

Pseudomonas aeruginosa TKU002所生產兒茶酚1,2-雙加氧?之純畫及定性

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摘要

本研究為以苯甲酸鈉為唯一碳源並且培養基為pH5.5進行篩選土壤中之菌株。在篩選出之菌株中挑選一株做為本實驗菌株，送經食品發展工業研究所鑑定並命名為Pseudomonas aeruginosa TKU002。此菌株轉化代謝培養基中之苯甲酸鈉並累積中間產物為兒茶酚 (catechol)，再由菌株生產的catechol 1,2-dioxygenase (C12O) 將之分解為cis,cis-muconic acid。菌株TKU002進行探討生長條件，最適氮源為添加0.05% urea及最適碳源為添加0.3% glycerol有最佳的菌株生長濃度。而C12O為胞內酵素，在大量培養菌株後，進行超音波破碎菌體以萃取其酵素進行酵素純化，經由DEAE-Sepharose、Sephacryl S-200及Sephacryl S-200等管柱層析純化出C12O，並經由電泳分析得知酵素分子量約為22 kDa。本實驗探討C12O酵素活性之生化性質，得知酵素以兒茶酚為基質之最適反應pH為7.5，在pH7~9有良好的安定性，酵素最適反應溫度為40，而在40以下則有相當好的熱安定性。而金屬離子對酵素之影響方面，Cu²⁺會降低酵素活性，而Fe²⁺則對酵素活性具有促進活性的效果。

關鍵詞：兒茶酚

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