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Biodegradation of N,N-dimethylacetamide by *Rhodococcus* sp. strain B83 isolated from the rhizosphere of pagoda tree

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ABSTRACT

The biodegradation characteristic and potential metabolic pathway for removal of environmental N,N-dimethylacetamide (DMAC) by *Rhodococcus* sp. strain B83 was studied. *Rhodococcus* sp. strain B83 was isolated from the rhizosphere of a pagoda tree and proved capable of utilizing DMAC as sole source of carbon and nitrogen. Batch culture studies showed that strain B83 could tolerate up to 25 g/L DMAC and showed distinct growth on possible catabolic intermediates except for acetate. The nitrogen balance analysis revealed that approximately 71% of the initial nitrogen was converted to organic nitrogen. DMAC degradation has led to accumulation of acetate and organic nitrogen, meanwhile traces of nitrate and ammonia was build-up but without nitrite. The growth of strain B83 could be inhibited by adding exogenous acetate. By means of the assay of enzymatic degradation of DMAC, several catabolic intermediates at different intervals were observed and identified. Based on the results obtained from culture solution and enzymatic degradation assay, a detailed pathway is proposed for DMAC biodegradation.

Introduction

N,N-dimethylacetamide (DMAC) is a water-miscible solvent being widely used for agrochemicals, pharmaceuticals, fine chemicals, man-made fibers, industrial coatings, films, paint strippers and other applications. Based on emission factors, a large amount of DMAC is released into the environment during manufacturing and application, even after recovery treatment. Considering its wide presence, toxicity, and slow rate of degradation, DMAC may have an adverse effect on the environment, public health and welfare. DMAC is readily absorbed by humans after oral, dermal, or inhalation exposure (Wexler, 2014). Some evidences reported that human DMAC exposure mainly causes liver toxicity, skin irritation, headache, in appetence, fatigue, and hepatic damage (Principalle et al., 2010; Buhler and Reed, 1990; Menegola et al., 1999; Oechtering et al., 2006). Recently, DMAC has been listed as a chemical known to cause reproductive toxicity (Wexler, 2014). Q2 Q3

Due to its adverse effects, many attempts have been made to develop technologies to remove DMAC contamination from industrial effluents. The chemical and physical technologies mainly include sorptive microextraction of titania and zirconia hollow fibers (Li et al., 2009), photocatalytic oxidation (Ge et al., 2012; Zhang et al., 2009), adsorption (Takatsuji and Yoshida, 1998) and internal microelectrolysis (Liu et al., 2012). The trickle-bed air biofilter (TBAB) has been proven to be an effective process treating DMAC waste gas, more than 90%

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and 80% DMAC removal efficiencies were achieved for influent DMAC loadings below 20.2 and 34.5 g/(m^3 hr), respectively (Lu et al., 2001). The biological degradation is undoubtedly a non-destructive, low-cost and environmentally-friendly technology as compared to chemical and physical methods and is worth exploring for removing DMAC contamination from industrial effluents. However, there has been no available report about the isolation of the bacteria and pure cultures, which can utilize DMAC as the sole carbon and energy source. Furthermore, the catabolic pathway involved in the biodegradation process of DMAC has not been elucidated. Therefore, it is important to screen DMAC-degrading bacterial strains from relevant environments, and to investigate the degradation characterization and metabolites for acquiring a more comprehensive understanding of the metabolic pathway of pollutant in the environment and for an effective bioremediation strategy of DMAC.

In this study we report isolation of a bacterial strain *Rhodococcus* sp. strain B83, which is capable of utilizing DMAC as the sole carbon, nitrogen and energy source. As one of the important bacteria which were extensively studied for degradation of organic pollutants, *Rhodococcus* sp. was distributed in various environments such as the water and soil. Therefore, this species have important research value and broad application prospects in environmental pollution treatment and bioremediation (Khan et al., 2013; Shen et al., 2009; Lu et al., 2009; Grishko et al., 2013; Homklin et al., 2012; Bajaj et al., 2014; Yi et al., 2011). The growth characteristics and degradation characteristics of *Rhodococcus* sp. strain B83 degrading DMAC are investigated in this communication. A detailed pathway is proposed for the biodegradation of DMAC by strain B83 based on the identification of the catabolic intermediates.

1. Materials and methods

1.1. Source of strain, chemicals, media and culture conditions

Soil samples were collected from the rhizosphere of a pagoda tree in Guanzhong region (Shaanxi, China). DMAC (99.5%) was purchased from the TJFCH Corporation (Tianjin, China). N-methylacetamide (99%) and acetamide (99%) were purchased from Aladdin Corporation (Shanghai, China). Unless otherwise stated, the organic solvents, media, salts and acids were purchased from various sources (Sigma, VWR and Fisher in USA or China). Beef extract-peptone medium containing filter-sterilized DMAC of different concentrations was used as enrichment medium. Minimal media (MM) were used for isolation and cultivation of DMAC-degrading bacteria; the MM without carbon and nitrogen are as follows (g/L distilled water): $Na_2HPO_4{\cdot}2H_2O$ 2.0, $MgSO_4$ 0.5, KCl 0.5, KH_2PO_4 1.0, $Fe_2(SO_4)_3{\cdot}H_2O$ 0.2, $CaCl_2{\cdot}2H_2O$ 0.1. The 100 mL MM was transferred to 250 mL Erlenmeyer flask and autoclaved at 121 °C for 20 min. Different concentrations of filter-sterilized DMAC (1–25 g/L) were added to the minimal medium as source of carbon and nitrogen.

1.2. Enrichment and isolation

For the purpose of enrichment 1 g soil sample was added to 100 mL of beef extract-peptone medium containing filter-sterilized DMAC (1 g/L); it was incubated for 5 days at 35 °C and shaker rate 120 r/min. Further enrichment was performed by transferring 10% bacterial suspension to fresh beef extract-peptone medium with the concentration of DMAC gradually increased to 10 g/L and the concentration of beef extract-peptone lowered. The isolation of DMAC-degrading bacteria was carried out by pipetting 10 mL enrichment bacterial suspension into 100 mL MM containing 5 g/L DMAC as the sole source of carbon and nitrogen for 5 days. After several repetitive inoculations of the culture, isolation was performed by serial dilution of the cultures and plating them on MM medium plates containing agar (DMAC 5 g/L). The purity of the culture was checked morphologically by microscopic observation. The strains obtained were separately inoculated in 250 mL Erlenmeyer flasks containing 100 mL of MM medium with 5 g/L DMAC for 5 days and the DMAC-degrading bacteria were found by determining the residual concentration of DMAC with high performance liquid chromatography (HPLC). The bacterial strain B83 utilizing solely DMAC as the source of carbon and nitrogen was selected for further research.

1.3. Identification

The colony morphology, cell morphology, Gram staining and other biochemical tests were carried out for the characterization of the strain as per standard procedures (Dong and Cai, 2001). Q4 Genomic DNA extraction of strain B83 was carried out using DNA isolation kit (MoBio Laboratories, USA) following manufacturer's recommendations, the concentration of extracted DNA was measured with a Nanodrop-2000 μ-spectrophotometer (Thermo Electron, USA) as per the manufacturer's instructions. The PCR amplification (Eppendorf Mastercyclerep, Germany) was carried out with the universal primers of both forward primer 27F (5′-AGAGTTTGATCCTGGCTCAG-3′) and reverse primer 1492R (5′-GGTTACCTTGTTACGACTT-3′) by following procedures (Moreno et al., 2002): initial denaturation at 95°C for 5 min, denaturation at 94°C for 30 sec, renaturation at 56°C for 30 sec, and elongation at 72°C for 80 sec. In total 35 thermal cycles and the final elongation was at 72°C for 10 min (Mehdi et al., 2012). The PCR-amplified 16S ribosomal ribonucleic acid (rRNA) Q5 gene fragments were purified by agarose gel electrophoresis (1.2%, V/V), and the purified product was detected by MultiImager (Syngene GBoxEF, USA) according to the manufacturer's instructions. Nucleotide sequences of the 16S rRNA genes were determined by Shanghai Sangon Biotech Ltd. (China). The BLAST was performed by searching for similar sequences at the National Center for Biotechnology Information (NCBI) site Q6 (http://www.ncbi.nlm.nih.gov/BLAST). Multiple sequence alignment was performed using ClustalX (Thompson et al., 1997). Phylogenetic analysis was performed though the neighbor-joining method using MEGA version 5.0 (Tamura et al., 2011; Saitou and Nei, 1987).

1.4. Growth on possible catabolic intermediates

To study the use of intermediates of DMAC degradation by the strain B83, N,N-dimethylformamide (DMF), dimethylamine, monomethylamine, N-methylacetamide, acetamide and formate were individually used as the sole carbon and nitrogen source in the minimal medium at an initial concentration of

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