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邻苯二酚2，3-双加氧酶在恶臭假单胞杆菌整细胞催化中的酶活检测方法

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摘要 为了定量了解整细胞催化过程中关键酶酶活的变化，以恶臭假单胞菌mt-2为模式菌株，研究其在芳香族化合物降解途径中的关键酶——邻苯二酚2，3-双加氧酶（C23O）的简易检测方法。首先确立了以苯甲酸钠为唯一碳源的培养基，在优化培养条件下，达到了细胞培养和C23O同时诱导表达的目的。进而通过用磷酸缓冲溶液重悬细胞，并用分光光度法监测产物的生成速率（375 nm），实现在整细胞的条件下，对C23O酶活进行快速准确的测量。在整细胞条件下，C23O的最适温度为35 ℃，最适pH 7.5，而在最适温度和pH下，酶与底物的动力学参数为Km=34.67 μmol•L^{-1}，Vmax=0.29 μmol•min^{-1} •mg干细胞$^{-1}$。这些动力学参数与纯酶的相差两到三个数量级。另外通过在细胞悬液中加入0.1 g•L^{-1} 阳离子表面活性剂（DTAB）作用30 min后，能有效的消除细胞膜的空间障碍，增加细胞膜的通透性，从而使测量的酶活更接近于最大酶活。本研究为整细胞催化及微生物的环境修复过程中酶活的快速检测提出了简易可行的方法。

关键词

邻苯二酚2,3-双加氧酶 整细胞催化 十二烷基三甲基溴化铵 环境修复

分类号

A simple assay of catechol 2，3-dioxygenase activity in whole-cell catalysis of Pseudomonas putida mt-2

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Abstract

Catechol 2，3-dioxygenase（C23O）is the key enzyme of aromatic substance degradation by Pseudomonas sp..In order to establish a simple assay of C23O activity during the whole-cell catalysis of Pseudomonas putida mt-2，C23O was induced by utilizing sodium benzoate acid as the sole carbon source，and its activity was determined in whole cells by the amended protocol of pure enzyme assay.After suspending the cells with potassium phosphate buffer，the substrate was added and the accumulation of 2-hydroxymuconic semialdehyde was measured by a UV757CRT spectrophotometer at 375 nm.The activity of C23O was evaluated by the climbing slope of time course curve of the UV absorption.By this means，the Km for catechol and C23O in whole cells was 34.67 μmol·L^{-1} ，while Vmax was 0.29 μmol·min^{-1} ·(mg dry cell)$^{-1}$ ，both of which differed from those for pure enzyme by 2—3 orders of magnitude.To eliminate the cell wall barrier for substrate permeation，a cationic surfactant， n-dodecyltrimethylammonium bromide，was used to pre-treat the cells.With 0.1 g·L^{-1} dodecyl trimethyl ammonium bromide(DTAB) treated for 30 min，the maximum C23O activity could be achieved，which was consistent with the result of treated cells by beads milling.In the present study，a feasible and simple method was put forward for the apparent enzyme activity assay intracells which could be conveniently applied to the whole-cell biocatalysis or to environmental bioremediation.

Key words

catechol 2,3-dioxygenase whole-cell catalysis DTAB environmental bioremediation

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