Degradation of Dimethoate by Cellulolytic Bacteria in Cotton Soils

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Abstract

The present experience has been aimed to isolate bacteria specifically cellulolytic bacteria, normally found in cotton soils and determine their role in degradation of the specific pesticide. Two cellulolytic bacterial isolates k1 k2 identified as Pseudomonas putida and Bacillus pumulus were capable of growing on dimethoate supplemented medium. P.putida exhibited maximum growth of 4.1x 106 cfu/ ml at 0.09 mg/ml while B.pumulus showed significant growth of 2.2 x107 cfu/ml at 0.06mg/ml of dimethoate after 72hrs of incubation at room temperature. Rate of utilization of dimethoate increased progressively with increase in the concentration of yeast extract added to the medium up to 0.56

Index terms— dimethoate, cellulolytic bacteria, degradation.

1 Introduction

otton, the most important fibre crop of India plays a dominant role in its agrarian and industrial economy. It is attacked by various sucking pestsjassids, aphids, thrips, whitefly, red spider, mite and mealy bug. Bollworms such as pink bollworm, spotted bollworm and American bollworm **??**Tomelin, 1995).

The progressive increase of pest problem and demand for agricultural products necessitated the application of agrochemicals and ensure high quality and crop yield **??**Graebing et.al.,2002). Among the xenobiotics used, organophosphorous are widely applied in Indian agricultural system.

The introduction of these pesticide in to the soil environment raises concern as to their effect on ecological balance in terms of soil fertility (Balwinder Singh 2002). Even though the pesticide degradation is under the impact of various physico-chemical parameters, literature survey reveals that the major pathway governing degradation and ecotoxicity of these compounds is mediated (Latha et al, 2001). Dimethoate, an organophosphate broad spectrum insecticide is of particular concern as it is widely used for controlling cotton pests. But in view of its

Author: e-mail: ravuri_jayamadhuri@rediffmail.com toxicity, it is important to remove dimethoate from the environment. The role of microorganisms in bioremediation is important because of their ability to degrade hazardous compounds into harmless ones. There is lack of information regarding biodegradation of dimethoate in cotton soils.

Hence the present work has been aimed to characterize the isolated cellulolytic bacteria from the cotton soil and for its ability to utilize dimethoate as the sole source of carbon and energy.

2 II.

3 Materials and Methods

For the isolation of bacteria, soil samples from cotton cultivated fields were collected from three different locations in Guntur District (Narsarao pet, Sattena Palli and LAM farm). All soil samples were sieved through a 2mm screen and then used.

4 a) Isolation and characterization of cellulolytic bacteria

The plates were incubated at 37 o C for 3-5 days and were flooded with 1% aqueous solution of hexadecyltrimethyl ammonium bromide, the isolates showing a clear zone of hydrolysis around the colony were selected for further study. These isolates were streaked on cellulose agar slants and maintained as pure cultures.

Biochemical characterization of isolated bacteria was done referring to the Bergey's manual of Systematic Bacteriology and named the two bacterial isolates producing maximum zone of hydrolysis as K1 and K2

5 b) Utilization of Dimethoate

The rate of dimethoate utilization in terms of growth by the isolates K1 and K2 was determined at 37 O C in dimethoate (0.1 mg/ml) mixed mineral salt solution for 140 hrs. The three soil samples were enriched with plant debris and incubated with 37 0 C for 15 days. After the incubation period, enriched soil samples were taken and serial dilutions (up to 10 -6) were made using sterile water. One ml aliquots of 10 -4 and 10 -5 dilutions were made to the sterile cellulose agar medium allowed to solidify. Triplicates for each dilution were maintained.

6 c) Dimethoate degradation test

The degradation of dimethoate was observed by bioassay method. 25 ml of MS solution was taken in 100 ml conical flask and 0.03, 0.06, 0.09, 0.12, 0.15, 0.18, 0.21 and 0.24 mg/ml of dimethoate were taken to which the bacterial inoculum of K1 and K2 separately were added and incubated at 37 0 C for 72 hrs and growth was studied by colony count technique.

7 d) Effect of yeast extract on the utilization of dimethoate

The effect of varying levels of yeast extract on the rate of dimethoate utilization was studied for the isolated bacteria K1 and K2. The bacterial isolates were allowed to grow for 24 hrs at 37 o C in MS solution amended with dimethoate(0.1 mg/ml) plus yeast extract (MSDY) and in MS solution supplemented with yeast extract(MSY) only as control. The results were tabulated and interpreted in terms of viable CFU/ml in MS solution.

Dimethoate degradation test : Bacterial cultures efficient in degradation of dimethoate were incubated for 72 hrs and the percentage of degradation as well as metabolites formed are analysed by HPLC technique using hexane as mobile phase and C18 column at IIHR, Bangalore.

8 III.

9 Results

10 a) Isolation and characterization

The K1 isolate obtained from cotton soil enriched with plant debris is a gram negative motile rod with 2.2µm length and 1.3µm width. It is aerobic and showed no spore formation. It is non spore forming, motile, catalase, oxidase and casein positive. Starch and gelatin are not hydrolyzed. IMVIC negative and nitrate is not reduced. According to Bergey's manual of classification, the K1 isolate was tentatively identified as Pseudomonas putida (Table 1).

The K2 isolate obtained is a gram positive rod with 3µm length and 0.6µm width. It is aerobic and a terminal ellipsoidal spore was observed. Utilization of urea, gelatin, citrate and nitrate reduction was observed. Hydrolysis of casein and starch was observed. The isolate was catalase positive and oxidase negative. Based on the above characteristics the K2 isolate was identified tentatively as Bacillus pumilis.

11 Name of the reaction K1 K2

12 b) Utilization of Dimethoate

The growth curve of K1 and K2 isolates cultivated in 0.1 mg/ml of dimethoate is shown in tables 2 and 3. The K1 isolate showed its maximum growth rate after 20 hrs in MS solution (Tab 2).

The K2 isolate showed its maximum growth rate after 40 hrs in MS solution (Tab 3).

13 c) Dimethoate tolerance and utilization test

Degradation of dimethoate provided as the sole carbon was studied in mineral salt solution. In K1 isolate it was found that maximum growth occurred at 0.09 mg/ml after incubation for 72 hrs at 37 0 C (Tab. 4). The K2 isolate was found tolerating dimethoate up to 0.24mg/ml in MS solution with maximum growth at 0.06mg/ml after an incubation for 72hrs at 37 0 C (Tab 5).

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15 d) Effect of Yeast Extract on the Utilization of Dimethoate

The K1 isolate when used for the utilization of dimethoate in the presence of yeast extract showed higher degrading activity. The rate of utilization of dimethoate increased progressively with the increase in concentration of yeast extract up to 0.56% but the growth was effected at a concentration of 0.7% and above (Tab 6).

The K2 isolate when used for the utilization of dimethoate in the presence of yeast extract showed higher degrading activity. The rate of utilization of dimethoate increased progressively with the increase in concentration of yeast extract up to 0.70% but the growth drastically effected at a concentration of 0.84% and above (Tab 7). IV.

16 Conc. of yeast extract

17 Mineral

18 Discussion

In the present study, two bacterial isolates K1 and K2 which are cellulolytic and capable of growth on dimethoate as a sole source of carbon and energy have been isolated from the cotton soils. [1]

Figure 1: C

1

Year

Figure 2: Table 1 :

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Figure 3: Table 2 :

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of k 2 in mineral salt solution
supplemented with 0.1mg/ml of dimethoate

Figure 4: Table 3 :

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	salts medium with dimethoate	Mineral salts medium with dimethoate and yeast extract
0.14	2.1 x 10 4	2.9 x 10 4
0.28	2.8 x 10 4	3.5 x 10 5
0.42	3.5 x 10 5	1.7 x 10 6
0.56	4.7 x 10 5	4 x 10 6
0.70	3.3 x 10 4	3.1 x 10 5
0.84	2.5 x 10 4	5.1 x 10 4
0.98	1.6 x 10 3	2.8 x 10 4

Figure 5: Table 4 :

5

Figure 6: Table 5 :

6

Figure 7: Table 6 :

7

Figure 8: Table 7 :

8

Figure 9: Table 8 :

9

Figure 10: Table 9 :

In this study the strain K1 showed maximum growth of 4.1x 10 6 cfu/ml at 0.09mg/ml and strain K2 with significant growth of 2.2x10 7 cfu/ml at 0.06 mg/ml. Bhadbade et al, **??**2002) reported that Bacillus licheniformis tolerated dimethoate upto the concen-tration of 2 mg/ml.

The K1 isolate showed maximum utilization of dimethoate at a concentration of 0.09 mg/ml and K2 isolate showed maximum utilization of dimethoate at a concentration of 0.06 mg/ml after 72 hrs at 37 0 C. In a previous study **??**andal et.al (2002) reported that Bacillus licheniformis showed a maximum utilization of dimethoate at 0.45 mg/ml

The K1 isolate when used for the utilization of dimethoate in the presence of yeast extract showed higher degrading activity. The rate of utilization of dimethoate increased progressively with the increase in concentration of yeast extract up to 0.56% but the growth was effected at a concentration of 0.7% and above .

The K2 isolate when used for the utilization of dimethoate in the presence of yeast extract showed higher degrading activity. The rate of utilization of dimethoate increased progressively with the increase in concentration of yeast extract up to 0.70% but the growth was drastically effected at a concentration of 0.84% and above. Sharmila et al, (1992a and b) also parathion and other organophosphates in soil was regulated by the amount of yeast extract in the medium. Similar evidence was provided by Despande et al, **??**2001). Accordingly, rate of utilization of dimethoate by Bacillus sps progressively increased with yeast extract concentration up to 0.15%. This was further supported by experimental results of Kadam (2003) at Sreerampore. India.

Even though previous evidence indicate rapid degradation of the pesticide by soil bacteria, **??**Dixit and Banerjee, 2000; **??**ernandez etal, 2002; **??**abik etal;2003).more than 90% of degradation rate was not reported. But in the present experiment, only trace amount of the pesticide residue was remaining after bacterial degradation indicating high efficiency of K 2 .

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