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Light-dependent transformation of anthranilate to indole by *Rhodobacter sphaeroides* OU5

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***Rhodobacter sphaeroides* OU5 transformed anthranilate (2 mM) to an indole (0.7 mM) in a light-dependent process. Photobiotransformation was enhanced by tricarboxylic acid cycle intermediates and the indole formed was identified as 2,3 dihydroxy indole.** *Journal of Industrial Microbiology & Biotechnology* (2000) **24,** 219–221.

Keywords: *Rhodobacter sphaeroides*; photobiotransformation; anthranilate; indole

Introduction

Interest has grown for the past few years in the metabolism of aromatic compounds by purple non-sulfur anoxygenic phototrophic bacteria from both an ecological and a biotechnological point of view [16]. Despite their limited ability to utilize aromatic compounds for growth [10,16], their ability to transform aromatic structures to value-added compounds is gaining importance [14,15]. Some of the transformations reported which do not support growth of purple non-sulfur bacteria include: (a) 4-phenyl butyrate, 6-phenyl hexanoate and 8-phenyl octanoate to phenyl acetate [5]; (b) 2,4-dinitrophenol to 2-amino 4-nitrophenol [2]; (c) phloroglucinol (1,3,5 trihydroxy benzoate) to dihydrophloroglucinol [17]; (d) indole to L-tryptophan and other derivatives [14]; and (e) indole to indole-3-acetic acid [15]. In this study we report extracellular transformation of anthranilate to indole by a purple non-sulfur bacterium, *Rhodobacter sphaeroides* OU5. Indole and a number of its derivatives are of high commercial significance because of their importance as phytohormones [15], for production of the essential amino acid L-tryptophan [13], as well as other industrial applications [3,4,6,11]. In this study an attempt was made to explore using purple non-sulfur bacteria for production of indole.

Materials and methods

Organism and growth conditions

Rhodobacter sphaeroides OU5 (ATCC 49885; DSM 7066) was grown photoheterotrophically (anaerobic/light) (2400 lux) in fully filled 500-ml reagent bottles on Biebl and Pfennig's [1] mineral medium with malate (22 mM) and ammonium chloride (7 mM) as carbon and nitrogen sources, respectively, at 30 ± 2°C.

Transformation studies

A log-phase culture of *R. sphaeroides* was harvested by centrifugation (16 000 × *g* for 15 min), washed (twice) and made into a thick suspension (0.1 OD at 660 nm = 1.6 mg dry wt ml^{-1}) with basal medium (devoid of nitrogen and carbon). One milliliter of this suspension was inoculated into the assay medium (taken in fully filled 15 × 125-mm screw cap test tubes; approximately 15 ml) with anthranilate (3 mM) and succinate (0.5% w/v) (unless otherwise mentioned) and the assay tubes were incubated at 2500 lux for 72 h at 30 ± 2°C.

Assay

After incubation, the cultures were centrifuged and the supernatant was used for assays. Anthranilate was estimated colorimetrically by the following procedure. To 5 ml of the sample, 0.2 ml of phenol alcohol (10% w/v phenol in ethanol), 0.2 ml of sodium nitroprusside (0.5% w/v) and 0.5 ml of oxidizing agent (20 g of tri-sodium citrate + 1 g of sodium hydroxide + 25 ml of 1.5 N sodium hypochlorite) were added, incubated for 30 min and the color developed was read at 640 nm against a blank. The concentration of anthranilate was calculated from the calibration curve prepared using anthranilate.

For analysis of mass, indole was extracted into ethyl acetate from the supernatant after adjusting the pH to 9 with saturated sodium bicarbonate solution. The ethyl acetate layer was concentrated and the concentrate was analyzed for mass using a Mass VG 70–70H mass analyzer.

Results and discussion

Anthranilate did not support phototrophic growth of *R. sphaeroides* OU5 when used either as sole source of carbon in the presence of 7 mM NH_4Cl or nitrogen in the presence of 22 mM malate, as observed earlier with *Rhodopseudomonas palustris* [10]. Moreover, anthranilate inhibited the growth of *R. sphaeroides* (with malate and ammonium chloride as carbon and nitrogen sources, respectively) and the IC_{50} was 1.8 mM. Though anthranilate did not support phototrophic growth of *R. sphaeroides* OU5, its disappearance from the medium was observed with time without any increase in biomass (data not shown). The supernatant after

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the assay gave a positive reaction with *p*-dimethylaminobenzaldehyde [PDAB] [12] and Salper's [8] reagents, which are specific for indole and its derivatives. An R_f of 0.96 by thin layer chromatography (acetone : isoproponol : H_2O : NH_4OH 5 : 4 : 0.7 : 0.3) corresponded to that of standard indole, confirming the product of photobiotransformation.

Disappearance of anthranilate and its biotransformation to indole were light-dependent (data not shown). The time course of disappearance of anthranilate from the medium and the simultaneous formation of indole are shown in Figure 1. The conversion of anthranilate to indole did not result in stoichiometric molar yields. From the consumption of 2 mM of anthranilate, only 0.7 mM of indole was obtained (Figure 1) which remained constant up to 120 h (data not shown in Figure 1). This might be due to a feedback inhibition of the product on anthranilate consumption/indole formation, detailed studies on which are in progress.

Since *R. sphaeroides* could photoproduce tryptophan from indole and serine [14], it was of interest to see if tryptophan could be produced directly from anthranilate. *R. sphaeroides* OU5 did not produce tryptophan either in the presence of anthranilate + succinate + serine, or indole (obtained from anthranilate) + serine. However, indole obtained from anthranilate did not inhibit tryptophan formation from pure commercial indole + serine (data not shown), indicating that the indole thus formed from anthranilate does not participate in tryptophan formation and does not inhibit biotransformation of pure commercial indole. Thus, this extracellular metabolism of indole must be different from intracellular synthesis since *R. sphaeroides* is known to synthesize its own tryptophan.

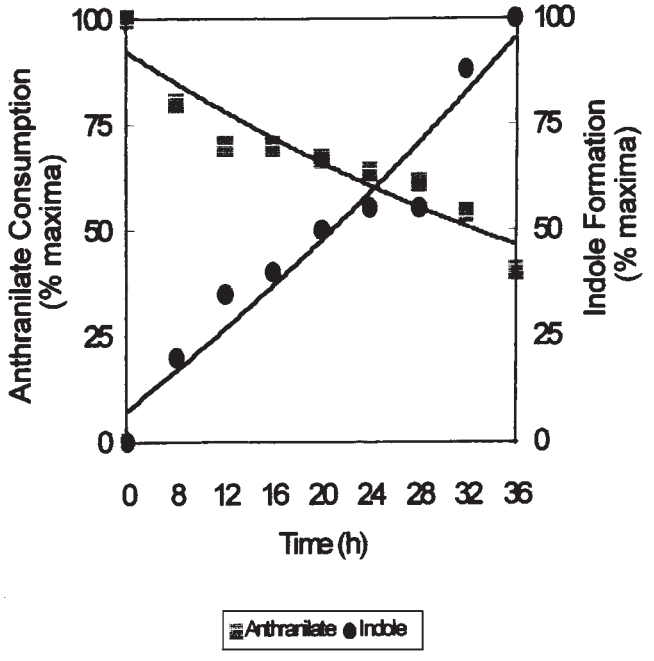

Figure 1 Time course of anthranilate consumption and indole formation by *R. sphaeroides*. Results expressed are average values of experiments done in triplicate assayed under light (2400 lux) anaerobic incubation at 30 ± 2°C. 100% anthranilate consumption = 2 mM, 100% indole formation = 0.7 mM.

Indole production was observed in the absence of externally supplied carbon (from 1 mM of anthranilate consumed, 0.3 mM of indole was formed without any additional substrates) and thus the additional carbons required for transformation may be provided from the cell itself. In the presence of a number of other carboxylic acids and a few carbohydrates the cell was able to synthesize indole (Table 1). Among the various organic substrates used, the tricarboxylic acid (TCA) intermediates resulted in higher uptake of anthranilate and considerable enhancement of indole production (except with citrate). The similar consumption of anthranilate observed with all the intermediates of TCA cycle used indicates that all these compounds may be transformed to a common end product before attaching themselves to anthranilate. Further, an increase in the efficiency of anthranilate transformation to indole was observed starting from citrate < α-ketogluterate < succinate = malate = oxaloacetate < fumarate. These results can be explained by assuming that fumarate is the substrate which is finally attached to anthranilate before forming indole. The indole thus formed had a mass of 149 (Figure 2) which may be from the molecular formula $C_8H_7NO_2$ and the compound as 2,3-dihydroxy indole (not as pure indole which has a mass of 117), an intermediate in indole biodegradation by some heterotrophic bacteria [7].

Table 1 Photoproduction of indole from anthranilate in the presence of various organic substrates by *R. sphaeroides* OU5

Substrates (0.5% w/v or v/v)	Biomass yield (mg dry wt ml^{-1})	Anthranilate consumed (mM)	Indole formation (mM)	% Conversion efficiency (over and above control)*
Monocarboxylates				
Formate	0.5	1.3	0.2	0
Acetate	0.7	1.0	0.3	0
Propionate	0.8	0.34	0.15	0
Pyruvate	1.1	2.1	0.4	10
Lactate	0.5	0.0	0.0	0
Butyrate	0.6	0.5	0.2	0
Valerate	1.0	1.1	0.4	10
Caproate	0.6	1.0	0.4	10
TCA intermediates				
Succinate	0.96	2.0	0.7	40
Malate	0.9	2.4	0.7	40
Fumarate	0.83	1.7	1.0	100
Oxaloacetate	0.7	2.3	0.7	31
Citrate	0.6	1.8	0.3	0
α-ketoglutarate	0.8	2.0	0.5	20
Carbohydrates				
Ribose	0.7	2.4	0.5	14
Glucose	0.8	1.35	0.0	0
Fructose	0.8	1.4	0.0	0
Control (without substrate)	0.5	1	0.3	NA

NA = Not applicable.
Results expressed are average values of two independent experiments done in triplicate. Experimental details as in Materials and methods with initial biomass of the assay being 0.9 mg dry wt ml^{-1} and the results expressed are after 72 h of light (2400 lux) anaerobic incubation at 30 ± 2°C, in the presence of anthranilate (3 mM) along with the specified substrates. *Data expressed are the values after subtracting from the control (1 mM anthranilate consumption and 0.3 mM indole formation).

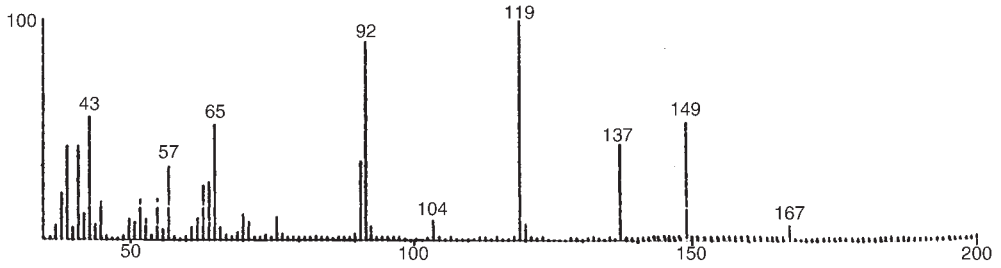

Figure 2 Mass spectrum of the indole derivative.

In conclusion, the study suggests the possibility of an alternate pathway of indole formation from anthranilate in anoxygenic phototrophic bacteria (Figure 3) (which metabolize carbohydrates poorly [14]) involving a TCA cycle intermediate instead of ribose phosphate [13] and its biochemical basis needs to the elucidated. The results suggest the probable role played by *R. sphaeroides* in transforming aromatic compounds, particularly formation of indole (this study) and its derivatives [14,15] under anoxic environments, thus benefiting paddy crop productivity [9] and support the possibility of commercial production of phytohormones [18].

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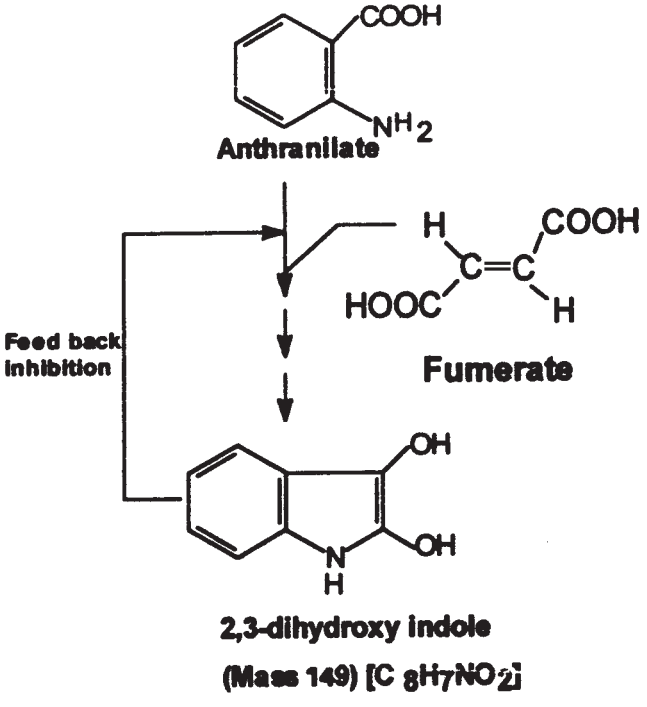

Figure 3 Photobiotransformation of anthranilate to indole derivative by *R. sphaeroides*.

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