Rhodopseudomonas blastica sp.nov.: a Member of the *Rhodospirillaceae*

By KEVIN ECKERSLEY AND CRAWFORD S. DOW*

Department of Biological Sciences, University of Warwick, Coventry CV4 7AL

(Received 6 December 1979; revised 23 February 1980)

Inoculation of samples from a small eutrophic pond into pyruvate/malate medium at neutral pH yielded a new species of the *Rhodospirillaceae* described here as *Rhodopseudomonas blastica*. The cells are ovoid to rod-shaped, 1 to 2.5 μm long and 0.6 to 0.8 μm wide, non-motile and multiply by a process of sessile budding. The photosynthetic membrane system consists of lamellae parallel to and underlying the cell membrane. The photopigments consist of bacteriochlorophyll *a* and carotenoids of the spheroidene group. Cells can grow anaerobically in the light using a wide range of organic compounds. Aerobic growth in the dark is also possible. Nicotinic acid and thiamin are required as growth factors. Good photolithotrophic growth with H_2 is possible, but sulphide and thiosulphate cannot be used as electron donors. As this species has properties lying between the two groups of existing *Rhodopseudomonas* species, i.e. those which show intercalary growth and those which multiply by budding (asymmetric polar growth), a new species is hereby proposed.

INTRODUCTION

Eutrophic freshwater systems are a rich source of phototrophic bacteria, particularly those belonging to the genus *Rhodopseudomonas* (Siefert *et al.*, 1978). Species within this genus exhibit such a morphological and ultrastructural diversity that it has been suggested that the genus be re-classified into two or three new groups (Gorlenko *et al.*, 1976; Pfennig, 1977) (Table 1).

From samples of a small eutrophic freshwater pond, a phototrophic bacterium was isolated by enrichment on pyruvate/malate medium at neutral pH. While characteristic of the genus *Rhodopseudomonas*, this organism was distinct from any one of the known species. It is described here as a new species of the *Rhodospirillaceae*: *Rhodopseudomonas blastica*.

METHODS

Source of organism. The organism was isolated from a small eutrophic freshwater pond on the campus of Warwick University.

Media. The mineral base used in all media contained (per litre distilled water): NH_4Cl , 0.5 g; $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$, 0.4 g; $\text{CaCl}_2 \cdot 2\text{H}_2\text{O}$, 0.05 g; NaCl , 0.4 g; 50 ml 0.1 M- $\text{NaH}_2\text{PO}_4/\text{Na}_2\text{HPO}_4$, pH 6.8, was added aseptically after autoclaving. The defined medium used routinely contained (per litre mineral base): sodium pyruvate, 1.5 g; sodium hydrogen malate, 1.5 g; thiamin.HCl, 0.02 mg; biotin, 0.02 mg; nicotinic acid, 0.1 mg; trace elements (Pfennig & Lippert, 1966) 1 ml; the pH was adjusted to 6.8 with KOH prior to autoclaving. Complex medium contained in addition 0.1 % (w/v) yeast extract. In the enrichment medium, 0.1 % (w/v) yeast extract replaced the vitamins and trace elements solution of the defined medium; in addition, MgSO_4 was omitted. When solid medium was required, agar was added to 1.5 % (w/v).

Enrichment and isolation. Enrichments were performed in screw-capped McCartney bottles completely filled with complex medium, incubated at 30 °C with an incident light intensity of 2000 lx from tungsten

Table 1. *Proposed grouping of the current species of the genus Rhodopseudomonas (after Pfennig, 1977)*

Species group	1	2	3
Species	<i>R. capsulata</i> <i>R. sphaeroides</i> <i>R. globiformis</i> <i>R. sulfidophila</i>	<i>R. gelatinosa</i>	<i>R. sulfoviridis</i> <i>R. acidophila</i> <i>R. viridis</i> <i>R. palustris</i>
Major morphological characteristics – cell shape	Spherical to ovoid cells showing intercalary growth	Slender rod-shaped to spirilloid cells showing intercalary growth	Rod shaped to elongated ovoid cells which multiply by budding (asymmetric polar growth)
Arrangement of intracytoplasmic membrane system	Vesicular	Single small tubular or lamellar intrusions of cell membrane	Lamellae parallel to and underlying the cell membrane

lamps. Isolation and purification was achieved by serial dilution and repeat restreaking on to complex agar medium which was incubated under phototrophic conditions at 30 °C with an incident light intensity of 2000 lx by the method of Westmacott & Primrose (1975) as modified by Dow & France (1980).

Growth conditions. Pure cultures of *R. blastica* were grown in 250 ml Erlenmeyer flasks containing 100 ml medium which were sealed with rubber serum caps (William Freeman & Co., Barnsley, Yorkshire), flushed with O₂-free N₂ and incubated, with shaking, at 30 °C under constant illumination (2000 lx). Chemoheterotrophic growth under aerobic conditions in the dark at 30 °C was examined using complex medium.

Substrate utilization. Substrate utilization was examined using mineral base supplemented with growth factors, NaHCO₃ (0.05 %, w/v) and the test substrate (0.15 %, w/v), the pH being adjusted to 6.8. However, utilization of methanol and ethanol were also examined at pH 7.5 since it has been reported that growth of certain *Rhodospirillaceae* on these substrates is pH-sensitive (Quayle & Pfennig, 1975).

The capacity for photolithotrophic growth was determined using the above medium to which thiosulphate or sulphide (sodium salts) was added. The latter experiments were repeated replacing the growth factors with yeast extract (0.01 %, w/v) as this has been shown to increase sulphide tolerance in other members of the *Rhodospirillaceae* (Hansen & van Gernerden, 1972). To examine the capacity for photolithotrophic growth with CO₂ and H₂, these were used at 20 and 80 % (v/v), respectively.

Utilization of nitrogen sources was investigated using defined medium from which NH₄Cl was excluded. The test substrate was added at 0.05 % (w/v) and the flasks made anaerobic by flushing with Ar. Nitrogenase assays, using whole cells grown under N₂ in defined medium lacking NH₄Cl, were performed as described by Zumft & Castillo (1978).

In all experiments growth was measured turbidimetrically at 540 nm.

Photopigments. The absorption spectrum was recorded for photosynthetically grown cells which had been centrifuged, washed and resuspended in 60 % (w/v) sucrose. Carotenoids from cells in the early-stationary growth phase were extracted according to the procedure of Schmidt (1971) and separated by thin-layer chromatography (t.l.c.) on silica gel F, using ethyl acetate/petroleum ether (b.p. 60 to 80 °C) (1:10, v/v) as solvent.

DNA isolation and purification. Late-exponential growth phase cultures were harvested by centrifuging (20000 g for 30 min) and washed in TES buffer (0.1 M-NaCl/0.05 M-Tris/0.001 M-EDTA, adjusted to pH 7.2 with HCl). Freshly prepared lysozyme (1 mg ml⁻¹) was added and the cells were incubated at 37 °C for 1 h. Sodium lauryl sarcosinate was added at 2 % (w/v) to bring about lysis. DNA was isolated by centrifuging the lysate at 120000 g for 36 h in a linear CsCl gradient of mean density 1.71 g cm⁻³. Fractions were collected and dialysed for 15 h at 4 °C against standard saline citrate (0.15 M-NaCl/0.015 M-sodium citrate, pH adjusted to 7.0). Sample purity was determined by reading A_{280}/A_{260} and the DNA concentration was calculated from the absorption coefficient.

DNA base composition. The buoyant density in neutral CsCl of the isolated DNA was determined using a Beckman model E Analytical Ultracentrifuge, operating at 140000 g and 25 °C for a minimum of 22 h. A cell with a 12 mm 4° Kel F centre piece was used and photographs were taken using ultraviolet absorption optics. Films were examined with a Joyce-Loebl recording microdensitometer. DNA from *Escherichia coli* ($\rho = 1.7100$ g cm⁻³) was used as a marker and the buoyant density and mol % G + C were calculated by the method of Mandel *et al.* (1968).

Fig. 1. Electron micrograph of *R. blastica* grown phototrophically on defined medium. Uranyl acetate stain. Bar marker represents 2 μ m.

Slide culture and light microscopy. Anaerobic slide cultures were as described by Dow & France (1980). Photomicrographs were obtained from an Olympus EHT microscope fitted with an Olympus PM-6 camera unit using Kodak Panatomic X film.

Electron microscopy. The fixation procedure of Ryter & Kellenberger (1958) was followed for ultrathin sections which were cut on a Reichert OMU2 ultramicrotome. Micrographs were taken with a Jeol JEM 100S instrument operating at 60 kV.

RESULTS

Enrichment and isolation

Mineral medium of neutral pH, lacking MgSO_4 and supplemented with pyruvate, malate and yeast extract, offers selective enrichment conditions for the *Rhodospirillaceae*. When such medium was inoculated with surface mud samples from a small eutrophic pond and incubated anaerobically under constant illumination one of two species became dominant: either a strain of *Rhodopseudomonas palustris* was found or, less frequently, enrichments became orange-brown in colour and were found to contain cells morphologically distinct from all documented species of *Rhodospirillaceae*. The latter was obtained in pure culture and maintained on complex medium.

Morphology and ultrastructure

The cells of this new species were short Gram-negative rods which immediately after cell division were spherical to ovoid (Fig. 1). Cells grown on complex medium were 0.6 to 0.7 μ m wide and 1.0 to 2.5 μ m long. Little variation of cell dimensions with growth medium composition was observed although in media containing yeast extract there was a tendency to form abnormally swollen cells and spheroplasts. Similar observations have been made with strains of *Rhodopseudomonas capsulata* (Weaver *et al.*, 1975). Cell growth occurred by budding (asymmetric polar growth); however, no tube or filament was formed between the cells and division occurred by binary fission when both cells were of equal size. Growth thereafter continued at the newly formed poles (Fig. 2). Although cells at division appeared morphologically similar they differed with regard to the time taken to reach the next division, i.e. the mother cell initiated cell growth immediately after division whereas the daughter cell had to undergo an obligate period of maturation (Fig. 2). Cells did not form chains nor did they form rosettes characteristic of cells possessing polar 'holdfast' material (Whittenbury & McLee, 1967). Motility was not observed under any growth conditions.

Ultrathin sections of photosynthetically grown cells revealed an intracytoplasmic system consisting of membranes parallel to and underlying the cytoplasmic membrane (Fig. 3).

Fig. 2. Sequential phase contrast photomicrographs of *R. blastica* grown phototrophically on complex medium. Photographs were taken at 20 min intervals. Bar marker represents 10 μ m.

These studies also suggested that the membrane system was formed *de novo* in the daughter cell.

Photopigments

Cultures grown phototrophically with pyruvate and malate as substrates were orange-brown. No colour change was observed when such cultures were shaken in air. Aerobically grown cultures were white to faint pink.

The absorption spectrum of whole cells (Fig. 4) showed the absorption maxima characteristic of bacteriochlorophyll *a* (378, 590, 795 and 862 nm) with carotenoid absorption maxima at 418, 476 and 506 nm.

A preliminary study of the extracted photopigments using t.l.c. indicated the presence of carotenoids of the spheroidene group (Liaaen-Jensen, 1963). Such extracts were compared with those from *R. capsulata* NCIB 8254, *R. sphaeroides* NCIB 8253 and *R. gelatinosa* NCIB 8290 (Fig. 5).

Physiology

The vitamins required for growth were nicotinic acid and thiamin. Biotin gave considerable growth stimulation. Addition of all three to the growth medium, however, did not achieve the same growth rate as the inclusion of 0.1 % (w/v) yeast extract.

A wide range of compounds supported photo-organotrophic growth (Table 2). Methanol

Fig. 3. Ultrathin longitudinal (*a*, *c*, *d*) and transverse (*b*) sections of *R. blastica* grown phototrophically on defined medium at 30 °C with an incident light intensity of 2000 lx. Bar markers represent 0.5 μ m (marker in *c* refers to *a*, *b* and *c*).

Fig. 4

Fig. 5

Fig. 4. Absorption spectrum of whole cells of *R. blastica* grown phototrophically on defined medium at 30 °C with an incident light intensity of 2000 lx. Cells were suspended in 60 % (w/v) sucrose. The peaks at 378, 590, 795 and 862 nm are characteristic of the bacteriochlorophyll and those at 418, 476 and 506 nm of the carotenoids.

Fig. 5. Absorption spectrum of extracted photopigments from *R. blastica*. Those of *R. capsulata* NCIB 8254, *R. sphaeroides* NCIB 8253 and *R. gelatinosa* NCIB 8290 were identical. The solvent was petroleum ether (b.p. 60 to 80 °C).

or ethanol were not utilized, irrespective of the pH. H₂ and CO₂ supported photolithotrophic growth, but sulphide and thiosulphate would not serve as electron donors.

Good aerobic, chemoheterotrophic growth on complex medium was possible in the dark at high oxygen tensions, both in liquid media and on agar plates, and was sustained in liquid media for several subcultures before the cells showed any tendency towards pleiomorphy. Attempts to grow the organism under strict anaerobic conditions in the dark, providing pyruvate and malate as substrates, were unsuccessful. Slow fermentation of these

Table 2. *Utilization of electron donors and organic compounds for phototrophic growth by R. blastica*

For comparison, results for *R. sulfoviridis* (Keppen & Gorlenko, 1975) and *R. sphaeroides* (Van Niel, 1944) are included.

Substrate	<i>R. blastica</i> *	<i>R. sulfoviridis</i> †	<i>R. sphaeroides</i> †
Pyruvate	3	—	+
Citrate	1	—	+
Succinate	3	+	+
Fumarate	3	+	+
Malate	3	+	+
Acetate	3	+	+
Butyrate	2	+	+
Lactate	2	+	NT
Formate	0	—	NT
Propionate	2	—	—
Tartrate	0	—	+
Glycerol	2	+	+
Glycollate	0	NT	NT
Benzoate	0	—	NT
Glutamate	2	NT	NT
Methanol	0	—	NT
Ethanol	0	+	+
Ribose	2	NT	NT
Glucose	3	+	+
Fructose	3	+	+
Mannitol	2	—	+
Sorbitol	3	+	+
Hydrogen	2	NT	+
Sulphide	0	+	—
Thiosulphate	0	+	—
Casamino acids	3	NT	NT
Yeast extract	3	NT	+

* A_{540} recorded after 1 week: 0, A as in the control without added substrate; 1, $A \leq 0.2$; 2, $0.2 < A \leq 0.5$; 3, $A > 0.5$.

† +, Growth; —, no growth; NT, not tested.

compounds under such conditions by some *Rhodopseudomonas* species has been reported (Uffen & Wolfe, 1970).

The optimal pH range for growth on pyruvate/malate medium was 6.5 to 7.5, and the optimal temperature range 30 to 35 °C.

Ammonium salts were the best nitrogen sources for growth, although a wide range of simple and complex organic nitrogen compounds could be utilized. Those utilized were glutamate, glutamine, aspartate, alanine, ornithine, tyrosine, thymine and urea. Nitrite and nitrate were not utilized. As is typical among purple bacteria, N_2 could serve as a sole nitrogen source. Cells could be grown and repeatedly subcultured in media in which the only nitrogen source was N_2 . Cells grown under such conditions had an active nitrogenase as judged by the acetylene reduction assay. The highest rate of acetylene reduction obtained was 2 nmol (mg protein)⁻¹ min⁻¹; this is a low rate compared with those for other *Rhodopseudomonas* species (Meyer *et al.*, 1978).

The mol % G + C, as determined by buoyant density, was 65.3 %.

DISCUSSION

Under all growth conditions *Rhodopseudomonas blastica* reproduced by asymmetric polar growth, a feature characteristic of the members of species group 3 (Table 1). Its relatedness

to this group was strengthened as it possessed an intracytoplasmic membrane system of the lamellar type, which also provides additional evidence to support the proposal by Whittenbury & McLee (1967) that a correlation exists between this type of membrane system and a budding mode of growth.

Associated with a budding mode of growth, cells produced at division are morphologically and physiologically distinct from one another, i.e. most budding bacteria, including the *Rhodopseudomonas* species of group 3, possess dimorphic life cycles (Whittenbury & Dow, 1977). In these organisms division is asymmetric giving rise to a non-motile mother cell and a motile daughter cell which differ in that the mother cell is capable of reproduction immediately after division whereas the daughter cell must undergo an obligate period of maturation. Budding in *R. blastica* is sessile, i.e. no tube or filament is formed between the mother and daughter cell, and in this respect it resembles *R. acidophila* (Pfennig, 1969) and *R. sulfoviridis* (Keppen & Gorlenko, 1975). However, at division *R. blastica* produces two identical cells indicating division to be symmetrical. Evidence that the cell cycle of *R. blastica* is, in fact, dimorphic has come from slide culture studies (Fig. 2). Although division is symmetrical, the daughter cell does not divide until after division has been accomplished by the mother cell, which suggests that, before it is reproductively competent, the daughter cell must pass through a phase of maturation – possibly *de novo* synthesis of the photosynthetic membrane system, as there are no obvious external morphological changes.

Rhodopseudomonas blastica and *Rhodocyclus purpureus* (Pfennig & Trüper, 1973) are the only species of *Rhodospirillaceae* in which motility has not been observed. In several *Rhodopseudomonas* species, motility has been reported to have been lost with changes in cultural conditions, e.g. *R. acidophila* and *R. palustris* (Tauschel & Hoeniger 1974) and *R. sulfidophila* (Hansen & Veldkamp, 1973); *R. blastica* was non-motile under all conditions tested.

The species of groups 1 and 2 (Table 1), except for *R. globiformis*, possess carotenoids of the spheroidene group, the distribution of which seems to be restricted to these four species. It appears, however, that *R. blastica* possesses carotenoids of this type. Though it is characteristic for anaerobic cultures of organisms possessing these carotenoids to change colour from brown to red in the presence of O₂, due to the formation of 2-oxycarotenoids, cultures of *R. blastica* did not show such a colour change. This may be due to the absence of the enzyme system responsible for the carotenoid oxidations (Schneour, 1962).

Physiologically, *R. blastica* possesses many properties characteristic of the *Rhodospirillaceae*, being able to utilize a wide spectrum of organic compounds. However, it is the ability to utilize monosaccharides which distinguishes it from the other budding *Rhodopseudomonas* species. The exception within the group 3 species is *R. sulfoviridis* (Keppen & Gorlenko, 1975) which can utilize sugars and, like *R. blastica*, possesses a comparatively simple cell cycle. In many respects *R. blastica* resembles *R. sulfoviridis*. Both grow by sessile budding and possess a lamellar membrane system, they are of the same dimensions and their spectrum of utilizable carbon compounds shows close similarity. They differ in photopigments (*R. sulfoviridis* possesses bacteriochlorophyll *b*, *R. blastica* bacteriochlorophyll *a*), in the ability to utilize sulphide, thiosulphate and pyruvate, and in motility (*R. sulfoviridis* is motile).

Morphologically and with respect to the mode of growth, *R. blastica* resembles the budding *Rhodopseudomonas* species of group 3. However, the simplicity of its cell cycle, its photopigments and its physiology suggests relatedness to group 1. Association with the latter has been strengthened by the observation that a bacteriophage isolated for *R. blastica* causes 'lysis from without' of *R. sphaeroides* NCIB 8253 (K. Eckersley & C. S. Dow, unpublished results).

An overview of the properties of this isolate indicates that, while it possesses the general characteristics of the genus *Rhodopseudomonas*, it is distinct from the described species and should be accommodated by the creation of a new species.

Rhodopseudomonas blastica sp. nov.

blast'ic.a. M.Gr. adj. *blastikos* apt to bud.

Cells are ovoid to rod-shaped, 1 to 2.5 μm long and 0.6 to 0.8 μm wide. Multiplication is by budding (asymmetric polar growth) without the production of a tube or filament between the mother and daughter cells. Division is symmetric, both cells being non-motile. Gram-negative.

The photosynthetic membrane consists of lamellae underlying and parallel with the cell membrane. The membrane system is formed *de novo* in the daughter cell.

Anaerobic liquid cultures are orange-brown, becoming dark brown. Aerobically grown cultures are white to pale pink. No colour change is observed on the admission of air into anaerobically grown cultures.

Photo-organotrophic, facultatively aerobic, growing either anaerobically in the light or aerobically in the dark. Optimal pH range, 6.5 to 7.5. Optimum temperature, 30 to 35 °C. Growth factors required are thiamin and nicotinic acid. Yeast extract and biotin stimulate growth appreciably.

A wide range of organic substrates are photoassimilated including most intermediates of the tricarboxylic acid cycle, sugars, sugar alcohols, several amino acids, lactate, glycerol and butyrate. Not utilized: alcohols, tartrate, formate and benzoate. Photolithotrophic growth using molecular hydrogen and carbon dioxide. Sulphide and thiosulphate cannot be used as electron donors.

Nitrogen sources: ammonium salts and amino acids; N_2 can be fixed. Nitrate or nitrite are not utilized.

Photopigments: absorption spectra of whole cells show the maxima characteristic of bacteriochlorophyll *a* (378, 590, 795 and 862 nm) and also carotenoid maxima at 418, 476 and 506 nm.

Unable to liquefy gelatin. Catalase present.

DNA base composition: 65.3 mol % G + C.

Source: small freshwater eutrophic pond.

Type strain: NCIB 11576.

K. Eckersley gratefully acknowledges the financial assistance of the Natural Environment Research Council (Research Studentship GT4/77/ALS/37).

REFERENCES

- DOW, C. S. & FRANCE, A. D. (1980). Simplified vegetative cell cycle of *Rhodomicrobium vannielii*. *Journal of General Microbiology* **117**, 47–55.
- GORLENKO, V. M., KEPPEN, O. I. & PUCHKOV, A. N. (1976). Morphological differentiation in non-sulphur purple bacteria. *Microbiology* (English translation of *Mikrobiologiya*) **45**, 705–710.
- HANSEN, T. A. & VAN GEMERDEN, H. (1972). Sulphide utilization by purple non-sulfur bacteria. *Archiv für Mikrobiologie* **86**, 49–56.
- HANSEN, T. A. & VELDAMP, H. (1973). *Rhodopseudomonas sulfidophila*, nov.spec., a new species of the purple non-sulfur bacteria. *Archiv für Mikrobiologie* **92**, 45–58.
- KEPPEN, O. I. & GORLENKO, V. M. (1975). A new species of purple budding bacteria containing bacteriochlorophyll *b*. *Microbiology* (English translation of *Mikrobiologiya*) **44**, 224–229.
- LIAAEN-JENSEN, S. (1963). Carotenoids of photosynthetic bacteria – distribution, structure and biosynthesis. In *Bacterial Photosynthesis*, pp. 19–34. Edited by H. Gest, A. SanPietro & L. P. Vernon. Yellow Springs, Ohio: Antioch Press.
- MANDEL, M., SCHILDKRAUT, C. L. & MARMUR, J. (1968). Use of CsCl density gradient centrifugation analysis for determining the guanine plus cytosine content of DNA. *Methods in Enzymology* **12B**, 184–195.
- MEYER, J., KELLEY, B. C. & VIGNAIS, P. M. (1978). Aerobic nitrogen fixation by *Rhodopseudomonas capsulata*. *FEBS Letters* **85**, 224–228.
- PFENNIG, N. (1969). *Rhodopseudomonas acidophila*, sp.n., a new species of the budding purple non-sulfur bacteria. *Journal of Bacteriology* **99**, 597–602.
- PFENNIG, N. (1977). Phototrophic green and purple bacteria: a comparative, systematic survey. *Annual Review of Microbiology* **31**, 275–290.
- PFENNIG, N. & LIPPERT, K. D. (1966). Über das Vitamin B₁₂-Bedürfnis phototropher Schwefelbakterien. *Archiv für Mikrobiologie* **55**, 258–266.
- PFENNIG, N. & TRÜPER, H. G. (1973). The Rhodospirillales (phototrophic or photosynthetic bacteria). In *Handbook of Microbiology*, vol. 1, pp. 17–27. Edited by A. I. Laskin & H. A. Lechevalier. Cleveland: CRC Press.

- QUAYLE, J. R. & PFENNIG, N. (1975). Utilisation of methanol by Rhodospirillaceae. *Archives of Microbiology* **102**, 193–198.
- RYTER, A. & KELLENBERGER, E. (1958). Étude au microscope électronique de plasmas contenant de l'acide désoxyribonucléique. I. Les nucléotides des bactéries en croissance active. *Zeitschrift für Naturforschung* **13b**, 597–605.
- SCHMIDT, K. (1971). Carotenoids of purple non-sulphur bacteria. *Archiv für Mikrobiologie* **77**, 231–238.
- SCHNEOUR, E. A. (1962). Carotenoid pigment conversion in *Rhodopseudomonas spheroides*. *Biochimica et biophysica acta* **62**, 534–540.
- SIEFERT, E., IRGENS, R. L. & PFENNIG, N. (1978). Phototrophic purple and green bacteria in a sewage treatment plant. *Applied and Environmental Microbiology* **35**, 38–44.
- TAUSCHEL, H. D. & HOENIGER, J. F. M. (1974). The fine structure of *Rhodopseudomonas acidophila*. *Canadian Journal of Microbiology* **20**, 13–17.
- UFFEN, R. L. & WOLFE, R. S. (1970). Anaerobic growth of purple non-sulfur bacteria under dark conditions. *Journal of Bacteriology* **104**, 462–472.
- VAN NIEL, C. B. (1944). The culture, general physiology, morphology and classification of the non-sulfur purple and brown bacteria. *Bacteriological Reviews* **8**, 1–118.
- WEAVER, P. F., WALL, J. D. & GEST, H. (1975). Characterization of *Rhodopseudomonas capsulata*. *Archives of Microbiology* **105**, 207–216.
- WESTMACOTT, D. & PRIMROSE, S. B. (1975). An anaerobic bag for photoheterotrophic growth of some Rhodospirillaceae in Petri dishes. *Journal of Applied Bacteriology* **38**, 205–207.
- WHITTENBURY, R. & DOW, C. S. (1977). Morphogenesis and differentiation in *Rhodomicrobium vannielii* and other budding and prosthecate bacteria. *Bacteriological Reviews* **41**, 754–808.
- WHITTENBURY, R. & McLEE, A. G. (1967). *Rhodopseudomonas palustris* and *R. viridis* – photosynthetic budding bacteria. *Archiv für Mikrobiologie* **59**, 324–334.
- ZUMFT, W. G. & CASTILLO, F. (1978). Regulatory properties of the nitrogenase from *Rhodopseudomonas palustris*. *Archives of Microbiology* **117**, 53–60.