Primary Study on the Characteristics of Crude Pyrethroid Pesticide-Degrading Enzyme Extracted from Pseudomonas diminuta Strain M5R14

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Abstract: One Pseudomonas diminuta strain M5R14 was selected as object in the present study, which was isolated from the tannage sludge in water course of Pesticide Factory of Hangzhou and could degrade a variety of strains of synthetic pyrethroid insecticides. The inner and extra-cell crude enzyme of Pseudomonas diminuta strain M5R14 prepared by ultrasonic break up and centrifugal separation methods, and the characteristics of crude pyrethroid pesticide-degrading enzyme were primary studied. The results showed that pyrethroid pesticides degrading enzyme excreted from the strain of was intracellular enzyme, the best pH for bifenthrin, fenpropathrin and cypermethrin degradation was 6.5, as well as the most appropriate temperature was 35°C. Besides, the Michaelis constant and maximum degradation rate varied due to pyrethroid species. The Michaelis constant to such 3 pyrethroid pesticides were 4.162.73, 1.092.57, 1, 171.97 μmol/L, respectively, and the maximum degradation rate to such 3 pyrethroid pesticides were 0.152, 0.038, 0.043 μmol/(min·mg) protein. The degradation process of crude degrading enzymes to such 3 pyrethroid pesticides was fitted for first-order kinetic equation and the half life (t1/2) were 119.51, 113.63, 108.30 min, respectively. Crude degrading enzymes for the three pyrethroid insecticides could be maintained high degradation activity when the pH ranging from 5.0 to 8.0, and the temperature was 20°C to 50°C, indicating the degrading enzymes were with large scope of pH stability and thermal stability. Therefore, there's higher potential in controlling the residuals of synthetic pyrethroid insecticides. 

Key words: pyrethroid pesticides; Pseudomonas diminuta; crude degrading enzyme
材料与方法

1.1 M5R14菌株

本实验室在以前的工作中从杭州农药厂污水排放的污泥中分离得到缺陷假单胞菌菌株M5R14[9]。携带农药储备液10 g，NH₄NO₃ 1.00 g，MgSO₄·7H₂O 0.5 g，(NH₄)₂SO₄ 0.5 g，KH₂PO₄ 0.5 g，NaCl 0.5 g，K₂HPO₄ 1.5 g，H₂O 1 000 mL，pH 7.0)。30°C、180 r/min[] [] 3 d,[] D[]，[] (NaCl 1.0 g，KH₂PO₄ 1.0 g，1 000 mL，[] [] 1.0 g)[]，30°C、180 r/min[] [] []。

1.2 M5R14菌株降解酶的粗提酶降解农药的米氏常数求得缺陷假单胞菌的粗提酶的米氏常数，95.8%[]，20%[] 94%[],[] 1.5[] ([] )[]。将活化过的缺陷假单胞菌10 g[] 50 mL，50 g/L[] [] 50 mL，[] 1 000 mL，[] 12 ~ 15 g[] 30°C、180 r/min[] [] 3 h,[] 0.1 MPa, 121°C, 30 min[] [] 。

1.3 Kₘ[] Vₘₐₓ[18,19]

100 mg/L[] 18 mL，[] () 70°C，10 min[] [] 10 min，[] 4°C[] 10,000 r/min[] 15 min[] [] . pH 7.0 0.02 mol/L[] []，[] (MWCO = 5 000)[] SO₄²⁻，[] 。[] pH 7.0[] 3 mL，[] 0.01 mol/L[] 1 g[] []，[] 400 W[]，[] 99%，5 s，[] 5 s，4°C[] 10,000 r/min[] 10 min[]，[] 。[] 3 mL[]，[] BS(A, sigma)[] 。

1.4 (50, 75, 100, 125, 150, 175, 200 mg/L)[] pH 7.0，18 mL，[] 35°C[] 5 min[]，[] 2 mL，[] 2 h，[] 3 mL[]，[] 4 mL[]，[] 4 mL[]，[] 2 ml[]，[] 3 mL，[] 4 mL[]，[] 10 mL，[] 。

1.5 M5R14[] [][]，[] Lineweaver-Burk[]，[]，[] M5R14[] Kₘ[] Vₘₐₓ[18,19]。
氯氰菊酯品残留量
除虫菊酯农药残留量

水

经无水硫酸钠除水后定容至冲液为对照

胞外粗酶液

并定容至

石油醚萃取的联苯菊酯

温度对降解粗酶活性的影响
拟除虫菊酯类农药残留量的测定
分析缺陷假单胞菌降解粗酶对联苯菊酯
氯氰菊酯降解动力学过程

值对降解粗酶活性的影响

表
缺陷假单胞菌
降解动力学方程

实验条件下

对联苯菊酯

甲氯菊酯

氯氰菊酯

结果与分析

外粗酶液对拟除虫菊酯类农药的降解效果

种拟除虫菊酯农药的降解率见

种拟除虫菊酯农

胞外和胞内降解粗酶对联苯菊酯

粗酶处理样

为

甲氰菊酯和氯氰菊酯的影响

比活力

甲氰

和

缺陷假单胞菌

降解粗酶的定位

M5R14 对 s 比活力

M5R14 对 bifenthrin, fenpropathrin and cypermethrin

表

M5R14
determination of the degradative inner and extra-cell enzyme of Pseudomonas diminuta strain

<p>| | | | | |</p>
<table>
<thead>
<tr>
<th></th>
<th></th>
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<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>%</td>
<td>µg·(mg·h)⁻¹</td>
<td>%</td>
<td>µg·(mg·h)⁻¹</td>
</tr>
<tr>
<td>1</td>
<td>44.56 ± 1.29</td>
<td>533.30 ± 15.44</td>
<td>45.45 ± 3.69</td>
<td>543.95 ± 44.16</td>
</tr>
<tr>
<td>2</td>
<td>6.54 ± 1.65</td>
<td>70.75 ± 17.85</td>
<td>7.13 ± 1.28</td>
<td>77.13 ± 13.85</td>
</tr>
<tr>
<td>3</td>
<td>2.28 ± 1.02</td>
<td>—</td>
<td>1.45 ± 0.84</td>
<td>—</td>
</tr>
</tbody>
</table>

1) —

2.1

2.3

pH4.0, 5.0, 6.0, 6.5, 7.0, 7.5, 8.0, 9.0, 10.0

M5R14

pH4.0 ~ 10.0

34.5% ~ 45.6%

pH6.5, 7.0

44.6% ~ 45.4%

pH7.0

M5R14

3 h

20 ~ 45℃
2.4 Effect of temperature on degradation rate of bifenthrin, fenpropathrin and cypermethrin by crude enzyme of *Pseudomonas diminuta* strain M5R14

![Graph showing degradation rate vs. temperature for different pesticides](image1)

**Fig. 2** Effect of temperature on degradation rate of bifenthrin, fenpropathrin and cypermethrin by crude enzyme of *Pseudomonas diminuta* strain M5R14

2.5 Effect of pH on degradation stability to bifenthrin, fenpropathrin and cypermethrin by crude enzyme of *Pseudomonas diminuta* strain M5R14

![Graph showing degradation stability vs. pH](image2)

**Fig. 3** Effect of pH on degradation stability to bifenthrin, fenpropathrin and cypermethrin by crude enzyme of *Pseudomonas diminuta* strain M5R14

2.6 Kinetic parameters for the degradation of bifenthrin, fenpropathrin and cypermethrin by crude enzyme of *Pseudomonas diminuta* strain M5R14

<table>
<thead>
<tr>
<th>Pesticide</th>
<th><em>K</em>&lt;sub&gt;m&lt;/sub&gt;</th>
<th><em>V</em>&lt;sub&gt;max&lt;/sub&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bifenthrin</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Fenpropathrin</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cypermethrin</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

**Fig. 4** Degradation stability to bifenthrin, fenpropathrin and cypermethrin under different temperature by crude enzyme of *Pseudomonas diminuta* strain M5R14

2.7 Effect of pH on degradation stability to bifenthrin, fenpropathrin and cypermethrin by crude enzyme of *Pseudomonas diminuta* strain M5R14

![Graph showing degradation stability vs. pH](image3)

**Fig. 5** Effect of pH on degradation stability to bifenthrin, fenpropathrin and cypermethrin by crude enzyme of *Pseudomonas diminuta* strain M5R14

...
2. **MSR14**

Lineweaver-Burk

Table 2  Correlation parameters Lineweaver-Burk of degradation to bifenthrin, fenpropidin and cypermethrin by crude enzyme of *Pseudomonas diminuta* strain MSR14.

<table>
<thead>
<tr>
<th>Enzyme</th>
<th>$k_m$ (μmol·L$^{-1}$)</th>
<th>$V_{max}$ (μmol·(min·mg)$^{-1}$)</th>
</tr>
</thead>
<tbody>
<tr>
<td>y = 27.474x + 0.006 6</td>
<td>4.162.73</td>
<td>0.152</td>
</tr>
<tr>
<td>y = 23.925x + 0.026 5</td>
<td>1.092.57</td>
<td>0.038</td>
</tr>
<tr>
<td>y = 29.053x + 0.023 3</td>
<td>1.171.97</td>
<td>0.043</td>
</tr>
</tbody>
</table>

3. **MSR14**

Table 3 Degradation kinetic parameters of crude enzyme of *Pseudomonas diminuta* strain MSR14 to bifenthrin, fenpropidin and cypermethrin.

<table>
<thead>
<tr>
<th>Enzyme</th>
<th>$y$ = 9.088 8e$^{-0.005 k}$</th>
<th>$t_{1/2}$ (min)</th>
<th>$R^2$</th>
</tr>
</thead>
<tbody>
<tr>
<td>y = 9.088 8e$^{-0.005 k}$</td>
<td>0.005 8</td>
<td>0.997 1</td>
<td>119.508 1</td>
</tr>
<tr>
<td>y = 9.382 8e$^{-0.005 k}$</td>
<td>0.006 1</td>
<td>0.980 6</td>
<td>113.630 7</td>
</tr>
<tr>
<td>y = 9.307 1e$^{-0.005 k}$</td>
<td>0.006 4</td>
<td>0.978 8</td>
<td>108.304 2</td>
</tr>
</tbody>
</table>

4. **MSR14**

（1）**M5R14** pH 6.5 35°C, pH 5.0~8.0 20~50°C, 3 μmol/L, 119.51, 113.63 108.30 min; **M5R14** 4.162.73, 1.092.57, 1.171.97 μmol/L; $V_{max}$ 0.152, 0.038, 0.043 μmol/(min·mg).
References:


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