学校编码：10384　　分类号＿＿＿密级＿＿＿

学号：20520061151896　　UDC＿＿＿＿＿＿＿＿＿＿

厦门大学

硕　士　学　位　论　文

海洋沉积物溴氰菊酯降解菌的鉴定及其降解酶的分离纯化与特性研究

Identification of the Deltamethrin-degrading Bacteria from Ocean Sediments and its Degrading Enzyme's Purification and Characterization

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论文提交日期：　2009 年　09　月

论文答辩日期：　2009 年　09　月

学位授予日期　2009 年　12　月

答辩委员会主席：＿＿＿＿＿＿

评　　阅　　人：＿＿＿＿＿＿

2009 年 09 月

Identification of the Deltamethrin-degrading Bacteria from Ocean Sediments and its Degrading Enzyme's Purification and Characterization

A Dissertation Submitted to the Graduate School in Partial Fulfillment of the Requirements for the Degree of Master Philosophy

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September, 2009

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摘 要

本论文着眼于对从海洋沉积物中筛选的溴氰菊酯降解菌菌株的鉴定；通过对高产酶菌株摇瓶培养基的优化，获得该菌株的最适培养基；在此基础上，对菌株产酶的酶液进行纯化，为降解酶的酶学研究做初步的准备。

首先研究了采用 PCR 技术进行溴氰菊酯降解菌 P1-1-B8 菌株的鉴定。通过革兰氏染色和 16S rDNA 测序，构建系统发育树，分析结果为：革兰氏阴性，P1-1-B8 菌株的 16S rDNA 基因序列和 *Pseudomonas xanthomarina* KMM1447(T) 菌株的 16S rDNA 基因序列具有最大相似性，同源性达 99%，确定菌株为 *Pseudomonas xanthomarina* P1-1-B8。

其次对 *Pseudomonas xanthomarina* P1-1-B8 菌株产酶条件进行了优化。考察了碳源、氮源、pH 值、培养温度、培养时间、接种量及溴氰菊酯对菌株产酶的影响。结果表明，降解菌产酶的最适条件为：以可溶性淀粉作为碳源、蛋白胨:酵母浸膏（2:1）为氮源、pH 7.5、温度 30 ℃、培养时间 2 d、接种量 3 %、溴氰菊酯含量 15 μ mol/L。在此条件下，菌株产酶的比活力最大为 113.3 U/mg。该降解酶对溴氰菊酯的降解，在 12 h 内降解率达 54.6 %。

在优化培养基的基础上，对溴氰菊酯降解酶的分离纯化及酶学性质。通过超声细胞粉碎制备降解酶，经硫酸铵粗提纯、DEAE-Sepharose Fast Flow 离子层析、Sephadex G-75 凝胶层析三步分离纯化降解酶，得到电泳级的纯化酶，分子量约为 5 KDa；该酶纯化效果较好，纯化倍数为 10.5，最终收率为 10.5 %。以 α-乙酸萘酯为底物测定纯化酶的动力学参数 Km 和 Vm 分别为 0.0645 mol/L、4055 mol/(L·s)。

关 键 词：溴氰菊酯；降解菌鉴定；降解酶产酶条件优化；降解酶的分离纯化

Abstract

Focus on the identification of deltamethrin-degrading bacteria; To obtain the high enzyme-producing strain by optimizing the cuture medium. This paper divided into five chapters on indentification of the marine bateria，and the purification and characterization of its degrading enzyme.

The dentification of P1-1-B3 strain which specificly degrade deltamethrin was reported by PCR technique. By using physiological and biochemical characteristics and 16S rDNA sequence and followed phylogenic tree, the results showed that the stain was negetive，and the 16S rDNA sequences of P1-1-B8 stain showd the maxium similarity about 99% to *Pseudomonas xanthomarina* KMM1447(T) strain. So the P1-1-B3 strain was named after *Pseudomonas xanthomarina* P1-1-B8 by the result of analysis of 16S rDNA sequence and phenotypic properties.

Then, some optimal conditions were studied on producing deltamethrin-degrading enzyme from *Pseudomonas xanthomarina* P1-1-B3 strain, which was obtained from marine sediment and showed high degradation efficiency on deltamethrin. These conditions were consisted of carbon sources, nitrogen sources, pH value, temperature, cultivation time, inoculation amount of bacteria and concentration of deltamethrin. The results indicated that the maxium enzymatic activity was 113.3 U/mg when the strain was cultured under the following conditions: soluble starch was chosen to be carbon source, the weight ratio of peptone to yeast extract was 2:1, pH 7.5, 30 ℃, 3 % inoculation amount of bacteria, 2 d of cultivation time, 15 μ mol/L of deltamethrin in media. Meanwhile, the degrading enzyme showed good degradation efficiency on deltamethrin with the degradation rate of 54.6 % in 12 h.

Subsequently, purification and characterization of the degrading-enzyme was reported. The degrading-enzyme was produced from the microorganism by ultrasonics, then purified by three steps, salted out by ammonium sulfate, DEAE-Sepharose Fast Flow ion exchange column and Sephadex G-75 gel column chromatography. The

molecular weight of purified enzyme was 5KDa by SDS-PAGE; The purification steps were very effective and gave the purification of 10.5 fold, and the activity yield of the purified enzyme were 10.5 %. The Km and Vm were 0.0645 mol/L and 4055 mol/(L·s) respectively for α-naphthyl acetate as substrate.

Key words: Deltamethrin; Identification; Optimization of conditions for enzymatic production; Separation and purification of degrading enzyme