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Properties of the *Rhodopseudomonas palustris* Strains Isolated From an Alkaline Lake in Turkey

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Abstract: 31 photoheterotrophic bacteria were isolated from Lake Akşehir sediment. Identification of the strains was based on morphological properties, motility and pigment composition, and also physiological properties such as carbon utilization and ability to respire anaerobically and aerobically in the dark. All strains were identified as *Rhodopseudomonas palustris*, but some strains were distinct from *R. palustris* with regard to vitamin requirement, malate utilization and aerobic growth in the dark. Until now, *R. palustris* had not been reported from an alkaline lake, and the strains growing alkaline pH (9) could be new strains of *R. palustris.*

Key Words: *Rhodopseudomonas palustris,* isolation, identification, Alkaline Lake.

Türkiye'deki Bir Alkali Gölden İzole Edilen *Rhodopseudomonas palustris* Suşlarının Özellikleri

Özet: Akşehir gölünden fotoheterotrofik bakteriler izole edilmiştir. Elde edilen 31 izolat, morfolojik özellikleri, hareketlilikleri, oluşturdukları pigmentleri, karbon kaynağı olarak kullandıkları substratlar ve karanlıkta aerobik gelişme özelliklerine göre identifiye edildiğinde, bu suşların *Rhodopseudomonas palustris* türüne ait olduğu belirlenmiştir. Ancak izole edilen bazı suşların, vitamin ihtiyacı, mannitol kullanımı ve karanlıkta aerobik gelişme yönünden *R. palustris*'den farklı özellikler gösterdiği tespit edilmiştir. Bu konuda zamanımıza kadar yapılan çalışmalarda, *R. palustris* şuşlarının alkali göllerden izole edilmediği görülmüştür. Alkali koşullarda (pH: 9) gelişen bu türlerin *R. palustris*'in yeni suşları olabileceği kanısına varılmıştır.

Anahtar Sözcükler: *Rhodopseudomonas palustris,* izolasyon, identifikasyon, alkali göl.

Introduction

The purple nonsulfur bacteria (Rhodospirillaceae) are the best studied and most diverse group of the phototrophic bacteria. All species grow best as anaerobic photoorganotrophs and have the capacity to grow also as facultatively microaerophilic to aerobic chemoorganotrophs (1, 2).

According to recent taxonomic studies, the anoxygenic phototrophic bacteria have been presented in seven subgroups that can be differentiated by the characteristics. The genera *Rhodopseudomonas* has been presented in subgroup 3 (Purple nonsulfur bacteria), and eight

species *(R. palustris, R. viridis, R. sulfoviridis, R. acidophila, R. blastica, R. julia, R. marina* and *R. rutila)* have been presented (3, 4). In addition, a new species, *R. rosea*, has been isolated by Jansen and Harfoot (5).

R. palustris is a very common species of the Purple nonsulfur bacteria in nature. A selective advantage for this species would be the use of benzoate as carbon source in the enrichment cultures. A number of aromatic compounds have also been used for growth of the *R. palustris* strains, which is of particular interest in terms of environmental pollution, with special respect to the production of industrially important organic compounds (6, 7).

R. palustris strains have been used in sewage treatment processes, biomass and molecular hydrogen production (7, 8).

R. palustris strains found in alkaline lakes have not yet been investigated. The pH range for growth of pure cultures of *R. palustris* is generally between pH 5.5 and 8.5 (3, 4, 9). The aim of the present study was to describe the properties of new *R. palustris* strains isolated from anoxic parts of the Akşehir Lake–Turkey.

Materials and Methods

Field samples: Three samples (1.5 m deep) were taken from the mud flat of the Akşehir Lake in Konya, Turkey, during 1993. The pH values of the samples ranged from 8.5 to 9.5.

Enrichment, isolation and purificiation: The Winogradsky column technique was used for enrichment of phototrophic bacteria in the samples, as described by van Niel (10). Columns were incubated until red growth on the walls was observed. Red growth zones were inoculated to enrichment media containing 0.01% w/v benzoate as substrates (11). Screw–cap bottles (30 ml) were used as culture vessels. The incubation temperature was about 20–25°C, and the light intensity was 50 ft–c from a 75 w tungsten lamp. After incubation, an agar shake dilution series (1.0% agar) with AT medium was set up with 1 drop of the growing cultures (11). Single red and brown colonies were present in the last positive tube of the agar shake dilution series. These colonies were transferred to the AT medium containing (g/l): $NaHCO_3$, 3; NH_4Cl, 1; $CaCl_2x2H_2O$, 0.1; NaCl, 1; KH_2PO_4, 1; $MgCl_2x6H_2O$, 0.5; Na–acetate, 1; Yeast extract, 0.1; Trace element solution SLA, 1 ml and vitamin solution VA, lml/l (3). The pH was adjusted to 9 by adding sterile NaOH. Pure cultures were obtained by repeated application of the agar–shake culture method, with AT medium.

Characterization: Isolates were characterized by morphology, photoassimilated substrates and vitamin requirement. Gram staining was performed by the Burke method (12). Cell size was measured and motility was determined by the hanging drop method and on the semisolid medium (AT medium containing 0.4% agar) (13). For photoassimilation experiments, sodium acetate–omitted AT medium was supplemented individually with the following compounds (final concentration, 0.2% w or v/v): glucose, fructose, glycerol, ethanol, mannitol, succinate, pyruvate, benzoate, lactate, citrate, malate, tartrate and casamino acids. Gelatin hydrolysis was tested by replacing the agar with 12.0% gelatin in the AT medium. Ability to grow in the dark

under aerobic conditions was tested in the AT medium (1.5% agar) in petri dishes. Whole–cell pigment scans were performed with cell pellets resuspended in sucrose (14). Scans were performed on a Hitachi model UV–visible recording spectrophotometer (200–20).

Results and Discussion

In this study, 31 strains were isolated and characterized according to their morphology, pigmentation and photoassimilated substrates. The cells of these strains were rod–shaped, 0.5 to 0.9 µm wide and 2.0 to 5.0 µm long. The cells of some strains were up to 10 µm in length (Fig. 1a). All strains showed polar growth and multiplied by budding. All of the strains were Gram negative and motile. Red cell suspensions were formed by pure cultures of strains grown under anaerobic photoheterotrophic conditions. Red–brown cell suspensions were formed by pure cultures of three strains (29, 30 and 31) under anaerobic conditions. These strains were ovoid, 0.4–0.7 µm in diameter (Fig. 1b).

Absorption spectra of living cell suspensions of all tested species show the maxima of bacteriochlorophyll a, and resembled the spectrum of the three species shown in Fig. 2. Absorption maxima at 854, 800, 587 and 373 nm for Fig 2a; 851, 800, 589 and 376 nm for

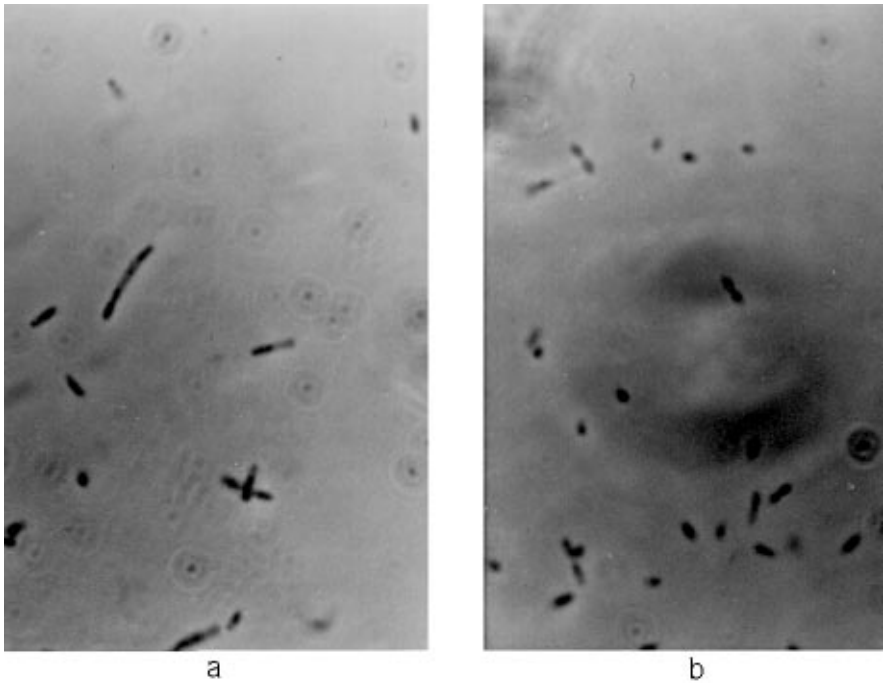

Figure 1. Phase–contrast photomicrograph of cells of strains 8 (a) and 30 (b) (x1000).

Fig 2 b; 861, 792, 584 and 391–358 nm for Fig 2 c pertain to bacteriochlorophyll a. Maxima corresponding to carotenoid peaks are detected at 501 nm for Fig 2a; 544, 510, 477 and 406 nm for Fig 2b; 504, 489, 468 and 421 nm for Fig 2c, but are poorly resolved.

These strains were grown on AT medium (omitted acetate and yeast extract) anaerobically in the light on the organic compounds. All of the strains photoassimilated acetate, succinate, lactate, pyruvate, benzoate, glycerol and casamino acids. Gelatin was not hydrolyzed. The strains were characterized by the inability to utilize glucose, fructose, citrate, mannitol, malate, ethanol and vitamin requirement for growth. Tartrate was not photoassimilated by the strains. The results of tests for photoheterotrophic growth of these strains on organic carbon sources and vitamins are summarized in Table 1.

R. palustris is able to utilize benzoate, a feature which has been used to distinguish it from other species of Purple nonsulfur bacteria. Only three species of Purple nonsulfur bacteria, *Rhodocyclus purpureus, Rhodospirillum fulvum* and *R. palustris,* are known to utilize benzoate. Citrate, glucose, fructose, mannitol and ethanol are used by some strains of *R. palustris* (3, 4, 9).

As a result of this study, all isolates were identified as *R. palustris,* but some strains (15, 16, 27, 28 and 31) showed different properties from *R. palustris.*

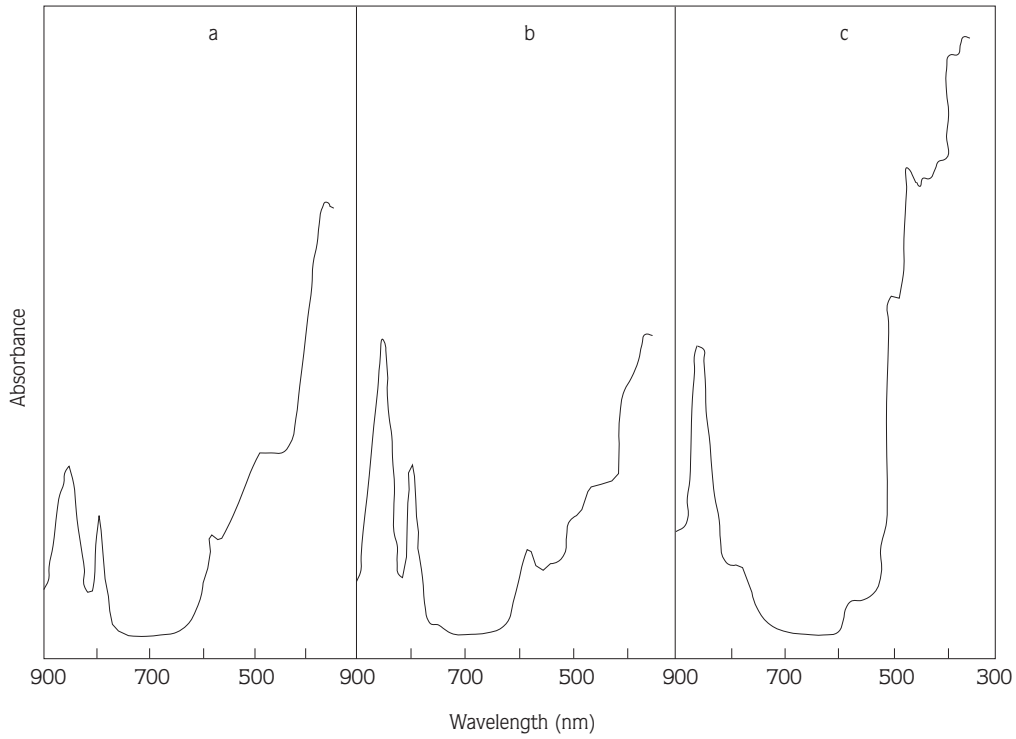

Figure 2. In vivo absorption spectrum of strain 28 (a), strain 29 (b) and strain 31 (c).

Table 1. Characteristic features of isolated *R. palustris* species[a].

Strains[b]	Aerobic dark growth	Vitamins[c] required	Malate[d] utilized	Ethanol[d] utilized	Glucose[d] utilized	Citrate[d] utilized	Fructose[d] utilized	Mannitol[d] utilized
1	+	–	+	+	–	–	+	–
2	+	–	+	+	–	–	+	–
3	+	–	+	+	–	–	+	–
4	+	–	+	+	–	–	+	–
5	+	–	+	+	–	–	+	–
6	+	–	+	+	–	–	+	–
7	+	–	+	+	–	–	+	–
8	+	–	+	+	–	–	+	–
9	+	–	+	+	–	–	+	–
10	+	–	+	+	–	–	+	–
11	+	–	+	+	–	–	+	–
12	+	–	+	+	–	–	+	–
13	+	–	+	+	–	–	+	–
14	+	–	+	+	–	–	+	+
15	+	+	+	+	–	+	+	–
16	+	+	+	+	–	+	+	–
17	+	–	+	+	+	+	+	–
18	+	–	+	+	+	+	+	–
19	+	–	+	+	–	+	+	+
20	+	–	+	+	–	+	+	–
21	+	–	+	+	–	+	+	–
22	+	–	+	+	–	+	+	–
23	+	–	+	+	–	+	+	–
24	+	–	+	+	–	+	+	–
25	+	–	+	+	–	+	+	–
26	+	–	+	+	–	+	+	–
27	+	+	+	+	–	–	+	–
28	–	–	+	+	–	+	–	–
29	+	–	+	+	+	+	+	+
30	+	–	+	+	+	+	+	+
31	+	–	–	–	+	+	+	+

[a] +, utilized; –, not utilized.

[b] All of the strains utilized acetate, succinate, lactate, pyruvate, benzoate, glycerol, casamino acids.

[c] Tests were performed in AT medium–omitted vitamin mixture and yeast extract.

[d] Tests were performed in AT medium–omitted acetate and yeast extract.

R. palustris strains required p–aminobenzoate and biotin as growth factors (4), but strains 15, 16 and 27 grew well AT medium without yeast extract and vitamin solution. These strains did not require growth factors.

Strain 28 was not able to grow in the dark under aerobic conditions on the AT solid medium, but *R. palustris* strains did not show this feature.

All the *R. palustris* strains were able to utilize malate, but No: 31 did not utilized it.

Growth of *R. palustris* strains occured pH 6.9 (pH range 5.5–8.5) (3, 4, 9). Only *Chloroflexus aurantiacus* (optimum at pH 7.6–8.4) and *Ectothiorhodospira* species (pH optima between 7.5 and 9.5) were grown at high pH vaules (7, 8). None of the Purple nonsulfur bcateria species grew in alkaline pH. All of the isolated strains in this study were grown pH 9. The strains could be new strains of *R. palustris.*

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