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Anoxygenic Degradation of Aromatic Substances by *Rhodopseudomonas palustris*

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Abstract. Three strains of the phototrophic purple nonsulfur bacterium *Rhodopseudomonas palustris* were isolated from different environments and were evaluated for their aromatic degradative potential under phototrophic conditions. All three strains (PFR1, PNR4, and MRL1) utilized benzoate, 4-hydroxybenzoate, 4-aminobenzoate, 4-aminophenol, cinnamate, ferulate, phloroglucinol, and 4-dimethylaminobenzaldehyde in the absence of exogenous CO_2. 4-Aminobenzoate and 4-aminophenol served as a carbon and nitrogen source for all the three strains. Utilization of 4-aminophenol was enhanced in the presence of 4-hydroxybenzoate. Salicylate was utilized by PFR1 and MRL1 strains, and phenol was utilized by the MRL1 strain only in the presence of exogenous CO_2.

Chemical and petroleum industries discharge a variety of toxic organic wastes, including many aromatic substances, which deplete oxygen in the receiving stream and result in highly anoxygenic conditions. Hence, many studies have been devoted to understanding the fate of aromatic substances under anoxygenic conditions [1, 3, 5, 15]. Phototrophic purple nonsulfur bacteria (PNSB) have been shown to efficiently degrade toxic aromatic substances, utilizing them as sole carbon sources under anoxygenic conditions, deriving energy from light for growth [4–7, 10, 13, 16, 20]. *Rhodopseudomonas palustris* has been acknowledged as the most versatile of all PNSB with respect to aromatic degradation [6, 10, 20]. This paper describes the evaluation of three strains of *R. palustris* isolated from different environments for anoxygenic aromatic degradation.

Materials and Methods

Bacterial strains. Three isolates of PNSB designated as PFR1, PNR4, and MRL1 were isolated from the samples collected from paddy field soil near Madras, an industrial wastewater disposal stream in Nagpur, and a petrochemical industry wastewater treatment plant near Madras, respectively, through an enrichment technique that used 4-hydroxybenzoate as the sole carbon source. Isolates were identified as different strains of *R. palustris* following Truper and Pfenning's procedure [17] and Bergey's *Manual of Systematic Bacteriology* [9].

Media. Composition (in wt/vol) of basal salts medium (BSM) was as follows: K_2HPO_4, 0.085%; KH_2PO_4, 0.015%; NH_4Cl, 0.1%; $MgCl_2 \cdot 6H_2O$, 0.02%; $CaCl_2 \cdot 2H_2O$, 0.02%; $Na_2S_2O_3 \cdot 5H_2O$, 0.004%; trace elements solution SLA [8], 1 ml/L. Yeast extract (100 mg/L) was added to the medium to suffice for vitamin requirements. Aromatic substances at 2 mM final concentration, unless otherwise mentioned, served as the sole source of carbon. Sodium bicarbonate was not added to the medium. Ammonium chloride was omitted when aminoaromatic substances were included in the BSM. The pH of the medium prior to inoculation was 7.2 ± 0.05.

Growth conditions. All cultures were grown photoheterotrophically under anaerobic light conditions in 30 ml screw-cap tubes and transparent glass bottles. Cells grown in BSM supplemented with sodium acetate (0.2% wt/vol) were harvested by centrifugation at 10,000 *g* (for 20 min) at 4°C, washed, and suspended in phosphate buffer to a final density of 30–40 μg cells dry weight/ml, and served as the inoculum. Inocula (5% vol/vol) were added to culture vessels containing BSM with 2 m*M* aromatic substance. Culture vessels were incubated at room temperature (30° ± 5°C) under continuous incandescent illumination of 1500 lx intensity.

Growth measurement. Growth was measured in terms of increase in both dry weight [12] and protein [11]. Total cell protein content was estimated after extraction by boiling in 1 N NaOH for 20 min.

Quantitation and catabolism of aromatic substances. Concentrations of all aromatic substances were estimated from their UV absorbance in growth medium by suitably diluting the culture filtrate in 50 mM phosphate buffer following the establishment of standard curves relating concentration to UV absorbance [20]. At different time intervals during growth, the utilization of aromatic substances was monitored by following the UV absorption spectrum of culture filtrates between 200 and 400 nm in a Hitachi 150-20 model spectrophotometer.

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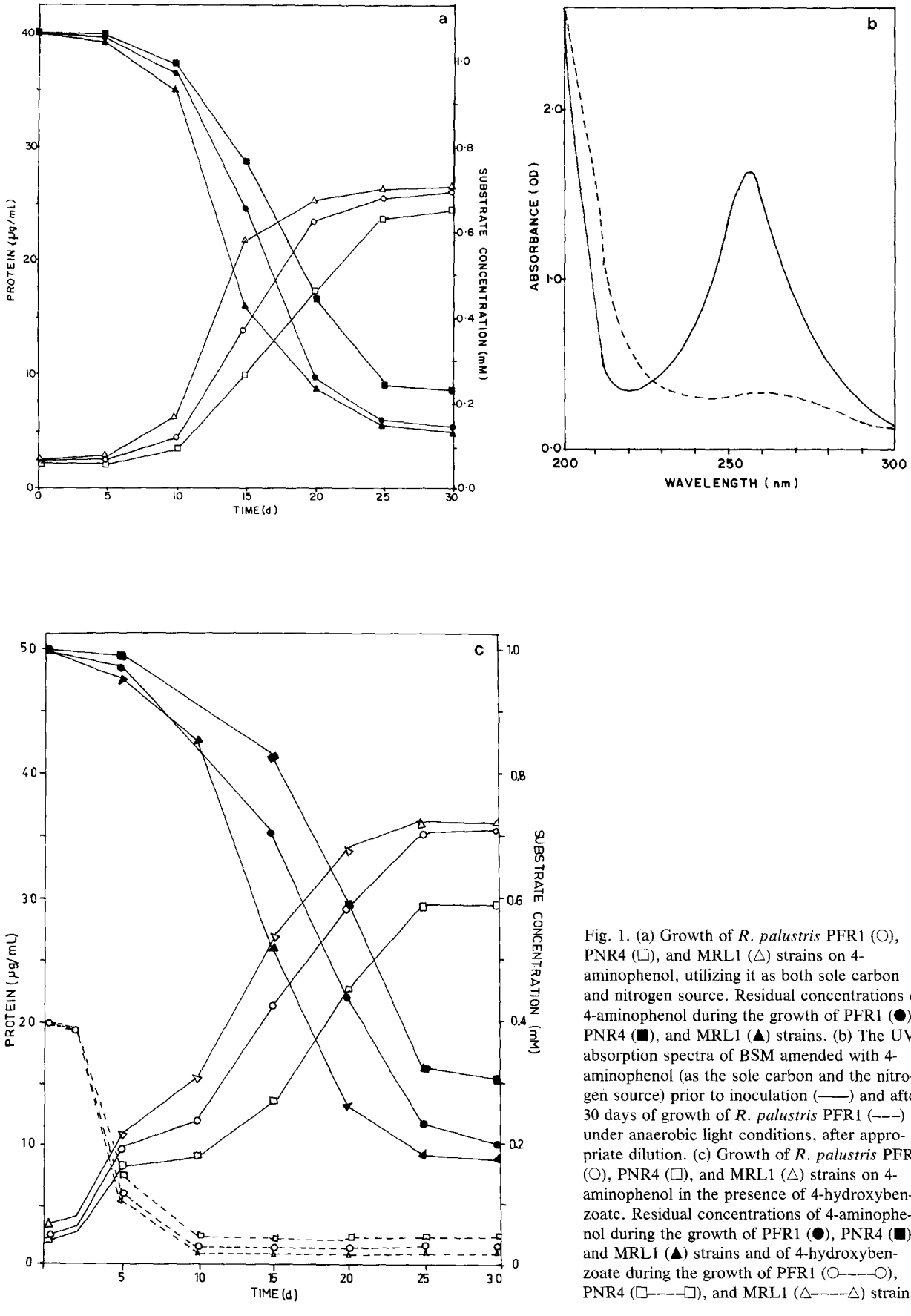

Fig. 1. (a) Growth of *R. palustris* PFR1 (○), PNR4 (□), and MRL1 (△) strains on 4-aminophenol, utilizing it as both sole carbon and nitrogen source. Residual concentrations of 4-aminophenol during the growth of PFR1 (●), PNR4 (■), and MRL1 (▲) strains. (b) The UV absorption spectra of BSM amended with 4-aminophenol (as the sole carbon and the nitrogen source) prior to inoculation (——) and after 30 days of growth of *R. palustris* PFR1 (---) under anaerobic light conditions, after appropriate dilution. (c) Growth of *R. palustris* PFR1 (○), PNR4 (□), and MRL1 (△) strains on 4-aminophenol in the presence of 4-hydroxybenzoate. Residual concentrations of 4-aminophenol during the growth of PFR1 (●), PNR4 (■), and MRL1 (▲) strains and of 4-hydroxybenzoate during the growth of PFR1 (○----○), PNR4 (□----□), and MRL1 (△----△) strains.

Results and Discussion

Three strains of *R. palustris* were isolated through an enrichment culture technique and were found to grow on aromatic substances that have not been reported previously. The results of various aromatic substances tested for anoxygenic aromatic degradation by the strains PFR1, PNR4, and MRL1 are presented in Table 1. This is the first report to show the utilization of phloroglucinol, salicylate, phenol, 4-aminophenol, 4-aminobenzoate, and 4-dimethylaminobenzaldehyde by *R. palustris*. Utilization of aminoaromatic substances 4-aminophenol (Fig. 1a and b) and 4-aminobenzoate (Fig. 2a and 2b) as sole carbon as well as nitrogen sources is an interesting metabolic capability of all three strains of *R. palustris* studied. 4-Dimethylaminobenzaldehyde was utilized by all three strains as a carbon source only, and not as a nitrogen source. Addition of 0.4 mM 4-hydroxybenzoate to a 4-aminophenol-supplemented medium increased the rate of utilization of 4-aminophenol (Fig. 1c). A 4-aminophenol concentration above 1 mM was inhibitory to the cultures.

Table 1. Utilization of aromatic substances for photoheterotrophic growth of strains of *R. palustris*

Growth substrate (mM)	PFR1	PNR4	MRL1
4-Aminobenzoate (2)	++	++	++
4-Aminophenol (1)	++	++	+
Aniline (1)	–	–	–
Benzoate (2)	+++++	+++++	+++++
Cinnamate (2)	+++++	+++++	+++++
4-Cresol (1)	–	–	–
4-Dimethylamino-benzaldehyde (2)	++	+	+++
2-4,Dimethylphenol (1)	–	–	–
Ferulate (2)	+++	+++	++
2-Hydroxybenzoate (2) [salicylate]	+	–	++
3-Hydroxybenzoate (2)	+	–	+
4-Hydroxybenzoate (2)	+++++	+++++	+++++
Phenol (1)	–	–	++
Phloroglucinol (2)	+++++	+++++	++++

The number of +s corresponds to increase in total cellular protein after 20 days of incubation under phototrophic conditions. +++++, >100 μg/ml; ++++, 75–100 μg/ml; +++, 50–75 μg/ml; ++, 25–50 μg/ml; +, 15–25 μg/ml; –, no growth.

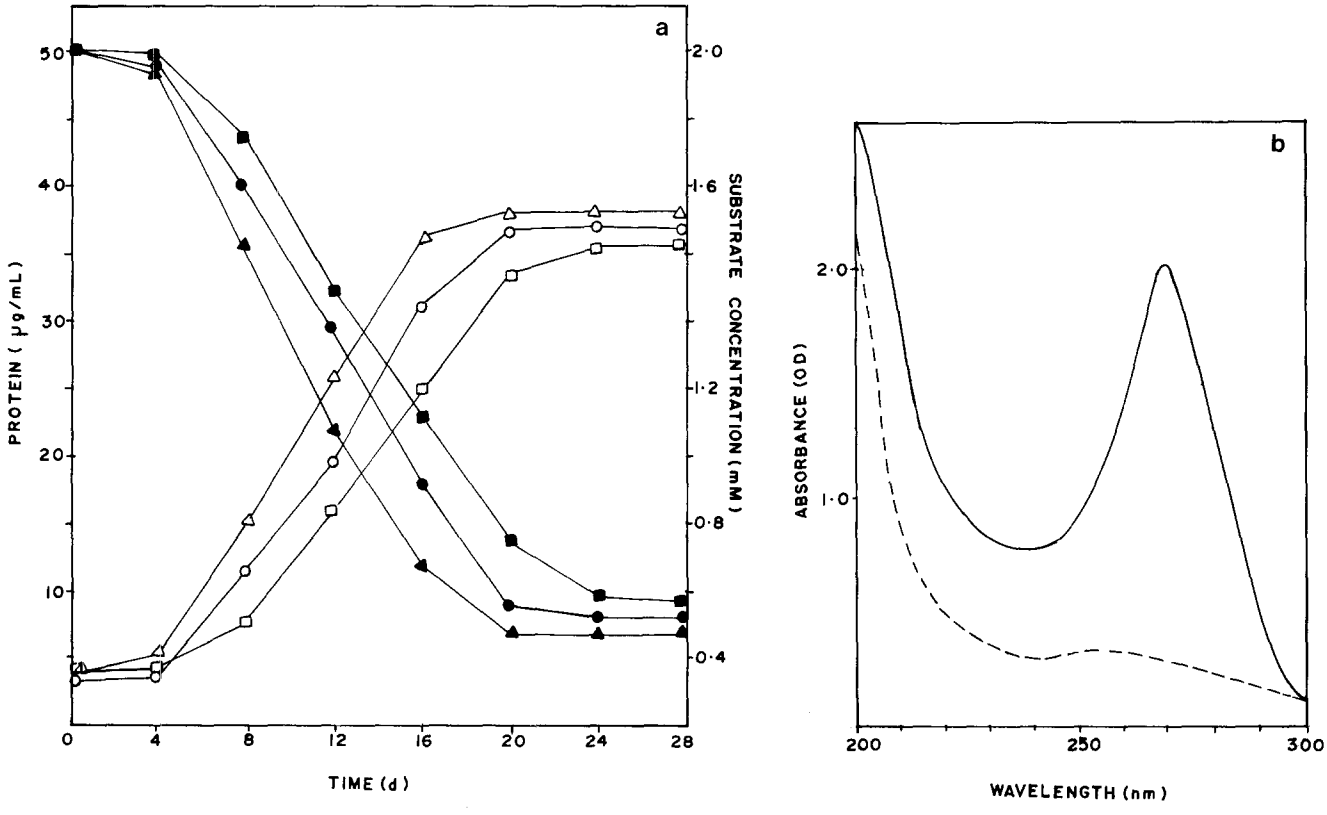

Fig. 2. (a) Growth of *R. palustris* PFR1 (○), PNR4 (□), and MRL1 (△) strains on 4-aminobenzoate utilizing it as both sole carbon and nitrogen source. Residual concentrations of 4-aminobenzoate during the growth of PFR1 (●), PNR4 (■), and MRL1 (▲) strains. (b) The UV absorption spectra of BSM amended with 4-aminobenzoate (as the sole carbon and nitrogen source) prior to inoculation (——) and after 30 days of growth of *R. palustris* PFR1 (– – –) under anaerobic light conditions, after appropriate dilution.

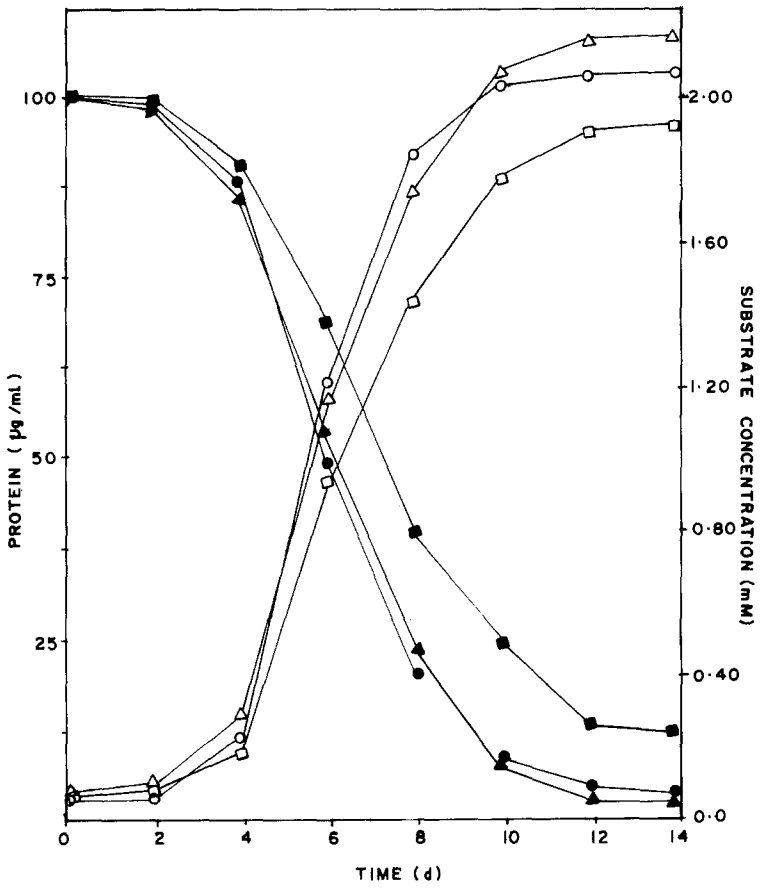

Fig. 3. Growth of *R. palustris* PFR1 (○), PNR4 (□), and MRL1 (△) strains on phloroglucinol. Residual concentrations of phloroglucinol during the growth of PFR1 (●), PNR4 (■), and MRL1 (▲) strains.

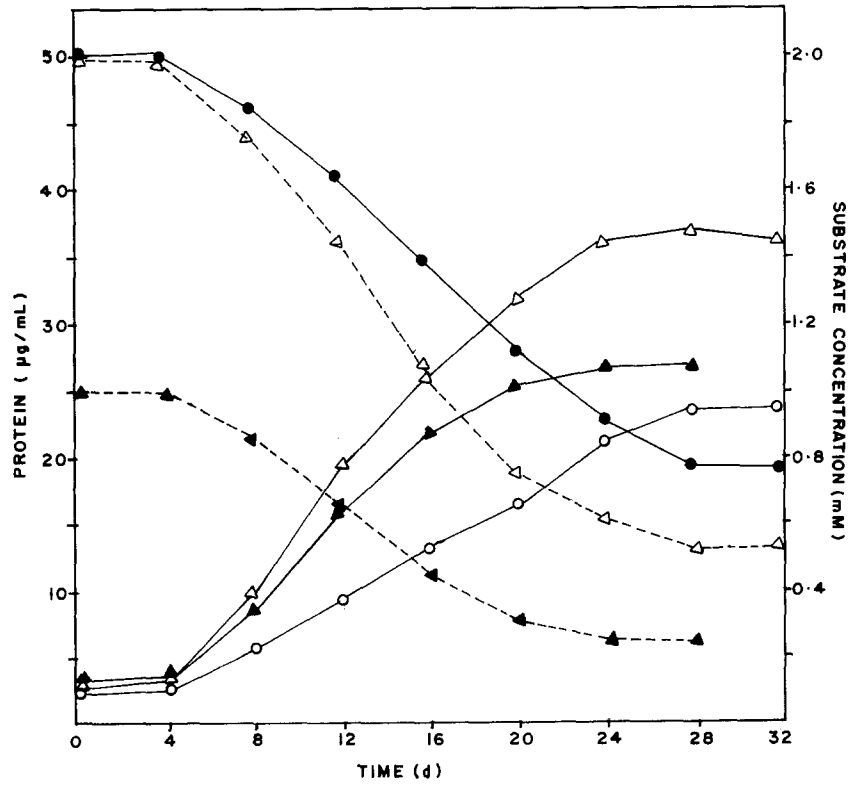

Fig. 4. Growth of *R. palustris* PFR1 (○) and MRL1 (△) strains on salicylate. Growth of MRL1 (▲) strain on phenol. Residual concentrations of salicylate during the growth of PFR1 (●) and MRL1 (△---△) strains, and of phenol during the growth of MRL1 (▲----▲) strain.

There has been no report on the utilization of 4-aminophenol and 4-aminobenzoate as sole carbon as well as nitrogen sources. However, there are reports on 2-aminobenzoate (anthranilic acid) degradation by denitrifying *Pseudomonas* sp. into NH_4^+ and CO_2 with simultaneous reduction of NO_3^- to NO_2^- to N_2 gas [2, 21]. NH_4Cl inhibited the utilization of aminoaromatic substances by all three strains. Probably this might be the reason for lack of growth on 4-aminobenzoate in an earlier study [6].

Phloroglucinol supported good growth of all three strains of *R. palustris,* as shown in Fig. 3. It was reported earlier [19] that phloroglucinol is catabolized via dihydrophloroglucinol to acetate in *R. gelatinosa,* a mechanism similar to that found in rumen bacteria [14, 18].

Salicylate supported the growth of PFR1 and MRL1 strains (Fig. 4), while phenol supported the growth of the MRL1 strain only. Phenol above 1 mM concentration completely inhibited the growth of the MRL1 strain.

All three strains did not require exogenous CO_2 for growth on tested aromatic substances (except for phenol utilization by the MRL1 strain), unlike the results mentioned in other reports [6, 13, 20].

The results of the present study confirm the potential of *R. palustris* to degrade several aromatic substances under the laboratory conditions as pure cultures, and indicate the possible role of PNSB in detoxifying and mineralizing toxic aromatic and other organic substances in the natural environment, where diverse physiochemical conditions and organisms are present. The utilization of aminoaromatic substances as sole carbon as well as nitrogen sources by all three strains of *R. palustris* reveals an important metabolic capability of the bacterium that should be further investigated in detail. The anoxygenic aromatic degradation potential of *R. palustris* could be effectively used for the development of an effluent treatment system for detoxifying industrial wastewater by use of sunlight as the energy source.

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