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Biodegradation of Homocyclic and Heterocyclic Aromatic Compounds by *Rhodopseudomonas palustris* Strains

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Abstract: Six strains were isolated from Lake Akşehir (Konya-Turkey) and identified as *Rhodopseudomonas palustris* on the basis of physiological and morphological characteristics. The strains grew phototrophically on a variety of aromatic compounds (under illuminated anaerobic conditions), namely, benzoate, hippurate, benzyl alcohol, 2-amino benzoic acid, phloroglucinol, phenylacetic acid, catechol, pyridine and pyrazoline. Biodegradation of 2-amino benzoate, phloroglucinol, catechol, pyridine and pyrazolin by *Rhodopseudomonas palustris* strains have not been reported in the literature.

Key Words: *Rhodopseudomonas palustris,* aromatic compounds, biodegradation.

Homosiklik ve Heterosiklik Aromatik Bileşiklerin *Rhodopseudomonas palustris* Suşları Tarafından Parçalanması

Özet: Akşehir Gölü'nden izolen edilen, fizyolojik ve morfolojik özelliklerine göre karakterize edilen altı suş *Rhodopseudomonas palustris* olarak tanımlanmıştır. Bu suşlar benzoat, hippurat, benzil alkol, 2-amino benzoik asit, floroglusinol, fenil asetik asit, kateşol, piridin ve pirazolin içeren besiyerlerinde fototrofik olarak geliştirilmiştir. Bu konuda yapılan çalışmalarda, *Rhodopseudomonas palustris* suşlarının 2-amino benzoat, floroglusinol, kateşol, piridin ve pirazolin'i parçaladığı belirtilmemiştir.

Anahtar Sözcükler: *Rhodopseudomonas palustris,* aromatik bileşikler, parçalanma.

Introduction

A diverse array of aromatic compounds exists in nature, primarily as substances released from dead plant material. In addition to biological sources of aromatic compounds, agricultural and industrial activities introduce a wide variety of xenobiotic aromatic compounds into the biosphere herbicides, insecticides, detergents and industrial waste products. These compounds are mostly phenolic derivatives of a general phenylpropane or benzyl structure(1, 2, 3).

Anaerobic catabolism of aromatic compounds occurs in several physiological groups of bacteria, including anoxygenic phototrophic bacteria (2, 4), which occur in all aquatic environments and in soils. Since their numbers increase with the increasing moisture content of soil, photometabolism could play a significant role in the dissimilation of simple aromatics in these environments (1).

According to recent taxonomic investigations, anoxigenic phototrophic bacteria are present in seven subgroups, and the genera *Rhodopseudomonas, Rhodocyclus, Rhodomicrobium, Rhodophila, Rhodospirillum* and *Rhodobacter* are present in subgroup 3 (5). It was concluded that biodegradation of aromatic compounds was widespread among the genera of subgroup 3 (purple nonsulfur bacteria).

This paper presents the results of the growth of *R. palustris* on different aromatic compounds. We report for the first time the growth of *R. palustris* on 2-amino benzoate, phloroglucinol, catechol, pyridine and pyrazolin as the sole organic carbon source.

Materials and Methods

Bacterial strains: Phototrophic purple nonsulfur bacteria were isolated from Lake Akşehir (Konya-Turkey) and identified as *R. palustris* (5, 6, 7, 8). Six *R. palustris* strains were used in the present investigation.

Media and growth conditions: Composition of the basal medium (AT medium) was as follows: NH_4Cl, 1g; KH_2PO_4, 1g; $MgCl_2x6H_2O$, 0.5g; Na-acetate, 1g; NaCl 1g; $CaCl_2x2H_2O$, 0.1g; trace element stock solution SLA, 1 ml; vitamen solution, 1ml; distilled water, 1L (7). The basal medium was supplemented with 0.1 g/L yeast extract and 3 g/L $NaHCO_3$ (sterilized by membrane filtration before use). The pH of the medium after autoclaving was 8.5-9.0 by adding sterile NaOH solution. Screw-cap tubes (30 ml) were used as culture vessels. All vessels were placed at room temperature (20-25°C) under light at an intensity of 1500 to 2000 lx of (incandescent) illumination.

Biodegradation studies and analytical measurement: Cultures (1% v/v) were added to AT medium (without acetate and yeast extract) supplemented individually with the following compounds: phloroglucinol (1,3,5-trihydroxybenzene) (1mM), pyrazoline (4-5 dihydroxyprazine) (1mM), phenyl acetic acid (1mM), 2-amino benzoic acid (1mM), pyridine (0.5mM), benzyl alcohol (3mM), benzoate (7mM), hippurate (N-benzylglycine) (10mM), cinnamic acid (1mM), catechol (1,2 dioxybenzol) (1mM) and phenol (0.3mM). Aromatic compounds were sterilized by membrane filtration before use. Pyrazoline and pyridine are heterocyclic and the others are homocyclic aromatic compounds. Screw-cap tubes were placed at room temperature (20-25°C) under light and incubated for 6 weeks. Growth was measured turbidimetrically in terms of cell density at 660 nm with a Labomed model spectronic 22.

Results and Discussion

In this study, 6 strains were isolated and characterized according to their morphology, pigmentation and photoassimilated substrates. Some of the physiological characteristics of the culture grown under anaerobic light conditions are given in table 1.

Table 1. Some differentiation characters of *R. palustris* strains[a].

Strains[b]	Aerobic dark growth	Vitamins[c] required	Glucose[d] utilization	Citrate[d] utilization	Fructose[d] utilization	Mannitol[d] utilization
1	+	-	-	+	+	+
2	+	+	-	-	+	-
3	-	-	-	+	-	-
4	+	-	+	+	+	-
5	+	-	+	+	+	+
6	+	-	+	+	+	+

[a]+: utilized, -: not utilized

[b]All of the strains utilized succinate, lactate, pyruvate, benzoate, ethanol, malate, glycerol, casamino acid.

[c]Test was performed in AT medium without vitamin mixture and yeast extract.

[d]Test was performed in AT medium withoud acetate and yeast extract.

All of the *R. palustris* strains were tested for utilization of homocyclic and heterocyclic aromatic compounds. As can be seen in table 2, all strains grew photoheterotrophically on benzoate as carbon soucres. These results are supported by other researchers. Thus, *R. blastica, Rhodospirillum rubrum* and similarly *Rhodomicrobium vannielli* (9), *R. palustris* (4), *Rhodocyclus purpureus* (10), *Rhodospirillum fulvum* (11), *Rhodobacter capsulata* (12), and *R. gelatinosa* (13) are anoxygenic phototrophic bacteria known to be capable of benzoate catabolism.

All the tested *R. palustris* strains degraded hippurate, benzyl alcohol, 2-amino benzoic acid and phloroglucinol, but benzyl alcohol was degraded more effectively by strains 1, 3 and 4 than benzoate (Table 2). Strain 5 showed good growth on benzoate. This strain exhibited limited growth on benzyl alcohol. Photocatabolism of benzyl alcohol by photosynthetic bacteria is not a common characteristic, and has been reported only for *Rhodomicrobium vannielii* (9), *R. palustris, R. blastica, Rhodobacter capsulata* and *Rhodospirillum rubrum* (12). It is thus possible that the *R. palustris* strains are ecologically significant as consumers of benzyl alcohol in nature.

Strain 4 exhibited limited growth on AT medium without acetate and yeast extract (A_{660}:0.2) (control[b], Table 2). Photoautotrophc growth of *Rhodopseudomonas* species has also been reported by Holt (5). The growth density on phloroglucinol, 2-amino benzoate and control[b] was the same. The strain exhibited decreased growth with regard to control[b] catechol (A_{660}:0.1) and phenylacetate (A_{660}:0.1) and degraded benzoate (A_{660}:0.4), hippurate (A_{660}:0.4) and benzyl alcohol (A_{660}:0.7).

Table 2. Utilization of Aromatic Compounds for Photoheterotrophic Growth of Different Strains of *R. palustris*[a].

Strain Numbers:	1	2	3	4	5	6
Control[b]	-	-	-	++	-	-
Control[c]	+++	+++	+++	+++	++	++
Benzoate	++	+++	++	++	++++	++
Hippurate	+++	+++	+++	++	++	++
Benzyl alcohol	+++	++	+++	+++	+	-
2-Amino benzoic acid	++	+	++	++	++	++
Phloroglucinol	++	++	++	++	+	+
Phenyl acetic acid	+	+	-	+	+	+
Catechol	+	-	-	+	+	-
Pyridine	+	-	+	-	+	-
Pyrazolin	+	-	-	-	-	-
Phenol	-	-	-	-	-	-
Cinnamic acid	-	-	-	-	-	-

[a]: Optical density at 660 nm

[b]: AT medium omitted acetate and yeast extract

[c]: AT medium including acetate and yeast extract

+: 0.1-0.06, ++: 0.2-0.4, +++: 0.5-0.7, ++++: 0.8-0.9 -: 0.05 and less (no growth)

The *R. palustris* strains exhibited limited growth on phenylacetic acid, catechol, pyridine and pyrazoline. Biodegradation of 2-amino benzoate, phloroglucinol, catechol, pyridine and pyrazoline by *R. palustris* strains have not been reported. Sasikala et al. (14) reported that *R. palustris* strains could degrade pyrazinoic acid and pyrazine but that pyrazoline degradation not detected. It was shown that *R. gelatinosa* grew anaerobically under light on phloroglucinol (3), but in this report, all tested *R. palustris* strains degraded phloroglucinol. None of the strains metabolized cinnamic acid, whereas biodegradation of cinnamic acid by *R. palustris* strains has been reported (15).

In summary, it is clear that the purple nonsulfur bacterium *R. palustris* is capable of photoheterotrophic (anaerobic) growth on both homocyclic and heterocyclic aromatic compounds. In this report we have shown that *R. palustris* can also utilize 2-amino benzoate, phloroglucinol, catechol, pyridine and pyrazoline.

Wastewaters held in lagoons which are open to sunglight and which receive high loadings of organic matter, such as pulp mill effluents, food manufacturing waste streams, and detergent wastes, often support copious mats of phototrophic bacteria. In these environments, complex mixtures of aromatic substrates may be important subsrates for phototrophic growth. *R. palustris* may contribute to the removal of aromatic substrates under these conditions.

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