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CHAPTER 3.1.1

The Phototrophic Alpha-Proteobacteria

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Introduction

The phototrophic purple α-Proteobacteria are purple nonsulfur bacteria able to perform anoxygenic photosynthesis. Owing to the presence of photosynthetic pigments, cell suspensions appear in various colors from beige, olive-green, peach-brown, brown, brown-red, red or pink and have characteristic absorption spectra. Photosynthetic pigments (bacteriochlorophyll *a* or *b* [esterified with phytol or geranylgeraniol] and various types of carotenoids) are located in the cytoplasmic membrane and internal membrane systems (vesicles, lamellae or membrane stacks).

Typically, phototrophic α-Proteobacteria grow under anoxic conditions in the light and their phototrophic growth, photosynthetic pigment synthesis and internal membrane formation are inhibited by oxygen but become derepressed at low oxygen tensions. Their metabolism is highly diverse and flexible. The preferred mode of growth is photoorganoheterotrophically, but many species also can grow photolithoautotrophically with molecular hydrogen, sulfide or thiosulfate as photosynthetic electron donor; some also can use ferrous iron. Growth factors are generally required, most commonly biotin, thiamine, niacin and *p*-aminobenzoic acid; growth of most species is enhanced by small amounts of yeast extract, and some have a complex nutrient requirement. Chemotrophic growth under microoxic to oxic conditions in the dark is common to most of these bacteria; some of them are very sensitive to minor levels of oxygen, while others grow equally well aerobically in the dark at the full atmospheric oxygen tension. Anaerobic dark growth by fermentation and oxidant-dependent growth also may occur.

Phylogeny

Phototrophic purple nonsulfur bacteria are a highly diverse and heterogeneous group of bacteria, both phenotypically and genetically. On the basis of 16S rDNA sequence similarities, phototrophic purple bacteria belong to the α-, β- and γ-Proteobacteria (Woese et al., 1984a; Woese et al., 1984b; Woese et al., 1985; Stackebrandt et al., 1988; Woese, 1987). While purple sulfur bacteria are γ-Proteobacteria, purple nonsulfur bacteria are found in the β- and α-Proteobacteria. The phototrophic purple β-Proteobacteria (including *Rhodocyclus* and relatives) are treated elsewhere in this volume (Imhoff). This chapter deals with the phototrophic purple α-Proteobacteria.

Three major phylogenetically distinct groups of phototrophic α-Proteobacteria are recognized (see Figs. 1–3). They are represented by *Rhodospirillum* and relatives (also called "α-1 Proteobacteria"; Fig. 1), by *Rhodopseudomonas* and relatives (also called "α-2 Proteobacteria"; Fig. 2) and by *Rhodobacter* and relatives (also called "α-3 Proteobacteria"; Fig. 3). While species of the α-3 group form a tight phylogenetic cluster, in the α-2 group *Rhodomicrobium* and *Rhodobium* species are at a greater distance to the other phototrophic species, and in the α-1 group, *Rhodovibrio*, *Rhodopila* and *Rhodothalassium* species form distinct lines separate from the cluster around *Rhodospirillum* and *Phaeospirillum* species. Close relatives of *Rhodovibrio* species and *Rhodothalassium salexigens* are not known and their assignment to the *Rhodospirillum* group is arbitrary. On the basis of 16S rDNA sequence similarities, chemotaxonomic characteristics and other properties, many representatives of the phototrophic α-Proteobacteria are very closely related to nonphototrophic, strictly chemotrophic bacteria. These similarities are taken as strong indication for the development of many nonphototrophic bacteria from phototrophic ancestors. A few examples of these relations are the following:

The acidophilic *Rhodopila globiformis* is closely related to other acidophilic bacteria including *Acidiphilium* species (Sievers et al., 1994).

Phaeospirillum species demonstrate a close relationship to *Magnetospirillum magnetotacticum* (Burgess et al., 1993).

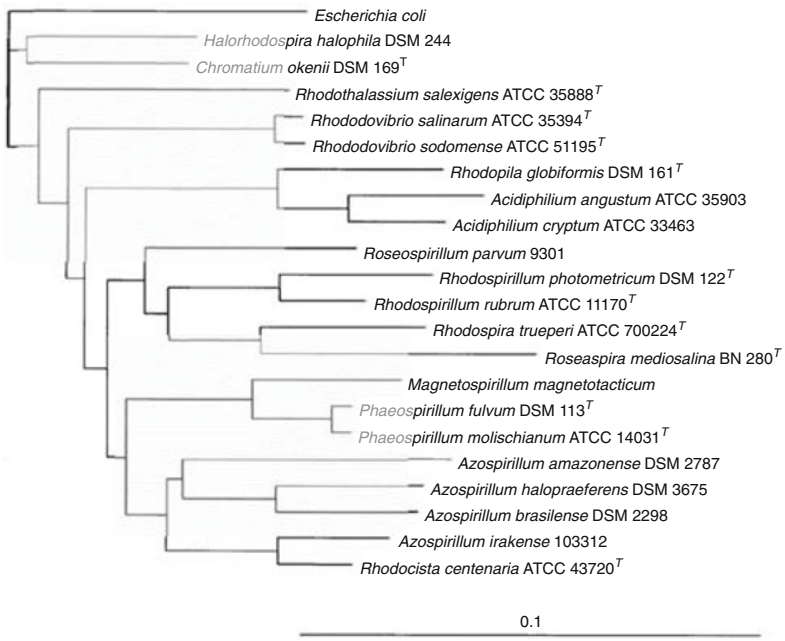

Fig. 1. Phylogenetic tree based on 16S rDNA sequences of the *Rhodospirillum*-group (α-1 Proteobacteria) in relation to other purple nonsulfur and purple sulfur bacteria and to representative close chemotrophic relatives.

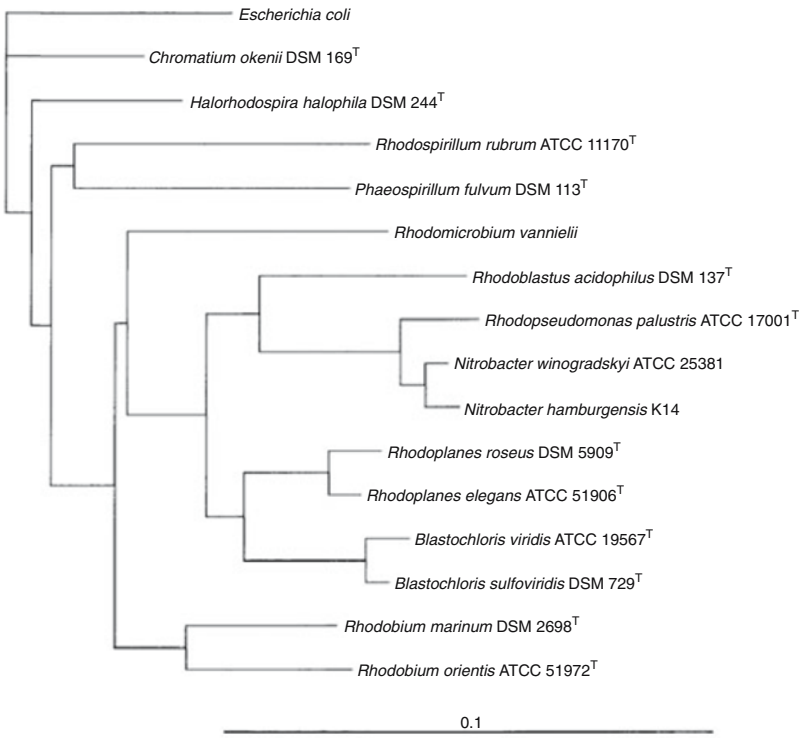

Fig. 2. Phylogenetic tree based on 16S rDNA sequences of the *Rhodopseudomonas*-group (α-2 Proteobacteria) in relation to other purple nonsulfur and purple sulfur bacteria and to representative close chemotrophic relatives.

Rhodocista centenaria has strong relations to *Azospirillum* species (Xia et al., 1994; Fani et al., 1995).

Within the α-2 Proteobacteria, *Rhodopseudomonas palustris* has strong relations to species of *Nitrobacter* (Seewaldt et al., 1982).

Rhodobacter and *Rhodovulum* species form a cluster closely associated to *Paracoccus denitrificans* (Imhoff, 1989a; Hiraishi and Ueda, 1994a).

Taxonomy

Since Molisch removed the purple sulfur bacteria from the Thiobacteria (Migula, 1900), pigmentation and ability to perform anoxygenic photosynthesis were considered of primary importance for assignment of bacteria to the Rhodobacteria (Molisch, 1907), later called "Rhodospirillales" (Pfennig and Trüper, 1971).

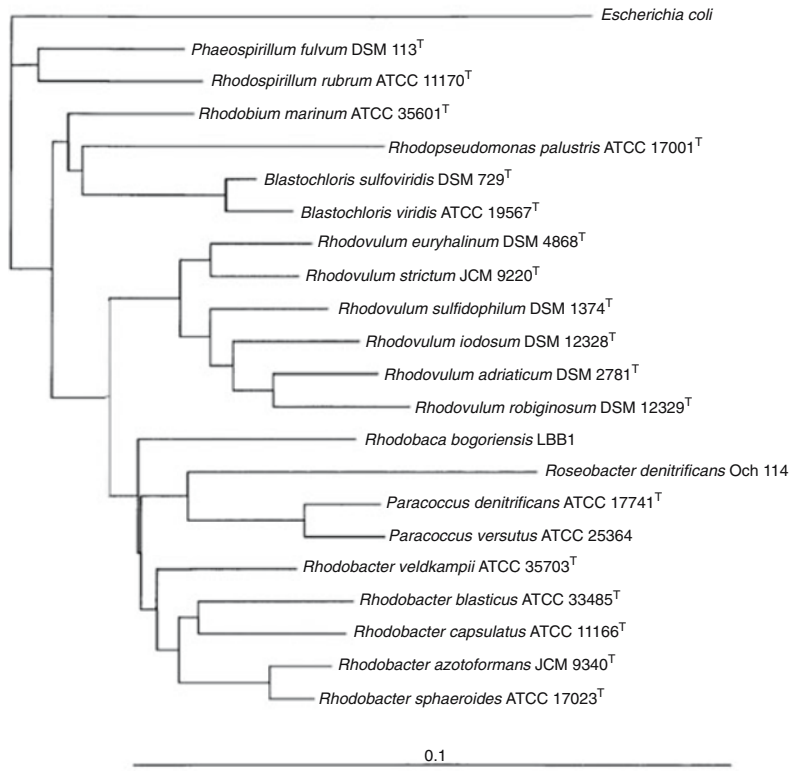

Fig. 3. Phylogenetic tree based on 16S rDNA sequences of the *Rhodobacter*-group (α-3 Proteobacteria) in relation to other purple nonsulfur and purple sulfur bacteria and to representative close chemotrophic relatives.

Because the Rhodospirillaceae (Pfennig and Trüper, 1971) do not represent a phylogenetically distinct group of bacteria, the use of the term "purple nonsulfur bacteria" (PNSB) was proposed for the α- and β-Proteobacteria that contain photosynthetic pigments and are able to perform anoxygenic photosynthesis under anoxic conditions (Imhoff et al., 1984b; Imhoff and Trüper, 1989b; Imhoff and Trüper, 1992b). Actual and historical aspects of the taxonomy of anoxygenic phototrophic purple bacteria have been discussed elsewhere (Imhoff, 1992a; Imhoff, 1995a; Imhoff, 1999; Imhoff, 2000).

Traditionally, purple nonsulfur bacteria have been classified into genera representing the rod-shaped *Rhodopseudomonas* species and the spiral-shaped *Rhodospirillum* species (Pfennig and Trüper, 1974b) and later into a third genus containing the half-circle- to circle-shaped *Rhodocyclus purpureus* (Pfennig, 1978). With the recognition of their genetic relationships and chemotaxonomic diversity, purple nonsulfur bacteria of the α- and β-Proteobacteria were taxonomically separated (Imhoff et al., 1984b; Imhoff and Trüper, 1989b). Later, bacteria within these groups were rearranged according to phylogeny, chemotaxonomic characteristics and ecophysiological properties. Despite the fact that many of the phototrophic purple nonsulfur bacteria are closely related to strictly chemotrophic relatives, the genus definitions of genera of the anoxygenic phototrophic bacteria still include phototrophic capability and content of photosynthetic pigments. At higher taxonomic ranks, phototrophic bacteria are treated together with nonphototrophic relatives.

Alpha-1 Proteobacteria

Most of the phototrophic bacteria that belong to the α-1 Proteobacteria (also known as the *Rhodospirillum* group) have been previously known as *Rhodospirillum* species and are of spiral shape. At present, the only nonspiral representative is *Rhodopila globiformis*. Genera included in this group are *Rhodospirillum*, *Phaeospirillum*, *Rhodospira*, *Roseospira*, *Rhodocista*, *Roseospirillum* and also *Rhodopila*, *Rhodothalassium* and *Rhodovibrio*.

In addition, 16S rDNA sequence data of purple nonsulfur bacteria implied that the spiral-shaped phototrophic α-Proteobacteria are phylogenetically quite distantly related to each other and do not warrant classification in one and the same genus (Kawasaki et al., 1993a; Imhoff et al., 1998). These bacteria also demonstrate great phenotypic diversity. Therefore, a reclassification of the spiral-shaped phototrophic α-Proteobacteria was proposed, based on distinct phenotypic properties and 16S rDNA sequence similarities. *Rhodospirillum centenum* was transferred to a new genus as *Rhodocista*

centenaria (Kawasaki et al., 1992). Other Rhodospirillum species were transferred to the new genera *Phaeospirillum*, *Rhodovibrio*, *Roseospira* and *Rhodothalassium* (Imhoff et al., 1998). Only *R. rubrum* and *R. photometricum* were maintained as species of the genus *Rhodospirillum*. In addition, new species were described of this group: *Rhodospira trueperi* was assigned to a new genus on the basis of significant phenotypic and genotypic differences from *Rhodospirillum rubrum* and other known PNSB (Pfennig et al., 1997); for similar reasons, the new bacterium *Roseospirillum parvum* was assigned to a new genus (Glaeser and Overmann, 1999).

Alpha-2 Proteobacteria

Most characteristic of phototrophic bacteria of the α-2 (*Rhodopseudomonas*) group is the budding mode of growth and cell division and the presence of lamellar internal membranes lying parallel to the cytoplasmic membrane. Most of these phototrophic bacteria have been previously known as *Rhodopseudomonas* species. Genera of this group now include *Rhodopseudomonas*, *Rhodoplanes*, *Rhodoblastus*, *Blastochloris*, *Rhodomicrobium* and *Rhodobium*.

After the removal of purple nonsulfur bacteria that contained vesicular internal photosynthetic membranes and those that were β-Proteobacteria from the genus *Rhodopseudomonas*, only those species remained within this genus that had lamellar internal membrane structures and grew and reproduced by budding (Imhoff et al., 1984b). The bacteria removed from *Rhodopseudomonas* are now recognized as species of *Rhodopila*, *Rhodobacter*, *Rhodovulum* and *Rubrivivax*. Thereafter, what remained of the genus *Rhodopseudomonas* (together with *Rhodomicrobium vannielii*) still represented a heterogeneous assemblage of species (Imhoff et al., 1984b) now recognized as genera of the α-2 Proteobacteria. Primarily due to the availability of sequence data of the 16S rDNA (Kawasaki et al., 1993a) and in part supported by the isolation and description of new species and additional data, the following proposals have been made. *Rhodopseudomonas marina* was transferred to the new genus *Rhodobium* as *Rhodobium marinum* together with the new species *Rhodobium orientis* (defined as the type species of this genus; Hiraishi et al., 1995b). *Rhodopseudomonas rosea* was transferred to the new genus *Rhodoplanes* and designated as the type species of this genus, *R. roseus* (Hiraishi and Ueda, 1994b). At the same time, *Rhodoplanes elegans* was described as a new species of this genus. *Rhodopseudomonas viridis* and *Rhodopseudomonas sulfoviridis* were assigned to the new genus *Blastochloris* as *B. viridis* and *B. sulfoviridis* (Hiraishi, 1997). Quite recently, *Rhodopseudomonas acidophila* was transferred to a new genus as *Rhodoblastus acidophilus* (Imhoff, 2001).

Rhodopseudomonas blastica was removed from this genus and transferred to *Rhodobacter blasticus* (Kawasaki et al., 1993b). Its 16S rDNA sequence is most similar to and clusters with those of the *Rhodobacter* species. *Rhodopseudomonas rutila* (Akiba et al., 1983) was considered as a later subjective synonym of *Rhodopseudomonas palustris* (Hiraishi et al., 1992). In addition to *Rhodopseudomonas palustris*, *Rhodopseudomonas julia* (Kompantseva, 1989) and *Rhodopseudomonas cryptolactis* (Stadtwald-Demchick et al., 1990) have been affiliated to this genus, though both species so far have not been validated and no 16S rDNA sequence of them is available.

Alpha-3 Proteobacteria

A characteristic feature of the phototrophic α-3 Proteobacteria (*Rhodobacter* group) is the presence of carotenoids of the spheroidene series and their extraordinary metabolic versatility and flexibility. These bacteria have been previously known as *Rhodopseudomonas* species and belong to the genera *Rhodobacter* and *Rhodovulum* (Pfennig and Trüper, 1974b; Imhoff et al., 1984b; Hiraishi and Ueda, 1994a). The former are freshwater bacteria and the latter true marine bacteria. Species of both genera have distinct 16S rDNA sequences (Hiraishi and Ueda, 1994a; Hiraishi and Ueda, 1995a; Hiraishi et al., 1996). Two new species, *Rhodovulum iodosum* and *Rhodovulum robiginosum*, have been described that use ferrous iron as photosynthetic electron donor (Straub et al., 1999). *Rhodobaca borogenensis*, a new isolate from an alkaline soda lake with low salt concentrations, has adapted in its salt response to this habitat (Milford et al., 2000).

Habitats

Ecological niches of phototrophic α-Proteobacteria are those anoxic parts of waters and sediments that receive light of sufficient quantity and quality to allow phototrophic development. Representatives of the purple nonsulfur bacteria are widely distributed in nature and are found not only in all kinds of stagnant water bodies, including lakes, waste water ponds, coastal lagoons, and in other aquatic habitats, but also in sediments, moist soils, and paddy fields. They live in aquatic habitats with significant amounts of soluble organic matter and low oxygen tension, but

rarely form colored blooms, like those of purple sulfur bacteria. However, often they are found accompanying the purple sulfur bacteria in stratified environments.

They have been found not only in freshwater, marine and hypersaline environments, and most frequently in habitats of moderate temperatures, but also in thermal springs and in cold polar habitats. Most purple nonsulfur bacteria have been isolated from all kinds of freshwater habitats where they also are most abundant. The greatest variety of species and the largest numbers of cells have been found in mud and water of eutrophic ponds, ditches and lakes. In the flat shore area of eutrophic lakes, 10^3 to more than 10^8 cells/ml of purple nonsulfur bacteria have been found in mud and water samples (Kaiser, 1966; Biebl and Drews, 1969). In pelagic water, the numbers usually are much lower (Biebl, 1973; Swoager and Lindstrom, 1971).

Quite a number of purple nonsulfur bacteria occur in marine and hypersaline environments. They are usually found in water bodies and sediments of intertidal flats, salt marshes, and polluted harbor basins, but not in the open sea. While freshwater isolates have a very low tolerance to sulfide, the sulfide tolerance of most marine species is much higher, and they even use sulfide and thiosulfate as photosynthetic electron donors. This is certainly an adaptation to this environment, which characteristically has a high activity of sulfate reduction and where consequently anoxic conditions are coincident with the presence of hydrogen sulfide. Although some isolates (strains of *Rhodopseudomonas palustris* and *Rhodomicrobium vannielii*) from marine habitats are not typical marine bacteria and do not require salt for optimum growth, most of the purple nonsulfur bacteria found in marine habitats are typical marine bacteria and are not found in freshwater habitats (Imhoff, 1988a). The marine forms include species of the genera *Rhodovulum* and *Rhodobium*, as for example *Rhodovulum sulfidophilum* (Hansen and Veldkamp, 1973), *Rhodovulum adriaticum* (Neutzling et al., 1984), *Rhodovulum euryhalinum* (Kompantseva, 1985), *Rhodobium marinum* (Imhoff, 1983a) and *Rhodobium orientis* (Hiraishi et al., 1995b). *Rhodospira trueperi* and *Roseospirillum parvum* are from a marine salt marsh and also represent typical marine bacteria. In addition, *Roseospira mediosalina* was isolated from a hot spring with low salt concentrations (2% salts) and is growing optimally between 5 and 7% NaCl (Kompantseva and Gorlenko, 1984).

Other halophilic species are well adapted to hypersaline environments. *Rhodothalassium salexigens* and *Rhodovibrio salinarum* are common to evaporated seawater pools and marine salterns and sometimes form colored layers in salt deposits or sediments (Drews, 1981; Nissen and Dundas, 1984; Rodriguez-Valera et al., 1985), whereas *Rhodovibrio sodomensis* is from Dead Sea sediments (Mack et al., 1993).

Some purple nonsulfur bacteria occur in acidic, boggy waters and soils. Most frequently, *Rhodoblastus acidophilus*, which grows optimally at pH 5.5–5.8, is found in such environments, often accompanied by *Rhodopseudomonas palustris* (Pfennig, 1969). *Rhodopseudomonas palustris* is very common and was isolated from all kinds of aquatic habitats (lakes, sewage and brackish waters), even from wet decaying leaves and from soils (Biebl and Pfennig, 1981; Imhoff and Trüper, 1992b). A preference for low pH values (pH 4.8-5.0) also is found in *Rhodopila globiformis* (Pfennig, 1974a).

Purple nonsulfur bacteria are found regularly in all stages of conventional sewage plants. Their numbers may increase dramatically from raw sewage to the activated sludge stage (from 2,000 to 100,000 cells/ml; Siefert et al., 1978). When anaerobic sewage is incubated in the light, it often spontaneously becomes red-brown in color owing to the presence of phototrophic bacteria. This suggests that it should be possible to easily direct the processes in the activated sludge towards photoassimilation of organic substrates by phototrophic bacteria. The first sewage treatment plant based on this principle was established in Japan (Kobayashi et al., 1971; see below).

Isolation

Two different strategies may be applied to isolate purple nonsulfur bacteria from their natural environment. Depending on the aims of the studies either the one or the other has clear preferences. According to classical enrichment techniques, phototrophic bacteria may be selectively enriched in suitable media under anoxic conditions and in the light; after visible growth, cells are separated in agar dilution series or on agar plates and isolated in pure culture. Alternatively pure cultures may be obtained by directly inoculating agar media from natural samples without prior enrichment.

The first strategy is the method of choice whenever the isolation of bacteria with particular physiological features is attempted. With a proper environmental sample and the right medium, bacteria with the desired properties may be selected and isolated. The second strategy has to be used whenever the natural diversity is to be analyzed and information on the natural abundance and distribution of the species in a sample is required. While specifically adapted

media are essential for the success of the first strategy, in the second one nonselective media are used with substrates (such as acetate, succinate or malate) that enable the development of most purple nonsulfur bacteria (Biebl and Pfennig, 1981; Imhoff and Trüper, 1992b). Even if the medium used can support only poor growth of a specific strain, after separation in or on agar and when the incubation time is adapted to the slow growth rates, it will grow out to a small colony and can be picked up for further transfers.

Selective Enrichment

Selective enrichment techniques for phototrophic bacteria were first used by Sergei Winogradsky (Winogradsky, 1888), and his technique is known as the "Winogradsky column." Variations of this column technique by other authors are discussed by Pfennig (1965) and Van Niel (1971). Van Niel (1944) elaborated the physiological basis for the enrichment of purple nonsulfur bacteria and was the first to develop a defined medium which could be used for their enrichment and cultivation.

For selective enrichment of purple nonsulfur bacteria, media have been used with lowered sulfate concentration to avoid production of sulfide by sulfate-reducing bacteria (Van Niel, 1944; Van Niel, 1971). An anaerobic enrichment culture using a common organic substrate and placed in the light usually will give rise to the development of purple nonsulfur bacteria. In many cases, in particular with marine samples, small Chromatiaceae—owing to their organotrophic capacity—compete with purple nonsulfur bacteria even if the sulfate concentration in the enrichment media is strongly reduced. The choice of the carbon source is not critical for the success of an enrichment culture because fermentative processes usually result in the formation of acetate or other acids (propionate, butyrate and lactate), which are good substrates for the majority of the purple nonsulfur bacteria. Some species of the purple nonsulfur bacteria, however, such as *Rhodovulum sulfidophilum* (Hansen and Veldkamp, 1973), *Rhodovulum adriacticum* (Neutzling et al., 1984) and *Blastochloris sulfoviridis* (Keppen and Gorlenko, 1975), tolerate and/or even require sulfide as a reduced sulfur source and/or photosynthetic electron donor.

Besides the mineral salts composition and the concentration of nutrients of the media, also the addition of vitamins, the pH, temperature, light intensity and light regime are of general importance for the selectivity of enrichment cultures. If samples from marine and hypersaline environments are investigated, the salinity and the mineral composition of the medium are of special importance.

A few examples of selective parameters for the enrichment of purple nonsulfur bacteria are the following:

Selective carbon sources for *Rhodopseudomonas palustris* are benzoate and formate (Qadri and Hoare, 1968). Because this species is very common in nature, many enrichments for purple nonsulfur bacteria will end up with the development of this species even without particularly selective conditions.

Enrichments under photoautotrophic conditions with hydrogen as electron donor favor growth of *Rhodobacter capsulatus*, which grows faster under these conditions than other phototrophic bacteria do (Klemme, 1968). Also this species often becomes predominant in unselective media.

Higher fatty acids like pelargonate and caproate (not more than 0.04% at pH 7.5) provide selective growth conditions for *Phaeospirillum fulvum* and *Phaeospirillum molischianum* (Pfennig, 1967; Van Niel, 1944).

For enrichment of *Rhodospirillum photometricum*, the anaerobic infusion method of Molisch (Giesberger, 1947) is still the best method (Biebl and Pfennig, 1981). Hay or other dried plant materials are suitable sources which may be used in large test tubes or cylinders under continuous illumination. *Rhodospirillum photometricum* also can be readily enriched from activated sludge incubated anaerobically in the light (1,000 lux, 30°C; Siefert et al., 1978).

The use of amino acids as carbon substrates favors the development of the *Rhodospirillum* species, in particular of *Rhodospirillum rubrum* (Biebl and Pfennig, 1981).

A succinate-mineral salts medium without growth factors and with an initial pH of 5.5 is highly selective for both *Rhodoblastus acidophilus* and *Rhodomicrobium vannielii* (Pfennig, 1969). If yeast extract or the required vitamins are present *Rhodopseudomonas palustris* may also develop under acidic conditions.

Enrichments in methanol bicarbonate medium select for *Rhodoblastus acidophilus* (and *Rubrivivax gelatinosus*).

Typical marine bacteria will not or only poorly grow in media without NaCl, though most freshwater species are inhibited by NaCl concentrations of sea water. Therefore, the addition of 3% NaCl is a selective factor for marine purple nonsulfur bacteria.

Salt concentrations of more than 10% are highly selective for moderately halophilic species like *Rhodothalassium salexigens*, *Rhodovibrio salinarum* and *Rhodovibrio sodomensis* that will not grow in media for freshwater or marine phototrophic bacteria.

Isolation Procedures

Direct Isolation. Methods of direct isolation of the phototrophic bacteria from a natural sample use agar dilution series or inoculation of agar plates to separate the cells prior to incubation. For isolation with solid media, water samples are most appropriate. A sample of mud, sludge, or soil may be used as a homogeneous suspension in medium or filter-sterilized water from the habitat. Samples containing less than 10 cells/ml need to be concentrated by centrifugation (agar dilution series) or filtration (agar plates). All methods for direct isolation are suitable for the determination of living cell counts, when known amounts of the sample are used in appropriate dilutions.

Preparation of Agar Dilution Series. Agar dilution series are prepared either with enrichment cultures or with promising natural samples by direct inoculation with an environmental sample. Selective media are not required and nonselective ones are preferred for a direct isolation without prior enrichment procedure.

In a modification of the method of Pfennig (Pfennig, 1965; Trüper, 1970), purified agar is dissolved (1.8%) and distributed in amounts of 3 ml into cotton-plugged test tubes. The agar is sterilized by autoclaving. The liquid agar is kept at 50°C in a water bath until use. A suitable medium is placed in the same water bath, and 6 ml of the prewarmed medium is added to each test tube. Medium and agar are mixed thoroughly by turning the tubes upside down and back and kept at 50°C. Eight tubes are sufficient for each dilution series. The first tube is inoculated with a natural sample or enrichment culture and mixed carefully; approximately 0.5 to 1.0 ml is transferred to a second tube, mixed carefully, and the procedure continued up to the last tube. The tubes are immediately placed into a cold water bath. After the agar has hardened, they are sealed with a paraffin mixture (3 parts paraffin oil and 1 part paraffin) and kept in the dark for several hours before they are incubated in the light (ca. 500–2,000 lux). After cells have grown to visible colonies, the paraffin layer is removed by melting, and well separated colonies are picked with a Pasteur pipette (the tip drawn out to a thin capillary) and transferred to a second dilution series. In general, three to four such dilution series are necessary to obtain pure cultures. When pure cultures have been obtained, single colonies are inoculated into liquid medium.

Cultivation on Agar Plates. Purple nonsulfur bacteria have quite often been successfully isolated on agar plates. When high numbers of phototrophic bacteria are present in the sample, streaking by conventional methods is appropriate. Samples containing low numbers of phototrophic bacteria can be easily concentrated on membrane filters (e.g., cellulose acetate or cellulose nitrate with 0.4 μm pore size; Biebl, 1973; Biebl and Drews, 1969; Swoager and Lindstrom, 1971; Westmacott and Primrose, 1975). The filter assembly is used after autoclaving, and the filters are transferred onto the agar surface with sterile tweezers. Samples with high numbers of purple nonsulfur bacteria (more than 100 cells/ml) may be diluted prior to filtration or distributed on the agar with a Drigalsky spatula (approximately 0.1 ml of a sample containing 100–300 cells/ml; Biebl and Drews, 1969).

Incubation of the plates is recommended in an anaerobic jar in which the air is replaced by an oxygen-free mixture of nitrogen with 5% carbon dioxide and 3% hydrogen. Remaining traces of oxygen are removed by reaction with hydrogen over a palladium catalyst. Alternatively the GasPak system (Becton, Dickinson and Co.) or comparable systems may be used. A more detailed description of these methods is given by Biebl and Pfennig (1981). Normally, an incubation time of five or more days is required before intensely colored colonies of purple nonsulfur bacteria become visible. Occasionally, unicellular green algae develop and form flat spots of grass-green color. Sometimes purple sulfur bacteria, in particular *Thiocapsa roseopersicina* and *Allochromatium vinosum*, also develop.

Media

A large number of media have been used for the enrichment and cultivation of purple nonsulfur bacteria (Biebl and Pfennig, 1981; Drews, 1965; Haskins and Kihara, 1967; Imhoff, 1982b; Imhoff and Trüper, 1976; Kaiser, 1966; Mack et al., 1993; Pfennig, 1969; Pfennig, 1978; Pratt and Gorham, 1970; Swoager and Lindstrom, 1971; Van Niel, 1944).

For the isolation and cultivation of purple nonsulfur bacteria from freshwater and marine sources, the AT medium (Imhoff and Trüper, 1976; Imhoff, 1982b; Imhoff, 1988c) with numerous slight modifications has been successfully used as a basic medium for more than 20 years in the author's laboratory. It can be used for cultivation of the great majority of the purple nonsulfur bacteria. It is also well suited for enrichment cultures of freshwater and marine purple nonsulfur bacteria. For the purpose of selective enrichment cultures, sulfate is omitted, a sulfate-free trace element solution (SLA) is used and selective carbon and nitrogen substrates are added. With nonselective carbon sources such as acetate, pyruvate, malate, succinate or fumarate, this medium is well suited for the isolation and enumeration of purple nonsul-

fur bacteria in agar dilution series or on agar plates. A vitamin solution (VA) is used that adds sufficient amounts of all vitamins required by known purple nonsulfur bacteria (Imhoff, 1988c; see below).

AT Medium

Dissolve in and dilute to 1 liter with distilled water:

KH_2PO_4	1.0 g
$MgCl_2 \cdot 6H_2O$	0.5 g
$CaCl_2 \cdot 2H_2O$	0.1 g
Na_2SO_4	0.7 g
NH_4Cl	1.0 g
$NaHCO_3$	3.0 g
NaCl	1.0 g
Na-acetate (or other carbon source)	1.0 g

Add: 1 ml of trace element solution SLA (see below)
1 ml of vitamin solution VA (see below)
10 ml of 5% sodium ascorbate
Adjust pH to 6.9.

The medium is filter-sterilized, collected in an autoclaved 2-liter Erlenmeyer flask with an outlet at the bottom and distributed from this vessel into sterile screw-cap bottles of desired volume (usually 50-ml and 1-liter bottles) under sterile conditions. These bottles are filled completely (not more than a pea-sized air bubble should be left) and can be stored for several months. To achieve anoxic conditions and to remove the oxygen from the medium, 0.05% sodium ascorbate is added. To avoid oxidation of the ascorbate prior to distribution into the bottles, it is added to the medium immediately before sterile filtration.

Yeast extract stimulates growth of most of the known purple nonsulfur bacteria. It is used as a source of growth factors and may be added at a concentration of 0.05%.

For *Phaeospirillum* and *Rhodospirillum* species addition of 0.01% Fe-citrate is growth stimulating. It may also be routinely added to the medium, except when sulfide is present.

For the cultivation of *Rhodoblastus acidophilus* and *Rhodomicrobium vannielli*, the pH is adjusted to 5.5.

For marine species, NaCl is added. A saline modification of the medium, the SAT medium, contains 3% NaCl.

For iron-oxidizing purple nonsulfur bacteria, 10 mM ferrous iron is added to completely anoxic media buffered with 20 mM bicarbonate at pH 7.0 (Ehrenreich and Widdel, 1994). An anoxic stock solution of $FeSO_4$ is autoclaved, maintained under nitrogen, and used to supplement the media. To increase the solubility of iron, chelators such as citrate or nitrilotriacetic acid may be added.

Some purple nonsulfur bacteria can tolerate sulfide to various degrees and/or use it as a photosynthetic electron donor. For these species, sodium sulfide is added at low concentrations of 0.4–1.0 mM (up to 2 mM for more tolerant strains). Some species even are dependent on the presence of reduced sulfur sources because they lack the ability to use sulfate as an assimilatory sulfur source. For these bacteria also low concentrations of sulfide may be used, or alternative sources of reduced sulfur such as thiosulfate, cysteine or methionine may be applied.

Vitamin Solution VA

(Modified after Imhoff and Trüper, 1977)

Biotin	10 mg
Niacin	35 mg
Thiamine dichloride	30 mg
p-Aminobenzoic acid	20 mg
Pyridoxolium hydrochloride	10 mg
Ca-panthothenate	10 mg
Vitamin B_{12}	5 mg

This vitamin solution meets the vitamin requirements of all purple nonsulfur bacteria. More specifically, for the culture of individual strains or species, the vitamins may be added individually or in desired combinations at the indicated concentration. Dissolve in 100 ml of distilled water, sterilize by filtration, keep refrigerated, and add 1 ml per liter of medium.

Trace Element Solution SLA

(Modified after Imhoff and Trüper, 1977)

$FeCl_2 \cdot 4H_2O$	1,800 mg
$CoCl_2 \cdot 6H_2O$	250 mg
$NiCl_2 \cdot 6H_2O$	10 mg
$CuCl_2 \cdot 2H_2O$	10 mg
$MnCl_2 \cdot 4H_2O$	70 mg
$ZnCl_2$	100 mg
H_3BO_3	500 mg
$Na_2MoO_4 \cdot 2H_2O$	30 mg

These salts are dissolved separately in a total of 900 ml of double-distilled water; the solutions are mixed, the pH is adjusted to about 2–3 with 1 N HCl, and the final volume is brought to 1 liter.

Medium for *Rhodopila globiformis*

(Modified after Pfennig, 1974a)

Mannitol	1.5 g
Gluconate	1.5 g
KH_2PO_4	0.4 g
NaCl	0.4 g
$CaCl_2 \cdot 2H_2O$	0.05 g
$MgCl_2 \cdot 6H_2O$	0.4 g
NH_4Cl	0.4 g
Na-thiosulfate	0.2 g

Dissolve the above in and dilute up to 1 liter with distilled water.

Add separately: 1 ml of SLA (or equivalent trace element solution)
1 ml of VA
5 ml of 0.1% Fe-citrate solution
Adjust pH to 4.9.

Complex Medium

(Modified after Nissen and Dundas, 1984)

$MgCl_2 \cdot 6H_2O$	3.5 g
KH_2PO_4	0.3 g
NaCl (depending on desired salinity)	100 g
Yeast extract (Difco)	1.5 g
Proteose peptone (Difco)	1.5 g
Na-malate	10 mM
Trace element solution SLA	1 ml

Dissolve the above in and dilute up to 1 liter of distilled water. Then adjust pH to 7.0. All strains of halophilic purple nonsulfur bacteria including our own isolates grow well in this medium. This and the complex medium described by Drews (1981) are suitable for growth of *Rhodothalassium salexigens* and *Rhodovibrio salinarum*.

Synthetic Medium for Halophilic (Purple Nonsulfur) Phototrophic α-Proteobacteria

(Modified from Imhoff, 1988c)

$NaHCO_3$	3.9 g
KH_2PO_4	0.5 g
KCl	1.0 g
$CaCl_2 \cdot 2H_2O$	0.05 g
$MgCl_2 \cdot 6H_2O$	3.5 g
Na_2SO_4	1.0 g
NaCl (depending on desired salinity)	40–150 g
Proline	5.0 mM
Acetate	2.0 mM
Pyruvate	2.0 mM
K_3 citrate	10.0 mM
Glycine betaine	10.0 mM
Na-glutamate	5.0 mM
Vitamin solution VA (see above)	1 ml
Trace element solution SLA (see above)	1 ml

Dissolve the above in and dilute up to 1 liter of distilled water. Adjust pH to 7.0. Sterilize by filtration.

For special biochemical and physiological studies, synthetic media (such as this one) are required. The NaCl concentration is varied according to the desired salinity.

Identification

Identification of new isolates can be obtained only by detailed studies of physiological and morphological properties together with chemotaxonomic information and genetic sequence data. To distinguish closely related strains and species of the same genus, often DNA-DNA hybridization studies are required.

Because of the great diversity of the phototrophic α-Proteobacteria, a tentative assignment to one of the known genera often is much easier. Owing to the different physicochemical requirements of the species (e.g., salinity, pH and possibly temperature) and their physiological potential, the choice of the medium and the culture conditions for enrichment and isolation restrict the number of possible species that will be able to develop. Important additional information can be obtained from the color, size and consistency of the colonies on agar plates, the color of cell suspensions, and microscopic examinations. The shape of the cells, cell width and length, motility, mode of division, formation of cell aggregates, and presence of slime capsules are relevant properties that can be recognized in the light microscope. The cell morphology of a few representative species using light microscopy is shown in Fig. 4. Ultrathin sections under the electron microscope reveal the fine structure of the cells, in particular the type of the internal membrane system.

In many species, the color of cell suspensions is indicative of the major type of carotenoids present (Schmidt, 1978). Spirilloxanthin as the major component gives a pink or red color, increasing amounts of additional rhodopin turn the color to brown-red, rhodopin without significant amounts of spirilloxanthin results in brown, okenone results in purple-red, and rhodopinal results in purple-violet. Carotenoids of the spheroidene series give colors from yellowish brown to brownish red (depending on the content of oxygenated derivatives formed in the presence of oxygen) and greenish to beige-brown under strongly reducing conditions. Carotenoidless mutants of *Rhodospirillum rubrum* and *Rhodobium marinum* are blue in color.

In addition to the color of cell suspensions, absorption spectra yield preliminary information on the predominant bacteriochlorophylls and on the kind of bacteriochlorophyll-protein complexes. The carotenoids absorb at 480–550 nm. Absorption bands of bacteriochlorophyll a in vivo are at 380, 590–600, and 800–900 nm. Owing to the formation of different light-harvesting complexes, absorption spectra show a remarkable variation in the long wavelength range from 800–900 nm. Absorption spectra of whole cells are measured with cell suspensions washed twice in medium or appropriate salt solutions and then suspended in 60% sucrose solution (Biebl and Drews, 1969). Better results are often achieved by using isolated internal membranes suspended in buffer. For this purpose, it is sufficient to break the cells by ultrasonication or with a French press and to separate whole cells and large cell fragments from the internal membranes by centrifugation at 15,000 g.

For the detailed description of a new bacterium, careful physiological studies are required, including the utilization of substrates, relations to oxygen, the ability to grow in darkness, respiratory and fermentative growth, vitamin requirement, and ranges and optima of salt concentration, pH and temperature.

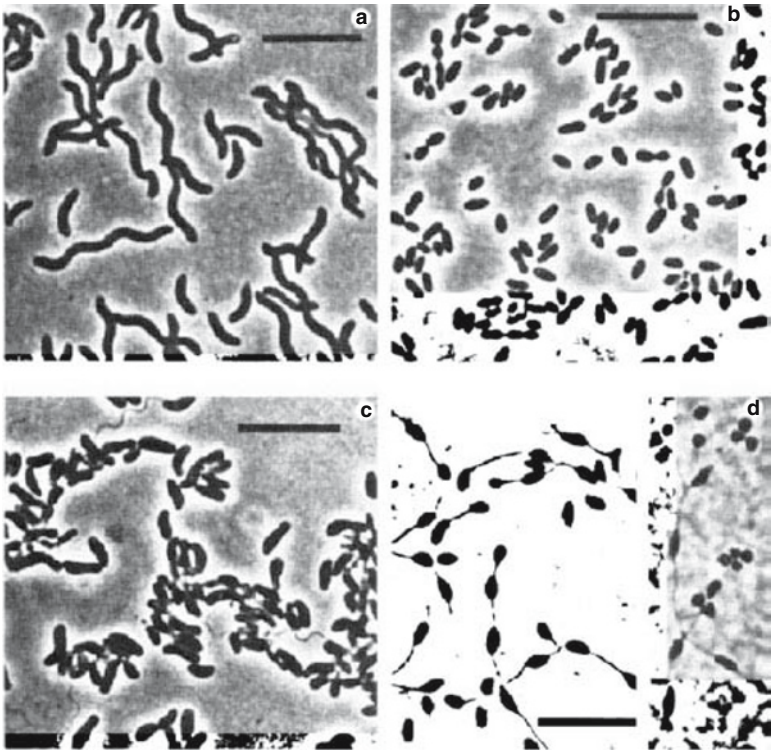

Fig. 4. Morphology of *Rhodospirillum rubrum* (a); *Rhodobacter sphaeroides* (b); *Rhodopseudomonas acidophilia* (c); *Rhodomicrobium vannielii* (d), left: Vegetative cells with polyhedral exospores; phrase contrast micrographs. (Bar = 10 μm.)

In addition to the phenotypic characterization, information on the genetic relatedness of a new isolate has to be obtained. To achieve a phylogenetic classification, 16S rDNA sequences are used and when the distinction of closely related strains and species is required (DeBont et al., 1981), DNA-DNA hybridization studies are performed. For the description of a new species, also the determination of the G+C content of the DNA is recommended.

Also chemotaxonomic properties have been found to be quite helpful to identify and classify new isolates of phototrophic bacteria (Hiraishi et al., 1984; Imhoff, 1984a; Imhoff, 1988b; Imhoff et al., 1982c; Imhoff et al., 1984b; 1998; Imhoff and Bias-Imhoff, 1995b; Thiemann and Imhoff, 1996; Imhoff and Süling, 1996; Pfennig et al., 1997). In particular the ring structure and the isoprenoid chain length of respiratory quinones and the fatty acid composition of the cell membranes are quite useful in identification (Hiraishi et al., 1984; Imhoff, 1984a). The structure and composition of polar lipids and lipopolysaccharides also are diagnostic properties of high value (Imhoff and Bias-Imhoff, 1995b; Weckesser et al., 1995). The need for sophisticated analytical methods, however, makes these properties less accessible for diagnosis.

Characteristic properties of presently known species of the phototrophic purple α-Proteobacteria that are of diagnostic value are shown in Tables 1–3. An arbitrary selection of outstanding properties of a few selected species follows:

Cultures of *Blastochloris viridis* and *Blastochloris sulfoviridis* are olive-green in color and contain bacteriochlorophyll β with an absorption maximum at 1020–1030 nm.

Cultures of *Rhodospira trueperi* are peach colored and reveal unusually low long-wavelength absorption maxima of bacteriochlorophyll b at 986 nm.

The in vivo absorption spectra of *Rhodospirillum rubrum* and *Rhodobium marinum* are characterized by an unusually low absorption maximum at approximately 803 nm and a single dominant peak without a shoulder (should be checked in derivative spectra) at 870–885 nm. This is taken as indication of the lack of peripheral light-harvesting complexes. Additional species with such spectra are *Rhodovibrio sodomensis*, *Rhodobaca bogoriensis* and *Rhodocista centenaria*.

For *Rhodomicrobium vannielii*, the peritrichous flagellation of swarmer cells, the formation of cell aggregates connected by thin filamentous tubes and the formation of exospore-like cysts of moderate heat resistance are typical.

Swarming motility on agar surfaces is characteristic for *Rhodocista centenaria*, which also forms desiccation- and heat-resistant cysts (Favinger et al., 1989).

A definitive growth requirement for salt is found in the marine species of *Rhodovulum* and *Rhodobium* and also in *Rhodospira trueperi*, *Roseospira mediosalina* and *Roseospirillum parvum*.

Table 1. Characteristic properties of the phototrophic *Rhodospirillum* and related phototrophic purple nonsulfur bacteria.

	Rhodospirillum		*Phaeospirillum*		*Rhodocista*	*Rhodovibrio*		*Rhodospira*		*Roseospira*		*Rhodopila*
Genus Species	*Rsp.* *rubrum*	*Rsp.* *photometricum*	*Phs.* *fulvum*	*Phs* *molischianum*	*Rci.* *centenaria*	*Rhv.* *salinarum*	*Rhv.* *sodomensis*	*Rsa.* *trueperi*	*Roseospirillum* *Reo. parvum*	*Ros.* *mediosalina*	*Rhodothalsassium* *Rts. salexigens*	*Rpi.* *globiformis*
Characteristic												
Cell diameter (μm)	0.8–1.0	1.1–1.5	0.5–0.7	0.7–1.0	1.0–2.0	0.8–0.9	0.6–0.7	0.6–0.8	0.4–0.6	0.8–1.0	0.6–0.7	1.6–1.8
ICM	Vesicles	Stacks	Stacks	Stacks	Lamellae	Vesicles	Vesicles	Vesicles	Lamellae	Vesicles	Lamellae	Vesicles
Motility	+	+	+	+	+	+	+	+	+	+	+	+
Color	Red	Brown	Brown	Brown	Pink	Red	Pink	Beige	Pink	Pink	Red	Purple-red
Bacteriochlorophyll	*a*	*a*	*a*	*a*	*a*	*a*	*a*	*b*	*a*	*a*	*a*	*a*
Vitamins required	b	n	paba	aa	b, B_{12}	c, n	c, n	b, t, pan	o	t, paba, n	glu	b, paba
Aerobic growth	+	–	–	–	+	+	+	–	+	(+)	+	(+)
Oxidation of sulfide	+	–	–	–	o	–	o	+	+	+	–	–
Salt requirement	None	None	None	None	None	4–6% (3–24)	10–14% (8–18)	2% (0.5–5)	1–2	4–7% (0.5–15)	6–8% (5–20)	None
Optimal temperature	30–35	25–30	25–30	30	40–45	42	35–40	25–30	30	30–35	40	30–35
Optimal pH	6.8–7.0	6.5–7.5	7.3	7.3	6.8	7.5–8.0	7	7.0–7.5	7.9	7	6.6–7.4	4.8–5.0
Habitat	Fresh water	Fresh water	Fresh water	Fresh water	Fresh water warm springs	Saltern	Salt lakes	Marine sediments	Marine sediments	Salty springs	Saltern	Fresh water acidic springs
DNA base ratio[a]	63.8–65.8	64.8–65.8	64.3–65.3	60.5–64.8	69.9	67.4	66.2–66.6	65.7	71.2	66.6	64.0	66.3
Cytochrome *c* size	Large	Large	Small	Small	o	None	None	o	o	o	o	Small
Major quinones	Q-10, RQ-10	Q-8, RQ-8	Q-9, MK-9	Q-9, MK-9	Q-9	Q-10, MK-10	o	Q-7, MK-7	o	o	Q-10, MK-10	Q-9/10, MK-9/10 RQ-9/10

Symbols: +, positive in most strains; –, negative in most strains; +/–, variable in different strains; o, not determined; (+), weak growth or microaerobic growth only.
Abbreviations: b, biotin; t, thiamine; n, niacin; paba, *p*-aminobenzoic acid; pan, pantothenate; glu, glutathione; ICM, internal cell membrane; Q-10, ubiquinone 10; MK-10, menaquinones 10; and RQ-10, rhodoquinone 10.
[a]Mol%.

Table 2. Characteristic properties of *Rhodopseudomonas* and related phototrophic purple nonsulfur bacteria.

	Rhodopseudomonas			*Rhodoblastus*	*Rhodoplanes*		*Blastochloris*			*Rhodobium*	
Genus Species	*Rps. palustris*	*Rps. julia*	*Rps. cryptolactis*	*Rbl. acidophilus*	*Rpl. roseus*	*Rpl. elegans*	*Blc. viridis*	*Blc. sulfoviridis*	*Rhodomicrobium Rmi. vannielii*	*Rbi. orientis*	*Rbi. marinum*
Characteristic											
Cell diameter (µm)	0.6–0.9	1.0–1.5	1	1.0–1.3	1.0	0.8–1.0	0.6–0.9	0.5–0.9	1.0–1.2	0.7–0.9	0.7–0.9
Type of budding	Tube	Sessile	Sessile	Sessile	Sessile	Tube	Tube	Sessile	Tube	Sessile	Sessile
Rosette formation	+	+	+	–	–	+	+	–	Large cell aggregates	–/+	–
Internal membrane system	Lamellae	Lamellae	Lamellae	Lamellae	Lamellae	Lamellae	Lamellae	Lamellae	Lamellae	Lamellae	Lamellae
Motility	+	+	+	+	+	+	+	+	+	+	+
Color of cultures	Brown-red to red	Pink	o	Red to orange-red	Pink	Pink	Green to olive-green	Olive-green	Orange-brown to red	Pink to red	Pink to red
Bacteriochlorophyll	*a*	*a*	*a*	*a*	*a*	*a*	*b*	*b*	*a*	*a*	*a*
Salt requirement	None	o	None	None	None	None	None	None	None	4–5%	1–5%
Optimum pH	6.9	6.0	6.8–7.2	5.5–6.0	7.0–7.5	7.0	6.5–7.0	7.0	6.0	7.0–7.5	6.9–7.1
Optimum temperature (°C)	30–37	25–35	40	25–30	30	30–35	25–30	28–30	30	30–35	25–30
Aerobic dark growth	+	+	+	+	+	+	(+)	(+)	+	+	(+)
Denitrification	+/–	–	o	–	+	+	–	–	o	+	–
Photoautotrophic growth with	H_2, TS, Sulfide	Sulfide, S^o	–	H_2	TS	TS	–	TS, Sulfide	H_2, Sulfide	TS	Sulfide
Vitamins required	Paba (biotin)	None	B_{12}, niacin, paba	None	Niacin	Thiamine, paba	Biotin, paba	Biotin, paba pyridoxine	None	Biotin, paba	o
Utilization of:											
Benzoate	+	–	–	–	–	–	–	–	–	–	–
Citrate	+/–	–	o	+	+	+	+/–	–	–	–	+/–
Formate	–	+	o	+	–	–	–	–	+/–	–	+
Tartrate	–	–	o	–	+	+	–	–	–	–	–
Glucose	–	+	–	–	–	–	(+)	+	–	+	+
Thiosulfate	+	o	o	–	+	+	–	+		+	–
Sulfide	+	+	o	–	–	–	–	+	+	–	(+)
Cytochrome c_2 size	Large	o	o	Small	o	o	Small	o	Small	o	o
Major quinones	Q-10	o	o	Q-10, MK-10, RQ-10	Q-10, RQ-10	Q-10, RQ-10	Q-9, MK-9	Q-8/10, MK-7/8	Q-10, RQ-10	Q-10, MK-10	Q-10, MK-10
Mol% G+C of the DNA	64.8–66.3 (Bd)	63.5	68.8	62.2–66.8 (Bd)	66.8 (HPLC)	69.6–69.7 (HPLC)	66.3–71.4 (Bd)	67.8–68.4 (CA)	61.8–63.8 (Bd)	65.2–65.7 (HPLC)	62.4–64.1 (HPLC)

Symbols: +, positive in most strains; –, negative in most strains; +/–, variable in different strains; o, not determined; and (+), weak growth or microaerobic growth only.
Abbreviations: paba, *p*-aminobenzoic acid; (biotin), biotin is required by some strains; Q-10, ubiquinone 10; MK-10, menaquinones 10; RQ-10, rhodoquinone 10; Bd, buoyant density; HPLC, high-pressure liquid chromatography; and TS, thiosulfate.

Table 3. Characteristic properties of *Rhodobacter* and related phototrophic purple nonsulfur bacteria.

	Rhodobacter					*Rhodobaca*	*Rhodovulum*					
Genus Species	*Rba. azotoformans*	*Rba. blasticus*	*Rba. capsulatus*	*Rba. sphaeroides*	*Rba. veldkampii*	*Rhb. bogoriensis*	*Rhv. strictum*	*Rhv. sulfidophilum*	*Rhv. adriaticum*	*Rhv. euryhalinum*	*Rhv. iodosum*	*Rhv. robiginosum*
Characteristic												
Cell diameter (µm)	0.6–1.0	0.6–0.8	0.5–1.2	2.0–2.5	0.6–0.8	0.8–1.0	0.6–1.0	0.6–0.9	0.5–0.8	0.7–1.0	0.5–0.8	0.5–0.8
Motility	+	–	+	+	–	+	+	+	–	+	–	–
Internal membrane system	Vesicle	Lamellae	Vesicle	Vesicle	Vesicle	Vesicle	Vesicle	Vesicle	Vesicle	Vesicle	Vesicle	Vesicle
Cell division	Binary fission	Budding	Binary fission	Binary fission	Binary fission	Binary fission	Binary fission	Binary fission	Binary fission	Binary fission	Binary fission	Binary fission
NaCl required (%)	–[a]	–	–[a]	–[a]	–	1–2	0.8–1.0	1–6	2.5–7.5	0.5–12	2.5–5	2.5–5
Sulfate assimilated	+	+	+	+	–	o	+	+	–	–	–	–
Oxidation products of sulfide	S^o	–	S^o	S^o	S^o/sulfate	o	Sulfate	Sulfate	S^o/sulfate	S^o/sulfate		
Aerobic dark growth	+	+	+	+	+	+	+	+	–	–	+	+
Vitamins required	b, n, t	b, n, t, B_{12}	t, (b, n)	b, t, n	b, paba, t	b, t, [B_{12}]	b, paba, t	b, n, paba, t	b, t	b, n, paba, t	b, n	b, n, B_{12}
Utilization of:												
Formate	+	–	+	–	–	–	+	+	+	+/–	–	–
Citrate	o	+	+/–	+	–	–	+	–	–	–	–	–
Tartrate	–	–	–	+	–	o	(+)	–	–	–	o	o
Mannitol	+	+	+/–	+	–	+	–	+/–	–	+/–	+	+
Glycerol	+	+	–	+	–	o	–	+	+	+	–	+
Ethanol	o	–	–	+	–	–	–	+/–	+	+/–	–	–
Hydrogen	o	+	+	+	–	o	–	+	–	+	+	+
Thiosulfate	–	–	–	–	+	o	+	+	+	+	+	+
Ferrous iron	o	o	o	o	o	o	o	–	–	–	+	+
Mol% G+C of DNA	69.5–70.2 (HPLC)	65.3 (Bd)	65.5–66.8 (Bd)	68.4–69.9 (Bd)	64.4–67.5 (Tm)	58–59	67.3–67.7 (HPLC)	66.3–66.6 (HPLC)	64.9–66.7 (Tm)	62.1–68.6 (Tm)	66 (HPLC)	69 (HPLC)

Symbols: +, positive in most strains; –, negative in most strains; +/–, variable in different strains; o, not determined; and (+), weak growth or microaerobic growth only.
Abbreviations: b, biotin; t, thiamine; n, niacin; paba, *p*-aminobenzoic acid; (biotin), biotin is required by some strains; [B_{12}], vitamin B_{12} growth stimulating but not absolutely required; Bd, buoyant density; Tm, thermal denaturation; and TS, thiosulfate.
[a]Optimal growth in the absence of NaCl, but able to grow at 3% NaCl.

The tolerance of salt concentrations of more than 10% and growth under these conditions are characteristic for the halophilic species *Rhodothalassium salexigens*, *Rhodovibrio salinarum* and *Rhodovibrio sodomensis*.

Preservation

For short-term preservation, well-grown cultures may be kept in closed, air-tight screw-cap bottles at 6–10°C in a refrigerator (or cold room) or at room temperature for several months, even years. Maintenance transfer of liquid cultures should occur at intervals of 2–6 months. In particular *Rhodopseudomonas palustris* and some *Rhodobacter* species have been shown to maintain viable cells over very long time periods. Stock cultures of the brown-colored *Rhodospirillum* and *Phaeospirillum* species and of other oxygen-sensitive species should be grown in the presence of 0.05% sodium ascorbate and transfers should be made every 1–2 months. Also, stock cultures of *Blastochloris sulfoviridis* and *Rhodopila globiformis* should be transferred more frequently. Stock cultures are incubated anaerobically in screw-cap 50-ml bottles in the described media at 25–30°C and 2,000 lux and then taken from the light during late exponential growth phase. Stock cultures also may be maintained for prolonged times in agar stab cultures or in appropriate dilutions of agar dilution series, sealed with paraffin and kept in the dark.

For long-term storage, preservation in liquid nitrogen is recommended. Well grown cultures are supplemented with 50% dimethyl sulfoxide to give a final concentration of 5%, thoroughly mixed, dispensed in plastic ampoules, sealed and frozen in liquid nitrogen. Preservation and storage in liquid nitrogen are possible with all strains tested with either dimethylsulfoxide (5%) or glycerol (10%) as a protecting agent.

Several purple nonsulfur bacteria were succsessfully preserved by freeze-drying (Biebl and Malik, 1976). The best protecting agent was skim milk (20%) supplemented with 10% sucrose, but most strains also survived in 10% sucrose alone. The brown-colored *Phaeospirillum* and *Rhodospirillum* species could not be lyophilized successfully (Biebl and Malik, 1976).

Physiology

A comprehensive treatment of the various aspects of the physiology of purple nonsulfur bacteria including structure, function and genetics of the photosynthetic apparatus is found in various chapters of *The Photosynthetic Bacteria* (Clayton and Sistrom, 1978) and *Anoxygenic Phototrophic Bacteria* (Blankenship et al., 1995). A short overview on physiology and photosynthesis is given by Drews and Imhoff (1991). In the following, the basic principles and a few specific examples of metabolic properties of the phototrophic α-Proteobacteria are presented.

Photosynthesis

Purple nonsulfur bacteria are anoxygenic phototrophic bacteria, growing phototrophically under anoxic conditions in the light without producing oxygen. All species can grow photoorganoheterotrophically using organic substrates as photosynthetic electron donors and carbon sources. Many representatives also grow under photolithoautotrophic conditions with reduced sulfur compounds, with hydrogen or ferrous iron ions as electron donor and CO_2 as sole carbon source.

Anoxygenic photosynthesis depends on the presence of a complex membrane-bound photosynthetic apparatus, which includes reaction center and light harvesting (antenna) pigment-protein complexes. The proteins of reaction center and antenna noncovalently bind bacteriochlorophyll, carotenoids and other cofactors in stoichiometric ratios. Most purple nonsulfur bacteria have two antenna complexes. The complexes of the reaction center are surrounded by core antenna (a B870 or B890 antenna complex with bacteriochlorophyll a and a B1020 complex with bacteriochlorophyll *b*) and additional peripheral antenna (B800-850 and B800-820 complexes with bacteriochlorophyll *a*). Species such as *Rhodospirillum rubrum* and *Rhodobium marinum* lack peripheral antenna complexes and can be recognized by their minor absorption maxima at 803 nm. Most purple nonsulfur bacteria have one type of peripheral antenna complex (B800-850). The synthesis of multiple forms of peripheral antenna polypeptides with varying proportions under different growth conditions (as found in *Rhodoblastus acidophilus* and *Rhodopseudomonas palustris*) and the formation of different peripheral antenna complexes result in a complex pattern of absorption maxima that is only resolved in derivative spectra (Brunisholz and Zuber, 1992; Zuber and Cogdell, 1995).

The principal function of the photosynthetic apparatus is the light-mediated excitation of a bacteriochlorophyll molecule in the reaction center followed by charge separation and resulting in electron transfer through the membrane. Most purple nonsulfur bacteria have an internal membrane system in which the photosynthetic apparatus is integrated. These internal membranes form vesicles, tubules or lamellar structures and are interconnected to the cytoplasmic

membrane. They can be isolated by cell rupture and fractionated centrifugation. Quite characteristically, the production of photosynthetic pigments, pigment-protein complexes and of the photosynthetic membrane structures is suppressed by oxygen. A notable exception is *Rhodocista centenaria* (Yildiz et al., 1991).

(Note: We distinguish between the anoxygenic phototrophic purple nonsulfur bacteria [which use photosynthesis as primary energy source and are well adapted to the anoxic way of life] and the aerobic bacteriochlorophyll-containing bacteria [which primarily gain energy by aerobic respiration but are unable to grow by anoxygenic photosynthesis under anoxic conditions]. The latter are treated elsewhere.)

Respiration

Chemoorganoheterotrophic growth in the presence of oxygen is common among purple nonsulfur bacteria and most of the known species are facultatively chemotrophic. While some species are very sensitive to oxygen, others grow equally well under oxic conditions in the dark at the full oxygen tension of air. Also chemolithoautotrophic growth with hydrogen or reduced sulfur compounds as electron donors and oxygen as electron acceptor has been demonstrated (Madigan and Gest, 1979; Siefert and Pfennig, 1979). Under anoxic dark conditions, growth of several species is also supported by respiratory electron transport in the presence of nitrate, nitrite, and nitrous oxide. Denitrification is induced by nitrate, either in the dark or in the light, but is suppressed by oxygen. A single strain of *Rhodobacter sphaeroides*, described as a subspecies, *Rhodobacter sphaeroides* f. sp. *denitrificans* (Satoh et al., 1976), but later recognized as a regular strain of *Rhodobacter sphaeroides* (De Bont et al., 1981; Imhoff, 1989a), was the first phototrophic purple bacterium known to use nitrate as an electron acceptor under anoxic dark conditions. Later, nitrate reduction was found in additional strains of *Rhodobacter sphaeroides* (Michalski and Nicholas, 1988), in strains of *Rhodopseudomonas palustris* (Klemme et al., 1980), and in *Rhodobacter capsulatus* (McEwan et al., 1984). Some of these strains could not grow with nitrate as sole nitrogen source, but dinitrogen produced during denitrification might be assimilated under these growth conditions (Dunstan et al., 1982). Also, *Rhodobacter azotoformans*, *Rhodobium orientis* and *Rhodoplanes roseus* and *Rhodoplanes elegans* are able to denitrify (Hiraishi and Ueda, 1994b; Hiraishi et al., 1995b; Hiraishi et al., 1996).

Anaerobic growth on sugars with dimethylsulfoxide (DMSO) or trimethylamine-*N*-oxide (TMAO) as an additional oxidant has been observed first in *Rhodobacter capsulatus* (Yen and Marrs, 1977) and later also in other species. Energy generation during growth with fructose and TMAO was proposed to be due to anaerobic respiration (Schultz and Weaver, 1982), and the generation of a membrane potential under these conditions has been demonstrated (McEwan et al., 1983). Later it was questioned that a true electron transport chain is involved in DMSO and TMAO reduction. The physiological role of these external oxidants was discussed in detail (Ferguson et al., 1987; Zannoni, 1995).

Fermentation

In the absence of external electron acceptors, a number of purple nonsulfur bacteria can use fermentative processes for energy generation (Uffen, 1978). During fermentative growth, substrates or storage products are oxidized incompletely, and reduced organic compounds as well as CO_2 and H_2 are produced. *Rhodospirillum rubrum* forms succinate, acetate, propionate, formate, CO_2 and H_2 during fermentation of fructose (Schön and Biedermann, 1973), whereas acetate, formate and equimolar amounts of CO_2 and H_2 are produced from pyruvate (Uffen, 1973). *Rhodobacter capsulatus* produces succinate, lactate, acetate, CO_2 and H_2 under identical conditions (Schultz and Weaver, 1982).

Carbon Metabolism

Organic carbon sources have quite different functions under phototrophic, respiratory and fermentative metabolism. Under phototrophic growth conditions, they serve primarily as a source of cellular carbon, but in addition may function as an electron source for photosynthetic electron transport; in the presence of inorganic electron donors, they may be exclusively photoassimilated. During respiratory growth, the major part of the carbon sources is completely oxidized and only a minor fraction is assimilated into cell substance. Enzymatic reactions of the citric acid cycle involved in substrate oxidation would be expected at elevated levels under these conditions and indeed have been found to be increased during respiratory growth of *Rhodobacter capsulatus* (Beatty and Gest, 1981). During fermentation, substrates or storage products are oxidized incompletely on a large scale, and reduced organic compounds and hydrogen are excreted to achieve a redox balance of the cells.

Most of the purple nonsulfur bacteria can use a variety of different organic carbon sources. Intermediates of the tricarboxylic acid cycle in addition to acetate and pyruvate are generally used. A number of purple nonsulfur bacteria use straight-chain saturated fatty acids with 5–18 car-

bon atoms (Janssen and Harfoot, 1987). Also organic acids, amino acids, alcohols and carbohydrates support growth of many of these bacteria. Carbon substrates such as citrate and aromatic compounds are used by a few species only. Citrate for instance is used by *Rubrivivax gelatinosus* (a β-Proteobacterium), *Rhodobacter sphaeroides*, *Rhodobacter blasticus*, *Rhodovulum strictum*, *Rhodothalassium salexigens*, *Rhodoblastus acidophilus*, *Rhodoplanes roseus*, *Rhodoplanes elegans* and by some strains of *Blastochloris viridis*, *Rhodobium marinum* and *Rhodopseudomonas palustris*. Some of these bacteria grow weakly with citrate.

A small number of species (*Rhodopseudomonas palustris*, *Rubrivivax gelatinosus*, *Phaeospirillum fulvum*, *Rhodocyclus purpureus*, *Rhodoblastus acidophilus* and *Rhodomicrobium vannielii*) can grow at the expense of aromatic organic compounds, such as benzoate, 3-hydroxybenzoate, 4-hydroxybenzoate, 1,3,5-trihydroxybenzene and other hydroxylated, methoxylated aromatic acids and aldehydes (Dutton and Evans, 1978). The ability to use aromatic compounds is best developed in *Rhodopseudomonas palustris*. This species also metabolizes phenolic acids, phenylalkane carboxylates, 4-hydroxy-cinnamate and related compounds (Harwood and Gibson, 1988; Gibson and Harwood, 1995). Most of these compounds support both phototrophic (anoxic conditions in the light) and chemotrophic (oxic conditions in the dark) growth; some are degraded only under the one or the other condition. During phototrophic growth, a reductive pathway for cleavage of the aromatic ring structure is used (Dutton and Evans, 1969; Gibson and Harwood, 1995).

Acetate is assimilated by almost all purple nonsulfur bacteria. The metabolic pathways of acetate assimilation, however, are quite different among the species. In many phototrophic purple bacteria, the primary reaction of acetate metabolism is the ATP-dependent formation of acetyl CoA, which is the substrate for further reactions. The two key enzymes of the glyoxylate cycle, isocitrate lyase and malate synthase, are present in most of these bacteria and acetate is assimilated via this pathway. There is, however, some variance in the literature regarding the presence of isocitrate lyase, in particular in *Rhodospirillum rubrum* and *Rhodobacter sphaeroides* (Tabita, 1995). Certainly, alternative pathways of acetate assimilation are possible. For *Rhodospirillum rubrum*, the conversion of acetate to oxalacetate by two carboxylation reactions from acetate to pyruvate and further to oxalacetate has been postulated (Buchanan et al., 1967). In *Rubrivivax gelatinosus* (a β-Proteobacterium), photoassimilation of acetate is possible via the serine-hydroxypyruvate pathway (Albers and Gottschalk, 1976).

For purple nonsulfur bacteria, CO_2 is an important carbon source. Under autotrophic growth conditions with CO_2 as sole carbon source, the Calvin cycle with ribulose bisphosphate carboxylase (RubisCO) as key enzyme is employed (Tabita, 1995). This enzyme is well studied and constitutes a major fraction of the cellular protein in bacteria that grow with CO_2 as sole carbon source and use the Calvin cycle. Also, CO_2 is required under heterotrophic growth conditions during assimilation of several reduced organic substrates. A number of carboxylating enzyme activities are responsible for this "heterotrophic CO_2 fixation" (see Kondratieva, 1979; Tabita, 1995). For instance, assimilation of propionate is connected with a carboxylation to succinate. Also, long-chain fatty acids and other highly reduced substrates such as methanol require CO_2 to elevate the oxidation-reduction level of these substrates to that of the cell material.

One-carbon compounds, such as methanol and formate, also are used by strains of a limited number of species. *Rhodomicrobium vannielii*, *Rhodobium marinum*, *Rhodoblastus acidophilus*, *Rhodospirillum rubrum*, *Rubrivivax gelatinosus*, *Rhodocyclus tenuis* and several *Rhodobacter* and *Rhodovulum* species use formate as carbon source. Reasonable growth rates with methanol were found only in strains of *Rhodoblastus acidophilus* (Douthit and Pfennig, 1976). Apparently, the RubisCO pathway is involved in carbon assimilation of *Rhodoblastus acidophilus* also during growth on methanol and formate; both substrates are used as electron donors and are oxidized to CO_2, which in turn is assimilated (Quale and Pfennig, 1975; Sahm et al., 1976). *Rubrivivax gelatinosus* is able to grow anaerobically in the dark with CO as sole source as carbon and energy. Under these conditions, CO is transformed into CO_2 and H_2, and RubisCO could be involved in assimilation of the latter (Uffen, 1983). In *Rhodospirillum rubrum*, CO is used under anoxic conditions during phototrophic growth and induces an oxygen-sensitive CO dehydrogenase (Bonam et al., 1989).

Sulfur Metabolism

The role of reduced sulfur compounds as photosynthetic electron donors for purple nonsulfur bacteria was realized together with the recognition of their importance in the marine environment. Purple nonsulfur bacteria vary greatly in their sulfur metabolism.

Most purple nonsulfur bacteria, in particular freshwater species, are inhibited by sulfide even

at low concentrations. Some species, however, are quite tolerant to this toxic compound and use it as a photosynthetic electron donor (Hansen and Imhoff, 1985; Hansen and van Gemerden, 1972; Imhoff, 1982a; Imhoff, 1983a; Neutzling et al., 1984). The tolerance of *Rhodovulum sulfidophilum* is high and comparable to that of *Allochromatium vinosum*. *Rhodomicrobium vannielii* tolerates concentrations of 2–3 mM, whereas growth of *Rhodobacter capsulatus* is completely inhibited at 2 mM. At low concentrations of sulfide (0.4–0.8 mM), however, growth of this species is quite rapid. Growth of *Rhodopseudomonas palustris*, as of most of the purple nonsulfur bacteria, is inhibited at concentrations as low as 0.5 mM sulfide (Hansen and van Gemerden, 1972). In general, small amounts of yeast extract in the media increase the tolerance towards sulfide.

In addition to sulfide, some purple nonsulfur bacteria can use thiosulfate as an electron donor. Extracellular elemental sulfur is the final oxidation product during sulfide oxidation of a number of purple nonsulfur bacteria, such as *Rhodospirillum rubrum*, *Rhodobacter capsulatus*, *Rhodobacter sphaeroides* (Hansen and Veldkamp, 1973) and *Roseospira mediosalina* (Kompantseva and Gorlenko, 1984). Elemental sulfur occurs outside the cells. Only with *Rhodopseudomonas julia* elemental sulfur was microscopically observed outside as well as inside the cells (Kompantseva, 1989). Oxidation of elemental sulfur is found in a limited number of species, as in *Rhodobacter veldkampii*, *Rhodovulum euryhalinum*, *Rhodovulum adriaticum* and *Rhodopseudomonas julia*. In these species, elemental sulfur is an intermediate product during oxidation to sulfate (Hansen, 1974; Hansen and Imhoff, 1985; Neutzling et al., 1984; Kompantseva, 1985; Kompantseva, 1989). Sulfate is formed from sulfide without formation of elemental sulfur in *Rhodovulum sulfidophilum*, *Rhodovulum strictum*, *Rhodopseudomonas palustris* and *Blastochloris sulfoviridis* (Hansen, 1974; Hiraishi and Ueda, 1995a; Neutzling et al., 1985). Tetrathionate is the only oxidation product of *Rhodomicrobium vannielii* grown in a chemostat, but in batch culture, sulfide reacts with the tetrathionate formed, so that thiosulfate is the major product (together with minor amounts of elemental sulfur) accumulated under these conditions; sulfate is not formed under either condition (Hansen, 1974).

With a few notable exceptions, the purple nonsulfur bacteria can use sulfate as an assimilatory sulfur source. Apparently two different pathways occur in these bacteria, involving either adenosine 5′-phosphosulfate (APS) or 3′-phosphoadenosine 5′-phosphosulfate (PAPS; Imhoff, 1982b). In the presence of reduced sulfur compounds, these are preferentially assimilated and the energetically expensive assimilation of sulfate is repressed (Imhoff et al., 1983b). Common alternative assimilatory sulfur sources include sulfite, thiosulfate, cysteine, glutathione, methionine and sulfide. *Rhodovulum adriaticum*, *Rhodovulum iodosum*, *Rhodovulum robiginosum*, *Rhodovulum euryhalinum*, *Blastochloris sulfoviridis*, *Rhodopseudomonas julia*, *Rhodobacter veldkampii* and *Roseospirillum parvum* require reduced sulfur sources and are unable to assimilate sulfate (Keppen and Gorlenko, 1975; Kompantseva, 1985; Kompantseva, 1989; Neutzling et al., 1984; Hansen and Imhoff, 1985; Pfennig, 1974a; Straub et al., 1999; Glaeser and Overmann, 1999). Growth of *Rhodopila globiformis* is inhibited by high concentrations of sulfate although at low concentration this can serve as an assimilatory sulfur source (Imhoff et al., 1981). Also *Rhodobium marinum* grows poorly with sulfate as an assimilatory sulfur source, but much better in the presence of low concentrations of reduced sulfur sources.

Nitrogen Metabolism

Ammonia, dinitrogen, and several organic nitrogen compounds (e.g., glutamate, aspartate or yeast extract) are the most appropriate nitrogen sources of most purple nonsulfur bacteria. Nitrate is assimilated only by a few species (strains of *Rhodospirillum rubrum*, *Rhodobacter sphaeroides*, *Rhodobacter capsulatus* and *Rhodopseudomonas palustris*) and growth yields with nitrate are considerably lower than with other nitrogen sources (Göbel, 1978; Imhoff, 1982a). Nitrate assimilation is inducible by nitrate but repressed by ammonia and glutamate.

Assimilation of ammonia is strongly regulated and two different major pathways occur. The primary pathway in purple nonsulfur bacteria is via glutamine synthetase and glutamate synthase, which is active if cellular concentrations of ammonia are low and if growth is occurring at low concentrations of ammonia or on dinitrogen and nitrate (Drews and Imhoff, 1991). The second pathway present in most purple nonsulfur bacteria is via glutamate dehydrogenase, which is active when ammonia concentrations are high.

The ability to fix dinitrogen is a common property of most phototrophic purple bacteria (Madigan, 1995). Dinitrogen fixation occurs under phototrophic and chemotrophic growth conditions, and the activity and expression of nitrogenase underlie a complex regulatory cascade (Drews and Imhoff, 1991; Haselkorn, 1986; Ludden and Roberts, 1995; Madigan, 1995). It has been most intensively studied in *Rhodospirillum rubrum*, where it was first discovered by Kamen and Gest (1949), and in *Rhodobacter capsulatus*.

Nitrogenase activity is repressed and inactivated by oxygen as in other dinitrogen-fixing bacteria. Its synthesis is derepressed at low concentrations of ammonia, i.e., under conditions of nitrogen limitation.

Hydrogen Metabolism

A great number of phototrophic purple bacteria can photoproduce hydrogen. With dinitrogen, glutamate or aspartate as nitrogen source, a number of carbon substrates (lactate, acetate, butyrate, malate and others) may be completely transformed to CO_2 and H_2, and these in turn may serve as substrates for photoautotrophic growth. After consumption of the organic substrate, cell suspensions of *Rhodobacter capsulatus* that produced, e.g., H_2 from lactate, rapidly started to consume the produced H_2 (Kelley et al., 1977). Similar observations were obtained with *Rhodospirillum rubrum* (Schick, 1971). Photoevolution of H_2 is catalyzed by nitrogenase, which is inhibited by ammonia, high concentrations of yeast extract and amino acids degraded to produce ammonia. The reaction is not reversible, insensitive to CO (a common inhibitor of hydrogenases) and independent of the partial pressure of H_2.

Hydrogen evolution in purple nonsulfur bacteria also occurs during fermentative growth under anoxic dark conditions. This hydrogen evolution is catalyzed by a reversible hydrogenase or by formate hydrogenlyase and underlies similar regulatory rules as in other fermenting bacteria, i.e., it is strongly inhibited by CO (Drews and Imhoff, 1991; Sasikala et al., 1993).

Hydrogen is not only produced, but also serves as an excellent photosynthetic electron donor for many purple nonsulfur bacteria and enables these bacteria to grow photolithoautotrophically. Hydrogen uptake is catalyzed by a reversible, membrane-bound hydrogenase, which is induced by hydrogen and independent of the nitrogen source. The membrane-bound hydrogenase is not inhibited by ammonia, but strongly inhibited by CO. During growth conditions of dinitrogen fixation, this uptake hydrogenase recycles the hydrogen produced by nitrogenase, and mutants lacking this hydrogenase demonstrate an increased hydrogen production during nitrogen fixation (Drews and Imhoff, 1991).

Applications

Most prominent examples of the application of phototrophic purple nonsulfur bacteria are their use in sewage treatment processes and for production of biomass, biopolymers and molecular hydrogen. They may be used as a source for cell-free systems performing photosynthesis and ATP formation and for the production of vitamins and other organic molecules.

Sewage contains a complex mixture of small organic molecules that are good substrates for purple nonsulfur bacteria. Phototrophic bacteria are regularly found in conventional sewage treatment plants (Holm and Vennes, 1971; Siefert et al., 1978). Facultative chemotrophic purple phototrophic bacteria compete best under such conditions. Because light-driven energy generation enables them to use compounds produced during anaerobic degradation processes, these bacteria are good candidates for application to the final stages of sewage treatment. A highly advanced system using phototrophic bacteria in the purification of municipal and industrial waste water is that developed by Kobayashi (Kobayashi et al., 1971; Kobayashi and Tschan, 1973; Kobayashi, 1977; Kobayashi and Kobayashi, 1995). This process uses the natural sequence of aerobic and anaerobic degradation, followed by culture of anoxygenic phototrophic bacteria and of green algae in separated reaction tanks (bioreactors). Phototrophic bacteria used in Kobayashi's system include *Rhodopseudomonas palustris*, *Rhodobacter capsulatus*, *Rhodobacter sphaeroides* and *Rubrivivax gelatinosus*. The use of phototrophic bacteria for sewage treatment was recently summarized by Kobayashi and Kobayashi (1995).

A number of different sources and processes have been used to produce bacterial biomass of phototrophic bacteria. Animal wastes (Ensign, 1977; Sasaki et al., 1990), soybean wastes (Sasaki et al., 1981), wheat bran (Shipman et al., 1975), municipal and industrial waste waters (Kobayashi, 1977), and clarified effluents of a biogas plant (Vrati, 1984) have been used. The produced biomass of phototrophic bacteria is a valuable source of animal feed; it is rich in vitamins and in essential and sulfur-containing amino acids (Vrati, 1984) and has been used in plankton production, in the culture of shrimp, and as food for fish and chicken (Kobayashi, 1977; Mitsui, 1979). Addition of phototrophic bacterial cells to the food increased the survival of fish as well as the production and quality of hens' eggs (Kobayashi and Tchan, 1973). With similar success the cell biomass of phototrophic bacteria has been used as fertilizer in agriculture (Kobayashi and Tchan, 1973). It may be used also for the production of hydrogen (Vrati and Verma, 1983; Bolliger et al., 1985), of biotin (Fillipi and Vennes, 1971), and of 5-aminolevulinic acid (Sasaki et al., 1990) and for other purposes.

Under nitrogen starvation, almost all phototrophic bacteria are able to produce molecular hydrogen. This process is mainly due to hydrogen evolution from nitrogenase. A large number

of substrates has been used by different research groups and with different purple nonsulfur bacteria to produce hydrogen (Kumazawa and Mitsui, 1982; Sasikala et al., 1993). Several attempts also have been made to produce hydrogen with immobilized cells of phototrophic bacteria (Francou and Vignais, 1984; Planchard et al., 1989; Vincenzini et al., 1982; von Felten et al., 1985; Weetall et al., 1981). Cells of *Rhodospirillum rubrum* (e.g., immobilized in a packed column) produced hydrogen with lactate as electron donor for 3,000 hours, with an activity loss of 60% after this time (von Felten et al., 1985). During the first days, mean rates of hydrogen production were 18–24 µl H_2 per mg dry weight and hour. Also cell-free systems were applied in hydrogen production (Mitsui, 1975). A recent comprehensive discussion of the technology of hydrogen production from phototrophic purple bacteria is given by Sasikala et al. (1993).

Poly-β-hydroxy-butyrate has been known for long as a storage product of phototrophic purple bacteria. In fact, an array of similar substances collectively termed "poly-3-hydroxyalkanoates" (PHAs) is accumulated, of which poly-3-hydroxybutyrate (PHB) is the most common. The PHAs occur as inclusion bodies of the cells visible in the light microscope and can account for a major fraction of the cell dry weight under appropriate growth conditions. Under strong nitrogen starvation or other conditions that restrict protein synthesis, excess carbon is converted into PHAs. In both *Rhodospirillum rubrum* and *Rhodobacter sphaeroides*, large amounts of storage PHAs can be synthesized under controlled growth conditions. *Rhodobacter sphaeroides* can produce more than 60% of its mass as PHAs of which 98% was PHB and the remainder was poly-3-hydroxyvalerate (Brandl et al., 1991). *Rhodospirillum rubrum* can produce a number of copolymers including an interesting tetrapolymer consisting of C_4, C_5 and C_6 repeating units (Brandl et al., 1989). A flexible polymerase which uses a variety of substrates yielding PHAs with different properties may be useful for the production of biodegradable thermoplastic polyesters of commercial value (Fuller, 1995). A copolymer of PHB and poly-3-hydroxyvalerate is commercially produced using cells of *Ralstonia eutropha*. A recent summary of aspects of biopolymer production by phototrophic purple bacteria is given by Fuller (1995).

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