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863课题进展

来源于假单胞菌4-硝基酚降解基因簇中的偏苯三酚1,2-双加氧酶基因克隆和功能鉴定

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摘要：

通过富集培养的方法从农药厂废水曝气池的活性污泥中筛选到了一株既具有甲基对硫磷降解活性又可以有效降解4-硝基酚的高效菌株1-7，经16S rDNA鉴定为假单胞菌（*Pseudomonas* sp.）。根据4-硝基酚降解相关基因保守区设计简并引物扩增小片段，再应用TAIL-PCR克隆得到偏苯三酚1,2-双加氧酶基因*dio1*，基因全长873 bp，编码290个氨基酸，酶蛋白理论分子量为32.8 kDa。 *doi1*基因在E. coli BL21中过量表达，并通过Ni-NTA亲和层析纯化，结果表明该酶具有正常的生物学活性，证明了假单胞菌1-7可以通过偏苯三酚途径降解4-硝基酚。初步探讨了假单胞菌1-7的4-硝基酚降解机制。

关键词： 甲基对硫磷；4-硝基酚；假单胞菌；TAIL-PCR；偏苯三酚1,2-双加氧酶

Cloning and Characterization of Hydroxyquinol 1, 2-dioxygenase from Pseudomonas sp.: A Novel Member of 4-Nitrophenol Degradation Gene Cluster

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Abstract:

A bacterium 1-7 with the capability of degrading both methyl parathion and 4-nitrophenol, identified as Pseudomonas sp. by 16S rDNA, was isolated by enrichment method from OP-polluted activated sludge. The degenerate primers were designed according to the conservative domains of hydroxyquinol 1, 2-dioxygenase to amplify the partial hydroxyquinol 1, 2-dioxygenase gene. The complete enzyme gene dio1 was obtained by TAIL-PCR subsequently and confirmed by DNA sequence analysis. The *dio1* gene is 873 bp long comprising one open reading frame encoding a polypeptide of 290 amino acids with a molecular weight of 32.8 kDa. The *dio1* gene was then over-expressed in *E.coli* BL21, and the recombinant protein Dio1 was further purified with Ni-NTA affinity chromatography. The hydroxyquinol 1, 2-dioxygenase Dio1 was proved to have catalytic activity. It was suggested that 4-NP was degraded via hydroxyquinol by *Pseudomonas* sp.1-7. 4-NP degradation pathway was preliminarily discussed in this research.

Keywords: methyl parathion;4-nitrophenol;*Pseudomonas* sp.;TAIL-PCR hydroxyquinol 1,2-dioxygenase

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