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Microbial metabolism of quinoline by Comamonas sp.

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Summary

An aerobic bacterial strain which can use quinoline as the sole carbon and energy source has been isolated from activated sludge and identified as *Comamonas* sp. The microbial metabolism of quinoline by this strain has been investigated. A pH 8 and a temperature of 30 °C were the optimum degradation conditions of quinoline. Five intermediates including 2-oxo-1,2-dihydroquinoline, 5-hydroxy-6-(2-carboxyethenyl)-1*H*-2-pyridone, 6-hydroxy-2-oxo-1,2-dihydroquinoline, 5,6-dihydroxy-2-oxo-1,2-dihydroquinoline, and 8-hydroxy-2-oxo-1,2-dihydroquinoline were found during quinoline biodegradation. The presence of these intermediates suggested that at least two pathways were involved for quinoline degradation by *Comamonas* sp. and a reasonable degradation route was proposed to account for the intermediates observed.

Introduction

In recent years, there has been increasing concern over the public health threat presented by the introduction of polycyclic aromatic nitrogen heterocycles (PANHs) into the environment (Sims & O'Loughlin 1989; Capel & Larson 2001). Quinoline and its derivatives, typical PANHs, are widely used as raw materials and solvents in the manufacture of dyes, paints, fungicides and wood-treatment chemicals (Johanson *et al.* 1997). They have already become common contaminants in ground water and soil, especially near landfills, solid wastes, as well as wood preservation and fossil fuel facilities (Stuermer *et al.* 1982; Fowler *et al.* 1994; Johansen *et al.* 1997). Many studies have shown that quinoline and its derivatives have toxic, carcinogenic and mutagenic activity to animals and humans (Birkholz *et al.* 1989; Shukla 1989). Thus, it is very important to learn the transformation and fate risk of quinoline in the environment and further develop effective strategies for remediation of polluted sites.

Biodegradation of quinoline has been extensively investigated (Al-Najjar & Al-Shakarchi 1980; Fetzner *et al.* 1998; Wang *et al.* 2002). Microorganisms capable of utilizing quinoline are identified mostly among *Pseudomonas* (Bennett *et al.* 1985; Brockman *et al.* 1989; Blaschke *et al.* 1991). However, species of *Burkhoderia* (Wang *et al.* 2001), *Desulfobacterium* (Johanson *et al.* 1997), *Moraxella* (Al-Najjar & Al-Shakarchi 1980), *Nocardia* (Shukla 1987), and *Rhodococcus* (O'Lougblin *et al.* 1996) can also degrade quinoline. In all cases 2-oxo-1,2-dihydroquinoline was found to be the first intermediate during quinoline degradation. Subsequent degradation may go through two different pathways. The first pathway involves preferential cleavage of benzene ring and results in the formation of 5-hydroxy-6-(3-carboxy-3-oxopropenyl)-1*H*-2-pyridone from 6-hydroxy-2-oxo-1,2-dihydroquinoline *via* 5,6-dihydroxy-2-oxo-1,2-dihydroquinoline (Schwartz *et al.* 1989; Rüger *et al.* 1993; Schwartz *et al.* 1993). In the second pathway the *N*-heterocyclic ring is cleaved prior to benzene ring cleavage to form 2,3-dihydroxyphenyl-propionic acid from 8-hydroxy-2-oxo-1,2-dihydroquinoline *via* 8-hydroxycoumarin (Schwartz *et al.* 1989; Kaiser *et al.* 1996; Fetzner 1998; Fetzner *et al.* 1998). In the former pathway 5,6-dihydroxy-2-oxo-1,2-dihydroquinoline was a presumed intermediate (Rüger *et al.* 1993; Kaiser *et al.* 1996).

In this study, a quinoline-degrading bacterium, *Comamonas* sp. was isolated from activated sludge and the metabolites of quinoline were analysed by GC/MS. We provide laboratory evidence that 5,6-dihydroxy-2-oxo-1,2-dihydroquinoline is indeed present during quinoline biodegradation, thus confirming the pathway of quinoline degradation *via* this important intermediate.

Materials and methods

Reagents and chemicals

Quinoline was purchased from Sigma. 2-Oxo-1,2-dihydroquinoline was obtained from Aldrich. 8-Hydroxy-2-oxo-1,2-dihydroquinoline was from Fluka. 8-Oxo-1,8-dihydroquinoline, 4-hydroxycoumarin, 7-hydroxycoumarin and 3-(2-hydroxyphenyl)propionic acid were purchased from Lancaster. *N,O*-bis-(Trimethylsilyl) trifluoroacetamide (BSTFA) was from Pierce. All other chemicals used were of the analytical grade commercially available.

Microorganism

The strain Q_{10} used in this study was isolated from an enrichment culture obtained from activated sludge of a gas-generating wastewater treatment plant. For the enrichment culture three times repeated supplementation of quinoline as sole carbon and nitrogen source was conducted and the culture was shaken at 30 °C and 120 rev/min for 3 weeks on a rotary shaker. The strain Q_{10} was purified by successive streak transfers on agar-plate medium. The bacterium was identified by both sequential analysis of 16SrDNA and API 20NE of the API System of BioMerieux Co. (French). The strain Q_{10} suspension was obtained by suspending the colonies from the enrichment culture into sterilized distilled water. The number of bacteria in the strain suspension was 1.9×10^9 ml^{-1}.

Media

The medium used for cultivation of the strain Q_{10} contained (g l^{-1}): 0.2 $MgSO_4 \cdot 7H_2O$; 0.5 KH_2PO_4; 0.5 $(NH_4)_2SO_4$; 1.5 K_2HPO_4 and 1% (v/v) stock trace element solution. The stock trace element solution contained (g l^{-1}): 12 $Na_2EDTA \cdot 2H_2O$; 2.0 $FeSO_4 \cdot 7H_2O$; 1.0 $CaCl_2$; 10 Na_2SO_4; 0.4 $ZnSO_4 \cdot 7H_2O$; 0.4 $MnSO_4$; 0.1 $CuSO_4 \cdot 5H_2O$; 0.1 $Na_2MoO_4 \cdot 2H_2O$; 5 ml concentrated H_2SO_4. The pH of the media was adjusted to 7.0 with 1 M H_2SO_4 and 1 M NaOH.

Biodegradation experiments

The strain suspension was inoculated into 250 ml Erlenmeyer flasks containing 100 ml medium in which qui noline was added. Except as indicated, general conditions were: 1% v/v of strain suspension; the pH value of the solution is 7.0; the initial concentration of quinoline was 100 mg/l; the flasks were incubated at 30 °C and 120 rev/min on a rotary shaker. During the period of batch growth, samples were taken at desired intervals for the analyses of quinoline and its degradation products. The control experiments were conducted with the same sterilized medium but without addition of the strain Q_{10}.

Analytical methods

The concentration of quinoline was analysed by high performance liquid chromatograph (HP1100, Agilent Co. Ltd.). Culture samples for HPLC analysis were filtered through 0.22 μm syringe filters. Five microlitre of the filtered solution was injected into the HPLC system equipped with a reversed-phase C18 column (Diamonsil™, 150 × 4.6 mm, 5 μm). The mobile phase was composed of methanol and water (70:30, v/v) and the flow rate set at 1 ml min^{-1}. Quinoline was detected at 275 nm.

Identification of metabolites was confirmed by thin-layer chromatography (TLC) on silica gel G plates with toluene/dioxane/acetic acid (72/16/1.6, vol/vol/vol) (Schwartz *et al.* 1989). Authentic quinoline and 2-oxo-1,2-dihydroquinoline were used as references. Metabolites in the mixture were observed under normal light and short-wave (254 nm) u.v. light and identified by the rate of flow (R_f). The separated products were also analysed by GC/MS. In addition, the Kovats indices, the retention indices, which are used to identify the unknown compounds, were based on the study of Lee & Vassilaros (1979) by comparison with authentic compounds naphthalene, phenanthrene, and pyrene.

Culture samples for metabolite analysis of quinoline were extracted with ethyl acetate. Part of the extracts was silylated with *N,O*-bis-(Trimethylsilyl)trifluoroacetamide (BSTFA) (Thomsen 1998) prior to GC/MS analysis. The authentic standards were analysed in the same manner by GC/MS after derivatization with BSTFA.

GC/MS analysis was performed by a Hewlett–Packard model 5890 GC with a 5972 MS detector at the following temperatures: injection port, 290 °C; detector, 300 °C; column temperature, 40 °C isothermal for 5 min and then 7 °C/min to 300 °C isothermal 20 min. The GC column used was a HP-5 fused silica capillary column (30 m by 0.25 mm).

Results and discussion

Morphological, physiological, and biochemical characteristics of strain Q_{10}

Strain Q_{10} was a Gram-negative, rod-shaped and oxidase-postive, aerobic bacterium. Colonies were light yellow, smooth on the surface and trim on the edge. Genomic DNA of the strain Q_{10} was purified by the method described by Zhou *et al.* (1996). The DNA was amplified by the polymerase chain reaction, using specific primers corresponding to bases F27 (5′-AGA-GTTTGATCCTG GCTCAG-3′) and R1522 (5′-AAG-GAGGTGATCCAGCCGCA-3′) (Edwards *et al.* 1989). The sequence of the amplified 16S ribosomal DNA were determined by direct sequencing method by TakaRa Biotechnology (Dalian) Co. Ltd. and compared with other available 16S rRNA sequences by using the BLAST search option of the GenBank database to

determine the closest phylogenetic neighbours. The 16S rRNA sequence of strain Q_{10} has been deposited in the GenBank nucleotide sequence database under accession number AF519533. Sequencing of the 16S rRNA gene and comparison with previously published 16S rRNA gene sequences resulted in classification of strain Q_{10} as a member of the genus *Comamonas* shared 99% 16S rRNA sequence homology with *Comamonas testosteroni* strain JCM 10170. This culture was maintained at Guangdong Key Laboratory Microbial culture collection and Breeding, Guangdong Institute of Microbiology, China.

The pH and temperature optima for cell growth in both nutrient broth and liquid quinoline-containing medium were pH 8 and 30 °C, respectively.

Mineralization of quinoline by strain Q_{10}

Strain Q_{10} can utilize quinoline as the carbon source and energy source for the cell growth. Decreasing the initial concentration of quinoline and increasing the cell concentration would be beneficial to the degradation of quinoline. However, the substrates were not always mineralized directly but proceeded *via* a number of intermediates. Therefore, the ultimate biodegradation or mineralization, i.e., the breakdown of a compound into inorganic substances or compounds making up the cellular biomass, will be an important indicator for the study of the organic pollutants biodegradation. In this study, TOC was chosen to monitor the mineralization extent of quinoline. As shown in Figure 1, it can be seen that the TOC decreased sharply at the beginning of quinoline degradation and nearly 70% of TOC was removed at 10 h, but then the TOC changed very slightly. This residual TOC may probably be some persistent metabolites, and maybe also included soluble polymers from the biomass.

It is interesting to note that there was a distinct colour change in the cultures. Production of a pink soluble pigment occurred and then the culture gradually turned to yellow during the degradation of quinoline by strain Q_{10}. Similar results were obtained during the degradation of quinoline by other bacteria (Schwartz *et al.* 1989). Shukla also reported the formation of pink pigments during the degradation of quinoline by *Nocardia* sp. (Shukla 1987). These phenomena also indicated that many intermediates were produced during the biodegradation of quinoline.

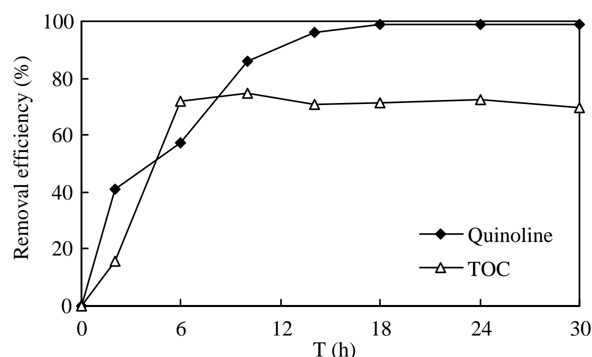

Figure 1. TOC removal during quinoline biodegradation.

Intermediates identification and proposed pathway

Five intermediates were identified in the bacterial culture during quinoline degradation (Table 1). These metabolites were not detected in sterile controls. 2-Oxo-1,2-dihydroquinoline was the first intermediate and was mainly found in extracts of neutral pH. The other four intermediates were detected only in the acidic fractions. From Table 1 it can be seen that production of 2-oxo-1,2-dihydroquinoline was up to 88.7% of all intermediate within the first 2 h of incubation. Its concentration decreased to 46.7% at 14 h incubation and to below detection limit after 24 h incubation. Meanwhile, the concentration of 6-hydroxy-2-oxo-1,2-dihydroquinoline increased from 2.6% at 14 h incubation to the maximum 24.3% at 24 h incubation and then decreased to 15.8% after 30 h incubation. Only a trace amount of 5,6-dihydroxy-2-oxo-1,2-dihydroquinoline was detected, suggesting that it may be unstable or rapidly transformed into 5-hydroxy-6-(2-carboxyethenyl)-2(1*H*)pyridone. The unstable nature or rapid turnover of 5,6-dihydroxy-2-oxo-1,2-dihydroquinoline may be one reason that this compound was not observed in previous studies (Schwartz *et al.* 1989; Rüger *et al.* 1993). A similar compound, 5,6-dihydroxy-3-methyl-2-oxo-1, 2-dihydroquinoline has been reported to be unstable and subject to oxidation in the biodegradation of 3-methylquinoline (Schach *et al.* 1993). Metabolites corresponding to authentic 4-hydroxycoumarin, 7-hydroxycoumarin, 3-(2-hydroxyphenyl) propionic acid, were not detected by GC/MS during the degradation of quinoline by strain Q_{10}.

Table 1. Metabolites formed during quinoline biodegradation.

Compound	Retention time (min)	Content (%)[a]				
		2 h	14 h	18 h	24 h	30 h
2-Oxo-1,2-dihydroquinoline	21.48	88.7	46.7	2.2	0	0
6-Hydroxy-2-oxo-1,2-dihydroquinoline	27.54	0	2.6	12.8	24.3	15.8
5,6-Dihydroxy-2-oxo-1,2-dihydroquinoline	30.39	0	0	Trace	Trace	0
5-Hydroxy-6-(2-carboxyethenyl)-2(1*H*)pyridone	27.82	0	0	5.3	11.4	8.1
8-Hydroxy-2-oxo-1,2-dihydroquinoline	27.07	0	0	0.2	Trace	Trace

[a] Percentage of the total area of all peaks present in the chromatograph.

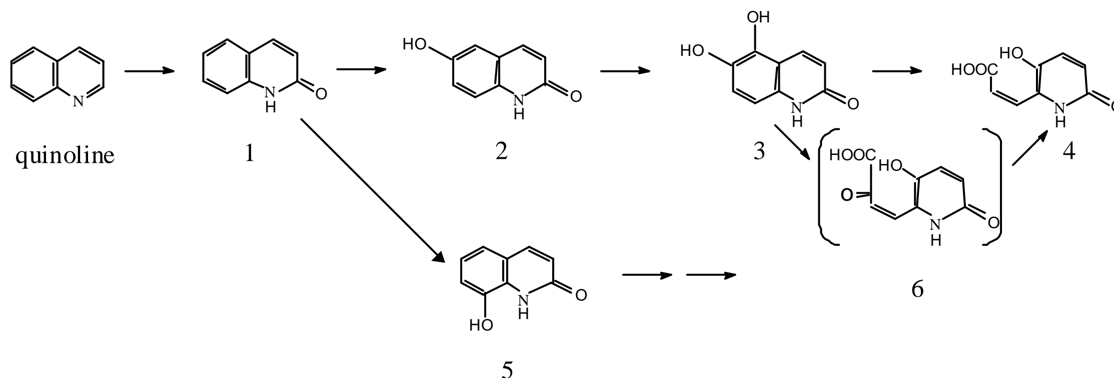

Figure 2. Pathway proposed for the degradation of quinoline: (1) 2-oxo-1,2-dihydroquinoline; (2) 6-hydroxy-2-oxo-1,2-dihydroquinoline; (3) 5,6-dihydroxy-2-oxo-1,2-dihydroquinoline; (4) 5-hydroxy-6-(2-carboxyethenyl)-1*H*-2-pyridone; (5) 8-hydroxy-2-oxo-1,2-dihydroquinoline; (6) 5-hydroxy-6-(3-carboxy-3-oxopropenyl)-1*H*-2-pyridone.

Based on the identified intermediates, the pathway for the degradation of quinoline is proposed as shown in Figure 2. In the proposed pathway, the degradation of quinoline is initiated by hydroxylation at position 2 to form 2-oxo-1,2-dihydroquinoline, which is then oxidized to polyhydroxylated compounds. The subsequent cleavage would yield 5-hydroxy-6-(2-carboxyethenyl)-1*H*-2-pyridone.

This pathway is similar to that proposed by Grant & Al-Najjar (1976) using a *Moraxella* sp. and by Schwartz *et al.* (1989) using a *Rhodococcus* sp. In these studies, however, 5,6-dihydroxy-2-oxo-1,2-dihydroquinoline was assumed to be a possible intermediate but not detected. Furthermore, none of these studies reported the presence of 5-hydroxy-6-(2-carboxyethenyl)-1*H*-2-pyridone during quinoline degradation. But Schwartz *et al.* (1989) reported the presence of 5-hydroxy-6-(3-carboxy-3-oxopropenyl)-1*H*-2-pyridone during quinoline degradation. It is possible that the C=O group in 5-hydroxy-6-(3-carboxy-3-oxopropenyl)-1*H*-2-pyridone was eliminated to form 5-hydroxy-6-(2-carboxyethenyl)-1*H*-2-pyridone.

Additionally, a small amount of 8-hydroxy-2-oxo-1,2-dihydroquinoline was also found during the degradation of quinoline, indicating that another pathway may be involved for the degradation of quinoline. However, other intermediates following 8-hydroxy-2-oxo-1,2-dihydroquinoline were not detected, suggesting that this pathway was not important for the degradation of quinoline.

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