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Rhodopseudomonas sulfidophila, nov. spec., a New Species of the Purple Nonsulfur Bacteria

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Summary. From marine mud flats a new type of photosynthetic purple bacterium was isolated. This type is described as a new species of the Rhodospirillaceae and is named *Rhodopseudomonas sulfidophila*. The cells are rod-shaped, 0.6 to 0.9 μ wide and 0.9 to 2.0 μ long, and motile by means of polar flagella. Cell division occurs by binary fission. The photosynthetic membrane system is of the vesicular type. The pigments consist of bacteriochlorophyll *a* and of carotenoids, most probably of the spheroidene group. A wide range of organic compounds can be utilized anaerobically in the light. Growth on organic compounds aerobically in the dark is also possible. Niacin, thiamin, biotin and p-aminobenzoic acid are required as growth factors. The new species needs 2.5% (w/v) sodium chloride for optimal growth. All strains show excellent photolithotrophic growth on hydrogen, hydrogen sulfide, and thiosulfate. They show a remarkably high sulfide tolerance. Sulfide and thiosulfate are oxidized to sulfate without an intermediate accumulation of elemental sulfur. The new species seems to be one of the most versatile types of photosynthetic bacteria isolated thus far.

Up till recently the purple nonsulfur bacteria were considered to be unable to utilize hydrogen sulfide as a photosynthetic electron donor (cf. Trüper, 1968; Pfennig and Trüper, 1969). However, experiments of Hansen and van Gemerden (1972) have shown that the purple nonsulfur bacteria *Rhodopseudomonas capsulata* SMG 155 and *Rhodopseudomonas palustris* SMG 124 can be grown in an inorganic medium (supplemented with the required organic growth factors) with sulfide as the photosynthetic electron donor. Since especially *Rps. palustris* SMG 124 was inhibited by relatively low sulfide concentrations, cultivation in a chemostat with sulfide as the growth-limiting substrate was the most appropriate way to obtain quantitative data on the utilization of sulfide by these strains. *Rps. capsulata* SMG 155 appeared to convert sulfide into extracellular elemental sulfur, whereas sulfate was the only conversion product in *Rps. palustris* cultures.

The above studies were initiated after the isolation of a new type of photosynthetic purple bacterium from marine mud flats. This type of

bacterium converts sulfide and thiosulfate into sulfate without an intermediate accumulation of elemental sulfur. Its sulfide tolerance is high. The need for organic growth factors and the ability to utilize organic compounds aerobically in the dark as carbon- and energy source suggest a relationship with purple nonsulfur bacteria such as *Rps. capsulata.*

In this paper we propose to accommodate the new strains in a new species of the purple nonsulfur bacteria (Rhodospirillaceae), named *Rhodopseudomonas sulfidophila.*

A short note on this type of bacterium was published earlier (Hansen and Veldkamp, 1972).

Materials and Methods

Media and Growth Conditions. The organisms were grown in a basal medium supplemented with sulfide, organic compounds, yeast extract, and growth factors as indicated. The composition of the basal medium was: NH_4Cl 1 g; KH_2PO_4 1 g; $MgCl_2 \cdot 6H_2O$ 0.5 g; $CaCl_2 \cdot 2H_2O$ 0.1 g; $NaHCO_3$ 2 g (sterilized separately); NaCl 25 g; trace elements solution 10 ml; water up to 1000 ml. The trace elements solution had the same composition, and the organic growth factors were added in the same concentrations as described by Hansen and van Gemerden (1972). In media in which sulfate or thiosulfate served as the electron donor the pH was adjusted to 7.2—7.4. In order to provide a sulfur source $MgCl_2 \cdot 6H_2O$ was replaced by $MgSO_4 \cdot 7\ H_2O$ in media without sulfide or thiosulfate.

The anaerobic utilization of organic compounds was tested in a mineral medium supplemented with growth factors. The pH of the medium was adjusted to 6.8 to 7.0. Growth was estimated by measuring the optical density at 660 nm with a Vitatron UC 200 colorimeter. Photolithotrophic growth with hydrogen as the electron donor was tested as described by Pfennig (1969). A hydrophilic suspension of elemental sulfur was prepared by acidifying a thiosulfate solution (Roy and Trudinger, 1970). After each washing step the sediment was suspended by sonic oscillation. The cultures in which sulfur had to serve as the electron donor were slowly agitated by a magnetic stirrer. The ability to utilize molecular nitrogen as a nitrogen source was investigated by employing a mineral medium with sulfide or malate from which ammonium chloride was omitted; 80 ml of medium were placed under a nitrogen atmosphere (50 ml) in a 130 ml screw-cap bottle. At least three transfers in the same medium were made.

Doubling times of anaerobic cultures were determined by measuring the optical density of cultures in 20 ml screw-cap tubes which could be placed in a special cuvet-holder of a Vitatron colorimeter. The doubling times of aerobic cultures were estimated by measuring the optical density of samples from cultures placed on a rotary shaker. For cultivation under aerobic conditions $NaHCO_3$ was omitted from the medium.

Absorption spectra of intact cells were recorded with a Cary 14 spectrophotometer. In order to reduce light scattering the cell suspensions were saturated with sucrose.

DNA Base Composition. DNA was isolated and purified according to Mandel *et al.* (1971). The moles percentage guanine plus cytosine was calculated from the melting point T_m of the DNA applying the formula of De Ley (1970).

Adenosine-5'-phosphosulfate (APS)-reductase was assayed after Trüper and Peck (1970) employing the spectrophotometric test. Cellfree extracts were prepared with a Ribi cell fractionator.

Results

Isolation of the Organisms. The strains of the new species originate from mud of the intertidal flats of the Dutch Waddenzee, north of the province of Groningen. The first strain (W 4) was isolated from an enrichment culture for purple and green sulfur bacteria employing hydrogen sulfide (2 mM) as the electron donor. This culture was incubated at room temperature at a light intensity of about 2000 lux. After one week purple sulfur bacteria similar to *Chromatium vinosum* were dominating. In addition, a relatively small number of short rods was present. We purified this type of bacterium by a repeated application of the agar shake culture method (Pfennig, 1965). The pure culture of strain W 4 grew in the liquid enrichment medium only after the addition of 0.01 % yeast extract. Additional strains were enriched in media with 2 mM of hydrogen sulfide supplemented with a growth factor mixture, in media with 2 mM of hydrogen sulfide and 0.01 % yeast extract and also in media with organic compounds (0.1 % w/v) such as formate and acetate or in media in which molecular hydrogen served as the electron donor. Such enrichment cultures were incubated at 30° C at 4000 lux. In addition to strain W 4, two other strains isolated on a sulfide-yeast extract medium were studied in detail. Since the differences between the latter strains were only small, the properties of only one of them (strain W 12) are described here. The enrichment methods used were not specific for *Rps. sulfidophila* and yielded varying results. Small *Chromatium* strains were present in large numbers in most cases, even in media with organic compounds and magnesium chloride instead of magnesium sulfate. Since sulfur globules were present in the *Chromatium* cells even when sulfide was not added, some sulfate reduction must have occurred. This is probably due to the high sulfate content of the mixture of seawater and mud which was used as the inoculum.

Attempts to isolate strains with the characteristic features of *Rps. sulfidophila* from water and mud from fresh water ditches were unsuccessful.

Morphology. The cells of the new species are short Gram-negative rods (Fig. 1). Immediately after the cell division the cells are almost spherical to ovoid. The dimensions were found to be rather independent of the composition of the growth medium. Sulfide- and malate-grown cells of strain W 4 are 0.6—0.8 μ wide and 0.9—1.3 μ long. The cells of strain W 12 are 0.6—0.9 μ wide and 1.2—2.0 μ long. Multiplication occurs by binary fission. Short, straight chains of up to four cells were sometimes present in the cultures, although in small numbers. In agar the strains formed lense-shaped colonies.

Very few cells were motile in media with yeast extract. Larger numbers of motile cells were present in cultures grown in minimal media, especially

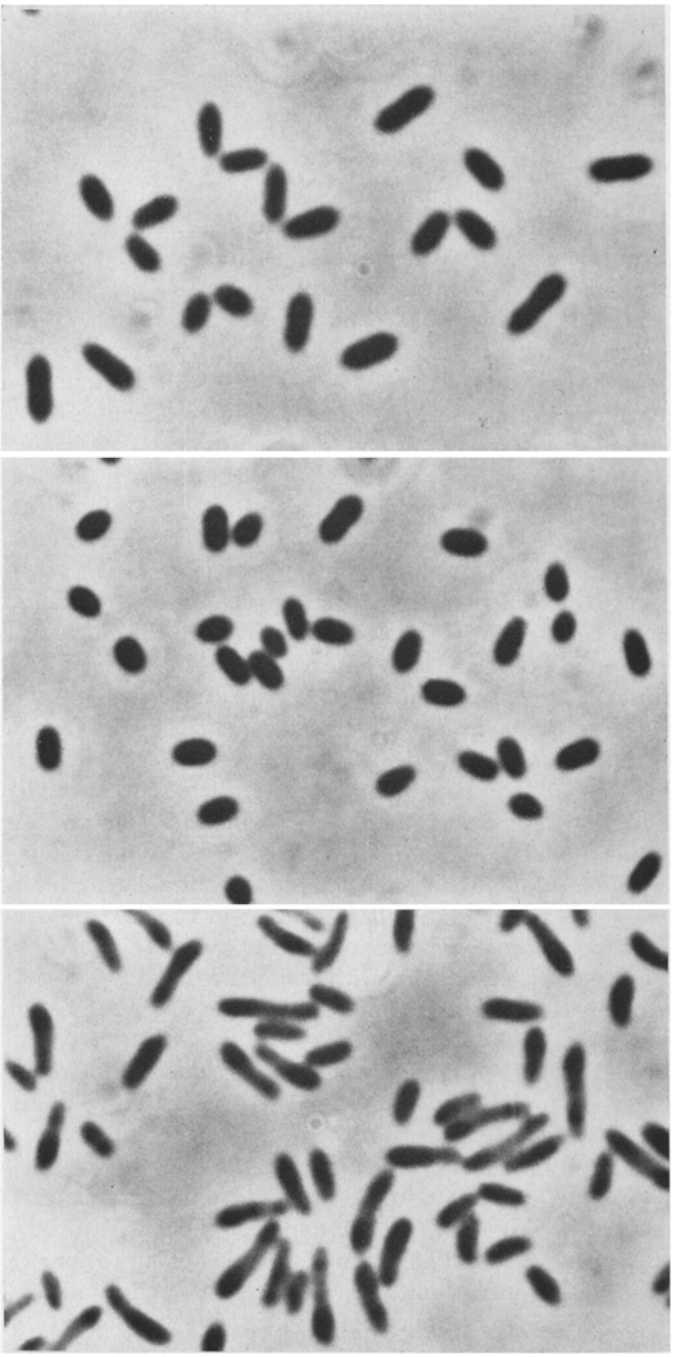

Fig. 1. Morphology of *Rhodopseudomonas sulfidophila* compared with *Rhodopseudomonas capsulata* and *Rhodopseudomonas palustris*. Center: *Rps. sulfidophila* strain W 4. Top: *Rps. capsulata* SMG 155. Note the zig-zag arrangement of the cells. Below: *Rps. palustris* SMG 124. All strains malate-grown. Phase contrast. 3900×

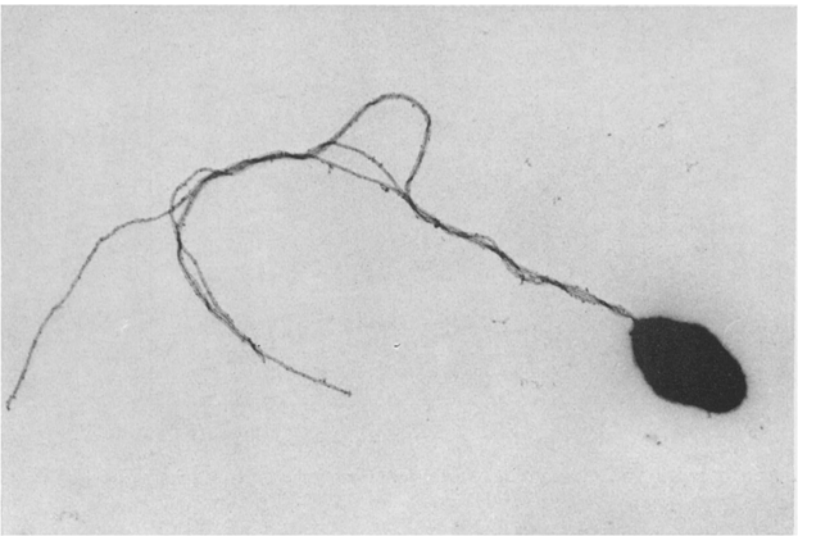

Fig. 2. Electron micrograph of a flagellated cell of strain W 12. Negative stain with uranyl acetate. 20650×

in cultures of strain W 12. The cells are polarly flagellated (Fig. 2). In electron micrographs the number of flagella attached to the cells varied from zero to four.

The photosynthetic membrane system is of the vesicular type as shown in thin sections of strain W 4 (W. E. de Boer, personal communication) and strain W 12 (M. Veenhuis, personal communication).

Pigments. The colour of photosynthetically grown cultures of the strains varied from yellowish-brown to red. Aerobically grown cultures were faintly red. The colour of photosynthetically grown cultures was found to be influenced by the presence of small amounts of oxygen, which caused a colour change from brown to red.

An absorption spectrum of whole cells of *Rps. sulfidophila* strain W 4 is shown in Fig. 3. The main near-infrared peak in the spectrum of strain W 4 is situated at a slightly higher wavelength (855 nm) than the corresponding peak in the spectrum of strain W 12 (850 nm). This absorption peak together with those at 803, 590 and 374 nm indicate the presence of bacteriochlorophyll *a*. The position of the peaks between 450 and 550 nm is similar to those encountered in *Rps. capsulata* strains (Biebl and Drews, 1969). This similarity and the identical influence of oxygen on the colour of *Rps. capsulata* and the new isolates are indications for the presence of carotenoids of the spheroidene group (Jensen, 1963).

Physiology. The growth factor requirement could be satisfied by the addition of niacin, thiamin, biotin and p-aminobenzoic acid. Yeast extract

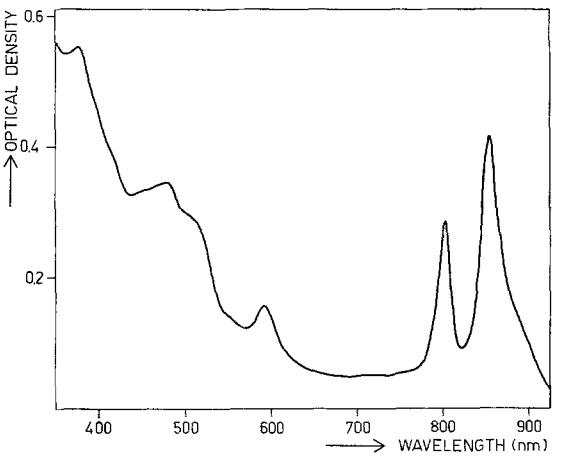

Fig. 3. Absorption spectrum of a brown, malate-grown culture of *Rhodopseudomonas sulfidophila* strain W 4

in a concentration of 0.01 % instead of the growth-factor mixture was satisfactory for strain W 4 but not for some other strains among which strain W 12. After a few transfers in a medium with yeast extract growth became scant. Good growth was restored by the addition of 10 µg/l of biotin to the yeast extract-containing medium. Obviously these strains need higher concentrations of biotin for optimal development than strain W 4.

The most remarkable properties of the newly isolated organisms are the ability to convert sulfide and thiosulfate into sulfate without an intermediate accumulation of elemental sulfur, and the relatively high sulfide tolerance. The term sulfide tolerance is used here in the same sense as it was used by Hansen and van Gemerden (1972). The sulfide tolerance of strain W 4 at pH 7.3 in a minimal medium was found to be about 6.3 mM (equivalent to 0.15 % $Na_2S \cdot 9H_2O$). Under the same conditions strain W 12 tolerated 0.125 % $Na_2S \cdot 9H_2O$ (5.2 mM total sulfide) as the highest concentration. The addition of 0.01 % yeast extract to the minimal medium increased the tolerance to 7 to 8 mM for both strains. For all strains the pH range for growth with sulfide as the electron donor is between 6.5 and 8.0.

Anaerobically in the light several organic compounds can be utilized (Table 1). As can be seen strain W 12 is able to utilize ethanol and propanol whereas strain W 4 is not. In minimal media the cells of strain W 12 had a tendency to clump. Generally strain W 4 did not form cell

aggregates. In media supplemented with 0.1 % yeast extract strain W 12 showed evenly dispersed growth. The pH range for growth on malate is 5.0 to 7.5, the optimum being between 5.5 and 7.0. The temperature optimum of both strains is 30—35° C. The specific growth rate of photosynthetically grown cultures increased with increasing light intensity up to about 4000 lux.

Molecular hydrogen was an excellent electron donor for both strains.

The new isolates grew well with ammonium salts as nitrogen source but could not utilize nitrate. Molecular nitrogen was a satisfactory nitrogen source for strain W 4 when grown on sulfide but not for strain W 12. When grown photosynthetically on malate, molecular nitrogen could not replace ammonium salts as a nitrogen source.

The new isolates grew well aerobically in the dark at full atmospheric oxygen tension both in liquid cultures and on agar slants. In these experiments pyruvate was added to the medium as the carbon- and energy source.

Table 1. Utilization of electron donors and organic compounds by *Rps. sulfidophila* strains grown anaerobically in the light at 30° C and 4000 lux

Substrate	Strain W 4	Strain W 12	Substrate	Strain W 4	Strain W 12
Sulfide	++	++	Propionate (0.05 %)	+÷	÷+
Thiosulfate	++	++	Butyrate	++	++
Sulfite (0.05 %)	–	–	Valerate (0.05 %)	++	÷+
Sulfur (0.2 %)	+	+	Caproate (0.05 %)	++	÷+
Hydrogen	÷+	++	Caprylate (0.05 %)	÷+	÷+
Formate	÷+	++	Pelargonate (0.025 %)	÷+	÷+
Acetate	++	÷+	Aspartate	–	+
Pyruvate	++	++	Alanine	÷	+
Succinate	++	++	Glutamate	÷+	++
Malate	++	++	Glucose	÷	+
Citrate	–	–	Fructose	–	+
Methanol	–	–	Mannose	–	+
Ethanol	–	++	Sorbitol	–	–
Propanol-1	–	++	Mannitol	–	–
Propanol-2	–	++	Casamino acids	++	++
Tartrate	–	–	Yeast extract	++	++
Lactate	++	++	Benzoate (0.05 %)	–	–
Glycerol	++	++			

Unless otherwise stated substrates were added to a concentration of 0.1 % (w/v). Acids were added as sodium salts.

++ = good growth. Optical density at 660 nm within one week > 0.250.
÷ = slow growth.
– = no growth.

Table 2. Doubling times (in hours) of *Rps. sulfidophila* strains under different conditions

Conditions and substrates	Strain W 4	Strain W 12
Anaerobically in the light		
4000 lux, 30° C		
with sulfide	7	7—10[a]
thiosulfate	8	8
formate	11	—[b]
malate	6	7
malate-yeast extract	2.4	2.8
Aerobically in the dark, 30° C		
with pyruvate	6	~8[a]
pyruvate-yeast extract	4.6	3.3

[a] No exact determination possible due to formation of cell aggregates.

[b] Specific growth rate upon visual inspection about the same as of strain W 4. Determination of doubling time impossible due to a pronounced tendency to form cell aggregates.

The doubling times of *Rps. sulfidophila* strains grown with different substrates and under different conditions are summarized in Table 2. The addition of 0.1 % yeast extract to minimal media markedly decreased the specific growth rates.

For optimal development the organisms require 2.5 % (w/v) sodium chloride in the medium. Lower concentrations result in slower growth. No growth could be obtained in growth media lacking NaCl. The cells do not lyse in the absence of sodium chloride.

Accumulation of storage polymers was observed microscopically in cells of both strains when grown anaerobically in the light with acetate. The sudanophilic storage polymer is most probably poly-β-hydroxybutyrate (Schlegel and Gottschalk, 1962).

APS-Reductase. Attempts to show the presence of APS-reductase in sulfide-grown cells of strain W 4 were unsuccessful. A low sulfite-oxidizing activity could be detected in the soluble cell fraction (0.016 µmole sulfite/min × mg protein). This activity, however, was not dependent on the addition of AMP and, therefore, cannot be ascribed to APS-reductase.

DNA Base Composition. The melting points T_m of the DNA of strains W 4 and W 12 in standard saline citrate (Marmur and Doty, 1962) were found to be 96.8° C and 98.4° C, respectively. From these values a guanine plus cytosine content of 67 moles-% for the DNA of strain W 4 and 71 % for the DNA of strain W 12 was calculated.

Discussion

In marine intertidal flats the oxidation of the sulfide which is produced mainly by the activity of sulfate reducing bacteria is brought about by several types of microorganisms, as well as by a direct chemical reaction with oxygen. Under aerobic conditions the oxidation of hydrogen sulfide is carried out by microbes such as *Thiovulum* (La Rivière, 1963), *Thiobacillus*, and *Thiomicrospira* (Kuenen and Veldkamp, 1972). Provided light is available the purple and green sulfur bacteria contribute to the oxidation of hydrogen sulfide under anaerobic conditions. The isolation of photosynthetic sulfur bacteria from marine mud of coastal areas has been described several times (cf. Matheron and Baulaigue, 1972). The physiological properties of *Rps. sulfidophila* indicate that this species may also play an active role in the conversion of sulfide into sulfate in this environment.

Taxonomy. As stated earlier (Hansen and van Gemerden, 1972) the utilization of sulfide by a purple bacterium can no longer be regarded as a primary criterion for its designation to the purple sulfur bacteria. *Rps. sulfidophila* is able to utilize sulfide, thiosulfate and, although slowly, also elemental sulfur. The utilization of extracellular elemental sulfur by purple sulfur bacteria was reported by Thiele (1968). However, *Rps. sulfidophila* lacks the characteristic ability of the Chromatiaceae to form elemental sulfur during growth on hydrogen sulfide and to oxidize it further to sulfate. Therefore, it seems most appropriate to regard our new isolates as members of the Rhodospirillaceae. We were unable to detect APS-reductase activity in extracts of *Rps. sulfidophila* strain W 4. This supports the suggestion made by Trüper and Peck (1970) that among the photosynthetic purple bacteria the presence of this enzyme is specific for organisms belonging to the Chromatiaceae.

In many respects *Rps. sulfidophila* resembles *Rps. capsulata*. The shape and dimensions of the cells of both organisms are very similar (Fig. 1). However, the tendency to form cell chains in a zig-zag arrangement which is characteristic for *Rps. capsulata* according to Van Niel (1944) is not found in *Rps. sulfidophila*. Good growth on H_2/CO_2 was found both for *Rps. capsulata* (Klemme, 1968) and *Rps. sulfidophila*. Most organic compounds that can be utilized by *Rps. sulfidophila* can also be utilized by *Rps. capsulata*. Exceptions are formate and glycerol (all strains of *Rps. sulfidophila*) and ethanol and propanol (some strains). Formate utilization was described for some strains of *Rps. gelatinosa* (Weckesser *et al.*, 1969) and for *Rps. palustris* (Quadri and Hoare, 1968). The ability to utilize mannitol, sorbitol, and tartrate, which is a characteristic property of *Rps. sphaeroides*, is not shown by any of our new strains.

Table 3. Characteristic features of *Rps. sulfidophila* and some other purple bacteria

	Rps. sulfidophila	*Rps. capsulata*	*Rps. palustris*	*Chromatium vinosum*	*Ectothiorhodospira* spp.
Morphology	Short rods	Short rods	Rods	Short rods	Short vibrios, spirilla
Mode of division	Binary fission	Binary fission	Budding	Binary fission	Binary fission
Photosynthetic membranes	Vesicular	Vesicular	Lamellar	Vesicular	Lamellar
Bacteriochlorophyll	*a*	*a*	*a*	*a*	*a*
Carotenoids	Spheroidene group	Spheroidene group	Spirilloxanthin series	Spirilloxanthin series	Spirilloxanthin series
Aerobic growth in the dark	+	+	+	−	− (+)[a]
Phototrophic growth with					
Organic compounds	+	+	+	+	+
Sulfide	+	+	+	+	+
Thiosulfate	+	−	+	+	+
Oxidation product of S^{2-}	SO_4^{2-}	e S	SO_4^{2-}	i S/SO_4^{2-}	e S/SO_4^{2-}
APS-reductase	Absent	Absent	Absent	Present	Present
Sulfide tolerance	High	Moderate	Low	Moderate to high	Moderate to high
Required growth factors	Niacin, Thiamin, Biotin, pABA	Thiamin[b]	pABA[c]	None	None or vitamin B 12
Moles % G + C in DNA	67—71	65.3—66.8	64.8—66.3	61.3—66.3	62.3—69.9

Based on information from Pfennig and Trüper (1969), Gusev *et al.* (1970), Trüper and Peck (1970), and Hansen and van Gemerden (1972). + = growth; − = no growth; e S = extracellular sulfur; i S = intracellular sulfur.

[a] Only *Ectothiorhodospira shaposnikovii*. [b] Some strains require in addition niacin and biotin. [c] Some strains require in addition biotin. pABA = p-aminobenzoic acid.

The conversion of hydrogen sulfide and thiosulfate into sulfate as shown by *Rps. sulfidophila* forms a marked difference with *Rps. capsulata* which can only produce extracellular elemental sulfur from hydrogen sulfide and which cannot utilize thiosulfate. The new species tolerates higher concentrations of hydrogen sulfide than *Rps. capsulata*. Since in addition differences exist in morphology (absence of zig-zag chains), the ability to utilize some organic compounds (formate and glycerol), in vitamin requirements and in the requirement for sodium chloride, it would not be appropriate to accommodate the new strains in *Rps. capsulata*.

With respect to the utilization of hydrogen sulfide, thiosulfate, and formate *Rps. sulfidophila* resembles *Rps. palustris* more closely. Important differences exist with this species with respect to the mode of cell division and the type of photosynthetic membrane structures (Table 3). *Rps. sulfidophila* grows faster on hydrogen sulfide than the *Rps. palustris* strain studied by Hansen and van Gemerden (1972). Growth of *Rps. palustris* on thiosulfate (Rolls and Lindstrom, 1967; Quadri and Hoare, 1968) and formate (Quadri and Hoare, 1968) is also relatively slow. Stokes and Hoare (1969) showed that the utilization of formate by *Rps. palustris* implies an autotrophic mode of life. They related the low growth rates under autotrophic conditions to a low level of carboxydismutase. Our strains do not require a long adaptation period for growth on thiosulfate as was reported for *Rps. palustris* by Knobloch *et al.* (1971).

In summary (cf. Table 3) we conclude that the creation of a new species of the Rhodospirillaceae to accommodate our new type of photosynthetic purple bacterium is justified. Since it is rod-shaped, it should be assigned to the genus *Rhodopseudomonas*. In view of its good growth on hydrogen sulfide and its high sulfide tolerance we propose *Rhodopseudomonas sulfidophila* as the name of the new species.

Species Description of *Rhodopseudomonas sulfidophila*, nov. spec.

sul. fi. do'phi. la. Gr. adj. *philus* loving; M. L. adj. *sulfidophila* sulfide-loving.

Cells spherical, ovoid to rod-shaped, 0.6—0.9 μ wide and 0.9—2.0 μ long; multiplication by binary fission. Motile by means of a polar tuft of flagella. Gram-negative.

Photosynthetic membrane system of vesicular type.

Anaerobic cultures yellowish-brown to red. Small amounts of oxygen cause a colour change from brown to red. Aerobic cultures faintly red.

Under anaerobic conditions either photolithotrophic or photoorganotrophic growth. Aerobic growth in the dark with organic compounds as carbon- and energy source is also possible.

Phototrophic growth occurs in mineral media with simple organic substrates and bicarbonate. Thiamin, biotin, niacin and p-aminobenzoic acid are required as growth factors. pH range from 6.5 to 8.0 on sulfide and from 5.0 to 7.5 on malate. Optimum temperature 30—35° C. Organic substrates photoassimilated: formate and other fatty acids up to pelargonate, lactate, glutamate, glycerol and some intermediates of the tricarboxylic acid cycle. Glucose is utilized slowly. Some strains utilize ethanol and propanol. Molecular hydrogen, hydrogen sulfide and thiosulfate are excellent electron donors. High sulfide tolerance. Sulfide and thiosulfate are converted to sulfate without an intermediate accumulation of elemental sulfur. Elemental sulfur can be oxidized slowly. Not photoassimilated: citrate, methanol, tartrate, benzoate, mannitol and sorbitol.

Capable of assimilatory sulfate reduction.

Nitrogen source: ammonium salts; nitrate not utilized. The type strain is able to utilize molecular nitrogen when grown on hydrogen sulfide but not when grown on malate.

Some strains do not utilize molecular nitrogen.

Pigments: bacteriochlorophyll *a* and carotenoids, most probably of the spheroidene group.

DNA base composition: 67 to 71 moles-% guanine plus cytosine.

Habitat: mud of marine intertidal flats.

Type: strain W 4.

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References

Biebl, H., Drews, G.: Das in-vivo-Spektrum als taxonomisches Merkmal bei Untersuchungen zur Verbreitung von Athiorhodaceae. Zbl. Bakt., II. Abt. **123**, 425—452 (1969).

De Ley, J.: Reexamination of the association between melting points, buoyant density, and chemical base composition of deoxyribonucleic acid. J. Bact. **101**, 738—754 (1970).

Gusev, M. V., Shenderova, L. K., Kondrat'eva, E. N.: Influence of the oxygen concentration on the growth and survival of photosynthesizing bacteria. Mikrobiologiya **39**, 562—566 (1970).

Hansen, T. A., Gemerden, H. van: Sulfide utilization by purple nonsulfur bacteria. Arch. Mikrobiol. **86**, 49—56 (1972).

Hansen, T. A., Veldkamp, H.: A new type of photosynthetic purple bacterium. Antonie v. Leeuwenhoek **38**, 629 (1972).

Jensen, S. L.: Carotenoids of photosynthetic bacteria—Distribution, structure and biosynthesis. In: Bacterial photosynthesis, pp. 19—34, H. Gest, A. San Pietro, and L. P. Vernon, Eds. Yellow Springs: Antioch Press 1963.

Klemme, J. H.: Untersuchungen zur Photoautotrophie mit molekularem Wasserstoff bei neuisolierten schwefelfreien Purpurbakterien. Arch. Mikrobiol. **64**, 29—42 (1968).

Knobloch, K., Eley, J. H., Aleem, M. I. H.: Thiosulfate-linked ATP-dependent NAD^+ reduction in *Rhodopseudomonas palustris*. Arch. Mikrobiol. **80**, 97—114 (1971).

Kuenen, J. G., Veldkamp, H.: *Thiomicrospira pelophila*, gen. n., sp. n., a new obligately chemolithotrophic colourless sulfur bacterium. Antonie v. Leeuwenhoek **38**, 241—256 (1972).

La Rivière, J. W. M.: Cultivation and properties of *Thiovulum majus* Hinze. In: Symposium on marine microbiology, pp. 61—72, C. H. Oppenheimer, Ed. Springfield: Ch. C. Thomas 1963.

Mandel, M., Leadbetter, E. R., Pfennig, N., Trüper, H. G.: Deoxyribonucleic acid base compositions of phototrophic bacteria. Int. J. Syst. Bact. **21**, 222—230 (1971).

Marmur, J., Doty, P.: Determination of the base composition of deoxyribonucleic acid from its thermal denaturation temperature. J. molec. Biol. **5**, 109—118 (1962).

Matheron, R., Baulaigue, R.: Bactéries photosynthétiques sulfureuses marines. Assimilation des substances organiques et minérales, et influence de la teneur en chlorure de sodium du milieu de culture sur leur développement. Arch. Mikrobiol. **86**, 291—304 (1972).

Pfennig, N.: Anreicherungskulturen für rote und grüne Schwefelbakterien. Zbl. Bakt., I. Abt. Orig., Suppl. **1**, 179—189, 503—505 (1965).

Pfennig, N.: *Rhodopseudomonas acidophila*, sp. n., a new species of the budding purple nonsulfur bacteria. J. Bact. **99**, 597—602 (1969).

Pfennig, N., Trüper, H. G.: Phototrophic bacteria. GSF-Bericht M 32 (1969).

Quadri, S. M., Hoare, D. S.: Formic hydrogenlyase and the photoassimilation of formate by a strain of *Rhodopseudomonas palustris*. J. Bact. **95**, 2344—2357 (1968).

Rolls, J. P., Lindstrom, E. S.: Effect of thiosulfate on the photoautotrophic growth of *Rhodopseudomonas palustris*. J. Bact. **94**, 860—866 (1967).

Roy, A. B., Trudinger, P. A.: The biochemistry of inorganic compounds of sulfur. Cambridge: University Press 1970.

Schlegel, H. G., Gottschalk, G.: Poly-β-hydroxybuttersäure, ihre Verbreitung, Funktion und Biosynthese. Angew. Chem. **74**, 342—347 (1962).

Stokes, J. E., Hoare, D. S.: Reductive pentose cycle and formate assimilation in *Rhodopseudomonas palustris*. J. Bact. **100**, 890—894 (1969).

Thiele, H. H.: Die Verwertung einfacher organischer Substrate durch *Thiorhodaceae*. Arch. Mikrobiol. **60**, 124—138 (1968).

Trüper, H. G.: *Ectothiorhodospira mobilis* Pelsh, a photosynthetic sulfur bacterium depositing sulfur outside the cells. J. Bact. **95**, 1910—1920 (1968).

Trüper, H. G., Peck, H. D.: Formation of adenylyl sulfate in photosynthetic bacteria. Arch. Mikrobiol. **73**, 125—142 (1970).

Van Niel, C. B.: The culture, general physiology, morphology and classification of the non-sulfur purple and brown bacteria. Bact. Rev. **8**, 1—118 (1944).

Weckesser, J., Drews, G., Tauschel, H. D.: Zur Feinstruktur und Taxonomie von *Rhodopseudomonas gelatinosa*. Arch. Mikrobiol. **65**, 346—358 (1969).

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