	−
Thiosulfate	+	+	n	−	+	±	+	+	−	−	−	−
Sulfur	+	+	n	+	+	n	+	−	−	−	−	−
Sulfite	+	+	n	n	−	±	n	−	−	−	−	−
Ethyl alcohol	−	n	n	n	−	n	−	+	+	+	+	+
Propionate	±	+	n	−	+	+	n	+	+	+	+	−
Citrate	−	n	+	−	±	−	n	n	±	±	+	n
Glucose	±	n	n	−	+	n	−	−	±	−	−	−
Fructose	+	n	n	−	+	n	n	±	−	−	−	−
Benzoate	−	n	n	n	n	n	n	+	±	−	n	−
Pigments												
Bacteriochlorophyll	*a*	n	n	n	*a*	*a*	*a*	*a*	*a*	*a*	*a*	*a*
Carotenoid group	1B	n	n	n	1B	n	1	1B	1C	1C	1	1A
Deoxyribonucleic acid base ratio	67.3	n	n	n	64.0	n	n	64.5–66	64–66	61–62.5	n	64–66

[a] n = definite information missing; pAB = *p*-aminobenzoate; bio = biotin.

authors (see *Bergey's Manual*) failed to mention the presence of sulfur globules in *T. rufum*, they were reported and photographed by Gietzen (14). Size and shape of the spiral of *T. rufum* is more similar to that of *Rhodospirillum rubrum* than to the short, slightly curved spiral of *E. mobilis*.

The extreme halophilic bacterium of Raymond and Sistrom (33) also belongs to the *Ectothiorhodaceae*, and should probably be assigned to the genus *Ectothiorhodospira*. It is definitely not identical with *E. mobilis*, differing by virtue of its extreme halophily (inability to grow in media with less than 4% NaCl, and preference for media with 14 to 22% NaCl); its single, probably sheathed, flagellum; and its inability to grow at the expense of organic carbon compounds as hydrogen donors.

Generic description of Ectothiorhodospira Pelsh

Ectothiorhodospira Pelsh, 1937 (The photosynthetic sulfur bacteria of the eastern reservoir of Lake Sakskoe [in Russian], Mikrobiologiya *6*, 1937, 1096.)

Ec.to.thi.o.rho.do.spi'ra. Gr. prep. *ecto* outside; Gr. noun *thium* sulfur; Gr. noun *rhodum* the rose; Gr. noun *spira* the spiral. M. L. fem. n. *Ectothiorhodospira* the rose spiral with sulfur outside.

Sulfur purple bacteria of spiral to vibrioid shape, dividing by binary fission, motile by means of polar flagella. Gram-negative. Contain bacteriochlorophyll and carotenoids. Photosynthetic in mineral media in the presence of extraneous oxidizable substances such as reduced sulfur compounds. Molecular oxygen is not produced. During growth with sulfide, globules of elementary sulfur are deposited extracellularly in the medium. No intracellular sulfur storage occurs. The sulfur may be further oxidized to sulfate.

The type species is *Ectothiorhodospira mobilis* Pelsh (monotype).

Species description of Ectothiorhodospira mobilis Pelsh

Ectothiorhodospira mobilis Pelsh, 1937 (Mikrobiologiya *6*, 1937, 1096; *Ectothiorhodospira mobile* [sic] Pelsh, Mikrobiologiya *6*, 1937, 1096.)

mo'bi.lis. L. adj. *mobilis* motile.

Morphology: Cells weakly curved in a short spiral. Width, 0.7 to 1.0 μ; length of young cells, 2.0 to 2.6 μ; of full spiral, 3.6 to 4.8 μ. Average width of the spiral, 1.4 μ. In old cultures, irregular cell shapes occur. Motile by means of a polar tuft of flagella. Cells contain several large stacks of lamellar membranes, carrying photosynthetic pigments.

Culture: Obligately photosynthetic, strictly anaerobic; photoautotrophic, facultatively photoheterotrophic. Optimal *p*H 7.5 to 8.0, growth temperature 25 C. Color of photoautotrophically grown suspensions, red; of photoheterotrophically grown suspensions, brownish red. Lens-shaped colonies in agar, dark red; young colonies in sulfide containing solid media are surrounded by a yellow halo of elementary sulfur which disappears during further growth. Obligately but not extreme halophilic, depending on at least 2% NaCl in the medium. Dependence on vitamin B_{12} may be type-specific, though not originally mentioned.

Pigments: Bacteriochlorophyll *a*, carotenoids of the spirillo xanthin series. Absorption spectra of living cell suspensions show characteristic maxima at 375, 590, 830–865 mμ (bacteriochlorophyll *a*), and 490, 520 mμ (carotenoids).

Photosynthetic hydrogen donors: Sulfide, sulfur, thiosulfate, sulfite, acetate, pyruvate, malate, fructose. Not utilized as such are: ethyl alcohol, citrate, benzoate. Citrate is strongly inhibitory.

Nitrogen sources: Ammonium salts. Nitrate is neither utilized nor reduced to nitrite. Molecular nitrogen is not utilized.

Storage materials: Polysaccharide, poly-β-hydroxybutyrate.

Enzymes present: Catalase, hydrogenase. Capable of assimilatory sulfate reduction.

Deoxyribonucleic acid base composition: 67.3 moles % guanosine plus cytosine.

Source: Hydrogen sulfide containing salt lakes and salt flats.

Holotype: Lost. Proposed neotype: strain 8112 (Santa Cruz I).

DISCUSSION

The basis for the differentiation between *Thiorhodaceae* and *Athiorhodaceae* has changed several times. Molisch (21), who first suggested the taxonomic recognition of two groups among the purple bacteria, considered as *Athiorhodaceae* those purple bacteria that do not store elementary sulfur globules inside their cells. Later, Buder (4) emphasized the basic physiological differences as more important, i.e., the *Athiorhodaceae* are heterotrophs, depending on organic carbon compounds, whereas the *Thiorhodaceae* are autotrophs, growing in the presence of sulfide and carbon dioxide. The extensive studies of van Niel (47) on 150 strains of *Athiorhodaceae* revealed that this group in general needed organic growth factors. These growth factor requirements were considered by *Bergey's Manual* (6th and 7th editions) and by Thimann (41) to constitute a basic difference from the *Thiorhodaceae*. Recent studies have shown that some members of the *Thiorhodaceae* have an absolute requirement for

vitamin B_{12} (28, 32, 42), whereas some members of the *Athiorhodaceae* do not need vitamins (12, 30). Accordingly, vitamin requirements can no longer be regarded as taxonomically valuable for the differentiation of *Thiorhodaceae* and *Athiorhodaceae*. Pfennig (30) stated in his review on photosynthetic bacteria that "the systematically important difference between purple sulfur and nonsulfur bacteria is nutritional and ecological: hydrogen sulfide is the critical factor which inhibits the growth of the Athiorhodaceae but is an electron donor for the photoautotrophic development of the Thiorhodaceae."

Although van Niel in *Bergey's Manual* (6th ed.) mentioned the similarity between his *Pseudomonas* type (45) and *Rhodopseudomonas palustris*, it is obvious from his paper in 1963 that he considers sulfide utilization by a red photosynthetic bacterium to provide the primary criterion for its recognition as a member of the *Thiorhodaceae* (49). The inclusion of the sulfide-utilizing species of Scardovi (37), Osnitskaya (26), Kondratieva (17), and Yang Hui-Fang (52) in the genus *Rhodopseudomonas* is thus not in agreement with the taxonomic proposals of Buder (4), van Niel (45, 49), Pelsh (27), and Pfennig (30): these organisms all appear to be purple sulfur bacteria. *Rhodopseudomonas vannielii* Scardovi, *Rhodopseudomonas issatchenkoi* Osnitskaya, and the autotrophic *Rhodopseudomonas* species of Kondratieva (17) were described as straight motile rods. In the illustrations of Scardovi (37) and Osnitskaya (26), however, their organisms appear rather pleomorphic. It seems possible that the authors did not achieve optimal growth conditions; hence, the morphological descriptions might be subject to revision. Van Niel stated that, despite several attempts, cultures sent by Scardovi failed to grow in his laboratory (48). To determine the final taxonomic status of these sulfide-utilizing "Rhodopseudomonads," careful reinvestigation is necessary. From their descriptions, it is definitely clear, however, that they belong to the *Ectothiorhodaceae* rather than the *Athiorhodaceae*.

The sulfide-utilizing *Athiorhodaceae* described by van Niel (46) and Nakamura (24, 25) may have been representatives of the *Ectothiorhodaceae*. Retrospectively, their taxonomic position is difficult to determine.

The recent finding of an extreme halophilic *Ectothiorhodospira*-like bacterium by Raymond and Sistrom (33) indicates that the subgroup *Ectothiorhodaceae* of the *Thiorhodaceae* might embrace a wide variety of organisms that occupy aquatic environments ranging from fresh-water (E. N. Kondratieva, *personal communication*) to highly saline brines.

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