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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% in P.putida and 0.7% in B.pumulus. The present findings indicate that among the two bacterial isolates, sps of Pseudomonas could degrade 88% of dimethoate while Bacillus sps exhibited high degradation rate of 92% which can be commercialized for bioremediation of dimethoate contaminated sites. Further, the rate of degradation is maximum at 72hrs of incubation.

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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% in P.putida and 0.7% in B.pumulus. The present findings indicate that among the two bacterial isolates, sps of Pseudomonas could degrade 88% of dimethoate while Bacillus sps exhibited high degradation rate of 92% which can be commercialized for bioremediation of dimethoate contaminated sites. Further, the rate of degradation is maximum at 72hrs of incubation.

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I. Introduction

Cotton, the most important fibre crop of India plays a dominant role in its agrarian and industrial economy. It is attacked by various sucking pests-jassids, 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 *microbial* 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 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.

II. 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.

a) *Isolation and characterization of cellulolytic bacteria*

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.

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

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.

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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.

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° 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.

III. Results

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.*

Table 1 : Morphological and biochemical characterization of K1 and K2

Name of the reaction	K1	K2
Gram reaction	Negative	Positive
Morphology	Rod	Rod
Cell length	2.2 μm	3 μm
Cell diameter	1.3 μm	μm
Pigment	Yellow	No pigmentation
Spore formation	Absent	Terminal, Ellipsoidal
Motility	Motile	Nonmotile
Starch hydrolysis	Negative	Positive
Catalase test	Positive	Positive
Gelatin hydrolysis	Negative	Positive
Oxidase test	Positive	Negative
Urease test	Negative	Positive
Casein hydrolysis	Positive	Positive
Nitrate reduction	Negative	Positive
Indole production	Negative	Negative
Voges Proskaeur	Negative	Negative
Citrate utilization	Negative	Positive

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).

Table 2 : Growth of k_1 in mineral salt solution supplemented with 0.1mg/ml of dimethoate

Hours	CFU/ml
0	1.2x 10^5
20	3 x 10^5
40	4.5 x 10^5
60	1.2 x 10^5
80	4 x 10^4
100	8 x 10^3
120	6 x 10^3

Table 3 : Growth of k_2 in mineral salt solution supplemented with 0.1mg/ml of dimethoate

Hours	CFU/ml
0	1.5 x 10^5
20	2.3 x 10^5
40	3.8 x 10^5
60	1.2 x 10^5
80	4.1 x 10^4
100	5.8 x 10^3
120	3.4 x 10^3

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).

Table 4 : Utilization and tolerance level of dimethoate by k_1 isolate

Dimethoate concentration (mg/ml)	CFU/ml
0	1.5×10^4
0.03	2×10^4
0.06	$2.7x\ 10^4$
0.09	4.1×10^6
0.12	2.4×10^4
0.15	1.3×10^3
0.18	8.8×10^2
0.21	No growth
0.24	No growth

Table 5 : Utilization and tolerance level of dimethoate by k_2 isolate

Dimethoate concentration (mg/ml)	CFU/ml
0	1.3×10^4
0.03	1.9×10^4
0.06	2.2×10^7
0.09	1.1×10^5
0.12	2.3×10^4
0.15	1.8×10^3
0.18	1.4×10^3
0.21	0.8×10^2
0.24	0.2×10^2

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 was drastically effected at a concentration of 0.84% and above (Tab 7).

Table 6 : Effect of yeast extract on k_1 utilization of dimethoate (0.1 mg/ ml) in mineral salts solution with initial inoculum of 1×10^3 CFU/ml

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

Table 7 : Effect of yeast extract on k_2 utilization of dimethoate (0.1 mg/ ml) in mineral salts solution with initial inoculum of 1×10^3 CFU/ml

Conc. of yeast extract	Mineral salts medium with dimethoate	Mineral salts medium with dimethoate and yeast extract
0.14	1.5×10^4	1.9×10^4
0.28	1.9×10^4	2.5×10^5
0.42	$3.1x\ 10^4$	1.5×10^7
0.56	5×10^4	3.9×10^7
0.70	1×10^5	3.1×10^8
0.84	1.5×10^5	7.9×10^5
0.98	2.5×10^5	3.9×10^4

Table 8 : Rate of degradation of dimethoate by Bacillus pumulis K_1 isolate

Incubation time	% of degradation
24 hr	38
48 hr	51
72hr	88

Table 9 : Rate of degradation of dimethoate by Bacillus pumulis K_2 isolate

Incubation time	% of degradation
24 hr	43
48 hr	78
72 hr	92

e) Degradation of dimethoate by selected bacterial cultures

Bacterial cultures obtained by enrichment technique were tested for dimethoate degradation. In the present experiment, at the end of incubation, HPLC analysis shows that 38% degradation occurred at 24 hrs. At 48 hrs time interval, 51% dimethoate disappeared. Finally after 72 hrs,only 12% of the parent residue was detected in the sample. That means 88% of compound was degraded with respect to k_1. (Tab 8)Regarding k_2, only 85% of the parent compound was detected at the end of incubation period. Even though previous evidence indicate rapid degradation of the pesticide by soil bacteria, 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 (. Morover, formation of extra peaks besides the parent compound indicate formation of intermediate products which means biotransformation of the parent insecticide. But due to non availability of compound, end product is not identified.

IV. 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.

In this study the strain K1 showed maximum growth of 4.1x 10^6cfu/ml at 0.09mg/ml and strain K2 with significant growth of 2.2x10^7cfu/ml at 0.06 mg/ml. Bhadbade et al, (2002) reported that Bacillus lichen-iformis 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 Mandal 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 reported earlier that rate of *microbial* degradation of 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; Fernandez etal, 2002; Zabik 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.

References Références Referencias

1. Balwindar Singh S. (2002). Pesticide contamination of the environment in Punjab. Indian J Ecol. 29: 189-198.
2. Bhadbhaden B.J., Dhakephalker, P.K., Sarnaik, S.S., and Kanekar P.P. (2002). Plasmid-associated biodegradation of an organophosphorous pesticide by Pseudomonas mendocina. Biotechnology Letters 24: 647-650.
3. Deb Mandal, M. Mandal S and Pal NK. 2002. Evaluation of bioremediation potential of organophosphorous pesticide dimethoate 30%. EC by heavy metal and antibiotic resistance proteous vulgaris isolated from Ganges at Sreerampore. Indian Res J. Chem Environ. 6: 49-52.
4. Deshpande, N.N. Dhakephalkar, P.K., and Kaniker, P.P. (2001). Plasmid mediated dimethoate degradation in Pseudomonas aeruginosa, MCMB-427. 33-275-279.
5. Grabing, P., Frank, M. and Chib J.S. (2002). Effects of fertilizer and soil components and pesticide photolysis. J. Agric. Food Chem. 50: 7332-7339.
6. Kadam, T.A., Jadhav, V.D. and Gyananath, G. (2003). *Microbial* degradation of dimethoate by gram negative soil isolated from cotton field. Polln Res. 22: 443-445.
7. Latha, M.R., Indiarani, R. and Kamaraj, S. (2001). Biodegradation of Polluted soils. J Environ. Res. 11:27-29.
8. Metin, D. and Ferdag, K. (2001). Effect of some organophosphorous insecticides on soil microorganisms. Turk j Biol. 25;51-58.
9. Sharmila, M. Ramananad, K. and Sethunathan, N. (1992). Effect of yeast extract on the degradation of organophosphorous insecticides by soil enrichment and bacterial cultures. Can J *Microbial*., 35:1105 - 1110.
10. Subhas and Singh. D.K. (2003). Utilization of monocrotophos as phosphorous source by Pseudomonas aeruginosa F10B and Clavibacter michiganense subsp. Insidiosum SBL 11 Can J. *Microbial* / Rev. Can*Microbial* . 49: 101-109.
11. Tomlin, C. (1995). The pesticide Manual (incorporating the Agrochemicals Hand book), 10th edn British Crop Protection Council (BCPC).
12. Zaidi BR and Imam SH. 1996. Inoculation of microorganisms to enhance biodegradation of phenolic compounds in industrial wastewater. Isolation and identification of three indigenous bacterial strains. J. Gen Appl Microbiology 42;249-256.