Biodegradation of 2,4,5-Trichlorophenoxyacetic acid (2,4,5-T) and Analysis of the Degradation Products (3)

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Introduction

Burkholderia cepacia AC1100 (BCAC1100) utilizes 2,4,5-trichlorophenoxyacetic acid (2,4,5-T) as a sole source of carbon and energy. A chromosomal deletion mutant of BCAC1100, known as PT88, was unable to grow on 2,4,5-T and accumulated the intermediate 5-chloro-1,2,4-trihydroxybenzene (5-CHQ). Only a few reports have dealt with subsequent enzymatic steps of the chlorohydroxyl hydroquinone pathway. For example, the enzyme 6-chlorohydroxyquinol 1,2-dioxygenase was purified from 2,4,5-trichlorophenol degrading bacteria Streptomyces rochei 303 and Azotobacter sp. strain GP1. To determine the low pathway of 2,4,5-T, 5-CHQ is prepared from 1,2,4-trihydroxybenzene. The genes involved in the low pathway of 2,4,5-T degradation have been cloned and sequenced. Two of the genes essential for the metabolism of the intermediate 5-CHQ were overexpressed in Escherichia coli (E. coli) and the gene products were purified.

Results and Discussion

5-CHQ, which is an intermediate biodegradation product of 2,4,5-T is a very unstable compound, so it decomposes by reaction with dissolved oxygen in the solvent and by light to give a dimer. However we treated it under a nitrogen gas atmosphere, and obtained pure compound (99.9%). Analytical results were:

MS m/z (rel.int. %): 160(M⁺, 100), 123(4), 89(5), 69(7), IR(KBr)cm⁻¹: 3378 (OH), 861(C-Cl), 1H-NMR(DMSO) δ: 9.10(s, C-OH), 8.98(s, C-OH), 8.55(s, C-OH), 6.62(s, CH), 6.43(s, CH). 13C-NMR (DMSO) δ: 143.52, 142.90, 136.44, 114.04, 105.94, 102.61.

The DNA sequence revealed the presence of six open reading frames (ORFs) for the metabolism of 2,4,5-T, designated ORF1 to ORF6. Four of these ORFs were essential for the complementation of PT88 for growth on 2,4,5-T. The enzymes encoded by these ORFs were overproduced in E. coli and biochemical assay were expressed to determine their function. The analytical results showed that the thiH gene product was a hydroxyquinol 1,2-dioxygenase enzyme which catalyzed ortho cleavage of hydroxyquinol to produce maleylacetate.

Methods

5-Chloro-1,2,4-trihydroxybenzene(5-chlorohydroxyquinol, 5-chlorohydroxyhydroquinone; 5-CHQ) was synthesized according to the procedure described by Paquette. 5-CHQ was purified by silica gel column chromatography using ethyl acetate and n-hexane (1:1) and recrystallized using ether and n-hexane twice. mp: 133-135°C (uncorrected, decomposed). The enzyme cofactors, NADH and NADPH were purchased from Aldrich Chemical Co. Maleyl acetate was synthesized by alkaline hydrolysis of a 1.0 ml, 5 mM dienelactone solution with 7.5 μl 2 M NaOH at 25°C.

References