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Degradation of quinaldine by *Alcaligenes* sp. and by *Arthrobacter* sp.

Gunnar Dembek, Theobald Rommel, Franz Lingens and Hartmut Höke*

Institut für Mikrobiologie der Universität Hohenheim, Garbenstr. 30, D-7000 Stuttgart 70 and **Rütgerswerke AG, Chemical Research/Tar Chemistry Branch, D-4620 Castrop-Rauxel, FRG*

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Two bacterial strains, which grew aerobically in mineral salt medium with quinaldine as sole carbon source, were isolated. These strains could be identified as *Alcaligenes* sp. and *Arthrobacter* sp. *Alcaligenes* sp. was adapted to higher concentrations of quinaldine in a continuous culture. Both strains accumulated one compound, identified as quinisatin, which decomposed chemically to anthranilic acid and isatin. Quinisatin spontaneously resulted from 3,4-dihydroxy-2-oxo-1,2-dihydroquinoline.

Quinaldine degradation; Quinisatin; Dihydroxy-2-oxo-1,2-dihydroquinoline, 3,4-

1. INTRODUCTION

In the course of our investigations on the microbial metabolism of quinoline and related compounds [1,2], we have studied the degradation of quinaldine (2-methylquinoline). All reports in the literature describe hydroxylation at the 2-position as yielding 2-oxo-1,2-dihydroquinoline (2-oxo-DHQ) as the first step in quinoline degradation [1,3–6]. In the degradation of quinaldic acid (quinoline-2-carboxylic acid) attack at the 2-position was impeded, with the benzene ring, and not the pyridine ring, being cleaved [7]. We expected that a quinoline compound substituted in the 2-position by a methyl group might also prevent attack at the 2-position. The present paper describes bacteria of the genus *Alcaligenes* sp. adapted to higher concentrations of quinaldine in a continuous culture and of the genus *Arthrobacter* sp., which both utilize quinaldine as sole carbon source. We isolated and characterized an oxidation product of a metabolite in the degradation of quinaldine. Furthermore, we obtained substances yielded by chemical decomposition of this oxidation product.

Dedicated to Professor Dr. M. Zander on the occasion of his 60th birthday

Correspondence address: F. Lingens, Institut für Mikrobiologie, Universität Hohenheim, Garbenstr. 30, D-7000 Stuttgart 70, FRG

Abbreviation: DHQ, 1,2-dihydroquinoline

2. MATERIALS AND METHODS

2.1. *Substrate and authentic compounds*

3,4-Dihydroxy-2-oxo-DHQ was prepared as described [8,9]. Quinisatin (2,3,4-trioxo-1,2,3,4-tetrahydroquinoline) was prepared by oxidation of 3,4-dihydroxy-2-oxo-DHQuinoline with $FeCl_3$ [10]. Quinaldine was obtained from Rütgerswerke (Castrop Rauxel) and isatin from EGA-Chemie (Steinheim/Albuch).

2.2. *Bacterial strains and culture conditions*

The bacterial strain Rü1, isolated from soil, and the strain Rü61, isolated from waste water were identified according to [11,12]. A mineral salt medium was used containing (per 1000 ml deionized water): 1.0 g K_2HPO_4, 0.5 g KH_2PO_4, 0.1 g $MgSO_4 \cdot 7H_2O$, 0.1 g NaCl, 1.0 g $(NH_4)_2SO_4$, 200 μl quinaldine and the following sources of trace elements and vitamins: 0.5 mg H_3BO_3, 0.04 mg $CuSO_4 \cdot 5H_2O$, 0.2 mg $FeCl_3 \cdot 6H_2O$, 0.4 mg $MnSO_4 \cdot 7H_2O$, 0.2 mg $(NH_4)_6Mo_7O_{24} \cdot 4H_2O$, 0.4 mg $ZnSO_4 \cdot 7H_2O$, 0.2 mg riboflavin, 0.2 mg pyridoxal, 0.2 mg thiamine, 0.02 mg vitamin B_{12} and 0.1 mg biotin. The pH was

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adjusted to 7.3. The complex medium contained (per 1000 ml deionized water): 5 g yeast extract, 5 g meat peptone and 5 g NaCl. Solid media contained 1.5% (w/v) agar (Merck).

2.3. *Continuous culture*

Bacteria of the strain Rü1 were adapted to higher concentrations of quinaldine in a continuous culture. The continuous culture device consisted of a chemostat (Julabo Labortechnik, Seelbach), the culture volume being 100 ml. Growth was at room temperature, and the medium [mineral salt medium with quinaldine as sole carbon source at 0.02% (w/v)] was introduced with a peristaltic pump. The initial flow rate was 0.5 ml/h. After degradation of quinaldine the flow rate was increased. Degradation of quinaldine was monitored by thin-layer chromatography (TLC). The continuous culture lasted for a total of 12 months. The final flow rate was 10 ml/h. One strain could be isolated (Rü1Ch), which appeared to be identical with the original strain.

2.4. *Isolation and identification of an accumulate and its decomposition products*

Quinaldine was added to the mineral salt medium at 0.03% (w/v) (strain Rü1Ch) and 0.075% (w/v) (strain Rü61). After incubation for 2–4 weeks, the medium was concentrated in vacuo and extracted at pH 7 with ethyl acetate. The aqueous phase was acidified and again extracted with ethyl acetate. The aqueous phase was discarded. The compounds were further purified by preparative TLC on SIL G-200/UV_{254} sheets (Macherey and Nagel). After development, the appropriate bands of the silica gel were scraped off and extracted with ethyl acetate. Analytical and preparative TLC was performed with the following solvent systems: (A) toluene/dioxane/acetic acid (72:16:1.6, v/v), (B) chloroform/acetic acid (35:1, v/v), (C) chloroform/acetone (1:1, v/v) and (D) toluene/dioxane (72:16, v/v). The products were identified by TLC, reactions with various reagents, mass spectroscopy and ^{1}H-NMR. Mass spectra were recorded on a Variant MAT 311 spectrometer (Bremen). ^{1}H-NMR spectra were taken with a Bruker NMR WM 250 spectrometer (Karlsruhe).

3. RESULTS

3.1. *Identification of strains Rü1 and Rü61*

3.1.1. Strain Rü1

The quinaldine-degrading bacteria were small rods (0.5–1 μm × 1–2 μm), gram-negative, motile with one to eight peritrichous or degenerate peritrichous flagella, obligately aerobic, nonfermentative, catalase-positive and cytochrome-*c* oxidase-positive. Cellulose, gelatin, esculin and DNA were not hydrolyzed. The urease reaction was negative. L-Arginine dihydrolase was not present. They did not utilize all carbohydrates. Growth was slow in complex media, very slow in chemically defined media, and required growth factors. They utilized acetate, propionate, succinate, fumarate, DL-β-hydroxybutyrate, anthranilic acid, *p*-aminobenzoic acid, 2-oxo-DHQuinoline, quinoline and quinaldic acid as sole carbon source.

3.1.2. Strain Rü61

A marked rod-coccus growth cycle occurred during growth in complex media. Older cultures were composed of coccoid cells. In young cultures the cells were irregular rods, and primary branching sometimes occurred. The bacteria were gram-positive, not acid-fast, nonsporing, nonmotile, obligately aerobic, and catalase-positive; metabolism was respiratory, never fermentative. They did not attack cellulose or gelatin, but did hydrolyze starch. They did not require growth factors and were sensitive to lysozyme. They could utilize a wide and diverse range of substrates as sole carbon sources (carbohydrates, amino acids, quinoline, 2-oxo-DHQuinoline, quinaldic acid).

3.2. *Continuous culture*

Via this experiment one strain (Rü1Ch) could be selected, which degraded 42 mg/dl quinaldine (strain Rü1: 20 mg/dl) and accumulated metabolites in contrast to the original strain.

3.3. *Characterization of a compound accumulated by strain Rü1Ch and Rü61 and its products by chemical decomposition*

In mineral salt medium with quinaldine [concentrations: 0.03% (w/v) quinaldine (Rü1Ch), 0.075% quinaldine (w/v) (Rü61)] as sole carbon source, slightly yellow coloration was observed. On TLC two compounds could be detected. One of these compounds (I) had the following chemical and physical data. Reaction with 2,4-dinitrophenylhydrazine yielded two hydrazones [13]. The hydrazones were intensely blue in color at alkaline pH values. ^{1}H-NMR of compound I showed signals corresponding to four aromatic protons and a broad peak corresponding to an N-H-proton: ^{1}H-NMR (250 MHz, DMSO) δ 6.9–7.7 ppm (4H,m), broad peak δ 10.7–11.1 ppm with a maximum at 10.9 ppm. These data and the R_f values were in accordance with the data of authentic quinisatin.

The other compound (II) was identified by TLC and from the mass spectrum. This compound gave the following mass spectral data: *m/e* 137 (69%), *m/e* 119 (100%) and *m/e* 92 (81%). The test with

Ehrlich's reagent [13] was positive. The R_f values and mass spectral data were identical with those of authentic anthranilic acid.

After several weeks a third, bright yellow compound (III) could be detected on TLC. This compound could be identified by TLC and by its mass spectrum. The data for the mass spectrum were as follows: *m/e* 147 (46%), *m/e* 119 (100%), *m/e* 92 (66%), *m/e* 76 (10%) and *m/e* 64 (33%). The R_f values and mass spectral data were identical to those of the same measurements performed on an authentic sample of isatin.

After shaking for several weeks at 30°C in sterile mineral salt medium with 3,4-dihydroxy-2-oxo-DHQ as substrate, compounds I–III were found. In sterile mineral salt medium with quinisatin (I) as substrate, compounds II and III were detected.

4. DISCUSSION

4.1. *Identification of strains Rü1 and Rü61*

Strain Rü1 could be identified as *Alcaligenes* sp. and strain Rü61 as *Arthrobacter* sp.

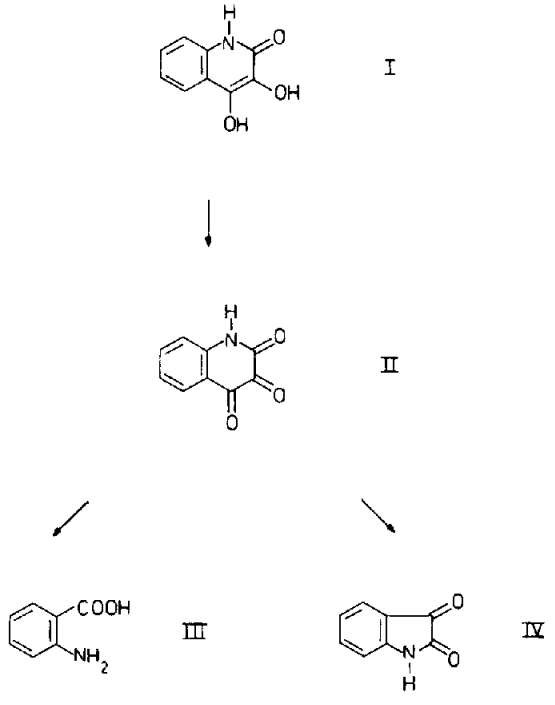

Fig.1. Chemical conversion of (I) 3,4-dihydroxy-2-oxo-DHQ [(II) 2,3,4-trioxo-1,2,3,34-tetrahydroquinoline (quinisatin); (III) anthranilic acid; (IV) isatin].

4.2. *Characterization of compound I, its products of chemical decomposition and the pathway proposed for degradation of quinaldine by* Alcaligenes *sp. and* Arthrobacter *sp.*

Strains Rü1Ch and Rü61 accumulated compound I, which yields compounds II and III on chemical decomposition.

Based on our results, compound I could be identified as quinisatin, compound II as anthranilic acid and compound III as isatin. As shown in fig.1, we can describe the chemical conversion of 3,4-dihydroxy-2-oxo-DHQ to the products mentioned above. Up to now the following intermediates in the degradation of quinoline and its derivatives have been found: 2-oxo-DHQ and 6-hydroxy-2-oxo-DHQ in *Bacillus* sp. and *Rhodococcus* sp. (Schwarz, G. et al., unpublished). Quinoline degradation in a *Pseudomonas* sp. proceeds via 2-oxoquinoline and 8-hydroxy-2-oxo-

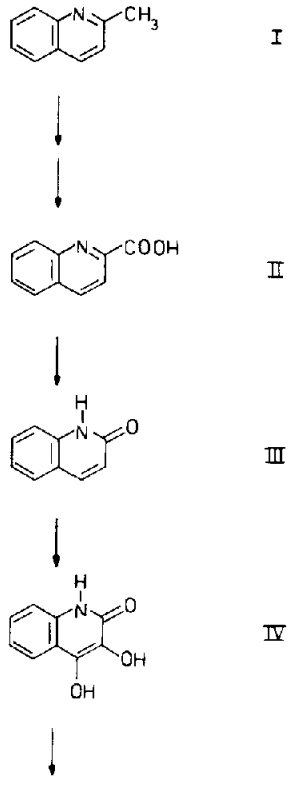

Fig.2. Proposed pathway for the degradation of quinaldine by *Alcaligenes* sp. and *Arthrobacter* sp. [(I) quinaldine, (II) quinaldic acid, (III) 2-oxo-DHQ, (IV) 3,4-dihydroxy-2-oxo-DHQ.

DHQ, 8-hydroxycoumarin and 2,3-dihydroxyphenylpropionic acid ([5] and Speer, M. et al. unpublished). In the degradation of quinaldic acid the product of cleavage at the *meta* position, 6-carboxy-3-(3'-carboxy-1'-hyroxy-3'-oxopropenyl)-1H-2-pyridone was accumulated by a mutant of *Azotobacter* sp. [7]. Hydroxylation at the 2-position and attack on the pyridine ring were prevented. Based on the structure of 3,4-dihydroxy-2-oxo-DHQ and on the fact that bacteria of strains Rü1 and Rü61 could utilize 2-oxo-DHQ, quinoline and quinaldic acid as sole carbon source, we propose a pathway for microbial degradation of quinaldine, as shown in fig.2. We suppose that in the degradation of quinaldine, the methyl group is oxidized to a carboxyl group. The intermediate is decarboxylated and hydroxylated at the 2-position. In contrast to the known degradation pathway of quinoline, 2-oxo-DHQ is hydroxylated at the 3- and 4-positions.

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