Biodegradation and Metabolism of Unusual Carbon Compounds by Anoxygenic Phototrophic Bacteria

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ABSTRACT

Anoxygenic phototrophic bacteria play an important role in anaerobic nutritional cycles. The most readily used and widely studied carbon sources for growth of these bacteria are organic acids and a few carbohydrates. In this review we survey the growing knowledge on the metabolism of a number of other carbon sources, particularly polymers (starch, poly(3-hydroxyalkanoates)), aromatic compounds (natural and xenobiotic), one-carbon compounds, alcohols, aliphatic hydrocarbons and higher fatty acids, and their influence on various cellular activities of purple non-sulfur bacteria. We also discuss the possible exploitations in various biotechnological processes of this group of microorganisms while metabolizing unusual carbon compounds.

ADVANCES IN MICROBIAL PHYSIOLOGY VOL 39
ISBN 0-12-027739-5

1. INTRODUCTION

Anoxygenic phototrophic bacteria (APB) are prokaryotic, unique photosynthetic microorganisms which lack photosystem II and thus carry out anoxygenic photosynthesis. They depend for photosynthetic electron donors on substrates of a lower redox potential (more reduced) than water, such as reduced sulfur compounds, elemental sulfur, molecular hydrogen or simple organic compounds. Organic compounds that can serve as photosynthetic electron donors include organic acids, simple sugars and sugar alcohols. The utilization of organic acids and simple sugars, and autotrophic carbon uptake by APB, have been studied extensively and reviewed (Tabita, 1994). However, with rapidly expanding knowledge about the capability of these bacteria to degrade many other organic compounds, such as polymers (starch, poly-β-hydroxyalkanoates), aromatic compounds (natural and xenobiotic), one-carbon compounds, alcohols, aliphatic hydrocarbons and higher fatty acids, there is need for a comprehensive review of the metabolic capabilities of this group of microorganisms.

1.1. Major Central Metabolic Pathways in Anoxygenic Phototrophic Bacteria

Organic acids are the preferred substrates for growth of APB and are assimilated by the citric acid cycle. In the absence of a nitrogen source (fixed nitrogen or N_2), these compounds are not assimilated for growth, but instead liberate H_2 and CO_2 which are formed via the anaerobic, light-dependent citric acid cycle. For utilization of simple sugars, APB use either the Embden–Meyerhof pathway or the Entner–Doudoroff pathway, depending on the substrate and growth conditions. Autotrophic

(both phototrophic and chemotrophic) carbon assimilation in APB is either by the pentose phosphate pathway, which is widespread among purple bacteria, or by the reductive tricarboxylic acid cycle, as observed in the green sulfur bacteria (Tabita, 1994).

2. METABOLISM OF AROMATIC COMPOUNDS

One of the major sources of combined carbon available in nature is the group of aromatic compounds. These are toxic environmental contaminants and pollutants, many of which are xenobiotic and recalcitrant. However, both synthetic and naturally occurring aromatic compounds can be catabolized by a few soil microorganisms under aerobic and anaerobic conditions and thus maintain the carbon cycle. Aromatic compounds are metabolized by microorganisms following two fundamentally different strategies. Under aerobic conditions, aromatic compounds are transformed by monooxygenases and dioxygenases into a few central intermediates such as catechol (Fig. 1), protocatechuate (Fig. 2) and gestisate (Lal *et al.*, 1995).

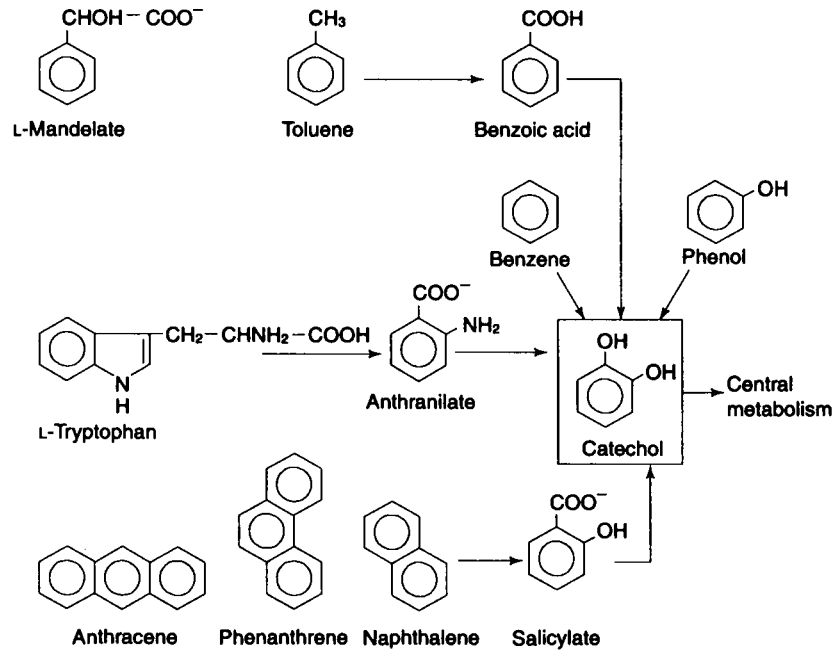

Figure 1 Channelling of different aromatic structures under aerobic conditions leading to the formation of catechol (after Cork and Krueger, 1991).

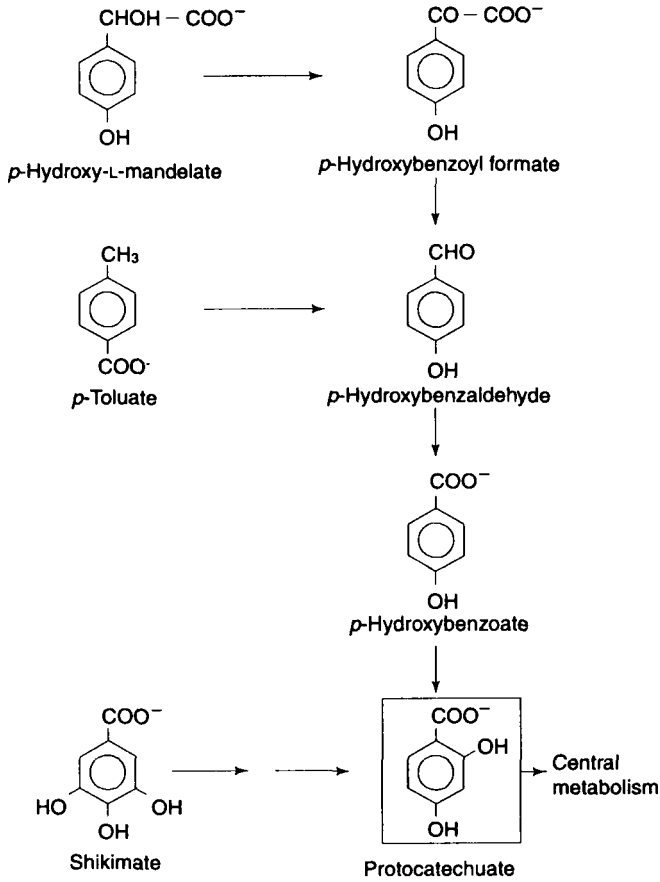

Figure 2 Channelling of different aromatic structures under aerobic conditions leading to the formation of protocatechuate (after Cork and Krueger, 1991).

These dihydroxylated compounds are suitable for oxidative cleavage of the aromatic ring. However, under anoxic (anaerobic) conditions, aromatic compounds have to be transformed by other means. It is now well established that numerous aromatic compounds of low molecular mass are degraded by different groups of anaerobic bacteria and the aromatic ring structures are reductively attacked, the important central aromatic intermediates being benzoyl CoA, resorcinol, phloroglucinol or 4-hydroxybenzoyl CoA (Fuchs *et al.*, 1994).

Catabolism of aromatic compounds by bacteria under anaerobic conditions can be categorized according to the energy-yielding process (Berry *et al.*, 1987; Fuchs et. al., 1994) as (i) photoreduction, (ii) denitrification, (iii) fermentation, (iv) sulfate

reduction, and (v) methanogenic fermentation. Anaerobic metabolism of aromatic compounds (which is a common phenomenon in flooded soils, sediments, landfills, lagoons, anaerobic fresh and ocean waters and ground waters) is restricted to a few anaerobic bacteria. Amongst these, APB function in the anaerobic disposal of aromatic hydrocarbons by photoreduction.

2.1. Anoxygenic Phototrophic Bacteria Capable of Catabolizing Aromatic Compounds

Anoxygenic phototrophic bacteria are separated into two cytologically different groups, the purple bacteria and the green bacteria. The purple bacteria are further separated into purple sulfur bacteria and purple non-sulfur bacteria (Imhoff, 1992). Among the known APB catabolizing aromatic compounds, all belong to the purple non-sulfur group, which may be due to their versatility to degrade many organic compounds.

The discovery of benzoate synthesis from phenylpropionate by a strain of purple non-sulfur bacterium *Rhodovibrio* (now *Rhodopseudomonas*) (Frank and Gaffron, 1941) opened up the field of photometabolism of aromatic compounds by APB. Later, Scher's experiments (Scher and Allen, 1960; Scher and Proctor, 1960) confirmed that *Rhodopseudomonas palustris* could grow using benzoate as sole carbon source. Today, we know that *Rhodopseudomonas palustris* (Dutton and Evans, 1967; Harwood and Gibson, 1988), *Rhodospirillum fulvum* (Pfennig *et al.*, 1965) and *Rhodocyclus purpureus* (Pfennig, 1978) metabolize benzoate, which differentiates these species taxonomically from others of the same genera. However, aromatic photocatabolism has also been reported in a few other species: *Rhodomicrobium vannielii* (Wright and Madigan, 1991); *Rhodocyclus gelatinosus* (Dutton and Evans, 1978) (now *Rubrivivax gelatinosus*, Willems *et al.*, 1991; Imhoff, 1995); *Rhodobacter capsulatus* (Blasco and Castillo, 1992); *Rhodopseudomonas acidophila* (Yamanaka *et al.*, 1983); and *Rhodopseudomonas blastica* (Ahmed and Mohamed, 1994). Many of the studies on the photometabolism of aromatic compounds are restricted to *Rhodopseudomonas palustris*, and this organism has served as a model for studies of anaerobic aromatic compound biodegradation (Perrotta and Harwood, 1994).

2.2. Catabolism of Aromatic Compounds for Growth by Purple Non-sulfur Bacteria

Though purple non-sulfur bacteria are known to metabolize aromatic compounds under both anaerobic (light) as well as aerobic (dark) conditions (Harwood and Gibson, 1988), photometabolism has received most attention. Benzoic acid is the most widely used aromatic substrate for growth by purple non-sulfur bacteria, and

trans-cinnamate was used as sole carbon source for the selective enrichment of *Rhodopseudomonas palustris* (Madigan and Gest, 1988). Apart from benzoate, methoxylated benzoates, vanillate, syringate (Harwood and Gibson, 1988; Wright and Madigan, 1991; Ahmed and Mohamed, 1994), phenol (Khanna *et al.*, 1992), amino- and nitro-substituted aromatic compounds such as aniline and nitrobenzene, (Sasikala *et al.*, 1994a; Blasco *et al.*, 1995), aromatic amines (Vijayalaxmi *et al.*, 1997), halogenated aromatics, (Geissler *et al.*, 1988; Kamal and Wyndham, 1990; van der Woude *et al.*, 1994), phenylalkane carboxylates (Madigan and Gest, 1988; Elder *et al.*, 1992a,b; Rahalkar *et al.*, 1993), hydroxybenzoate (Hegeman, 1967; Harwood and Gibson, 1988; Khanna *et al.*, 1992), and some of the intermediates in benzoate photometabolism, e.g. cyclohex-1-ene-1-carboxylate (Perrotta and Harwood, 1994) and cyclohexane carboxylate (Hutber and Ribbons, 1983), are known to support growth of *R. palustris*. Amino aromatic compounds – 4-aminobenzoate and 4-aminophenol – could serve as both carbon and nitrogen source for the growth of *R. palustris*, and such growth is inhibited in the presence of ammonia (Khanna *et al.*, 1992).

Apart from the photometabolism of homocyclic aromatic compounds, purple non-sulfur bacteria are also known to metabolize a few heterocyclic aromatic compounds. Purines (Goodwin and Passon, 1961; Aretz *et al.*, 1978; Busse *et al.*, 1984; Kaspari and Werner, 1986), pyrimidines (Goodwin and Passon, 1961; Kaspari, 1979), pyridine derivatives and pyrazines (Sasikala *et al.*, 1994b) and methylbenzimidazol-2-ylcarbam (carbendazim, a synthetic fungicide) (Rajkumar and Lalithakumari, 1992), are the only heterocyclic aromatic compounds known to be photometabolized for growth as either sole nitrogen or carbon source. Although *R. palustris* could not photometabolize thiophene-2-carboxylate, it could transform this compound to (+)-3-hydroxytetrahydrothiophene-2-carboxylate and tetrahydrothiophene-2-carboxylate (Tanaka *et al.*, 1982).

2.3. Channelling Reactions to Benzoyl-coenzyme A

Two major routes were proposed by Harwood and Gibson (1988) for the photobiodegradation of diverse homocyclic aromatic compounds: one via benzoate, and the other via 4-hydroxybenzoate prior to cleavage of the aromatic ring (Fig. 3). During the anaerobic photodegradation of *trans*-cinnamate and ω-phenylalkanecarboxylates by *Rhodopseudomonas palustris*, the shortening of aliphatic side-chains proceeds through β-oxidation (Elder *et al.*, 1992a). Benzoate was the final product during the β-oxidation of 3-phenylpropionate, 5-phenylvalerate and 7-phenylheptanoate. Phenylacetate accumulated and persisted in the culture medium during growth on 4-phenylbutyrate, 6-phenylhexanoate, and 8-phenyloctanoate (Elder *et al.*, 1992a).

With most work on the photometabolism of aromatic compounds being

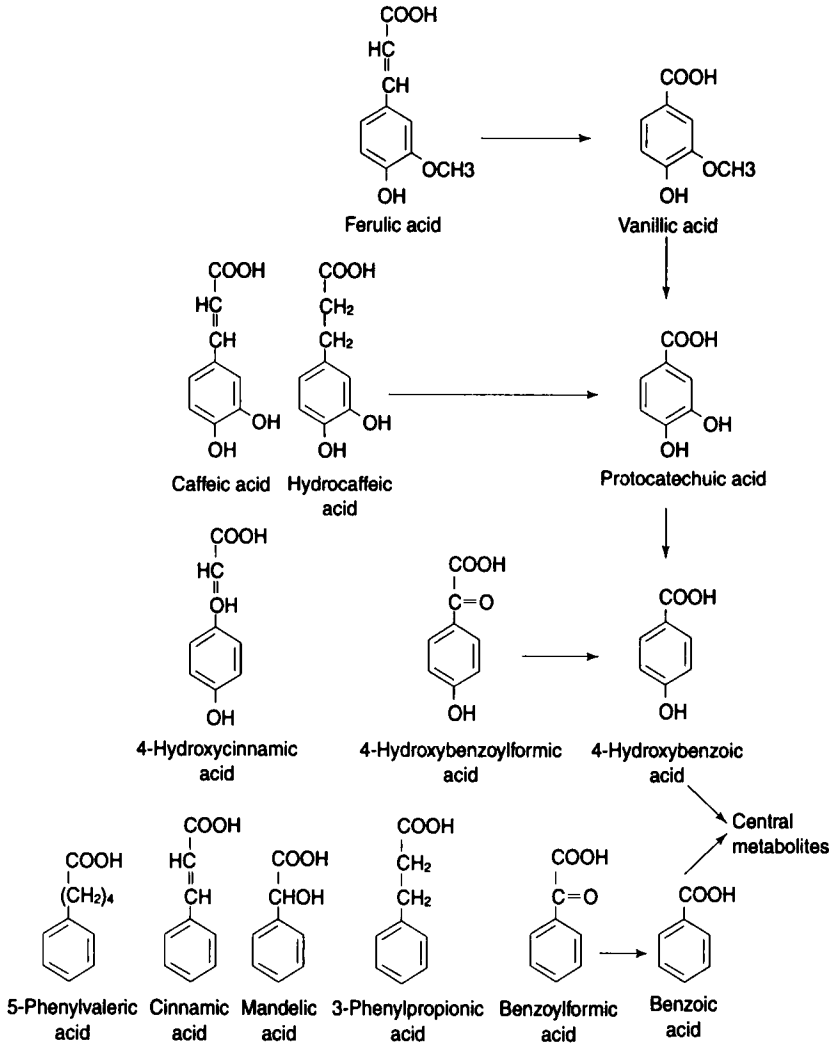

Figure 3 Channelling of different aromatic structures under photoanaerobic conditions leading to the formation of 4-hydroxybenzoate and benzoate by *Rhodopseudomonas palustris* (after Harwood and Gibson, 1988; Elder *et al.*, 1992a; Rahalkar *et al.*, 1993).

restricted to their utilization for growth, little had been done on the channelling of different aromatic structures during aerobic or anaerobic metabolism to central aromatic intermediates like benzoate in this group of microorganisms. In general, anaerobic breakdown of different aromatic structures in various bacteria leads to the formation of central aromatic intermediates such as benzoyl-CoA, resorcinol

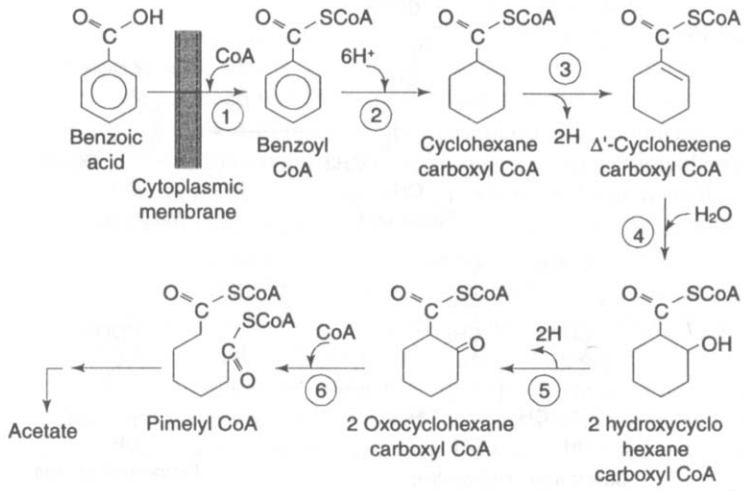

Figure 4 Reductive pathway for the photocatabolism of benzoate by *Rhodopseudomonas palustris* (after Dutton and Evans, 1970). Complete mineralization of benzoate results in the formation of CO_2 and H_2O.

and phloroglucinol, by β-oxidation of C_6–C_3 compounds, decarboxylation/carboxylation reactions, *O*-methylation, α-oxidation, oxidative decarboxylation, aromatic alcohol and aldehyde dehydrogenation, reductive dehalogenation, reductive deamination, nitro group reduction, and many other reactions. These are discussed in detail by Fuchs *et al.* (1994).

2.4. Photoanaerobic Ring Reduction and Cleavage

Catabolism of the homocyclic aromatic ring during anaerobic photocatabolism of benzoate in *Rhodopseudomonas palustris* (Fig. 4) was studied by incubating unlabelled test intermediates with whole cell suspensions actively photometabolizing C^{14}-labelled benzoate (Dutton and Evans, 1968a; 1969). However, confirmation of the reductive pathway was achieved by isolating mutant strains of *R. palustris* and testing their ability to grow on the proposed intermediates (benzoate, cyclohexanecarboxylate, cyclohex-1-ene carboxylate, 2-hydroxycyclohexane carboxylate, pimelate and acetate) in the light (Evans and Fuchs, 1988; Elder and Kelly, 1994).

The first step in the anaerobic pathway of benzoate degradation by *R. palustris* is catalysed by benzoyl-CoA ligase. The factors influencing the synthesis of this enzyme were studied with the help of polyclonal antiserum which was used in

immunoblot assays (Kim and Harwood, 1991). This enzyme was synthesized when *R. palustris* cells were grown with benzoate, as well as with hydroxyl- and methyl-substituted benzoates as observed with the chemotrophic bacterium *Pseudomonas* sp. (Altenschmidt *et al.*, 1991). The diversity of inducers of benzoyl-CoA ligase was consistent with the observation that benzoate is a central intermediate in the degradation of a variety of aromatic acids with more complex structures (Kim and Harwood, 1991; Egland *et al.*, 1995).

Benzoyl-CoA ligase purified from *R. palustris* has a molecular mass of 60 kDa, is oxygen-labile and catalyses Mg^{2+}, ATP-dependent formation of acyl-CoA from carboxylate and free reduced CoA. The enzyme has high specificity for benzoate and 2-fluorobenzoate (Geissler *et al.*, 1988). Further, only carboxylates with a close resemblance to benzoate were active in the ligase reaction (Kim and Harwood, 1991. The most effective analogue was 2-fluorobenzoate, the reaction rate being similar to that of benzoate. Substituents at the *ortho* position of the aromatic ring reduced rates more than substituents present at the *meta* or *para* position (Geissler *et al.*, 1988). Benzoate thioesterification occurs in the cytoplasmic compartment of the cell and follows, rather than accompanies, passage of the substrate across the cytoplasmic membrane (Fig. 4).

Apart from benzoyl-CoA ligase, *R. palustris* has two other enzymes, 4-hydroxybenzoate-CoA ligase (Gibson *et al.*, 1994) and cyclohexane carboxylate-CoA ligase (Kuever *et al.*, 1995). Gibson and his co-workers (Gibson *et al.*, 1994) isolated 4-hydroxybenzoate-CoA ligase from *R. palustris* when cells were incubated in the presence of 4-hydroxybenzoate. This enzyme was purified, and the genes were isolated and sequenced. Growth on cyclohexane carboxylate by *R. palustris* occurred with doubling times of about 7–8 h, and a CoA ligase was purified that thioesterified cyclohexane carboxylate but had little or no activity on benzoyl-CoA or 4-hydroxybenzoyl-CoA (Kuever *et al.*, 1995). Two cyclic dienes, cyclohexa-2,5-diene-1-carboxylate and a 1,4-diene isomer, were proposed as early intermediates in the anaerobic photometabolism of benzoate by *R. palustris* (Gibson and Gibson, 1992).

2.5 Photometabolism of Heterocyclic Aromatic Compounds

Not many heterocyclic aromatic compounds are known to be photometabolized by purple non-sulfur bacteria. Photometabolism of purines (Busse *et al.*, 1984) and pyrimidines (Kaspari, 1979) occurs by pathways similar to those of chemotrophs (Vogels and van der Drift, 1976; Berry *et al.*, 1987). Though a number of pyrazines are known to be converted to pyrazinoic acid during their assimilation (Fig. 5) (Sasikala *et al.*, 1994b), their catabolism has not been studied either in purple bacteria or in other microorganisms, though many of these compounds are known to have antimicrobial properties.

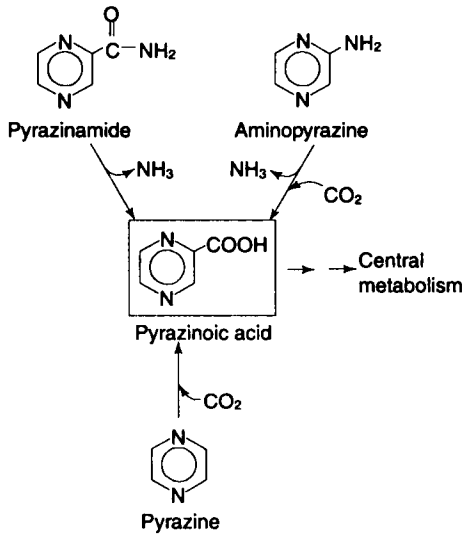

Figure 5 Channelling of different pyrazines to the formation of pyrazinoic acid during the photometabolism by *Rhodopseudomonas palustris* OU11 (after Sasikala *et al.*, 1994b).

2.6 Biotransformation of Aromatic Compounds by Purple Non-sulfur Bacteria

Among purple non-sulfur bacteria, most of the work on aromatic compound metabolism is restricted to *Rhodopseudomonas palustris*, and little information is available on aromatic compound metabolism by bacteria that cannot use aromatic structures for growth. Cell-free extracts of *Rhodocyclus gelatinosus* transformed phloroglucinol (1,3,5-trihydroxybenzoate) to dihydrophloroglucinol in the presence of cystine, NADPH and EDTA (Whittle *et al.*, 1976), while *Rhodobacter capsulatus* could photobiotransform 2,4-dinitrophenol to 2-amino-4-nitrophenol (Blasco and Castillo, 1992).

Rhodobacter sphaeroides is an important purple non-sulfur bacterium with proven biotechnological applications. It is the most abundant purple bacterium in paddy-field soils (Sasikala and Ramana, 1995b) and waste waters (Sasikala *et al.*, 1995) but lacks the ability to photometabolize aromatic compounds for growth. Aromatic photobiotransformation studies using this organism have revealed that benzoate is not the final product of photometabolism of diverse aromatic compounds (Nanda, 1996). This indicates that not only the benzoyl-CoA ligase, but also the reactions leading to the formation of benzoic acid themselves, are absent in this organism. The two major end-products of photobiotransformation of aromatic compounds by *R. sphaeroides* OU5 are salicylate and nitrobenzene (Nanda, 1996).

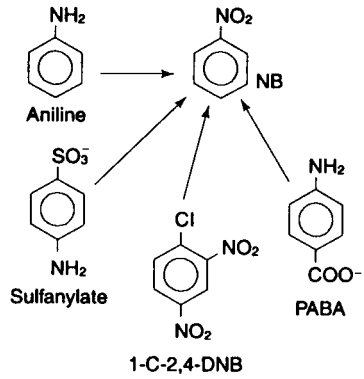

Figure 6 Formation of nitrobenzene as an end-product of photobiotransformation of some of the aromatic compounds with nitrogen substitutes by *Rhodobacter sphaeroides* OU5 (Nanda, 1996). NB, nitrobenzene; PABA, *p*-aminobenzoic acid; 1-C-2,4-DNB, 1-chloro-2,4-dinitrobenzene.

While aromatic compounds like phenylalanine and 4-hydroxybenzoate are transformed to salicylate, all aromatic compounds with N-containing functional groups are converted to nitrobenzene (Fig. 6). A process of aromatic nitrification (for aminobenzenes) may also occur in this group of microorganisms (Nanda, 1996), as observed earlier with a strain of *Nitrosomonas europaea* (Keener and Arp, 1994).

Studies on the photobiotransformation of aromatic compounds by *R. sphaeroides* OU5 suggest the capability of the organism for biotransformation of anthranilate to indole (Fig. 7a) and the formation of tryptophan from indole (Fig. 7b) either by tryptophanase or by tryptophan synthase as observed in many bacteria (Laszlo *et al.*, 1983).

2.7. Excretion of Aromatic Compounds by Purple Non-sulfur Bacteria

Apart from photobiodegradation and transformations of aromatic compounds, purple non-sulfur bacteria are also known for their ability to synthesize and excrete several aromatic compounds including ubiquinone, hopanoids and porphobilinogen. *Rhodospirillum rubrum*, grown with an excess of malate and with nitrogen deficiency, excretes considerable amounts of *n*-oxybenzaldehyde, *n*-oxyphenylacetic acid and *n*-oxybenzoic acid (Iwasaki *et al.*, 1962). In addition, APB can also excrete or accumulate under certain nutritional conditions large quantities of tetrapyrroles (Tanaka *et al.*, 1993), ubiquinones (Uragami and Yoshida, 1986a,b), nucleic acids (Kobayashi, 1977), corrinoids (John, 1968), hopanoids (Nagumo *et*

al., 1991) and brassinosteroids (Yamaji *et al.*, 1994), which are of commercial importance.

Ubiquinones ('coenzymes Q') are a family of 5,6-dimethoxytoluquinones with polyisoprenoid side-chains of various lengths at the 6-position of the benzene ring. Five naturally occurring ubiquinones have been detected, i.e. Q-6, Q-7, Q-8, Q-9, Q-10, where the number refers to the number of isoprene units in the side-chain. These compounds, which are widely distributed in bacteria, play an important role in respiratory or photosynthetic electron transport in bacterial cytoplasmic membranes and have been extensively studied, not only from a biochemical and taxonomical point of view, but because ubiquinone is effective in medicine (Sasikala and Ramana, 1995a).

The quinone composition of different species of anoxygenic phototrophic bacteria is shown in Table 1. *Rhodopseudomonas viridis* (Korsunskii, 1987), *Rhodobacter sulfidophilus* (Uragami and Yoshida, 1986a), *Rhodobacter sphaeroides* (Kyowa Hakko Kogyo Co. Ltd, 1982; Kyowa, 1983; Uragami and Yoshida, 1986b), *Rhodocyclus gelatinosus* (Sasaki and Nagai, 1979), *Rhodobacter capsulatus* (Mochida *et al.*, 1973) and *Rhodospirillum rubrum* (Roeper, 1985) are some of the anoxygenic phototrophic bacteria which can produce Q_{10} in large quantities. Rhodoquinone (RQ), a derivative of ubiquinone in which one of the methoxyl groups is replaced by an amino group, plays a significant role in the modern chemotaxonomic classification of this group of microorganisms (Hiraishi and Hoshino, 1984), along with ubiquinones (Imhoff, 1995; Hiraishi *et al.*, 1984).

Table 1 Summary of quinone systems in purple non-sulfur bacteria (Sasikala and Ramana, 1995a).

Species	Presence of			Major homologues
	Q	RQ	MK	
Purple non-sulfur bacteria				
Rhodospirillum				
R. rubrum	+	+	–	10
R. photometricum	+	+	–	8
R. fulvum	+	–	+	9
R. molischianum	+	–	+	9
R. salexigens	+	–	+	10
R. salinarum	+	–	+	10
Rhodobacter				
R. capsulatus	+	–	–	10
R. sphaeroides	+	–	–	10
R. sulfidophilus	+	–	–	10
R. adriaticus	+	–	–	10
R. veldkampii	+	–	–	10
Rhodopseudomonas				
R. palustris	+	–	–	10
R. rutila	+	–	–	10

Table 1 *continued*

Species	Presence of Q	RQ	MK	Major homologues
R. blastica	+	–	–	10
R. acidophila	+	+	+	10
R. viridis	+	–	+	9
R. marina	+	–	+	10
R. sulfoviridus	+	–	+	8 +10
Rhodomicrobium				
R. vannielli	+	+	–	10
Rhodopila				
R. globiformis	+	+	+	9 + 10
Rhodocyclus				
R. purpureus	+	–	+	8
R. tenuis	+	–	+	8
R. gelatinosus	+	–	+	8
Rhodoferax				
R. fermentans	+	+	–	8
Purple sulfur bacteria				
Chromatium sp.	+	–	+	7
C. vinosum	+	–	+	8
C. warmingii	+	–	+	8
Thiocapsa roseopersicina	+	–	+	8
Thiocystis gelatinosa	+	–	+	8
Ectothiorhodospira				
E. mobilis	+	–	+	7
E. shaposhnikovii	+	–	+	7
E. vacuolata	+	–	+	7
E. halophila	+	–	+	8
E. halochloris	+	–		8
E. abdelmalekii	+	–	+	8
Green sulfur bacteria				
Chlorobium				
C. thiosulfatophilum	+	–	+	Different types
C. limicola	–	–	+	7
Filamentous green bacteria				
Chloroflexus				
C. aurantiacus	–	–	+	10
Heliobacteriaceae				
Heliobacterium				
H. chlorum	–	–	+	7–10

Key: +, present; –, absent. MK, menaquinone; Q, ubiquinone; RQ, rhodoquinone.

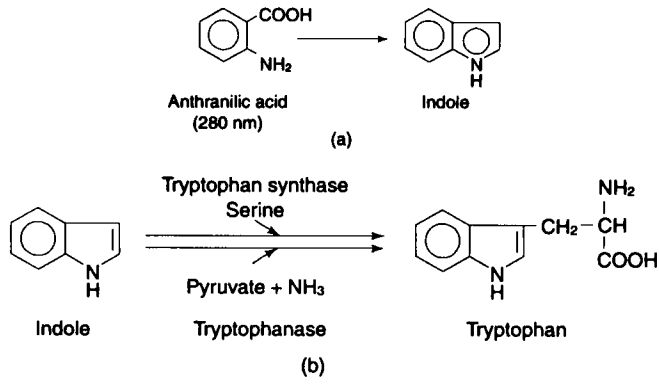

Figure 7 (a) Photobiotransformation of anthranilic acid to indole by *Rhodobacter sphaeroides* OU5 in the presence of organic acids. (b) Photobiotransformation of indole to tryptophan by *Rhodobacter sphaeroides* OU5 (authors' unpublished results).

Anoxygenic phototrophic bacteria are also capable of producing phytohormones involved in the regulation of photosynthesis (Serdyuk *et al.*, 1993). Hormones isolated from *Rhodospirillum rubrum* had high physiological activity in the cytokinin bioassay. Three cytokinins were isolated which were adenine derivatives, and one of them was identified as 6-(4-hydroxy-3-methyl-2-*trans*-2-butenyl-amino)-9-β-D-ribofuranosylpurine (zeatinriboside) (Serdyuk *et al.*, 1993). In a patented process, kinetin (4.7 $g\,l^{-1}$) and zeatin (2 $g\,l^{-1}$) were isolated from *Rhodobacter sphaeroides* from organic waste as growth substrates (Kuroda, 1990). Auxins like indole-3-acetic acid (IAA) and indole-3-butyric acid (IBA) were also produced by *R. sphaeroides* IFO 12203 (Yokoyama, 1990); however, the auxins may not be synthesized by this organism, and the process may be a simple biotransformation of indole as shown with *R. sphaeroides* OU5 (discussed above).

2.8. Factors Regulating Biodegradation of Aromatic Compounds

2.8.1. *Concentration of the Aromatic Compounds*

Little is known of the effects of the concentrations of different aromatic compounds on growth of purple non-sulfur bacteria, most of the aromatic compounds being tested only at low concentrations (1–3 mM) (Harwood and Gibson, 1988; Kamal and Wyndham, 1990; Elder *et al.*, 1992a; Khanna *et al.*, 1992; Sasikala *et al.*, 1994 a,b). Cinnamate could be tolerated up to 1–6 mM by *Rhodopseudomonas palustris*

(Madigan and Gest, 1988), while benzoate concentrations of above 5 mM completely inhibited growth of *Rhodomicrobium vannielii* (Wright and Madigan, 1991). Phenol and 4-aminophenol concentrations above 1 mM were inhibitory for *Rhodopseudomonas palustris* (Khanna *et al.*, 1992). Growth-inhibitory concentrations determined for various aromatic compounds for *R. palustris* are: benzoate, 8.9 mM; *p*-hydroxybenzoate, 8.5 mM; cinnamate, 9 mM; *p*-coumarate, 7.7 mM; phenylacetate, 9.9 mM; and cinnamyl alcohol, 2.4 mM (Rahalkar *et al.*, 1993).

2.8.2. *Co-metabolism of Aromatic Compounds*

An interesting phenomenon observed during the photocatabolism of mono- and dinitrophenols by *Rhodobacter capsulatus* (Blasco and Castillo, 1992) and 3-chlorobenzoate by *Rhodopseudomonas palustris* (Kamal and Wyndham, 1990) was the obligate requirement for acetate and benzoate respectively. These results indicate a requirement for reducing equivalents (Dolfing and Tiedje, 1991) for aromatic ring reduction, which greatly depends on the nature of the aromatic compound and/or the organism photometabolizing such compounds.

2.8.3. *Position of the Substitutions*

Aromatic compounds not commonly photometabolized by many strains of *Rhodopseudomonas palustris* are the benzenoid compounds substituted at the *ortho* position (Table 2). It still remains unclear why *ortho*-substituted benzenoid compounds cannot be photometabolized by *R. palustris*, although they are metabolized anaerobically by many other bacteria (Fuchs *et al.*, 1994; Elder and Kelly, 1994). Reduction in the rates of benzoyl-CoA formation in *R. palustris* occurred with *ortho*-substituted aromatic compounds, rather than those having *para* or *meta* substitutions (Geissler *et al.*, 1988). This may contribute to the inability to metabolize *ortho*-substituted aromatic compounds.

2.8.4. *Effect of Fatty Acids and Alcohols*

Photometabolism of aromatic acids by *Rhodopseudomonas palustris* was sensitive to the presence of exogenous fatty acids (Dutton and Evans, 1968b, 1970). This was explained as due to competition for enzymes or cofactors involved in the photometabolic pathway. Acetate, malonate or crotonate showed no inhibition of benzoate photoassimilation; monocarboxylic acids such as butyrate and propionate were effective inhibitors. Addition of aliphatic alcohols (ethanol, methanol) (Tanaka *et al.*, 1982) and carboxylates (acetate) (Blasco and Castillo, 1992) stimulated the reduction of aromatic substrates by *Rhodobacter capsulatus* E1F1. However, carboxylates such as lactate and succinate inhibited the reduction of thiophene-2-carboxylate (Tanaka *et al.*, 1982).

Table 2 Aromatic compounds that can and cannot be photometabolized by *Rhodopseudomonas palustris* and other purple non-sulfur bacteria.

Compounds utilized	Compounds not utilized
Benzoic acid	2-Amino, 4-nitrophenol
3-Chlorobenzoate	2-Fluorobenzoate
3,4-Dimethylbenzoate	2-Hydroxybenzoate (salicylate)
3-Hydroxybenzoate	2,4-Dimethylphenol
3-Methoxybenzoate	Phthalic acid
p-Nitrophenol	
syringate	

See Harwood and Gibson (1988), Kamal and Wyndham (1990), Wright and Madigan (1991), Khanna *et al.* (1992), Blasco and Castillo (1992) and Sasikala *et al.* (1994a).

2.8.5. *Carbon Dioxide Requirement*

Carbon dioxide, provided as bicarbonate, presumably acts as a redox sink for the disposal of excess reducing equivalents during growth of *Rhodopseudomonas palustris* with ω-phenylalkane carboxylates with chain lengths greater than that of phenylacetate (Elder *et al.*, 1992a). While growth on benzoate and a few other aromatic compounds (Khanna *et al.*, 1992; Sasikala *et al.*, 1994a) did not require exogenous carbon dioxide, its stimulation of the photometabolism of cinnamate by *R. palustris* (Madigan and Gest, 1988) was evident. Carbon dioxide is also essential for those aromatic structures (e.g. aniline, pyrazine) which are carboxylated before ring cleavage.

2.8.6. *Aerobic and Anaerobic Conditions*

Harwood and Gibson (1988) indicated that separate metabolic pathways mediate aerobic and anaerobic breakdown of diverse aromatics in *Rhodopseudomonas palustris*. Some compounds (benzaldehyde, benzoate, benzoylformate, 4-hydroxybenzoate and DL-mandelate) supported anaerobic but not aerobic growth, while others (homogentisate, isovanillate, phenylacetate, protocatechuate and vanillate) supported aerobic but not anaerobic growth.

3. PHOTOMETABOLISM OF ALCOHOLS

Utilization of alcohols is not a widespread property among APB. Alcohols (methanol, ethanol, *n*-propanol and *n*-butanol) are assimilated by an NAD-linked alcohol dehydrogenase in a few purple bacteria (Quayle and Pfennig, 1975; Siefert and Pfennig, 1979; Fujii *et al.*, 1982, 1983, 1987; Hirayama *et al.*, 1986). Growth

on alcohols was optimum at higher pH (Tabita, 1994) and dependent on the simultaneous presence of bicarbonate (Quayle and Pfennig, 1975; Siefert and Pfennig, 1979; Fujii *et al.*, 1982), which functions as an electron acceptor. *Rhodopseudomonas acidophila* oxidizes methanol to CO_2 with methanol dehydrogenase and used the reducing equivalents for CO_2 fixation via the ribulose bisphosphate cycle (Sahm *et al.*, 1976). The methanol dehydrogenase of purple bacteria resembles numerous previously reported methanol dehydrogenases from methylotrophic organisms (Bamforth and Quayle, 1978).

4. PHOTOMETABOLISM OF FORMATE AND CARBON MONOXIDE

Growth of purple non-sulfur bacteria on one-carbon compounds such as formate or carbon monoxide (CO) is exceedingly slow and not many species are known to use these compounds for growth. An inducible formate hydrogen lyase (catalysing the formation of CO_2 and H_2) is documented among a few purple bacteria (Qadri and Hoare, 1968; Voelskow and Schoen, 1978). Formate hydrogen lyase isolated from purple bacteria appears to have properties similar to that from heterotrophic bacteria (Qadri and Hoare, 1968). The hydrogen and carbon dioxide formed are then assimilated autotrophically. The presence of hydrogenase along with formate hydrogen lyase is a prerequisite for autotrophic growth on formate. Apart from the autotrophic assimilation of formate, heterotrophic (direct) assimilation of formate via the serine pathway, as found in *Pseudomonas* (Large and Quayle, 1963), must also be present in purple bacteria. This is evident from a strain of *Rhodobacter sphaeroides* OU 001 which was found capable of growth with formate as the sole carbon source (Sasikala, 1990) with apparently no capability for autotrophic growth (Sasikala and Ramana, 1990).

Utilization of carbon monoxide by purple bacteria was accidentally discovered by Hirsch (1968) during a study on CO-oxidizing bacteria. *Rhodopseudomonas* sp. (Hirsch, 1968), *Rhodocyclus gelatinosus* (Uffen, 1976, 1981, 1983; Dashekvicz and Uffen, 1979; Wakim and Uffen, 1983; Champine and Uffen, 1987) and *Rhodospirillum rubrum* (Bonam *et al.*, 1989) are some of the purple bacteria known so far to assimilate CO.

Assimilation of CO can occur under both anaerobic light (Uffen, 1983) or aerobic dark (Kerby *et al.*, 1995) conditions. Carbon monoxide dehydrogenase, an enzyme responsible for CO oxidation in purple bacteria (Bonam *et al.*, 1984, 1988, 1989; Bonam and Ludden, 1987) resembles that of the homoacetate-fermenting bacteria (Diekert *et al.*, 1979; Drake *et al.*, 1980; Ragsdale *et al.*, 1983). This enzyme is nickel-dependent (Diekert *et al.*, 1979; Ragsdale *et al.*, 1983; Bonam and Ludden, 1987; Ensign *et al.*, 1989a), oxygen-labile (Bonam *et al.*, 1984),

heat-labile (Ensign and Ludden, 1991), has optimum activity at pH 8.4 (Ragsdale *et al.*, 1983) and is inhibited by cyanide (Ensign *et al.*, 1989b). Carbon monoxide induces two enzymatic activities in *Rhodospirillum rubrum*, CO-dehydrogenase and a CO-insensitive hydrogenase, which appear to function together to accomplish the oxidation of CO to CO_2 and H_2, which are then assimilated autotrophically.

The genes coding for carbon monoxide assimilation have been extensively studied. It was found that CO stimulates the expression of the *coo* regulon (Shelver *et al.*, 1995), which consists of at least two transcriptional units in *R. rubrum*. The products of this regulon are a carbon monoxide dehydrogenase (CooS), an Fe–S protein (CooF) and a hydrogenase (CooH) (He *et al.*, 1996). The first two proteins (Bonam *et al.*, 1988, 1989; Ensign *et al.*, 1989a,b; Ensign and Ludden, 1991), and a CO-insensitive hydrogenase have been purified and characterized (Fox *et al.*, 1996).

When grown on producer gas (CO plus H_2), a *Rhodopseudomonas* sp. can accumulate a storage polymer, poly(3-hydroxyalkanoate) (PHA), at up to 18% of cell mass (Maness and Weaver, 1994). The product is a copolymer (poly(3-hydroxybutyrate-co-3-hydroxyvalerate) with a composition of 70% 3-hydroxybutyrate (3HB) and 30% 3-hydroxyvalerate (3HV).

5. METABOLISM OF HIGHER FATTY ACIDS, ALIPHATIC HYDROCARBONS AND ACCUMULATION OF POLY(HYDROXYALKANOATES)

Utilization of higher fatty acids is not restricted to one or two strains but is a common phenomenon among many purple non-sulfur bacteria. Out of 31 strains of purple non-sulfur bacteria tested for their ability to use *n*-fatty acids with chain lengths of 5–18 carbon atoms, 27 strains were able to utilize the entire range (Janssen and Harfoot, 1987). For growth on long-chain fatty acids, the medium also contained 0.005% (v/v) Tween-80 as an emulsifying agent. The ability to utilize particular fatty acids is species-specific; *Rhodocyclus gelatinosus* and *R. tenuis* were able to grow on C_5 to C_{18} fatty acids, while *R. gelatinosus* showed some restricted ability to use C_{20} (eicosanoate) and C_{22} (docosanoate) fatty acids (Janssen and Harfoot, 1985). *Rhodocyclus purpureus* has a limited ability to use *n*-fatty acids of chain length longer than caprylate (octanoate) (Pfennig, 1978; Harfoot and Janssen, 1985).

A concentration of 0.4 g l^{-1} of cecanoate (C_{10}) was growth-inhibitory, and optical densities at 680 nm (growth yields) as high as 1.0 to 1.4 were measured at lower concentrations (Janssen and Harfoot, 1987). *Rhodobacter sphaeroides* (Haruki *et al.*, 1971) and *R. capsulatus* (Mochida *et al.*, 1973) could grow on *n*-tridecane or *n*-tetradecane, and genetically altered *R. sphaeroides* could utilize C_{12}–C_{16} *n*-alkanes (Lee *et al.*, 1993).

With high concentrations of fatty acids (150 mM) APB are known to accumulate polymers, poly(β-hydroxyalkanoates) (PHAs), which are found as inclusion bodies. Poly(hydroxyalkanoates) are a group of biopolymers of commercial importance as biodegradable plastics (Anderson and Dawes, 1990; Brandl *et al.*, 1990; Poirier *et al.*, 1995; Sasikala and Ramana, 1996). Anoxygenic phototrophic bacteria can accumulate PHAs in large quantities (Table 3), and produce unusual polymers other than poly(β-hydroxybutyric acid) when grown on some of the fatty acids. These bacteria can accumulate three different types of PHAs: (i) homopolyesters, where the monomers are 3-hydroxyalkanoates (3HA) of various chain lengths (Liebergesell *et al.*, 1991; Ulmer *et al.*, 1994); (ii) copolymer P(3HB-co-3HV), where the monomers are 3HB and 3HV (Brandl *et al.*, 1989); and (iii) terpolyester P(3HB-co-3HP-co-3HV), where the monomers are 3HB, 3HP (3-hydroxypentanoate) and 3HV (Ballistreri *et al.*, 1995).

When grown on unusual carbon sources, particularly the higher fatty acids, *n*-alkanes and 1-alkenes, many bacteria are known to incorporate longer chain lengths into the polyester (Sasikala and Ramana, 1996). Chain lengths of 4–7 carbon atoms are known in the purple non-sulfur bacteria *Rhodospirillum rubrum* (Brandl *et al.*, 1989) and *Rhodobacter sphaeroides* (Brandl *et al.*, 1991), while longer carbon lengths (4 to 12) were found in the chemotrophic bacteria *Pseudomonas oleovorans* (Lageveen *et al.*, 1988) and C-8 in *Bacillus megaterium* (Findlay and White, 1983).

When *Rhodospirillum rubrum* was grown on various *n*-alkanoic acids as carbon source, the PHA formed contained up to 30% (mol %) of units with vinyl pendant group (3-hydroxy-4-pentanoate), along with 3HB (11%) and 3HV (59%) was incorporated (Ulmer *et al.*, 1994). Longer growth times and lower cell and polymer yields obtained with *R. rubrum* grown on higher fatty acids, when compared with other bacteria, may make this bacterium a poor candidate for the production of large amounts of PHAs from higher fatty acids (Ulmer *et al.*, 1994).

In most of the organisms so far investigated, poly(β-hydroxybutyrate) (PHB) is synthesized from acetyl-CoA by a sequence of three reactions catalysed by 3-ketothiolase (acetyl-CoA acetyltransferase, EC 2.3.1.9), acetoacetyl-CoA reductase (hydroxybutyryl-CoA dehydrogenase, EC 1.1.1.36) and PHB synthase. However, purple non-sulfur bacteria (and the purple sulfur bacterium *Chromatium vinosum*; Liebergesell and Steinbuchel, 1992) differ from other bacteria in which two stereospecific enol-CoA hydratases are also involved (Sasikala and Ramana, 1996). The enol-CoA hydratases catalyse the conversion of L-(+)-3-hydroxybutyryl-CoA via crotonyl-CoA to D-(–)-3-hydroxybutyryl-CoA which is polymerized to yield PHB. The overall pathway which leads to PHB synthesis from acetate by *Rhodospirillum rubrum* can be represented as follows:

acetate ⟶ acetyl-CoA ⟶ acetoacetyl-CoA ⟶ L-(+)-β-hydroxybutyryl-CoA ⟶ crotonyl-CoA ⟶ D-(–)-β-hydroxybutyryl-CoA ⟶ poly-β-hydroxybutyrate

Table 3 Poly(β-hydroxyalkanoate) content of anoxygenic phototrophic bacteria grown with different carbon sources.

Organism	Carbon source	PHA content (% of cellular dry wt)	Composition (%)	
			3HB	3HV
Purple non-sulfur bacteria				
Rhodobacter				
R. capsulatus	Acetate	33.7	97.0	2.1
R. sphaeroides	Acetate	69.9	92.7	7.3
Rhodopseudomonas				
R. palustris	Acetate	15.2	100	0.0
R. acidophila	Valerate	51.8	8.9	91.1
R. blastica	Acetate	31.5	96.0	4.0
R. viridis	Acetate	2.0	100	0.0
Rhodospirillum				
R. fulvum	Acetate	37.2	100	0.0
R. molischianum	Propionate	16.5	100	0.0
R. rubrum	Acetate	41.2	95.8	4.2
R. centenum	Butyrate	30.0		
Rhodomicrobium vannielii	Valerate	12.2	32.4	67.6
Rhodocyclus				
R. gelatinosus	Acetate	34.1	97.4	2.6
R. tenuis	Valerate	22.1	29.4	70.6
Purple sulfur bacteria				
Chromatium				
C. vinosum	Acetate	58.0	100	0.0
C. minutissimum	Acetate	36.0	100	0.0
C. purpuratum	Acetate	8.9	100	0.0
C. okenii	Acetate	12.8	100	0.0
C. warmingii	Acetate	22.9	100	0.0
Lamprocystis roseoper-sicina	Acetate	27.1	100	0.0
Thiocapsa pfennigii	Acetate	36.2	100	0.0
Amoebobacter				
A. roseus	Acetate	32.6	100	0.0
A. pendens	Acetate	30.8	100	0.0
Thiocystis violacea	Acetate	83.0	100	0.0
Ectothiorhodospira				
E. mobilis	Acetate	57.5	100	0.0
E. vacuolata	Acetate	35.6	100	0.0
E. shaposhnikovii	Acetate	29.3	100	0.0

Data from Brandl *et al.* (1989, 1991); Liebergesell *et al.* (1991); Philippis *et al.* (1992); Ulmer *et al.* (1994).

3HB, 3-hydroxybutyrate; 3HV, 3-hydroxyvalerate; PHA, poly(β-hydroxyalkanoate).

In general, the PHA biosynthetic route from fatty acids can be linked to (i) β-oxidation, (ii) *de novo* fatty acid biosynthesis, or (iii) a process of direct oxidation (Sasikala and Ramana, 1996). The key enzymes involved in PHA biosynthesis are 3-ketothiolase, acetoacyl-CoA reductase and PHA synthase. The enzymes 3-ketothiolase and acetoacyl-CoA reductase have been purified and characterized from various PHA-synthesizing bacteria. The PHA synthase (polymerase) enzyme is associated with PHA granules isolated from different bacteria; it helps in the polymerization reaction from hydroxyalkanoic acids and determines the type of monomer unit of PHAs (Gerngross *et al.*, 1994).

At present it is not clear whether only one polymerase functions in the formation of different PHAs, or whether different polymerases exist. The PHB synthase is strictly specific for 3-hydroxyacyl-CoA in anoxygenic phototrophs (Liebergesell *et al.*, 1991).

Three different types of PHA synthases have been distinguished (Steinbuchel *et al.*, 1992). Type I PHA synthases are of high molecular mass (about 61 kDa and above) and use hydroxy fatty acids of short chain lengths (C_3 to C_5) as substrates; type II synthases use medium chain lengths (C_6 to C_{14}) as substrates; and type III enzymes of low molecular mass (less than 40 kDa) use short-chain fatty acids as substrates. The PHA biosynthetic genes have been cloned, and analysed at a molecular level, from *Chromatium vinosum* (Liebergesell and Steinbuchel, 1992), *Rhodospirillum rubrum* and *Rhodobacter sphaeroides* (Hustede *et al.*, 1992; Eilert and Alexander, 1993; Liebergesell *et al.*, 1993).

Granules of PHB from *Chromatium vinosum* are associated with at least four proteins (Liebergesell *et al.*, 1992, 1994). Two proteins are required for the expression of PHA synthase activity, while the functions of the others are not clear. Three major classes of PHA granule-associated proteins have been identified in bacteria: these are PHA synthase, PHA depolymerase and phasins. Other proteins which constitute a major proportion of the total protein surrounding the surface of PHA granules have been loosely grouped into a fourth class (Steinbuchel *et al.*, 1995). Phasins are a newly defined class of proteins thought to have similar functions to oleosins of triacylglycerol inclusions in seeds and pollen of plants. This type of protein has been identified from *Alcaligenes eutrophus* (Wieczorek *et al.*, 1995), *Rhodococcus ruber* (Pieper-Furst *et al.*, 1994, 1995) and *Acinetobacter* spp. (Schembri *et al.*, 1995), and has been shown to influence the size of intracellular PHA granules.

Poly(β-hydroxybutyric acid) granules of *Rhodospirillum rubrum* undergo a rapid enzymatic digestion when incubated either in the soluble fraction of cell extracts or in buffer, yielding β-hydroxybutyric acid, suggesting that depolymerizing enzymes, as well as biosynthetic enzymes, are associated with PHB granules (Merrick and Doudoroff, 1964). However, extracellular biodegradation of PHAs by APB has not been reported either in the laboratory or under field conditions. Further details on the biodegradation of PHA by other bacteria and fungi are given in Sasikala and Ramana (1996).

6. PHOTOBIODEGRADATION OF POLYMERS

Studies on biodegradation and metabolism of polymers are mostly restricted to biopolymers: starch and poly(hydroxyalkanoates). To the best of our knowledge, nothing is known of the biodegradation of other natural and synthetic polymers by APB.

Starch is the substrate of choice for the photoproduction of hydrogen by a few purple bacteria (Buranakarl *et al.*, 1985; Sing *et al.*, 1994). *Rhodocyclus gelatinosus* (Buranakarl *et al.*, 1988) and *Chloroflexus aurantiacus* (Ratanakhanokchai *et al.*, 1992) can grow on a medium containing starch as carbon source. Unlike other amylases, which produce glucose and maltose from starch as their main products, APB amylase produces maltooligosaccharides (maltotetraose and maltotriose) in addition to glucose; these maltooligosaccharides are of potential use in the food, pharmaceutical and fine chemical industries because of their unique nature and specific properties (Ratanakhanokchai *et al.*, 1992). Apart from APB, such maltooligosaccharide-producing amylases are restricted to very few organisms, such as *Pseudomonas stutzeri* (Robyt and Ackerman, 1971), *Bacillus circulans* (Takasaki, 1983), *Bacillus subtilis* (Takasaki, 1985) and *Streptomyces griseus* (Wako *et al.*, 1978).

At least two types of amylase were found in *Rhodocyclus gelatinosus* grown on raw cornstarch; one of them when purified had a molecular mass of about 56 kDa with optimum activity at 40 °C and pH 6.0 (Buranakarl *et al.*, 1988). A heat-stable amylase was isolated from a thermophilic *Chloroflexus aurantiacus* showing optimum amylase activity at 71 °C with a pH optimum at 7.5 (Ratanakhanokchai *et al.*, 1992).

Gelatin liquefaction is a common property of *Rhodocyclus gelatinosus*, while only 2 out of 12 strains of *Rhodopseudomonas palustris* and none of *Rhodobacter sphaeroides* were proteolytic (Klemme and Cornelia, 1977). A protease produced by *Rhodocyclus gelatinosus* was inactivated at 70 °C, was not affected by phenyl methane sulfonyl fluoride (PMSF) or *N*-tosyl-L-lysyl-chloromethyl ketone, but was activated by EDTA, citrate and *o*-phenanthroline (Klemme and Cornelia, 1977). Hyun *et al.* (1989) purified a neutral serine protease from a purple non-sulfur bacterium and characterized it. A heat-stable protease resistant to denaturing agents was isolated from *Chloroflexus aurantiacus* J-10-fl (Watanabe *et al.*, 1993). Two types of thermostable proteases (protease I and protease II) were isolated; protease I, a monomer with a molecular mass of 66 kDa, was inhibited by metalloprotease inhibitors such as EDTA, ethyleneglycol-bis(β-aminoethyl ether)-*N*-*N*′-tetraacetic acid (EGTA) and *o*-phenanthroline. The optimum pH for activity was 8.0 (the enzyme being stable between pH 4.0 and 11.0); the temperature optimum was 70 °C (stable up to 75 °C); and the enzyme was resistant to treatment by denaturing reagents (8 M urea or 1% sodium dodecylsulfate).

Although there is a report on the use of cellulose as electron donor for the

photoproduction of hydrogen by *Rhodospirillum rubrum* (Vatsala and Balaji, 1990; Vatsala, 1991), further confirmation of its photobiodegradation by other workers with pure cultures of APB is awaited. However, there is a report of using cocultures for the photoproduction of hydrogen from cellulose by a heterotrophic bacterium (*Cellulomonas* sp.) and *Rhodobacter capsulatus* (Odom and Wall, 1983).

7. EFFECT OF UNUSUAL CARBON COMPOUNDS ON VARIOUS CELLULAR CONTENTS AND ACTIVITIES OF PURPLE NON-SULFUR BACTERIA

A number of unrelated compounds (Table 4) greatly influence one or more physiological activities of purple non-sulfur bacteria. Though long-chain alcohols such as triacontanol could not support growth of *Rhodobacter sphaeroides* as sole source of carbon, they stimulated diazotrophic growth of *R. sphaeroides* when malate was used as a carbon source and in their presence altered bacteriochlorophyll-a (BChl-a) synthesis was suggested (Uma *et al.*, 1997). Stimulation of growth of purple non-sulfur bacteria was also observed with trimethylamine-*n*-oxide (Roldan *et al.*, 1994a).

The presence of aromatic compounds inhibited growth of *R. sphaeroides* on malate and ammonium chloride as carbon and nitrogen sources, respectively (authors' unpublished data). Studies on the biopolymer composition of *R.*

Table 4 Carbon compounds (mostly xenobiotics) that influence physiological activities of purple non-sulfur bacteria.

1-Chloro-2,4-dinitrobenzene
4-Chlorophenol
2,4-D (2,4-dichlorophenoxyacetic acid)
2,4-Dinitrophenol
Anthranilic acid
Atrazine
Captan
Carbendazim
Diphenylamine
Gabaculine
Imidazole
Indole
Monocrotophos
p-Chlorobenzoic acid
Phenanthroline
Quinalphos
Syringic acid
Vanillic acid

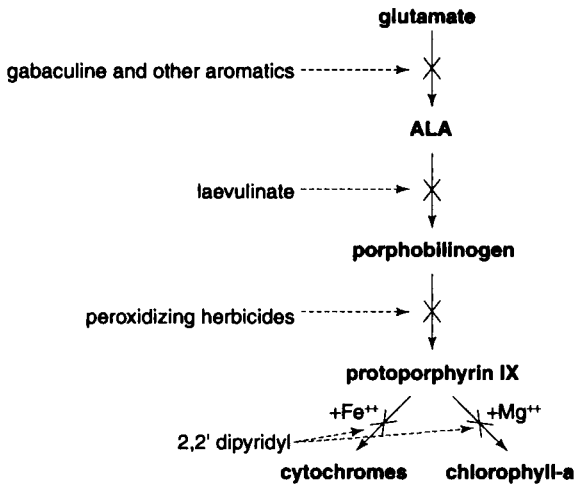

Figure 8 Porphyrin synthesis pathway (Sasikala *et al.*, 1994c) and possible site of inhibition by various aromatic compounds and other modulators (Nanda, 1996).

sphaeroides in the presence of aromatic compounds revealed enhancement of the protein and carbohydrate content of the cells, suggesting that the aromatic compounds are not inhibitors of their synthesis (Nanda, 1996). Furthermore, enhanced PHA accumulation was observed when cells of *R. sphaeroides* OU5 were exposed to a variety of homocyclic aromatic compounds. This suggests that their synthesis under stress in this organism helps to overcome unfavourable conditions by means of accumulating cellular reserves (Dawes and Senior, 1973).

The most affected cellular component was bacteriochlorophyll a (Bchl-a), whose synthesis is inhibited in *R. sphaeroides* OU5 (authors' unpublished data; Hatch and Lascelles, 1972) and in *Rhodomicrobium vannielii* (Wright and Madigan, 1991) by methoxylated (syringate and vanillate) and *o*-phenanthroline aromatic compounds, suggesting that growth inhibition was due to the inhibition of a biosynthetic step in Bchl-a synthesis. Gabaculine (3-amino-2,3-dihydrobenzoic acid) is one such aromatic compound which blocks the conversion of glutamate 1-semialdehyde (GSA) to 5-aminolaevulinic acid (ALA) by inhibiting the enzyme GSA aminotransferase (Sasikala *et al.*, 1994c). Other related aromatic compounds may have similar effects on *Rhodobacter sphaeroides* OU5 (Fig. 8).

Unlike Bchl-a synthesis, which was inhibited by most of the aromatic compounds tested, carotenoid biosynthesis was in general stimulated in *Rhodobacter sphaeroides* OU5 (Nanda, 1996). The fact that the stimulation in carotenoid

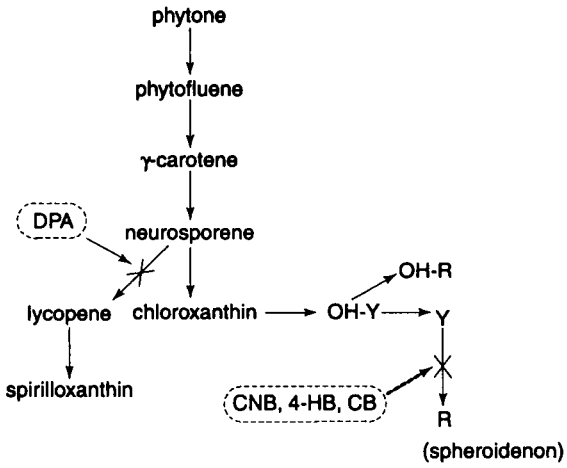

Figure 9 Carotenoid biosynthesis and the site of inhibition by some of the aromatic compounds (Nanda, 1996). CB, *p*-chlorobenzoate; CNB, 1-chloro-2,4-dinitrobenzene; DPA, diphenyl amine; 4-HB, 4-hydroxybenzoate; R, red carotenoid; Y, yellow carotenoid.

synthesis in *R. sphaeroides* was accompanied by reduced levels of Bchl-a suggests that carotenoids are serving functions other than photosynthetic ones, and may be protective as observed not only in purple bacteria (Cohen-Bazire and Stanier, 1958) but also in other bacteria (Mathews and Sistrom, 1959) and green plants (Goodwin, 1960).

While yellow carotenoid synthesis by *R. sphaeroides* OU5 was not inhibited by many of the aromatic compounds tested, reduced levels of red carotenoid content alone in the presence of 1-chloro-2,4-dinitrobenzene and *p*-chlorobenzoate indicate that these compounds affect conversion of yellow to red carotenoid and not carotenoid biosynthesis *per se* (Fig. 9). On the other hand, diphenylamine (DPA), a known inhibitor of carotenoid synthesis in *Rhodospirillum rubrum* and *Rhodopseudomonas palustris* (Goodwin and Osman, 1953; Cohen-Bazire and Stanier, 1958) which blocks the conversion of neurosporine to lycopene in the synthesis of carotenoids of the spirilloxanthin series, does not inhibit carotenoid synthesis of the sphaeroidene series (Goodwin, 1955) even in *Rhodobacter sphaeroides* OU5.

Some of the pesticides such as 2,4-dichlorophenoxyacetic acid (2,4-D), captan (*N*-trichloromethyl mercapton-4-cyclohexene-1,2-dicarboximide), carbendazim (2-benzimidazole carbamic acid methyl ester), quinolphos (*O,O*-diethyl-*O*-quinoxy-alinyl-2-thiooxophosphate) and monocrotophos (ε-phosphoric acid dimethyl-1-methyl-3-(methylamino)-3-oxo-1-propenyl ester) greatly influence the diazotrophic growth and nitrogenase activity of purple non-sulfur bacteria (Chalam *et al.*, 1997). It appears that APB can resist higher concentrations of pesticides than oxygenic phototrophic bacteria (cyanobacteria) (Chalam *et al.*, 1997). Growth of

Rhodobacter capsulatus was affected by the presence of nitroaromatic compounds, which was further dependent on the nitrogen source used during phototrophic growth (Roldan *et al.*, 1994b). Phototrophic growth on glutamate or ammonium was not inhibited by 2,4-dinitrophenol, 4-nitrophenol, 2-amino-4-nitrophenol, 4-aminophenol or 4-chlorophenol, whereas 2,4-dinitrophenol and 4-chlorophenol partially inhibited bacterial growth on nitrate, nitrite, and dinitrogen (Roldan *et al.*, 1994b).

Atrazine-resistant strains of *Rhodobacter sphaeroides* (Sutton *et al.*, 1984; Brown *et al.*, 1990) have been shown to produce certain polypeptides in large quantities (Brown *et al.*, 1994). Flocculation of *R. sphaeroides* was commonly observed when cells are exposed to high concentrations of yeast extract. Flocculation in *R. sphaeroides* can be induced when cells are exposed to unusual carbon compounds such as aromatic compounds, aliphatic chlorinated compounds (authors' unpublished data) or formaldehyde (Chang *et al.*, 1995). Vitamins with aromatic structures present in the yeast extract may play an important part in flocculation.

8. BIOTECHNOLOGICAL APPLICATIONS OF APB WITH UNUSUAL CARBON COMPOUNDS

Although the activities of APB have been known for about two centuries, their potential for various biotechnological applications has only recently been recognized (Sasikala and Ramana, 1995a,b). Some of their applications in the field of biotechnology are summarized in Table 5. The rapidly growing knowledge of the metabolism of unusual carbon compounds will surely contribute to making the potentials of APB for various biotechnological applications more attractive, technically feasible and economically viable.

The value of APB in waste-water treatment is not restricted to their photobiodegradation capabilities alone. Their ability to produce bacteriocins and antiviral substances makes them ideal candidates for treatment of wastes which can then be recycled for other purposes. Antiviral substances from *Rhodobacter capsulatus, Rhodospirillum rubrum* and *Chromatium vinosum* (Kobayashi *et al.*, 1975) have been isolated. *Rhodobacter capsulatus* produces an antiviral substance which inactivates poliovirus, Sindbis virus, fish viruses and coliphages without causing damage to the host cells (Kobayashi and Hirotani, 1987). The purified compound was identified as a fatty acid, *cis*-vaccenic acid (Hirotani *et al.*, 1991). The antiviral properties of *R. capsulatus* have been exploited for waste treatment and it was found that the organism could effectively remove up to 97% of coliphages from waste water from a pig-pen. Of special interest is the degradation/transformation of aromatic compounds (a major part of industrial effluents), which makes this group of microorganisms valuable in the anaerobic disposal of wastes.

Table 5 Commercial applications of purple non-sulfur bacteria.

Product or process	Application	Reference
Single-cell protein	Protein source	Shipman *et al.* (1975)
s-Adenosyl-L-cystine	Precursor useful in therapeutic manufacturing	Yamada *et al.* (1986)
Vitamin B_{12}	Vitamin	Yasumasa (1972); Florent and Ninet (1979); Sasaki and Nagai (1979); Takeuchi and Hamada (1985)
Ubiquinone Q-10	Clinical medicine	Mochida *et al.* (1973); Sasaki and Nagai (1979); Japan Synthetic Rubber Co. Ltd (1982); Uragami and Yoshida (1986a,b)
Hydrogen	Fuel	Sasikala *et al.* (1993)
Antiviral substances	Waste treatment	Hirotani *et al.* (1991)
Carotenoid	Natural dye	Noparatnaraporn *et al.* (1987)
5-Aminolaevulinic acid	Herbicide	Sasaki *et al.* (1990)
Biodegradation of organic and inorganic compounds	Waste treatment	Sawada and Rogers (1977a,b)
Hopanoid	Therapeutic agent	Nagumo *et al.* (1991)
Poly(β-hydroxyalkanoates)	Biodegradable plastic	Brandl *et al.* (1989, 1991)
Enzymes	Food preparations	Izaki and Kamiom (1992)
Cytokinin	Plant hormone	Kuroda (1990); Serdyuk *et al.* (1993)
Auxins	Phytohormone	Yokoyama (1990)

During waste treatment, valuable by-products such as hydrogen, biomass (which can be employed as biofertilizer or single-cell protein, SCP) and 5-aminolaevulinic acid (a natural herbicide) can be obtained. The APB are considered to be ideal candidates for use as SCP (Shipman *et al.*, 1975), since their nutritional value is superior to other protein sources of microbial and non-microbial origin (Table 6) with respect to the range and quantity of essential amino acids, as well as the vitamin content, including vitamin B_{12}. In addition, APB can be used in the industrial production of vitamin B_{12} (see Sasikala and Ramana, 1995a).

The unique capability of APB to fix atmospheric nitrogen using aromatic compounds as electron donors, coupled to their tolerance to various pesticides and stimulation by plant growth promoters such as triacontanol, make APB ideal candidates for field application as biofertilizers in submerged paddy-fields, where they are naturally distributed. The biotransformation capabilities of APB in transforming anthranilate, indole and its derivatives have value in the production of essential amino acids, such as tryptophan, and plant growth hormones like indoleacetic acid.

Table 6 Amino-acid composition of different proteins.

Protein source	Amino acid (% protein)						
	Ile	Leu	Lys	Met	Phe	Thr	Val
Photosynthetic bacteria							
Rhodobacter capsulatus	5.2	8.0	5.4	3.2	5.2	5.1	7.2
Rhodobacter sphaeroides	3.8	7.1	5.6	3.0	4.7	5.0	6.5
Rhodopseudomonas palustris	4.3	7.2	5.2	3.3	4.2	4.8	6.5
Rhodopseudomonas acidophila	4.4	7.0	4.8	3.4	4.4	4.8	7.0
Rhodocyclus gelatinosus	4.0	7.0	5.0	3.0	4.8	5.0	6.4
Rhodospirillum rubrum	4.1	6.6	5.0	3.0	5.1	5.4	7.0
Rhodocyclus tenue	4.3	7.7	5.0	3.4	5.2	4.8	7.3
Other protein sources							
FAO reference protein	4.0	7.0	5.5	3.5	6.0	4.0	5.0
Chlorella sp.	4.4	8.0	5.0	0.5	4.8	4.1	5.4
C. vulgaris	2.4	4.4	2.7	0.3	2.6	2.3	3.0
Scenedesmus obliquus	3.8	8.4	5.7	1.7	5.1	5.1	5.7
Spirulina maxima	6.0	8.0	4.6	1.4	5.0	4.6	6.5
Cellulomonas sp.	4.7	11.2	6.8	1.9	4.4	5.4	10.7
Lactobacillus fermenti	7.0	7.5	6.9	1.3	4.1	4.9	6.8
Pseudomonas sp.	3.9	7.0	5.3	1.8	4.2	4.5	5.9
Yeast protein	5.2	7.0	7.4	1.0	4.3	5.2	6.3
Meat protein	3.4	6.4	5.0	1.3	3.6	3.4	5.0
Egg protein	6.6	8.8	6.4	3.1	5.8	5.0	7.4
Soybean protein	5.4	7.7	6.3	1.3	4.9	3.9	5.2
P-SPI	3.0	2.5	11.7	0.0	1.4	4.6	4.6
Wheat flour	4.2	7.0	1.9	1.5	5.5	2.7	4.1
Esso-Nestlé SCP (yeast)	2.6	3.5	3.8	0.5	2.2	2.6	3.2

Ile, isoleucine; Leu, leucine; Lys, lysine; Met, methionine; Phe, phenylalanine; Thr, threonine; Val, valine.

FAO, Food and Agriculture Organization; P-SPI, phosphorylated soybean protein isolate; SCP, single-cell protein.

For references see Sasikala and Ramana (1995a).

The ability of APB to produce PHA with unusual monomers in the presence of higher fatty acids and *n*-alkanes/alkenes makes them worth exploiting for the production of bioplastics with special applications. The property of PHA accumulation by *Rhodobacter sphaeroides* exposed to aromatic compounds can be exploited for commercial production. Furthermore, the production of thermotolerant and unique amylases by APB is of great commercial significance. Production of Q-10 by *Rhodobacter capsulatus* and *R. sphaeroides* when grown on molasses (Kyowa, 1983), corn-steep liquor (Takara Sake Brewery Co. Ltd and Taiko Pharmaceutical Co. Ltd, 1982), alcohols (Uragami and Yoshida, 1986a), *n*-alkanes (tetradecane; Mochida *et al.*, 1973), starch (Sasaki and Nagai, 1979) and sewage

(Hirayama *et al.*, 1972) makes the process economically attractive. The presence of isopentenyl alcohol and/or dimethylaryl alcohol (4%) (Japan Synthetic Rubber Co. Ltd, 1982), and amino acids such as proline (Kyowa Hakko Kogyo Co. Ltd, 1983) stimulates Q-10 production by *R. sphaeroides*.

ACKNOWLEDGEMENTS

The authors wish to thank the UGC and CSIR, Government of India, New Delhi, for the awards of a Research Scientistship and Pool Scientistship, respectively. Financial support received from the Department of Science and Technology to Dr Sasikala under the Young Scientist programme is gratefully acknowledged.

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