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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

刘丽花

指导教师姓名: 陈小兰 副教授

王兆守 助理教授

专业名称: 无机化学

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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

By Liu Lihua

Supervised by

Prof. CHEN XIAOLAN

WANG ZHAOSHOU

Department of Chemistry

Xiamen University

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摘 要.....	i
Abstract.....	ii
第一章 前言.....	1
1.1 溴氰菊酯简介.....	1
1.2 溴氰菊酯农药残留及危害.....	1
1.2.1 农药残留.....	1
1.2.2 对鱼类的毒性.....	2
1.2.3 对哺乳动物毒性.....	3
1.3 降解方式.....	4
1.3.1 光化学降解.....	5
1.3.2 生物降解.....	5
1.4 酶工程.....	6
1.4.1 酶的制备--细胞裂解方法.....	6
1.4.2 沉淀技术.....	6
1.4.3 生化分离色谱技术.....	9
1.4.4 SDS 聚丙烯酰胺凝胶电泳.....	14
1.4.5 蛋白含量的测定.....	14
1.4.6 酶活力.....	16
1.5 本文研究内容及研究意义.....	17
参 考 文 献.....	19
第二章 溴氰菊酯降解菌的鉴定.....	27
2.1 材料及方法.....	27
2.1.1 菌种.....	27
2.1.2 主要试剂.....	27
2.1.3 细菌 16S rDNA 引物:.....	28
2.1.4 主要实验仪器.....	28

2.1.5 主要分析软件	28
2.1.6 培养基	28
2.1.7 溶液的配置	28
2.1.8 降解菌的活化和纯化	29
2.1.9 降解菌的形态及生理生化特性	29
2.1.10 革兰氏染色法	30
2.1.11 降解菌株的 16S rDNA 分析	31
2.1.12 构建系统发育树	32
2.1.13 菌株的生长曲线	32
2.1.14 菌株降解溴氰菊酯的紫外吸收测定	32
2.1.15 菌株降解溴氰菊酯气相色谱测定	32
2.2 结果与讨论	33
2.2.1 菌体形态	33
2.2.2 降解菌 P1-8-B3 的 16S rDNA 序列分析	34
2.2.3 系统发育树	35
2.2.4 菌株的生长曲线结果	37
2.2.5 菌株降解溴氰菊酯的紫外吸收曲线	37
2.2.6 菌株降解溴氰菊酯的气相色谱曲线	38
2.3 本章小结	40
参 考 文 献	41
第三章 溴氰菊酯降解菌产酶条件优化	44
3.1 材料及方法	44
3.1.1 菌种	44
3.1.2 材料	44
3.1.3 主要仪器	44
3.1.4 实验方法	45
3.2 结果与讨论	49
3.2.1 酶含量标准工作曲线	49
3.2.2 酶活力标准工作曲线	50
3.2.3 不同碳源及不同含碳量下菌体产酶的酶活力的测定	50
3.2.4 不同氮源及不同氮含量下菌体产酶的酶活力的测定	51

3.2.5 不同 pH 菌体产酶的测定	52
3.2.6 不同温度下菌体产酶的酶活力的测定	52
3.2.7 不同培养时间下菌体产酶的酶活力测定	53
3.2.8 不同接种量下菌体产酶的酶活力的测定	54
3.2.9 溴氰菊酯含量对菌体产酶的酶活力的测定	55
3.2.10 降解酶降解溴氰菊酯的紫外吸收曲线	56
3.2.11 降解酶降解溴氰菊酯的气相色谱曲线.....	56
3.3 本章小结	57
参 考 文 献.....	59
第四章 降解酶的分离纯化及酶学性质	61
4.1 材料及方法	61
4.1.1 材料.....	61
4.1.2 主要仪器	61
4.1.3 溶液的配制	62
4.1.4 实验方法	64
4.2 结果与讨论	68
4.2.1 粗分离酶的酶活力测定	68
4.2.2 DEAE-Sepharose Fast Flow 离子层析纯化的酶含量和酶活力的测定 (pH 8.0)	68
4.2.3 Sephadex G-75 凝胶层析纯化酶液 (pH 8.0)	69
4.2.4 K_m 和 V_m 计算.....	71
4.2.5 酸度对酶活力影响及 pH 稳定性	72
4.2.6 温度对酶活力的影响及热稳定性	73
4.2.7 干扰物质对酶活力的影响	74
4.3 本章小结	75
参 考 文 献.....	76
第五章 小结与展望.....	77
5.1 论文小结	77
5.1.1 菌株鉴定及产酶条件优化	77

5.1.2 酶的分离纯化	77
5.2 展望.....	77
5.2.1 酶分析	77
5.2.2 固定化酶	78
硕士期间发表论文.....	79
致 谢	80
附 录.....	81

厦门大学博硕士学位论文摘要库

Table of Contents

Abstract in Chinese	i
Abstract in English	ii
Chapter1 Introduction	1
1.1 Deltamethrin	1
1.2 Residue and toxicity of deltamethrin insecticide	1
1.2.1 Residue	1
1.2.2 Toxicity to fish	2
1.2.3 Toxicity to mammalian	3
1.3 Degradation	4
1.3.1 Photochemical degradation	4
1.3.2 Biological degradation	5
1.4 Enzyme engineering	6
1.4.1 Production of enzyme	6
1.4.2 Precipitation technology	6
1.4.3 Chromatographic technique for biochemical separation	9
1.4.4 Gel electrophoresis of SDS-PAGE	14
1.4.5 Calculation of enzymatical contentation	14
1.4.6 Enzyme activity	16
1.5 Contents and significances of this paper	17
References	19
Chapter 2 Indentification of microorganism	27
2.1 Materials and methods	27
2.1.1 Microorganism strain	27
2.1.2 Main reagents	27
2.1.3 16S rDNA primers of microorganism	28

2.1.4 Main instruments	28
2.1.5 Analysis software	28
2.1.6 Culture medium	28
2.1.7 Solution preparation	28
2.1.8 Activation and purification of microorganism strain	29
2.1.9 Morphology and physiological and biochemical characteristics of this microorganism strain	29
2.1.10 Gram staining.....	30
2.1.11 16S rDNA of degradation strain	31
2.1.12 Construction of phylogenetic tree.....	32
2.1.13 Construction growth curve of strain	32
2.1.14 Adsorption of deltamethrin degraded by strain.....	32
2.1.15 GC of deltamethrin degraded by strain.....	32
2.2 Result and discussion	33
2.2.1 Morphology	33
2.2.2 16S rDNA sequence analysis of P1-1-B3 strain	34
2.2.3 Phylogenetic tree	35
2.2.4 Result of growth curve.....	37
2.2.5 UV-Vis Spectra	37
2.2.6 Gas Chromatography	38
2.3 Summarization of this chapter	40
References.....	41
Chapter 3 Condition optimization for enzyme production.....	44
3.1 Materials and methods	44
3.1.1 Microorganism strain	44
3.1.2 Materials	44
3.1.3 Main instruments	44
3.1.4 Methods.....	45
3.2 Results and discussions.....	49
3.2.1 Standard curve of enzymatic contentation	49
3.2.2 Standard curve of enzymatic activity.....	50

3.2.3 Activity of enzyme produced from bacteria cultured by different carbon sources and concentrations	50
3.2.4 Activity of enzyme produced from bacteria cultured by different nitrogen sources and concentrations	51
3.2.5 Activity of enzyme produced from bacteria cultured in different pH	52
3.2.6 Activity of enzyme produced from bacteria cultured in different temperature	52
3.2.7 Activity of enzyme produced from bacteria cultured in different time	53
3.2.8 Activity of enzyme produced from bacteria cultured in different inoculation	54
3.2.9 Activity of enzyme produced from bacteria cultured in different concentrations of deltamethrin	55
3.2.10 UV-Vis Spectra	56
3.2.11 Gas Chromatography	56
3.3 Summarization of this chapter	57
References	59
Chapter 4 Purification and characterization of enzyme-degrading ...	61
4.1 Materials and methods	61
4.1.1 Materials	61
4.1.2 Main instruments	61
4.1.3 Solution preparation	62
4.1.4 Methods	64
4.2 Results and discussions	68
4.2.1 Purification by coarse separation	68
4.2.2 Purification of enzyme by DEAE-Sepharose FF ion exchange (pH 8.0)	68
4.2.3 Purification of enzyme by Sephadex G-75 column (pH 8.0)	69
4.2.4 Calculation of K_m and V_m	71
4.2.5 Effect of pH on enzyme activity and stability	72
4.2.6 Effect of temperature on enzyme activity and stability	73
4.2.7 Effect of interfering substances	74

4.3 Summarization of this chapter	75
References.....	76
Chapter 5 Conclusions and prospects	77
5.1 Conclusion	77
5.1.1 Identification and enzyme production	77
5.1.2 Purification of enzyme.....	77
5.2 Prospects	77
5.2.1 Enzymatic analysis.....	77
5.2.2 Enzymatic immobilization.....	78
Publications during Master study.....	79
Acknowledgement.....	80
Appendices	81

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 °C、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 K_m 和 V_m 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 culture medium. This paper divided into five chapters on identification of the marine bacteria, and the purification and characterization of its degrading enzyme.

The identification of P1-1-B3 strain which specifically degrades deltamethrin was reported by PCR technique. By using physiological and biochemical characteristics and 16S rDNA sequence and followed phylogenetic tree, the results showed that the strain was negative, and the 16S rDNA sequences of P1-1-B3 strain showed the maximum similarity about 99% to *Pseudomonas xanthomarina* KMM1447(T) strain. So the P1-1-B3 strain was named after *Pseudomonas xanthomarina* P1-1-B3 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 consisted of carbon sources, nitrogen sources, pH value, temperature, cultivation time, inoculation amount of bacteria and concentration of deltamethrin. The results indicated that the maximum 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 °C, 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 K_m and V_m 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

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