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Photobiotransformation of indole to its value-added derivatives by *Rhodobacter sphaeroides* OU5

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A purple non-sulfur anoxygenic phototrophic bacterium, *Rhodobacter sphaeroides* OU5 was able to photobiotransform indole in the presence of various organic substrates to its value-added derivatives tryptophan, tryptamine, indole lactic acid and indigo, which are of high commercial value. The product formed varied with the precursors provided in the medium.

Keywords: *Rhodobacter sphaeroides*; photobiotransformation; indole; tryptophan; indigo

Introduction

Anoxygenic phototrophic bacteria, particularly the purple nonsulfur bacteria, play a vital role in the biodegradation and biotransformations of aromatic structures under anaerobic environments [10]. Among the purple non-sulfur bacteria, the most widely studied species with such metabolic potential is *Rhodopseudomonas palustris* whose biochemistry and physiology of aromatic photometabolism is widely studied [10]. In contrast, not much information is available on the photometabolism of aromatic compounds by other purple non-sulfur bacteria. It appears from the literature [10] that other purple non-sulfur bacteria do not have the capability to biodegrade most of the aromatic structures; however, their role in the biotransformation of these structures is quite evident [3,11] In the present communication we discuss an important potential of *Rhodobacter sphaeroides* in the biotransformation of indole to value-added derivatives such as tryptophan, tryptamine, indole lactic acid and indigo which are of high commercial value.

Materials and methods

Organism and growth conditions

Rhodobacter sphaeroides OU5 (ATCC 49885; DSM 7066) was grown photoheterotrophically (anaerobic/light [2400 lux]) on Biebl and Pfennig's [2] mineral medium with malate (0.3% w/v) and ammonium chloride (7 mM) as carbon and nitrogen source respectively at 30 ± 2°C.

Biotransformation studies

Logarithmically growing cultures of *Rb. sphaeroides* OU5 were harvested by centrifugation (at $16\,000 \times g$ for 10 min), pellet washed (twice) in basal medium (without carbon and nitrogen) and suspended into an assay medium containing Biebl and Pfennig's mineral salts with indole (1.5 mM) and other organic compounds (1% w/v or v/v). After 48 h of light anaerobic (in fully filled 15×125 mm screw cap test tubes) incubation, growth was measured turbidometrically at 660 nm. Biomass yield was estimated using an OD *vs* dry weight graph (0.1 OD at 660 nm = 0.15 mg dry wt ml^{-1}). The sample was then centrifuged ($19\,000 \times g$ for 15 min) and the supernatant was used for the assay of indole and tryptophan [4]. Other derivatives of indole were detected by using both thin layer chromatography [1] and spectrophotometry (U.V. Chemito 2000) by UV absorption in 12.7 M H_2SO_4 [5] along with standards. Uninoculated culture controls were used to check for any possible photochemical reactions.

Results and discussion

To the best of our knowledge there are no reports on the photometabolism of indole by purple bacteria, though some of these bacteria are known to metabolise a few other heterocyclic aromatic compounds *viz* purines, pyrimidines and a number of pyrazines [10]. During our studies on the photobiodegradation of indole by the purple non-sulfur bacteria *Rhodopseudomonas palustris* and *Rhodobacter sphaeroides*, the latter alone showed indole photobiodegradation capability in the absence of an organic substrate and the product formed was anthranilate [7]. The product of microbial biotransformation of indole is known to vary with the precursors provided [6]. Hence, an experiment was devised to find out indole photobiotransformation capability of *Rb. sphaeroides*. This organism was able to biotransform indole into tryptophan as the major end product in the presence of various organic and amino acids (Table 1) in a light-dependent process (data not shown). We rule out the possibility of simple photochemical transformation of indole to tryptophan for our observations using uninoculated culture controls (Table 1).

The conversion efficiency of the substrates to the products varied with different precursors used in the present study (Table 1). Though utilization of indole was more in the presence of pyruvate/lactate + NH_4Cl, its conversion to tryptophan was much less compared with that in the presence of serine or glycine. Though the specific enzyme activities involved in indole photobiotransformation to tryp-

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Table 1 Photobiotransformation of indole to tryptophan by *Rhodobacter sphaeroides* OU5 using different precursors

Indole + organic substrate	Biomass yield (mg dry wt ml^{-1})	Indole consumption (mM)	Tryptophan formation (mM)	Conversion efficiency (%)
Pyruvate + NH_4Cl	0.19	0.80 ± 0.2	0.35 ± 0.01	44
Lactate + NH_4Cl	0.15	0.90 ± 0.4	0.34 ± 0.05	38
Serine	0.15	0.69 ± 0.04	0.65 ± 0.01	95
Glycine	0.15	0.55 ± 0.02	0.53 ± 0.02	96
Control (– organic substrate)	0.18	0.09 ± 0.01	0.09 ± 0.01	100
Uninoculated culture control	NA	0.0	0	NA

NA = Not applicable.
Washed cell suspensions of logarithmically growing cultures of *Rhodobacter sphaeroides* were suspended in the basal medium supplemented with yeast extract (0.02% w/v), indole (1.5 mM) and organic substrates (1% w/v or v/v). Ammonium chloride was added at a concentration of 7 mM where indicated. Results pertain to that after 24 h of light (2400 lux) anaerobic incubation and are the average of three independent experiments done in triplicate. For other details see Materials and Methods.

tophan were not measured in the present study, synthesis of tryptophan from indole with pyruvate + NH_4Cl as well as indole + serine indicates the presence of both the enzymes tryptophanase and tryptophan synthase [6] respectively in *Rb. sphaeroides*.

The rate of production of tryptophan from indole + serine (27 nM h^{-1}) was much higher than that from indole + pyruvate + NH_4Cl (14 nM h^{-1}) (Figure 1), which may explain the observed differences in the conversion efficiencies (Table 1). In fact, with indole + pyruvate + NH_4Cl, a diphasic pattern of tryptophan production was observed with an initial low tryptophan production rate (Figure 1) which may be due to the contribution of precursors from intracellular reserves and/or from yeast extract during the initial period of photobiotransformation. This first phase of tryptophan synthesis was followed by a lag in tryptophan production (and not in the indole consumption). During this period the organism may be converting the precursors provided to those compounds which can be attached to indole nucleus for tryptophan formation. Subsequent to this phase, the rates of tryptophan production were identical to that in the presence of serine (Figure 1).

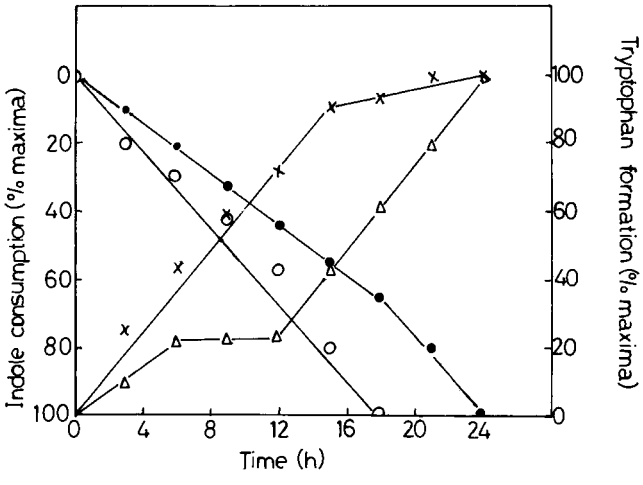

Figure 1 Photoproduction of tryptophan using various precursors. (○–○) Indole consumption in the presence of serine (100% consumption = 0.69 mM). (×–×) Tryptophan formation in the presence of serine (100% formation = 0.65 mM). (●–●) Indole consumption in the presence of pyruvate + ammonium chloride (100% consumption = 0.80 mM). (△–△) Tryptophan formation in the presence of pyruvate + ammonium chloride (100% formation = 0.35 mM). Experimental details as in Table 1 except that the analyses were done at regular time intervals as indicated. Initial biomass of assay was 0.69 mg dry wt ml^{-1} (OD at 660 nm = 0.46) and biomass yields are given in Table 1.

Though yeast extract was a component of the medium in these experiments, the concentration used was only 0.02% (w/v) and thus its contribution for tryptophan content of the medium was negligible. In fact, tryptophan was not detectable in the 0-h sample. It is well established that production of tryptophan from pyruvate or serine has an obligate requirement of pyridoxal phosphate [6]. In order to check for biotransformations of indole by *Rb. sphaeroides* OU5 in the absence of pyridoxal phosphate, yeast extract was omitted from the medium. This resulted in the formation of indole lactic acid as the major end product with pyruvate, tryptamine with glycine, while with serine it was indigo (Table 2). Supplementing this medium with pyridoxal phosphate resulted in the formation of tryptophan (Table 2), thus proving its requirement for tryptophan formation even by *Rb. sphaeroides* OU5.

Rhodobacter sphaeroides OU5 was able to produce 0.612 g L^{-1} tryptophan in about 48 h under photoanaerobic conditions from indole (3 mM) and serine (1% w/v). These results are in comparison with those produced by wild strains of different microorganisms [6]. In the presence of a number of other organic substrates *viz*, dl-alanine, *β*-

Table 2 Photobiotransformation of indole to its derivatives by *Rhodobacter sphaeroides* OU5 in the absence of yeast extract

Indole + organic substrate	Biomass yield (mg dry wt ml^{-1})	Indole consumption (mM)	Product of biotransformation
Pyruvate + NH_4Cl	NG	NT	Indole lactic acid
Serine	0.15	1.5	Indigo
Glycine + NH_4Cl	NG	0.2	Tryptamine
Serine + pyridoxal-5-phosphate	0.15	0.6	Tryptophan

NT = Not tested; NG = No growth.
Experimental details as in Table 1, except that yeast extract was not added. Pyridoxal-5-phosphate was used at a concentration of 15 *μ*g L^{-1}.

alanine, glutamate, acetate, formate and propionate, indole was photobiotransformed to anthranilic acid (data not shown).

The observations made in the present study suggest that a number of indole derivatives can be produced by *Rb. sphaeroides* OU5 by manipulating the media composition. Production of indole derivatives, particularly compounds like tryptophan (an essential amino acid) and indigo (a biodegradable natural dye) are of high commercial significance. *Rhodobacter sphaeroides* OU5 can provide an alternate stable strain to solve the existing strain-related problems [6] for the production of tryptophan and other indole derivatives. This organism can also provide additional benefits compared to the chemotrophs, which can be listed as:

(1) tolerates high concentrations of indole (3 mM) tested in the present study;
(2) very high conversion efficiency;
(3) light being the source of energy, *Rb. sphaeroides* does not use the precursors as a source of energy. In addition, these precursors are also not used as sole carbon and nitrogen sources for growth in the presence of indole (Table 1). Indole is known to inhibit growth of *Rb. sphaeroides* OU5 [7] and thus the entire indole and substrates are made available for such biotransformations.

The potential of *Rb. sphaeroides* OU5 in photobiotransforming indole to some of the value-added derivatives adds to the list of biotechnological applications of this group of microorganisms [8,9] and opens up scope for further detailed studies on the biochemistry and production of these industrially important compounds. It is still not known if this property is restricted to this strain alone or is a general property of other strains of *Rhodobacter sphaeroides*.

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