

## Reductive Dehalogenation of Halocarboxylic Acids by the Phototrophic Genera *Rhodospirillum* and *Rhodopseudomonas*

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**Type strains of the purple nonsulfur species *Rhodospirillum rubrum*, *Rhodospirillum photometricum*, and *Rhodopseudomonas palustris* grew phototrophically on a number of two- and three-carbon halocarboxylic acids in the presence of CO<sub>2</sub>, by reductive dehalogenation and assimilation of the resulting acid. Strains of each of these species were able to grow on chloroacetic, 2-bromopropionic, 2-chloropropionic, and 3-chloropropionic acids at a concentration of 2 mM. Only *R. palustris* DSM 123 was able to grow on bromoacetic acid and then only at a reduced concentration of 1 mM. *R. palustris* ATCC 33872 (formerly *R. rutila*) was unable to grow on any of the substrates tested. The ability of these organisms to utilize halocarboxylic acids indicates that they may have a significant role to play in the removal of these environmental pollutants from illuminated anaerobic habitats such as lakes, waste lagoons, sediments of ditches and ponds, mud, and moist soil.**

Halogenated aliphatic compounds form an important group of chemicals produced by industrial processes. Due to the halogen substituent, many of these compounds are poorly degraded and hence persist in the environment.

A number of bacteria have been shown to grow aerobically at the expense of halogenated two- and three-carbon carboxylic acids (4–6); however, much less is known of the degradation of these compounds under anaerobic conditions. Of the groups of bacteria inhabiting anaerobic sediment, a group of phototrophic organisms known as the purple nonsulfur bacteria are among the most prevalent and metabolically diverse. Certain species of this group have been shown to utilize a wide range of aliphatic and aromatic compounds as sole carbon sources or electron donors for phototrophic growth in the absence of oxygen. The ability of a small number of species of the purple nonsulfur bacteria to reductively remove the halogen from certain compounds has been shown (10, 11, 13, 14); however, little is known of how widespread this ability is within the group and of the diversity of halogenated compounds that can be catabolized.

We begin to address this gap in the knowledge with a study of the degradation of low-molecular-weight halocarboxylic acids by the purple nonsulfur genera *Rhodospirillum* and *Rhodopseudomonas*. Species from each of these genera were screened for the ability to grow anaerobically on a range of these compounds. Once species with this ability were identified, we followed the degradation of the substrate to establish the mode of metabolism.

The results reported in this paper show that reductive dehalogenation and subsequent catabolism of low-molecular-weight halocarboxylic acids by strains within the two genera studied occur in a number of species.

**Bacterial strains.** All bacteria used in this study were strains of species from the purple nonsulfur genera *Rhodospirillum* and *Rhodopseudomonas*. The strains used were *Rhodospirillum photometricum* DSM 122, *R. rubrum* DSM 467, and *Rhodopseudomonas palustris* DSM 123 and ATCC 33872.

**Media and growth conditions.** For growth tests on halocarboxylic acids, a basal salts medium (3) was used, with the addition of 0.15% (wt/vol) sodium bicarbonate (growth of these bacteria on reduced substrates is dependent on the availability of CO<sub>2</sub> for disposing of excess reducing equivalents [15]), 0.002% (wt/vol) yeast extract, and a 1 to 2 mM final concentration of halocarboxylic acid. All cultures were grown under illuminated anaerobic conditions in serum bottles with the headspace flushed with oxygen-free nitrogen gas. After inoculation with a 2% inoculum, all tubes were placed in darkness for a 24-h period to allow any residual oxygen to be used, thus avoiding possible photo-oxidative damage to cells when they were placed at 30°C under 5,000 lx of incandescent illumination.

**Measurement of growth.** Growth was measured turbidimetrically at 660 nm on a Shimadzu UV-250 spectrophotometer.

**Measurement of halide ion release.** The amount of chloride and bromide released was determined by the method of Bergmann and Sanik (2), with uninoculated medium as the blank.

**Quantitation of halocarboxylic acids and degradation products.** Because these bacteria readily consume acetic and propionic acids, detection of the dehalogenated products required allowing them to accumulate in nongrowing cultures. Each of the strains tested was grown under the conditions described above with the added halocarboxylic acid. The cells were then centrifuged in a Beckman Induction Drive centrifuge at 5,000 × g for 15 min and resuspended in a modified basal salts medium with the same halocarboxylic acid substrate described above except that it contained no nitrogen source and the sodium chloride was removed to keep the background halide levels as low as possible. The cells were incubated as described above, but under an argon atmosphere. The cells were able to convert the haloacid substrate but with no nitrogen source were unable to grow on the resulting products; hence, the products accumulated and were detectable. The disappearance of substrate and the accumulation of product were monitored by gas chromatography. At intervals, 1 ml of culture fluid was removed from the culture vessel and centrifuged in an Eppendorf centrifuge (model 5415C) at 9,900 × g for 10 min. The concentrations of substrate and product in the sample supernatant were measured on a Hewlett-Packard 5890 Series II gas chromatograph equipped with a flame ionization detector. An SGE BP-21, 25-m-long fused silica capillary column with a

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TABLE 1. Utilization of halocarboxylic acids by *Rhodospirillum* and *Rhodopseudomonas*<sup>a</sup>

Substrate <sup>b</sup>	Growth response <sup>c</sup> (μg/ml of protein) of species or strain:			
	<i>R. rubrum</i>	<i>R. photometricum</i>	<i>R. palustris</i> DSM 123	<i>R. palustris</i> ATCC 33872
Control (acetate)	170	167	107	110
Control (propionate)	185	180	116	115
Control (no substrate)	10	11	15	20
Bromoacetic acid	9	11	37	15
Chloroacetic acid	70	116	39	17
2-Bromopropionic acid	151	143	81	22
2-Chloropropionic acid	107	111	86	14
3-Chloropropionic acid	99	151	79	11

<sup>a</sup> Incubation time was 20 days.

<sup>b</sup> All substrates were supplied at a final concentration of 2 mM, except for acetate and propionate (10 mM) and bromoacetic acid (1 mM).

<sup>c</sup> Growth was measured by the Lowry protein assay.

bonded FFAP phase and 0.25-μm film thickness was used isothermally at 210°C for detection of all halocarboxylic acids. The injection volume was 2 μl.

**Confirmation of degradation products.** The results of gas chromatography detection of the products of degradation were confirmed by electrospray mass spectrometry. A 20-μl sample of culture supernatant was diluted 1:1 with a 50:50 (vol/vol) mixture of acetonitrile and water and analyzed on a VG Platform II electrospray mass spectrometer at a cone voltage of 20 V, with a 50:50 (vol/vol) mixture of acetonitrile and water as the mobile phase.

**Chemicals.** All chemicals were obtained from BDH Chemicals Ltd. (Poole, England), except for mercuric isothiocyanate, which was obtained from Sigma Chemical Co. (St. Louis, Mo.).

**Utilization of two- and three-carbon halocarboxylic acids by pure cultures of *Rhodospirillum* and *Rhodopseudomonas*.** Two species of *Rhodospirillum*, *R. rubrum* and *R. photometricum*, along with two strains of the species *R. palustris* (DSM 123 and ATCC 33872) were tested for utilization of five halocarboxylic acids (bromoacetic, chloroacetic, 2-bromopropionic, 2-chloropropionic, and 3-chloropropionic acids). All strains, with the exception of *R. palustris* ATCC 33872, showed the ready capacity to grow phototrophically on the majority of these compounds at a 2 mM concentration (Table 1). Only *R. palustris* DSM 123 was able to grow on bromoacetic acid and then only at a reduced concentration of 1 mM. The generation time of *R. photometricum* growing on 2 mM 2-chloropropionic acid was about 36 h (Fig. 1); generation times of 12 to 48 h were typical for both *Rhodospirillum* strains on each of the substrates they utilized. The generation times for the growth of *R. palustris* DSM 123 tended to be longer, 60 to 72 h being typical.

**Degradation of the substrate, release of halide, and formation of corresponding nonhalogenated acids in nongrowing cultures.** In all cases, the degradation of each of the substrates was accompanied by the release of the associated halogen, as halide, and the formation of the corresponding nonhalogenated acid (Table 2). The appearance of the halide and the acid product can be claimed to be reasonably stoichiometric with the disappearance of the halogenated substrate. Control vials containing a 2 mM concentration of the substrate in the same medium but with no inoculum were set up to test for possible spontaneous dehalogenation during incubation. Levels of free halide were shown not to increase above background

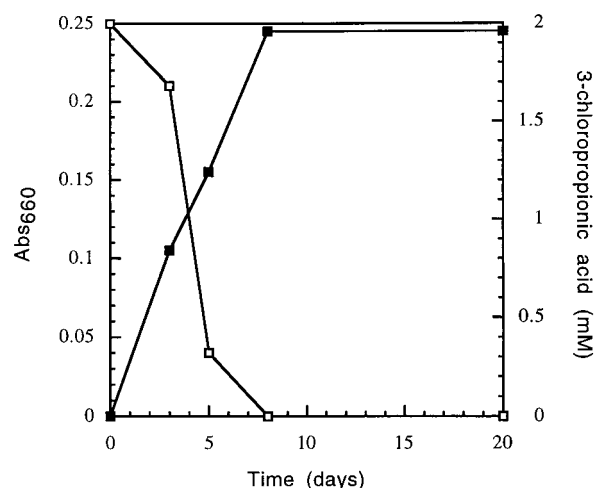


FIG. 1. Growth of *R. photometricum* DSM 122 phototrophically on 3-chloropropionic acid. The medium contained 2 mM 3-chloropropionic acid and 0.15% (wt/vol) bicarbonate. ■, growth (A<sub>660</sub>); □, 3-chloropropionic acid concentration.

throughout the incubation period, and all of the substrate was found to be present at the end of incubation (data not shown).

**Identification of degradation products.** Electrospray mass spectrometry analysis of the products of dehalogenation of the halocarboxylic acids by the strains tested showed that in all cases the corresponding nonhalogenated acids were formed. Thus, the dehalogenation of haloacetic yielded acetic acid and the dehalogenation of the halopropionic acids yielded propionic acid. Both of these acids are readily photometabolized by each of the strains (9).

Bacteria with the ability to reductively dehalogenate haloalkyl compounds are both numerous and diverse (11). Included in these bacteria are several anaerobes; *Clostridium* species and methanogenic bacteria are examples (12). The experiments described in this paper show that this dehalogenating ability also occurs within the purple nonsulfur bacteria. Under illuminated anaerobic conditions, strains from the genera *Rhodospirillum* and *Rhodopseudomonas* were shown to grow phototrophically on chlorinated and brominated acetic and propionic acids by the reductive removal of the halogens and subsequent utilization of the acids for growth. Of the strains tested, only *R. palustris* DSM 123 showed growth on all of the substrates tested. Both strains of *Rhodospirillum* grew on all substrates except for bromoacetic acid, whereas *R. palustris* ATCC 33872, formerly known as *R. rubra* (1, 8), was unable to utilize any of the substrates. Each substrate was supplied at a final concentration of 2 mM except for bromoacetic acid which was supplied at 1 mM. At these concentrations, false-negative growth results due to substrate toxicity were avoided.

When testing for the dehalogenating ability of organisms it is important to take into account the possibility of the nonmicrobial or spontaneous removal of the halogen from the substrate during the incubation period. It has been shown that some halogenated fatty acids can be chemically unstable during aerobic incubation (12). However, control experiments carried out under the illuminated anaerobic incubation conditions used for the work described here showed no dehalogenation of the substrate in uninoculated controls. Hence, it can be concluded that the dehalogenating activity is due only to microbial action.

In summary, it is clear that the strains of purple nonsulfur

TABLE 2. Substrate utilization, halide release, and dehalogenated acid formation from the growth of *Rhodospirillum* and *Rhodopseudomonas* strains on halocarboxylic acids

Species or strain	Substrate <sup>a</sup>	Concentration (mM) of:		
		Substrate utilized	Halide detected	Free acid detected
<i>R. rubrum</i>	2-Bromopropionic acid	1.3	1.3	1.3
	2-Chloropropionic acid	0.9	0.8	0.9
	3-Chloropropionic acid	1.0	1.0	0.9
<i>R. photometricum</i>	Chloroacetic acid	1.6	1.5	1.5
	2-Bromopropionic acid	2.0	1.8	2.0
	2-Chloropropionic acid	1.2	1.1	1.2
	3-Chloropropionic acid	2.0	2.0	2.0
<i>R. palustris</i> DSM 123	2-Bromopropionic acid	1.4	1.3	1.3
	2-Chloropropionic acid	1.6	1.6	1.5
	3-Chloropropionic acid	2.0	1.9	1.8

<sup>a</sup> Each substrate was supplied at an initial concentration of 2 mM to individual serum bottles.

bacteria tested in this study are capable of reductive dehalogenation of two- and three-carbon carboxylic acids. Knowledge of the range of halogenated compounds that can be degraded by this group of bacteria is extremely limited. However, from the results shown here and the work done by others (10, 13, 14) it seems likely that many of these types of compounds, both aliphatic and aromatic, may be susceptible to degradation by the purple nonsulfur bacteria. These bacteria have been shown to utilize a wide range of aromatic (7, 13, 16) and aliphatic compounds (9); this, in addition to their ability to dehalogenate, suggests that these bacteria may have an important role to play in the removal of many halogenated contaminants in anaerobic sediments. Much work is still to be done to establish the range of halo-organic compounds that can be used and the number of species of these bacteria that can use them.

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