Biodegradation **5**: 71–76, 1994.

Utilization of aromatic compounds by phototrophic purple nonsulfur bacteria

Ahmed A.M. Shoreit & Mohamed S.A. Shabeb
Botany Department, Faculty of Science, Assiut University, Assiut, Egypt

Received 29 April 1993; accepted 29 October 1993

Key words: purple nonsulfur bacteria, utilization of aromatic compounds, benzoate, benzyl alcohol, vanillate

Abstract

Biodegradation of aromatic compounds by *Rhodopseudomonas blastica* and *Rhodospirillum rubrum* appears to be lacking in the literature. The above species grew phototrophically (illuminated anaerobic conditions) on a variety of organic compounds. They were found to degrade benzoate, benzyl alcohol, 4-hydroxy-3,5-dimethoxybenzoate (Syringate) and 4-hydroxy-3-methoxybenzoate (vanillate). The ability of the above species to photocatabolize aromatic compounds indicates that these organisms may be ecologically significant as scavengers of aromatic derivatives in illuminated anaerobic habitats in nature.

Introduction

A diverse array of aromatic compounds exists in nature, primarily as substances released from decaying plant material (Sleat & Robinson 1984; Berry et al. 1987; Leahy & Colwell 1990; Wright & Madigan 1991). Methoxylated aromatic compounds are structural components of peats, coal and lignin; the second most abundant organic residue on Earth (Sarkanen & Ludwig 1971). Purple nonsulfur bacteria occur in a variety of habitats where they carry out an oxygenic photosynthesis (Shoreit et al. 1989, 1992; Imhoff & Trüper 1991). Recently numerous studies have shown that substituted benzoates including chlorinated, methoxylated, nitro-, and aminoaromatics and also aromatic hydrocarbons and phenolic compounds, can be broken down under anaerobic conditions by microorganisms in sediments from the Atlantic Coastal Plain and isolates of phototrophic bacteria (Evans & Fuchs 1988; Harwood & Gibson 1988; Kamal & Wyndham 1990; Wright & Madigan 1991; Blasco & Castillo 1992; Liu & Suflita 1993). In this paper, some isolates of Rhodospirillaceae resistant to aromatic compounds are described and characterized. They were isolated from Aswan-High-Dam-Lake (Egypt). The utilization of benzoate, benzyl alcohol and vanillate by *Rhodopseudomonas blastica* and *Rhodospirillum rubrum* is particularly noteworthy. This is the first report of photocatabolism of these substrates.

Materials and methods

Culture and cultural conditions

Phototrophic purple nonsulfur bacteria were isolated from the Aswan High Dam Lake and identified as *Rhodopseudomonas blastica, Rps. palustris, Rhodobacter capsulata* and *Rhodospirillum rubrum* (Shoreit et al. 1989, 1992). All isolates were maintained on modified synthetic medium RCVB (Tayeh & Madigan 1987) in which acetate replaced malate as sole carbon source. For testing the bacterial growth on aromatic compounds, the medium RCVB was modified by omitting acetate, EDTA, vitamins and adding 0.35 %, wt/vol (Final concentration) sodium bicarbonate, 0.05 % sodium ascorbate and an aromatic compound at 1 to 3 mM final concentration. Inoculated liquid cultures (3 to 5 % inocula), in completely filled screw-cap test tubes, were placed in darkness for 8 to 12 hours to allow for reduction of the medium and then incubated at 35°C in light at an intensity of 4500 to 5000 lux.

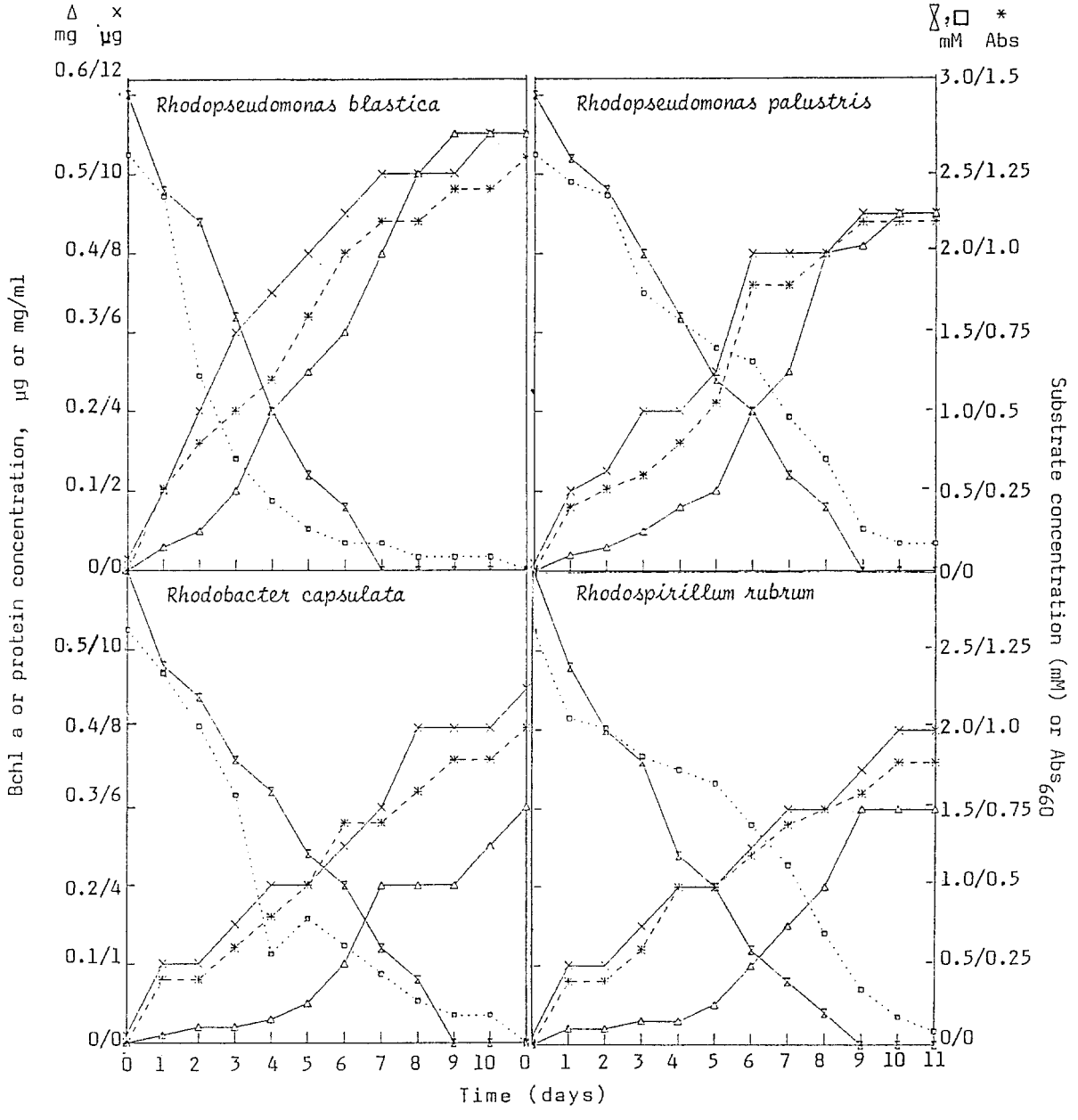

Fig. 1. Growth of *Rhodospirillaceae* on benzoate plus growth limiting levels of acetate. ⧖, acetate concentration (mM). □, benzoate concentration (mM). *, growth (A_{660}). △ biomass (mg protein/ml). ×, Bchl a concentration (µg/ml).

Analyses

Bacterial growth was measured turbidimetrically and increases in both protein and dry weight were estimated. Cell densities were measured at 600 nm with spectronic-601-spectrophotometer. Changes in the dry weight were followed as described by Madigan & Gest (1979). Cell protein content, were estimated by the method of Lowry et al. (1951), following extraction by boiling in 1 N NaOH for 20 min. Concentrations of all aromatic compounds were estimated from their UV absorption in the growth medium following the establishment of standard curves relating concentrations to UV absorbance. The concentration of benzoate was determined by using its absorption maximum of 276 nm, the concentration of vanillate was estimated

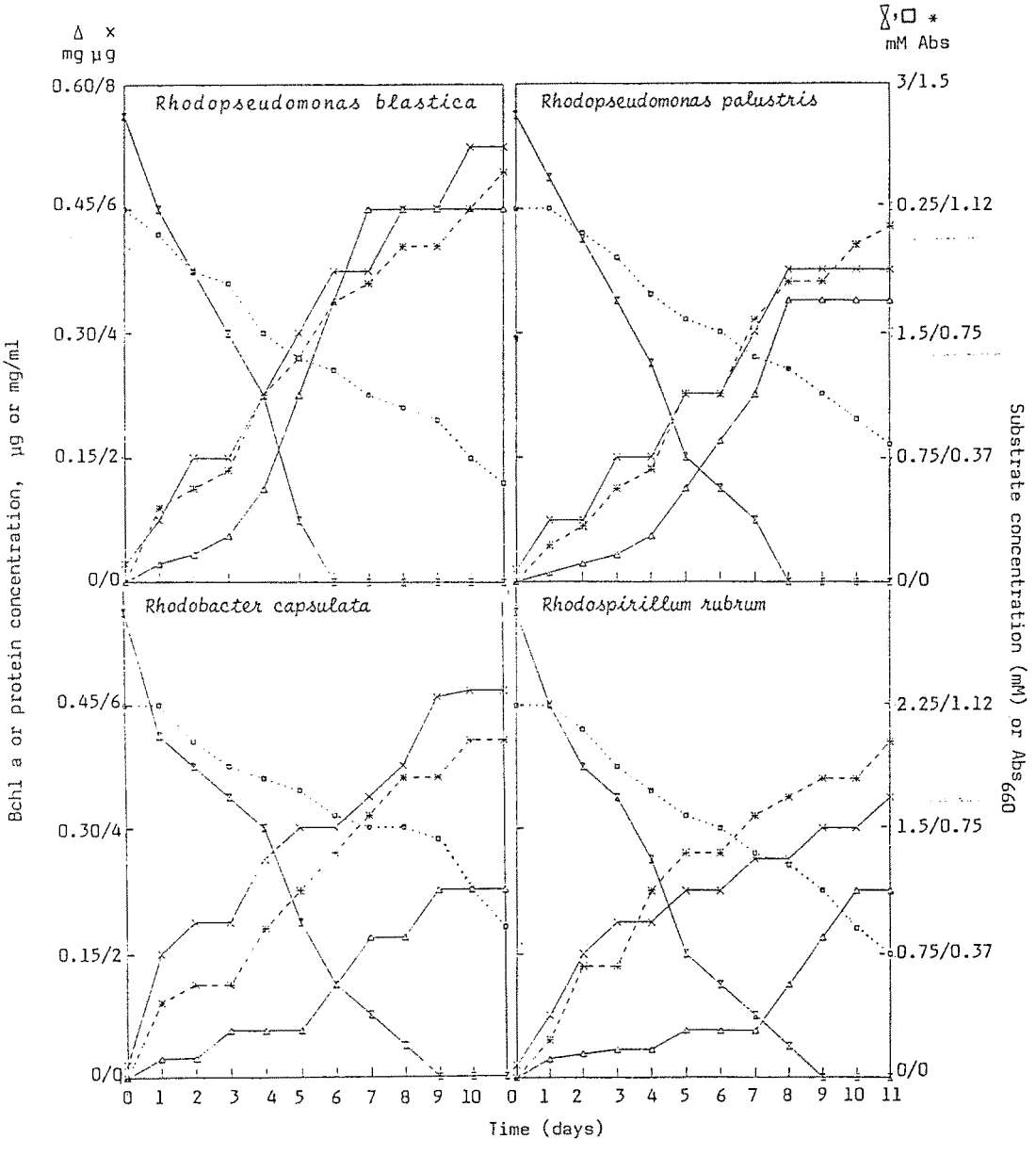

Fig. 2. Growth of *Rhodospirillaceae* on vanillate plus growth limiting levels of acetate. ⧖, acetate concentration (mM). □, vanillate concentration (mM). *, growth (A_{660}). △, biomass (mg protein/ml). ×, Bchl a concentration (μg/ml).

from the absorption maximum at 285 nm, and the concentration of acetate was estimated by its absorption at 265 nm (Boltz et al. 1978). Bacteriochlorophyll a content was determined by extraction of cell pellets with ice-cold methanol for 1 h at - 20°C (in darkness), followed by centrifugation of the mixture, and finally reading the absorption of the methanol extract at 770 nm. Absorbance was converted to concentrations of Bchl. by using the extinction coefficient of 46.11 g^{-1} cm^{-1} for Bchl a in methanol (Smith & Benitez 1955).

Table 1. Utilization of aromatic compounds for photoheterotrophic growth of different genera and species of Rhodospirillaceae*.

Substrate[a]	Isolates and growth response[b]			
	Rps. blastica	*Rps. palustris*	*Rhodobacter capsulata*	*Rhodospirillum rubrum*
Control (Acetate)	++++	++++	++++	++++
Control (no substrate)	-	-	-	-
Benzoate	++++	++++	+++	+++
Benzyl alcohol	++	++	++	++
3-chlorobenzoate	+	+	-	-
3,4-Dimethoxybenzoate	-	-	-	-
3-methoxybenzoate	-	-	-	-
N-benzylglycine (Hippurate)	+	+	-	-
4-hydroxybenzoate	-	-	-	-
4-hydroxy-3,5-di-methoxybenzoate (syringate)	++	++	++	+
3,4,5-trimethoxybenzoate	-	-	-	-
4-hydroxy-3-methoxybenzoate (Vanillate)	++++	++++	++	++

* All isolates was grown in media containing bicarbonate.

Incubation time was 30 days.

a: All substrates were supplied at a final concentration of 3 mM, except for acetate (at 30 mM).

b: Optical density at (660 nm) were as follows

-, 0.05; +, 0.06 to 0.1; ++, 0.11 to 0.3; +++, 0.31 to 0.6; ++++, > 0.61.

Results and discussion

Utilization of aromatic compounds by pure cultures of different genera and species of Rhodospirillaceae

Four species belonging to three genera were tested for utilization of benzoate and different benzoate derivatives, at 3 mM each, (benzyl alcohol, 3-chlorobenzoate, 3,4-dimethoxybenzoate, 3-methoxybenzoate, N-benzylglycine (hippurate), 4-hydroxybenzoate, 4-hydroxy-3,5-dimethoxybenzoate (syringate), 3,4,5-trimethoxybenzoate and 4-hydroxy-3-methoxybenzoate (vanillate)). The results in Table 1 show that the growth of *Rps. blastica, Rps. palustris, Rhodobacter capsulata* and *Rhodospirillum rubrum* was supported photoheterotrophically on benzoate as a sole carbon source.

Thus, *Rps. blastica, Rhodospirillum rubrum* and similarly *Rhodomicrobium vannielii* (Wright & Madigan 1991), *Rps. palustris* (Harwood & Gibson 1988), *Rhodocyclus purpureus,* (Pfennig 1978), and *R. fulvum* (Pfennig et al. 1965) can now be regarded as the anoxygenic phototrophic bacteria known to be capable of benzoate catabolism. Benzyl-alcohol appears to be used by all species tested in this report, but less efficiently than benzoate. This represents, to our knowledge the second report after Wright & Madigan (1991), who stated that benzyl alcohol was photocatabolized by only one out of five *R. vannielii* strains tested. Anaerobic benzyl-alcohol catabolism by bacterial pure cultures is not common and has been reported for only one other bacterium, a denitrifying *Moraxella* species isolated from soil (Williams & Evans 1975). It is thus possible that *Rps. blastica* and *Rhodospirillum rubrum* are ecologically significant as consumers of benzyl-alcohol in nature. Of the other aromatic compounds tested, vanillate and syringate were better growth substrates for the two species of *Rhodopseudomonas* (OD_{660}>0.6, 0.1–0.3) than for the *Rhodobacter* strain (OD_{660} 0.1–0.3, 0.1–0.3) and the *Rhodospirillum* isolate (OD_{660} 0.1–0.3, 0.06–0.1). When the other derivatives of benzoate (3,4-dimethoxybenzoate, 3-methoxybenzoate, 4-

hydroxybenzoate and 3,4,5-trimethoxybenzoate) were offered as a carbon source, no growth was observed by the three representatives of the Rhodospirillaceae. However, *Rhodopseudomonas* species exhibited limited growth on 3-chlorobenzoate and on hippurate each (OD_{660} 0.06–0.1).

Growth and pigment synthesis by different genera and species of Rhodospirillaceae grown on benzoate and vanillate

The growth on acetate-benzoate or acetate-vanillate was examined in the cultures of *Rps. blastica, Rps. palustris, Rhodobacter capsulata* and *Rhodospirillum rubrum* in which acetate was present in growth limiting amounts. Figure 1, shows that in most cases benzoate was apparently catabolized faster than acetate. *R. rubrum,* however catabolized benzoate less efficiently than acetate. The pattern of growth on acetate-vanillate showed that acetate utilization was initiated before the utilization of the aromatic compound vanillate (Fig. 2). However, as the vanillate catabolism almostly commenced, the rate of Bchl a synthesis reached the steady state (4–6 μg/ml) and accompanied by almost constant value in protein contents and turbidity. However, at the end of the experiment, substantial amounts of vanillate still remained in the medium ($\sim$ 0.75 mM) (Fig. 2). The above results suggest that benzoate degrading enzymes are present in vanillate grown cells of all cultures tested. Organotrophic anaerobes, e.g. *Acetobacterium woodii,* catabolize methoxylated aromatic compounds (syringate & vanillate) via demethoxylation, yielding free methanol which is then used as an acetogenic substrate (Bache & Pfennig 1981). This conclusion was also reached by Frazer & Young (1986) in experiments involving the utilization of a ^{14}C-labled methoxy substituent of vanillic acid under anaerobic conditions. If the above tested phototrophic bacteria, catabolize vanillate or syringate via demethoxylation, they may, in addition, utilize methanol as a photoheterotrophic growth substrate (Quayle & Pfennig 1975). Exogenously supplied methanol (up to 7 mM final concentration) had no inhibitory effect on the growth of the above tested species with benzoate or vanillate. Also, the presence of ribulose diphosphate carboxylase in the methanol-grown organisms at specific activity of fourth-fold that in the acetate grown organisms is consistent with this interpretation (unpublished results).

In conclusion it is clear that the purple nonsulfur bacteria *Rhodopseudomonas blastica* and *Rhodospirillum rubrum* were capable of photoheterotrophic (anaerobic) growth on certain benzoate derivatives. Further work on the metabolism of methoxylated aromatic compounds by these organisms is required to trace the biodegradable products at different cultural conditions.

Acknowledgements

The authors are deeply indebted to Prof. Dr. A.M. Hamad, Head of Botany Department, Faculty of Science, Assiut University for valuable discussion and reading the manuscript.

References

Bache R & Pfennig N (1981) Selective isolation of *Acetobacterium woodii* on methoxylated aromatic compounds and determination of growth yields. Arch. Microbiol. 130: 255–261

Berry DF, Francis AJ & Bollag JM (1987) Microbial metabolism of homocyclic and heterocyclic aromatic compounds under anaerobic conditions. Microbiol. Rev. 51: 43–59

Blasco R & Castillo F (1992) Light-dependent degradation of nitrophenols by the phototrophic bacterium *Rhodobacter capsulatus* EI F1. Appl. Environ. Microbiol. 58: 690–695

Boltz DF, Holland WJ & Howell JA (1978) Methods of determination. In: Colorimetric Determination. John Wiley & Sons, New York

Evans WG & Fuchs G (1988) Anaerobic degradation of aromatic compounds. Annu. Rev. Microbiol. 42: 289–317

Frazer AC & Young LY (1986) Anaerobic C_1 metabolism of the O-methyl-^{14}C-labeled substituent of vanillate. Appl. Environ. Microbiol. 51: 84–87

Harwood CS & Gibson J (1988) Anaerobic and aerobic metabolism of diverse aromatic compounds by the photosynthetic bacterium *Rhodopseudomonas palustris.* Appl. Environ. Microbiol. 54: 712–717

Imhoff JF & Trüper HG (1991) The genus *Rhodospirillum* and related genera. In: Balows A, Trüper HG, Dworkin M, Harder W & Schleifer KH (Eds) The Prokaryotes, Vol. III. 2nd edn. (pp 2141–2155) Springer-Verlag Inc., New York

Kamal VS & Wyndham RC (1990) Anaerobic phototrophic metabolism of 3-chlorobenzoate by *Rhodopseudomonas palustris* WS 17. Appl. Environ. Microbiol. 56: 3871–3873

Leahy JG & Colwell RR (1990) Microbial degradation of hydrocarbons in the environments. Microbiol. Rev. 54: 305–315

Liu S & Suflita JM (1993) H_2-Co_2-dependent anaerobic O-demethylation activity in subsurface sediments and by an isolated bacterium. Appl. Environ. Microbiol. 59: 1325–1331

Lowry OH, Rosebrough NI & Randall RS (1951) Protein measurement with the Folin phenol reagent. J. Biol. Chem. 193: 265–275

Madigan MT & Gest H (1979) Growth of the photosynthetic bacterium *Rhodopseudomonas capsulata* chemo autotrophically in darkness with H_2 as the energy source. J. Bacteriol. 137: 524–530

Pfennig N, Eimbjellen KE & Jensen SL (1965) A new isolate of the *Rhodospirillum fulvum* group and its photosynthetic pigments. Arch. Microbiol. 51: 258–266

Pfennig N (1978) *Rhodocyclus purpureus* gen. nov. and sp. nov., a ring-shaped, vitamin B_{12}-requiring member of the family Rhodospirillaceae. Int. J. Syst. Bacteriol. 28: 283–288

Quayle JR, Pfennig N (1975) Utilization of methanol by Rhodospirillaceae. Arch. Microbiol. 102: 193–198

Sarkanen KV & Ludwig CH (1971) Lignins: Occurrence, formation, structure and reactions. Wiley-Interscience, New York

Shoreit AAM, Ahmed AA & Shabeb MSA (1989) Field and laboratory studies on some photosynthetic bacteria in Aswan High Dam Lake II. Isolation and identification of purple nonsulfur bacteria. Int. Revue. Ges. Hydrobiol. 74: 579–583

Shoreit AAM, Abd-Alla MH & Shabeb MSA (1992) Acetylene reduction by Rhodospirillaceae from the Aswan High Dam Lake. World J. of Microbiol. and Biotechnol. 8: 151–154

Sleat R & Robinson JP (1984) The bacteriology of anaerobic degradation of aromatic compounds. J. Appl. Bacteriol. 57: 381–394

Smith JHC & Benitez A (1955) Chlorophylls: analysis in plant materials. In: Paechk & Tracey MV (Eds) Moderne Methoden der Pflanzen Analysis, Vol. IV. (pp 142–196) Springer-Verlag, Berlin

Tayeh MA & Madigan MT (1987) Malate dehydrogenase in phototrophic purple bacteria: purification, molecular weight, and quaternary structure. J. Bacteriol. 169: 4196–4202

Williams RJ & Evans WC (1975) The metabolism of benzoate by Moraxella spp. through anaerobic nitrate respiration. Biochem. J. 148: 1–10

Wright G & Madigan MT (1991) Photocatabolism of aromatic compounds by the phototrophic purple bacterium *Rhodomicrobium vannielii*. Appl. Environ. Microbiol. 57: 2069–2073