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Light-Dependent Degradation of Nitrophenols by the Phototrophic Bacterium *Rhodobacter capsulatus* E1F1

R. BLASCO AND F. CASTILLO*

Departamento de Bioquímica, Biología Molecular y Fisiología, Facultad de Ciencias, Universidad de Córdoba, Avenida San Alberto Magno s/n, 14071 Cordoba, Spain

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***Rhodobacter capsulatus* E1F1, a phototrophic purple nonsulfur bacterium capable of photoassimilating nitrate or nitrite, grew phototrophically in the presence of mono- and dinitrophenols with acetate as a carbon source, the highest growth levels being obtained under microaerobic conditions. Utilization of 2,4-dinitrophenol was strictly light dependent, was inhibited by O_2 and by ammonium, and took place with the simultaneous and stoichiometric production of 2-amino-4-nitrophenol, which accumulated in the medium and was poorly used for further growth in anaerobiosis. Metabolism of mononitrophenols was also light dependent but was activated by O_2 and by ammonium. Metabolism of nitrophenols seemed to depend on inducible systems which were repressed in nitrogen-starved cells. Induction of the in vivo 2,4-dinitrophenol reducing system was strongly inhibited by chloramphenicol.**

Chemical industries synthesize a great variety of nitroaromatic derivatives for use as components of manufactured chemicals throughout the world. These compounds, which also result from natural processes in the biosphere, are common pollutants in several ecosystems in developed countries (13, 20). Nitroaromatic pesticides such as parathion, widely used in agricultural practices, are degraded in soils by microorganisms (21, 24), and several parathion hydrolases have been purified from gram-negative bacteria (20). As a result, there exist many studies on the microbial degradation of nitroaromatics, especially with *Pseudomonas* strains. These bacteria can use nitrophenols as well as nitrobenzoates (1), and a nitrophenol oxygenase that transforms 2-nitrophenol (2-NP) to catechol and nitrite was recently purified (27). On the other hand, reductive pathways related to nitrophenol degradation have also been described (8), and several nitroreductases have been purified from some microorganisms (14). Degradation of 2-NP by *Pseudomonas putida* B2 is influenced by *p*-substituents, which are incapable of inducing nitrophenol oxygenase (28). In addition, secondary substrate utilization enhances biodegradation of 4-nitrophenol (4-NP) (22). A recent study on the capacity of immobilized *Pseudomonas* spp. to metabolize 4-NP has also been reported (11).

Anaerobic phototrophic bacteria are also capable of breaking down aromatic compounds such as benzoate (5, 9, 10) and benzoate derivatives (26). The involvement of coenzyme A cofactors and a coenzyme A ligase in the anaerobic degradation of benzoate has been recently reported (7, 17). However, nothing is known about the degradation of nitroaromatic compounds by phototrophic bacteria. In this work, we present a light-dependent microaerobic degradation of nitrophenols by the phototrophic nitrate-assimilating bacterium *Rhodobacter capsulatus* E1F1. A novel anaerobic photoreduction of 2,4-dinitrophenol (2,4-DNP) by *R. capsulatus* E1F1 cells is also described.

* Corresponding author.

MATERIALS AND METHODS

Chemicals. The nitrophenols 2-NP, 3-NP, 4-NP, and 2,4-DNP were purchased from Sigma Chemical Co. (St. Louis, Mo.). 2-Amino-4-nitrophenol (ANP) was purchased from Fluka Chemie AG (Buchs, Switzerland). Acetic acid, methanol, and triethylamine were purchased from Merck (Darmstadt, Germany). The remaining chemicals were obtained from Panreac (Barcelona, Spain).

Organisms and culture conditions. *R. capsulatus* E1F1 (a gift from W. G. Zumft, Karlsruhe University, Karlsruhe, Germany) was cultured in RCV medium (25) containing the following (per liter): 200 mg of $MgSO_4 \cdot 7H_2O$, 75 mg of $CaCl_2 \cdot 2H_2O$, 12 mg of $FeSO_4 \cdot 7H_2O$, 20 mg of EDTA, 1 mg of thiamine pyrophosphate, 10 mmol of phosphate buffer (pH 6.8), and 1 ml of a solution containing trace elements [in 250 ml, 400 mg of $MnSO_4 \cdot H_2O$, 700 mg of H_3BO_3, 10 mg of $Cu(NO_3)_2 \cdot 3H_2O$, 60 mg of $ZnSO_4 \cdot 7H_2O$, and 190 mg of $Na_2MoO_4 \cdot 2H_2O$]. Sodium acetate (10 mM) and nitrophenols (0.1 mM) were used as carbon and nitrogen sources, respectively. Where indicated, 6 mM NH_4Cl was used as a supplementary nitrogen source. In the absence of added nitrogen, dissolved N_2 (up to 16 mg/liter at 30°C under air) could contribute to bacterial growth.

Rhodobacter sphaeroides DSM 158 and *Rhodopseudomonas palustris* DSM 123 were cultured in the same RCV medium supplemented with vitamins (5 ml/liter of culture of a solution containing, per liter, 165 mg of thiamine, 550 mg of nicotinic acid, 0.6 mg of biotin, 100 mg of pantothenate, 160 mg of *p*-aminobenzoate, 400 mg of mesoinositol, and 40 mg of vitamin B_{12}) and trace elements (1 ml/liter of culture of a solution containing, per liter, 100 mg of $ZnSO_4 \cdot 7H_2O$, 30 mg of $MgCl_2 \cdot 4H_2O$, 300 mg of H_3BO_3, 200 mg of $CoCl_2 \cdot 6H_2O$, 10 mg of $CuCl_2 \cdot 2H_2O$, 20 mg of $NiCl_2 \cdot 6H_2O$, and 300 mg of $NaMoO_4 \cdot 2H_2O$).

Bacteria were cultured at 30°C under any of the following conditions: (i) light-anaerobiosis, which involved the use of screw-cap 500-ml Pyrex bottles completely filled with culture medium under saturating light (240 W/m^2) provided by tungsten lamps; (ii) light-microaerobiosis, which involved the use of screw-cap 500-ml Pyrex bottles partially filled with culture medium (up to one-half total volume) under saturating light (240 W/m^2) provided by tungsten lamps; (iii) dark-

aerobiosis, which involved the use of cotton-stoppered 500-ml Erlenmeyer flasks partially filled with culture medium and shaken continuously (140 rpm) in a Gallenkamp orbital incubator; or (iv) dark-anaerobiosis, which involved the use of screw-cap 500-ml Pyrex bottles filled with culture medium under dark conditions.

When necessary, the cultures were placed in 14-ml Erlenmeyer flasks capped with rubber stoppers under an Ar or N_2 atmosphere containing variable amounts of pure O_2.

The purity of the cultures was checked by plating them on solid agar media containing 1.8% Difco Bacto Agar and 0.3% yeast extract.

Analytical determinations. Nitrophenols were measured at 275 nm by using a UV detector coupled to a reverse-phase high-performance liquid chromatograph (HPLC) column (15 by 0.4 cm; Analytical-Tracer column Spherisorb ODS-2; particle diameter, 5 μm). Nitrophenols were eluted by using a mixture of methanol, H_2O, acetic acid, and triethylamine (50:50:0.6:0.008, by volume).

Light intensity was measured by using a LI-100 Dataloger photometer.

Bacterial growth was determined turbidimetrically at 680 nm. A linear relationship between A_{680} and cell dry weight values was observed up to an A_{680} of 1.0, which was equivalent to approximately 0.5 mg (dry weight) of bacteria, obtained as previously described (16). Cell growth and nitrophenol consumption experiments were repeated four to five times with less than 5% variation in the data presented.

Nitrite was measured colorimetrically at 540 nm (23).

Protein was estimated by the method of Lowry et al. (15).

Nitrogen fixation was measured in whole cells resuspended in the culture medium under saturating light (240 W/m^2) and an atmosphere of Ar, with continuous shaking (140 rpm) at 30°C. Nitrogenase activity was determined by the acetylene reduction technique in a gas chromatograph with a Porapak-N column as previously described (29).

ANP was extracted with ethyl acetate from acidified supernatants (pH 2.0) of centrifuged cultures. The organic phase was then treated with 0.1 N NaOH, and the aqueous phase was reacidified and then reextracted with ethyl acetate. This organic solution was dried at 40°C, and the compound was dissolved either in 0.1 M KOH for UV-visible spectroscopy, in deuterated acetone for nuclear magnetic resonance (NMR) studies, or in methanol for mass spectrometry.

UV-visible spectra were recorded in a Beckman DU-70 spectrophotometer, mass spectra were recorded in a Hewlett-Packard 5988 A spectrometer, and NMR spectra were obtained by use of an ACE 200 Brücker spectrometer.

RESULTS

Anaerobic degradation of nitrophenols. *R. capsulatus* E1F1 cells grew under light-anaerobiosis in the presence of acetate and 2,4-DNP (Fig. 1), but little growth and high nitrite production (>30 μM) were observed when this compound was used as a C or C-N source. The bacterium also grew phototrophically in the presence of acetate and mononitrophenols but, contrary to observations in the case of 2,4-DNP, 2-NP and 4-NP were only partially consumed under anaerobiosis whereas 3-NP showed a higher resistance to anaerobic degradation (Table 1). Bacterial phototrophic growth in the presence of 2,4-DNP with N_2 bubbling was about twofold higher than that observed under anaerobiosis with dissolved N_2, and no growth was observed under an Ar atmosphere. None of the nitrophenols used in this study was consumed under dark-anaerobiosis.

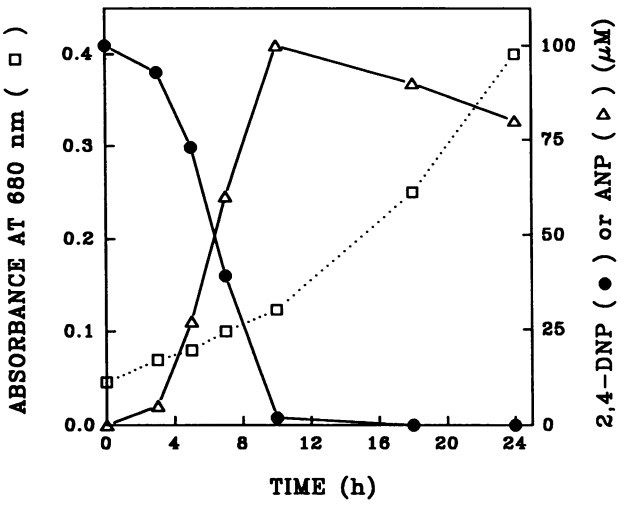

FIG. 1. Time course of 2,4-DNP consumption, excretion of ANP, and cell growth of *R. capsulatus* E1F1 cultured phototrophically with 2,4-DNP. Cells grown on acetate and 2,4-DNP were inoculated into the same medium and cultured under light-anaerobiosis. Cell growth, 2,4-DNP, and ANP were determined at the indicated times as described in Materials and Methods. Nitrite in the medium was never detected under these growth conditions.

Light-anaerobic growth of *R. capsulatus* E1F1 with nitrophenols was dependent on the concentration of these compounds in the culture medium. Bacterial growth was observed with mononitrophenol concentrations of up to 0.5 mM. At higher concentrations, a sudden drop in growth levels and nitrophenol utilization as well as an increase in nitrite excretion (60 to 70 μM) could be observed. In contrast, 2,4-DNP was totally consumed under light-anaerobic conditions at low concentrations of the compound (up to 0.2 mM). Some bacterial assimilatory processes such as N_2 fixation and nitrate assimilation were severely inhibited in vivo by nitrophenols (not shown).

Anaerobic utilization of 2,4-DNP was followed by the stoichiometric production of a nonmetabolizable intermediate which was excreted into the medium (Fig. 1 and 5). This metabolite exhibited a different retention time in HPLC,

TABLE 1. Effect of O_2 on nitrophenol consumption by *R. capsulatus* E1F1[a]

Compound	Nitrophenol consumption (%) at an O_2 pressure of:		
	0 atm[b]	0.1 atm	0.2 atm
2,4-DNP	100	95	60
2-NP	63	77	77
3-NP	8	65	65
4-NP	22	55	85

[a] The experiments were performed with cell suspensions (A_{680} = 0.8) kept under a high-purity argon atmosphere in the presence of 0.1 mM nitrophenol. Oxygen was injected into the gas phase, and, after cultures had been maintained under saturating light with continuous shaking (140 rpm) for 3 h, cell suspensions were centrifuged and the nitrophenol concentration was determined in the supernatants. Data correspond to an average of four different experiments.

[b] 1 atm = 101.29 kPa.

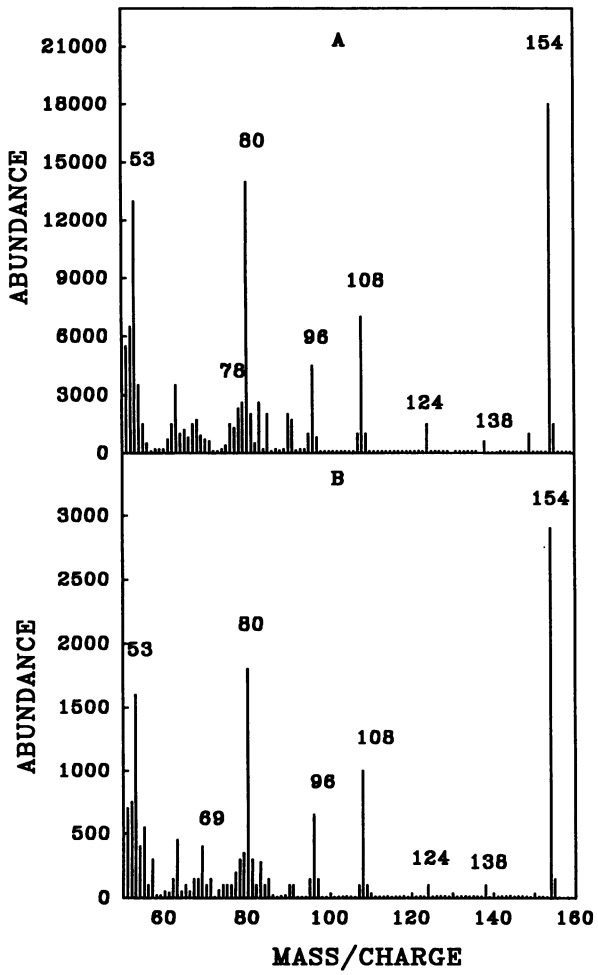

FIG. 2. Mass spectra of synthetic ANP (A) and of the intermediate (B) isolated from *R. capsulatus* E1F1 cultured with 2,4-DNP under light-anaerobiosis.

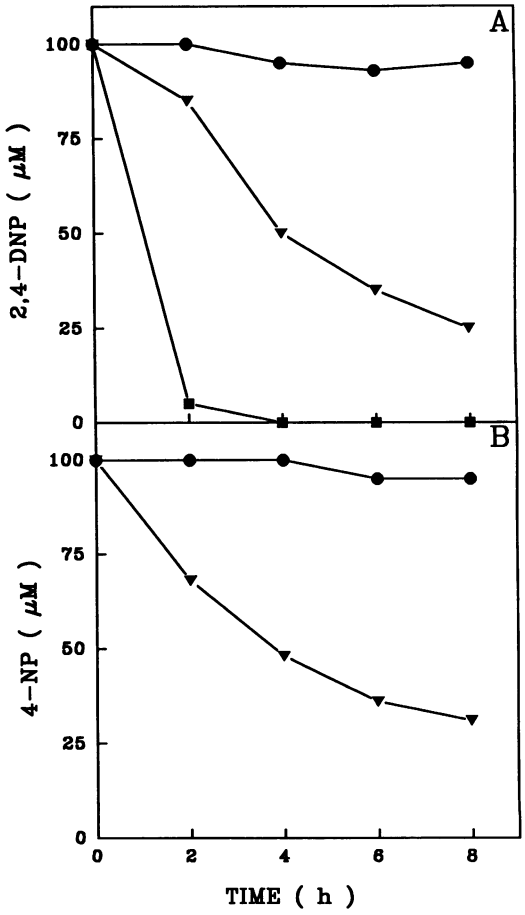

FIG. 3. Induction of 2,4-DNP- and 4-NP-degradative pathways of *R. capsulatus* E1F1 cultured in the presence of several nitrogen sources. (A) Cells cultured in the absence of fixed nitrogen (●), with 10 mM nitrate (▼), or with 0.1 mM 2,4-DNP (■) were washed and cultured under light-anaerobiosis in media containing 0.1 mM 2,4-DNP. (B) Cells cultured in the absence of fixed nitrogen (●) or with 10 mM nitrate (▼) were washed and cultured under light-microaerobiosis in media containing 0.1 mM 4-NP. The concentration of nitrophenol in the media was measured at the times indicated in the figure. The initial A_{680} of the cultures was 0.7.

corresponding to a more hydrophilic structure, and was identified as ANP from its UV-VIS spectral properties as well as H^+-NMR spectroscopy, ^{13}C-NMR spectroscopy, and mass spectrometry. Figure 2 shows mass spectra of ANP from a commercial source (Fig. 2A) and of the intermediate isolated from cultures of *R. capsulatus* E1F1 grown anaerobically on 2,4-DNP (Fig. 2B). Synthetic ANP was not used as a carbon or nitrogen source for photoanaerobic growth of *R. capsulatus* E1F1.

Other phototrophic purple nonsulfur bacteria such as *R. sphaeroides* DSM 158 and *R. palustris* DSM 123, incapable of assimilating nitrate, were unable to use nitrophenols as a N or C source for phototrophic growth although both strains exhibited a weak nitrophenol-degrading activity in vivo which resulted in nitrite ejection to the medium (results not shown).

In vivo, the 2,4-DNP reducing system was fully expressed in cells cultured anaerobically in the light with 2,4-DNP, whereas it was induced to a lesser extent in cells grown in nitrate, nitrite, or mononitrophenols and was absent in nitrogen-starved and ammonium- or glutamine-grown cells. Figure 3A shows 2,4-DNP utilization by cells grown on limited nitrogen, nitrate, and 2,4-DNP. When these cells were washed and inoculated into media containing 2,4-DNP, the compound was rapidly consumed only by 2,4-DNP-grown cells. Induction of the 2,4-DNP reductive pathway did not take place in the presence of chloramphenicol (Fig. 4). Neither chloramphenicol, rifampin, nor 6-methylpurine caused short-term inhibition of 2,4-DNP utilization by induced cells (not shown).

Ammonium also inhibited 2,4-DNP consumption by induced cells. The addition of 6 mM NH_4Cl to *R. capsulatus* E1F1 cells growing on 2,4-DNP immediately stopped both 2,4-DNP utilization and the appearance of ANP in the medium (Fig. 5).

Anaerobic reduction of 2,4-DNP was strictly light dependent. A typical saturation curve demonstrated that anaerobic 2,4-DNP degradation required low-intensity light levels (up to 15 W/m^2) (results not shown).

Microaerobic degradation of nitrophenols. *R. capsulatus* E1F1 cells showed good growth levels in the presence of nitrophenols under microaerobic conditions with acetate as a

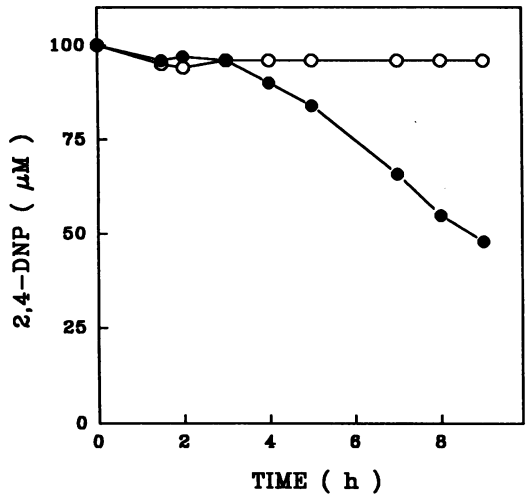

FIG. 4. Effect of chloramphenicol on the induction of the 2,4-DNP-reducing system in *R. capsulatus*. Cells cultured under light-anaerobiosis with ammonium chloride as a nitrogen source were washed and inoculated in media containing 0.1 mM 2,4-DNP in the presence (○) and in the absence (●) of chloramphenicol (50 μg/ml of culture). The concentration of nitrophenol was determined at the times indicated. The initial A_{680} of the cell suspensions was 0.5.

carbon source. Contrary to what was observed with 2,4-DNP, which was rapidly consumed only under anaerobic conditions in the light, 3-NP and 4-NP utilization required microaerobic conditions whereas 2-NP metabolism was relatively independent of O_2 pressure (Table 1). Figure 6 shows growth levels, 4-NP utilization, and nitrite excretion in *R. capsulatus* E1F1 cells cultured with 4-NP and acetate under microaerobic conditions. On the other hand, growth levels were similar when the bacteria were cultured in the light with 4-NP under an atmosphere of either Ar + 20% O_2 or N_2 + 20% O_2. The bacterium exhibited neither growth nor nitrophenol consumption under dark-aerobiosis.

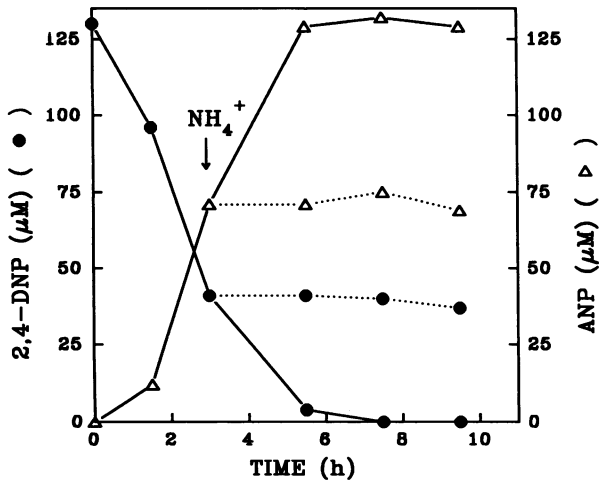

FIG. 5. Effect of ammonium on 2,4-DNP metabolism by *R. capsulatus* E1F1 cells. At the time indicated by the arrow, 6 mM NH_4Cl was added to cells growing with 0.13 mM 2,4-DNP as a nitrogen source under light-anaerobiosis. Solid lines, cultures in the absence of ammonium; dotted lines, cultures in the presence of ammonium. 2,4-DNP and ANP were determined at the indicated times as described in Materials and Methods. The initial A_{680} was 0.65.

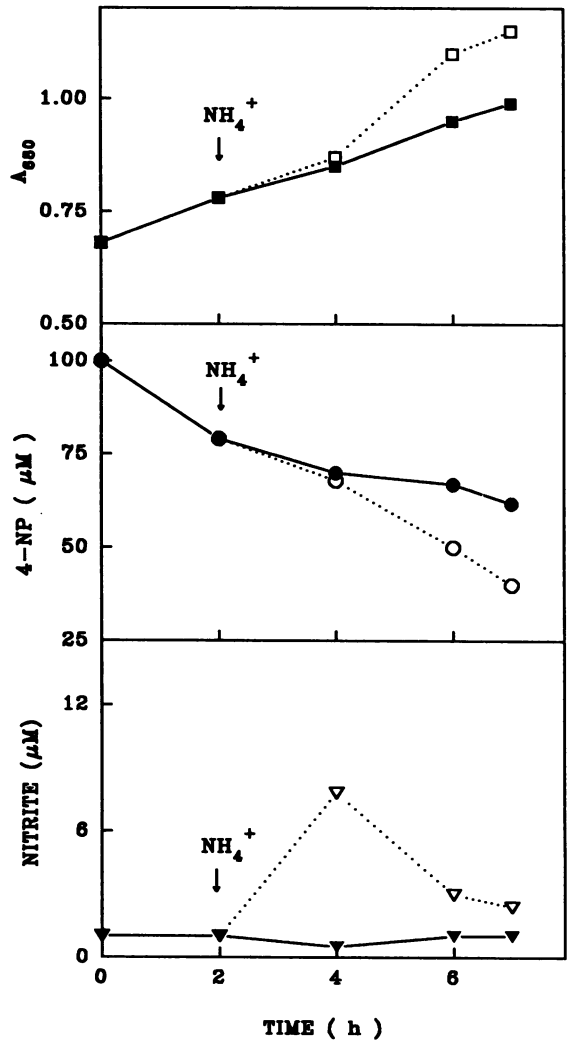

FIG. 6. Effect of ammonium on 4-NP metabolism by *R. capsulatus* E1F1. Cells growing under light-microaerobiosis in the presence of 4-NP were washed and cultured in the same medium. At the time indicated by the arrow, ammonium chloride was added to the culture to a concentration of 6 mM. Cell growth, concentration of 4-NP, and nitrite excretion were measured at the times indicated in the figure. Solid lines and filled symbols, cultures in the absence of ammonium; dotted lines and empty symbols, cultures in the presence of ammonium.

When *R. capsulatus* E1F1 was grown under microaerobic conditions in the light, 2,4-DNP was not stoichiometrically converted into ANP. Besides this compound, other nonidentified and more-hydrophilic intermediates which presented different retention times in HPLC appeared in the medium. Under these conditions, mononitrophenols were also transformed in more-hydrophilic unidentified compounds which also appeared in the culture medium.

Microaerobic growth of *R. capsulatus* E1F1 in the presence of nitrophenols showed a dependence on the nitrophenol concentration similar to that described for anaerobic conditions, with about 0.5 mM mononitrophenol and 0.2 mM 2,4-DNP as the upper limits of tolerance.

Induction of mononitrophenol metabolism under light-microaerobiosis also required a source of fixed nitrogen. When cells growing in nitrate were washed and inoculated in medium containing 4-NP, the compound was degraded, whereas nitrogen-starved cells did not metabolize it at least for 8 h (Fig. 3B). Similar results were obtained when cells growing in nitrite, ammonium, or nitrophenols were washed and inoculated in media containing 4-NP or other mononitrophenols. Contrary to what was observed with 2,4-DNP, 4-NP consumption was enhanced in the presence of ammonium, which also increased nitrite excretion into the medium (Fig. 6).

DISCUSSION

Heterotrophic bacteria can use mononitrophenols as a carbon and energy source for growth (21), whereas 2,4-DNP is only degraded in nitrogen-limiting cultures in the presence of a suitable carbon source (1). Recently, a bacterial consortium of an actinomycete and a *Janthinobacterium* sp. has been reported to degrade 2,4-DNP in the presence of a supplementary substrate (12). However, there are no previous reports on nitroaromatic degradation by phototrophic anaerobes.

R. capsulatus E1F1, a phototrophic bacterium capable of fixing N_2 and photoassimilating nitrate and nitrite (3, 19), exhibited notable resistance to nitrophenols at a wide concentration range (up to 0.5 mM) under either anaerobic or microaerobic culture conditions in the light. However, nitrophenol tolerance of *R. capsulatus* E1F1, especially towards 2,4-DNP, was lower than that previously reported in *P. putida* B2 (27) but considerably higher than that reported in other heterotrophic bacteria (12).

R. capsulatus E1F1 showed no growth under an Ar atmosphere with 2,4-DNP as a nitrogen source which, taken together with the stoichiometric production of ANP from 2,4-DNP under light-anaerobiosis, indicates that 2,4-DNP was not used as a nitrogen source. Besides, no nitrite excretion was observed under these conditions (Fig. 1) unless the 2,4-DNP concentration reached toxic levels (0.2 mM). Under anaerobic conditions without Ar, dissolved N_2 could be an alternative nitrogen source but, since N_2 fixation was severely inhibited by 2,4-DNP, N_2 could not contribute significantly to bacterial growth until the 2,4-DNP concentration was lowered (Fig. 1). In fact, cell growth levels were considerably increased under an atmosphere of N_2. In contrast, under light-microaerobiosis, 2,4-DNP was not stoichiometrically reduced to ANP, which appeared in the medium together with unidentified compounds as well as low amounts of nitrite. Under these conditions (20% O_2 atmosphere), nitrogenase activity was switched off so that the observed initial growth had to be supported by 2,4-DNP.

The 2,4-DNP reducing system was not expressed in nitrogen-limited cultures or in cells growing in ammonium or glutamine (Fig. 3A). In addition, both 2,4-DNP consumption and ANP excretion were strongly inhibited by ammonium (Fig. 5). Taking into account that the addition of ammonium represses and inactivates some enzymatic systems related to nitrogen metabolism in *R. capsulatus* E1F1 (18), a mechanism connected to this "nitrogen regulon" might control the reductive pathway of 2,4-DNP. Other factors controlling 2,4-DNP reduction seem to be dioxygen tension, which decreased the rate of 2,4-DNP consumption, and light intensity, which appeared to be essential for the reduction to take place. Reduction of 2,4-DNP in vivo was strictly light dependent, even though it showed saturation at low light intensities (15 W/m^2), conditions which can prevail in the natural environments where these bacteria usually thrive. A similar photoreduction of 2,4-DNP to ANP has been described for chloroplasts (4).

When ammonium-grown cells were washed and inoculated into media with 2,4-DNP as a nitrogen source, expression of the 2,4-DNP reducing system was severely inhibited by chloramphenicol (Fig. 4), thus indicating that induction of the system required de novo protein synthesis.

Mononitrophenols were degraded to a lesser extent than 2,4-DNP under light-anaerobiosis with the exception of 2-NP, which was considerably metabolized in the absence of O_2 probably because of anaerobic reduction of the nitro group (Table 1). Weak growth and no nitrite excretion were also observed under these conditions. No growth and high nitrite excretion were also observed in the presence of toxic levels of mononitrophenols (0.5 mM), thus suggesting that the elimination of nitro groups can also occur under anaerobiosis.

Degradation of 3-NP and 4-NP was activated by O_2 (Table 1). The compounds formed from these degradative pathways remain unidentified, but the appearance of nitrite in the medium and the dependence of 3-NP and 4-NP on O_2 pressure suggest the involvement of an aerobic pathway as described for some heterotrophic bacteria whose nitrophenol oxygenase has been characterized (27, 28). When N_2 was substituted for Ar (both in the presence of 20% O_2), no stimulation of growth was observed in cultures with 4-NP, which confirms that the observed growth was due not to N_2 fixation but to nitrophenol utilization.

Mononitrophenol degradative pathways were not expressed in nitrogen-starved cells but were induced in illuminated cells growing in several nitrogen sources, including ammonium (Fig. 3B). In addition, the addition of ammonium chloride enhanced 4-NP utilization by *R. capsulatus* E1F1 (Fig. 6). A similar secondary substrate effect but concerning carbon metabolism was found in a *Pseudomonas* sp., whose simultaneous utilization of 4-NP and glucose has been kinetically characterized (22). In the presence of ammonium, nitrite excretion to the medium was increased (Fig. 6), which is consistent with the ammonium inhibition of nitrite assimilation previously reported for *R. capsulatus* E1F1 (2). Since mononitrophenol consumption was directly inhibited by chloramphenicol (not shown), an effect of this compound on the induction of degradative pathways of mononitrophenols cannot be clearly concluded.

The above discussion can be summarized as follows. (i) 2,4-DNP was not used as a nitrogen or carbon source under light-anaerobic conditions. Stoichiometric production of ANP might serve as a protective process against nitrogenase inhibition by 2,4-DNP as well as a mechanism to dissipate an excess of reducing power. Actually, several reductases acting as a valve of reducing power excess have been described for *R. capsulatus* (6). 2-NP could probably be metabolized under anaerobiosis by a similar reaction. (ii) At high concentrations of nitrophenols (0.2 mM 2,4-DNP and 0.5 mM mononitrophenols), the bacterium did not grow but excreted nitrite, probably as a defensive mechanism against the extreme toxicity of these compounds. Under these conditions, nitrite cannot be assimilated because its assimilatory pathway is strongly inhibited by nitrophenols. (iii) 2,4-DNP and mononitrophenols could contribute as nitrogen sources to bacterial growth under light-microaerobic conditions probably because of nitrite production from nitro groups (note that bacterial strains unable to assimilate nitrite did not grow with nitrophenol as a nitrogen source). With the

exception of ANP, the compounds formed from nitrophenol metabolism under light-microaerobiosis remain unidentified. (iv) Low light intensities were absolutely required to metabolize nitrophenols either in the absence or in the presence of O_2. (v) Low O_2 tensions were required to degrade 3-NP and 4-NP, whereas 2-NP utilization was only slightly activated by O_2 which, in turn, decreased the rate of 2,4-DNP metabolism and prevented its stoichiometric reduction to ANP. (vi) The 2,4-DNP reducing system was induced in cells growing in 2,4-DNP and was not expressed in nitrogen-starved or ammonium-grown cells. Induction of this system seemed to be dependent on de novo protein synthesis. (vii) Microaerobic degradative pathways of mononitrophenols were expressed in cells growing in fixed nitrogen. Ammonium not only allowed this expression but increased the rate of mononitrophenol utilization.

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REFERENCES

1. **Bruhn, C., H. Lenke, and H.-J. Knackmuss.** 1987. Nitrosubstituted aromatic compounds as nitrogen source for bacteria. Appl. Environ. Microbiol. **53**:208–210.
2. **Caballero, F. J., C. Moreno-Vivian, F. Castillo, and J. Cárdenas.** 1986. Nitrite uptake system in photosynthetic bacterium *Rhodopseudomonas capsulata* E1F1. Biochim. Biophys. Acta **848**: 16–23.
3. **Castillo, F., and J. Cárdenas.** 1982. Nitrate reduction by photosynthetic purple bacteria. Photosynth. Res. **3**:3–18.
4. **Del Campo, F. F., J. M. Ramírez, A. Paneque, and M. Losada.** 1966. Ferredoxin and the dark and light reduction of dinitrophenol. Biochem. Biophys. Res. Commun. **22**:547–553.
5. **Dutton, P. L., and W. C. Evans.** 1978. Metabolism of aromatic compounds by *Rhodospirillaceae*, p. 719–726. *In* R. K. Clayton and W. R. Sistrom (ed.), The photosynthetic bacteria. Plenum Press, New York.
6. **Ferguson, S. J., J. B. Jackson, and A. G. McEwan.** 1987. Anaerobic respiration in the *Rhodospirillaceae*: characterization of pathways and evaluation of roles in redox balancing during photosynthesis. FEMS Microbiol. Rev. **46**:117–143.
7. **Geissler, J. F., C. S. Harwood, and J. Gibson.** 1988. Purification and properties of benzoate-coenzyme A ligase, a *Rhodopseudomonas palustris* enzyme involved in the anaerobic degradation of benzoate. J. Bacteriol. **170**:1709–1714.
8. **Hallas, L. E., and M. Alexander.** 1983. Microbial transformation of nitroaromatics in sewage effluent. Appl. Environ. Microbiol. **45**:1234–1241.
9. **Harwood, C. S., and J. Gibson.** 1986. Uptake of benzoate by *Rhodopseudomonas palustris* grown anaerobically in light. J. Bacteriol. **165**:504–509.
10. **Harwood, C. S., and J. Gibson.** 1988. Anaerobic and aerobic metabolism of diverse aromatic compounds by the photosynthetic bacterium *Rhodopseudomonas palustris*. Appl. Environ. Microbiol. **54**:712–717.
11. **Heitkamp, M. A., V. Camel, T. J. Reuter, and W. J. Adams.** 1990. Biodegradation of *p*-nitrophenol in an aqueous waste stream by immobilized bacteria. Appl. Environ. Microbiol. **56**:2967–2973.
12. **Hess, T. F., S. K. Schmidt, J. Silverstein, and B. Howe.** 1990. Supplemental substrate enhancement of 2,4-dinitrophenol mineralization by a bacterial consortium. Appl. Environ. Microbiol. **56**:1551–1558.
13. **Keith, L. H., and W. A. Telliard.** 1979. Priority pollutants. I. A perspective view. Environ. Sci. Technol. **13**:416–423.
14. **Kinouchi, T., and Y. Ohnishi.** 1983. Purification and characterization of 1-nitropyrene nitroreductases from *Bacteroides fragilis*. Appl. Environ. Microbiol. **46**:596–604.
15. **Lowry, O. H., N. J. Rosebrough, A. L. Farr, and R. J. Randall.** 1951. Protein measurement with the Folin phenol reagent. J. Biol. Chem. **193**:265–275.
16. **Madigan, M. T., and H. Gest.** 1979. Growth of the phototrophic bacterium *Rhodopseudomonas capsulata* chemoautotrophically in darkness with H_2 as the energy source. J. Bacteriol. **137**:524–530.
17. **Merkel, S. M., A. E. Eberhard, J. Gibson, and C. S. Harwood.** 1989. Involvement of coenzyme A thioesters in anaerobic metabolism of 4-hydroxybenzoate by *Rhodopseudomonas palustris*. J. Bacteriol. **171**:1–7.
18. **Moreno-Vivián, C., F. J. Caballero, M. Martínez-Luque, J. Cárdenas, and F. Castillo.** 1990. Regulation of inorganic nitrogen metabolism in the phototrophic bacterium *Rhodobacter capsulatus* E1F1, p. 145–150. *In* Inorganic nitrogen in plants and microorganisms: uptake and metabolism. Springer-Verlag KG, Berlin.
19. **Moreno-Vivián, C., F. Castillo, and J. Cárdenas.** 1982. Effect of light and darkness on nitrate assimilation by *Rhodopseudomonas capsulata* E1F1. Photosynth. Res. **3**:313–319.
20. **Mulbry, W. W., and J. S. Karns.** 1989. Purification and characterization of three parathion hydrolases from gram-negative bacterial strains. Appl. Environ. Microbiol. **55**:289–293.
21. **Raymond, D. G. M., and M. Alexander.** 1971. Microbial metabolism and cometabolism of nitrophenols. Pestic. Biochem. Physiol. **1**:123–130.
22. **Schmidt, S. K., K. M. Scow, and M. Alexander.** 1987. Kinetics of *p*-nitrophenol mineralization by a *Pseudomonas* sp.: effects of second substrates. Appl. Environ. Microbiol. **53**:2617–2623.
23. **Snell, F. D., and C. T. Snell.** 1949. Colorimetric methods of analysis, 3rd ed., vol. 2, p. 804–805. Van Nostrand Reinhold, Princeton, N.J.
24. **Sudhakar-Bakir, R., R. Siddaramappa, and N. Sethunathan.** 1976. Metabolism of nitrophenols by bacteria isolated from parathion-amended flooded soil. Antonie van Leeuwenhoek J. Microbiol. **42**:461–470.
25. **Weaver, P. F., J. D. Wall, and H. Gest.** 1975. Characterization of *Rhodopseudomonas capsulata*. Arch. Microbiol. **105**:207–216.
26. **Wright, G. E., and M. T. Madigan.** 1991. Photocatabolism of aromatic compounds by the phototrophic purple bacterium *Rhodomicrobium vannielii*. Appl. Environ. Microbiol. **57**:2069–2073.
27. **Zeyer, J., and H. P. Kocher.** 1988. Purification and characterization of a bacterial nitrophenol oxygenase which converts *ortho*-nitrophenol to catechol and nitrite. J. Bacteriol. **170**:1789–1794.
28. **Zeyer, J., H. P. Kocher, and K. N. Timmis.** 1986. Influence of *p*-substituents on the oxidative metabolisms of *o*-nitrophenols by *Pseudomonas putida* B2. Appl. Environ. Microbiol. **52**:334–339.
29. **Zumft, W. G., and F. Castillo.** 1978. Regulatory properties of the nitrogenase from *Rhodopseudomonas palustris*. Arch. Microbiol. **117**:53–60.