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ANALYSIS OF DIMETHOATE DEGRADATION BY *KOCURIA TURFANENSIS* USING GC-MS

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Abstract – Pesticide is an essential factor in agricultural fields of India. Because of its beneficial properties dimethoate is most commonly used organophosphorus pesticide. At the same time unwise use of dimethoate exerts hazardous effects on ecosystem via bioaccumulation and biomagnifications. However, effective removal or degrading technique has to be identified. Hence, the present study was aimed to carry out dimethoate degradation by potential microorganism. A novel dimethoate degrading bacterial strain was isolated from agriculture soil. The strain utilizes dimethoate as sole carbon source. On the basis of morphological, biochemical characteristic and 16S rRNA gene sequence analysis the strain was identified as *Kocuria turfanensis*. Dimethoate contaminated soil was treated with *Kocuria turfanensis* for degradation and degradation products were identified using gas chromatography – mass spectrometry (GC-MS) analysis. The result indicated that the *Kocuria* strain degraded 78.22% dimethoate.

INTRODUCTION

Organophosphorus (OP) pesticides are most widely used in agriculture as insect control agents. Dimethoate was first introduced in the year 1956. It was patented by American Cyanamid. Dimethoate is a widely used organophosphorus insecticide, chemically termed as O,O-dimethyl - S-methyl carboxyl-methyl phosphorodithioate (Chen *et al*., 2007). The Environment Protection Agency (EPA) classifies dimethoate as class II toxicity - moderate toxic compound. Dimethoate (30% E.C.) was registered in 1962 and used against a broad range of insects such as aphids, mites, and whiteflies and on a number of crops including citrus, cotton, fruit, olives, potatoes, tea, tobacco and vegetables (Deshpande *et al*., 2001). Dimethoate acts by interfering with the activities of cholinesterase, an enzyme essential for the proper functioning of the nervous system of insects and humans.

Dimethoate also acts by interfering with the activities of cholinesterase, an enzyme essential for the proper functioning of the nervous system of insects, other animals and humans too (Megeed and El-Nakieb, 2008; Shinde *et al*., 2015; Karishma and Hari, 2014; Van Scoy *et al*., 2016). Organophosphorus pesticides have been thoroughly reviewed in recent years because of their adverse effects in environment. The extensive use of pesticide kills the target organism and remaining contaminates the air, soil, surface and ground water, ultimately resulting in adverse affect on environment. Therefore the removal of pesticide from environment becomes an important goal. Many research studies indicated that soil bacteria have ability to degrade dimethoate (Deshpande *et al*., 2001; Liu *et al*., 2001; Jiang *et al*., 2007; Debmandal *et al*., 2008).

MATERIALS AND METHODS

Soil collection

To investigate the enhanced biodegradation phenomena at laboratory level soil samples were used in this investigation. All soil samples were collected from the top 10-15 cm of soil in fields which is having a long history of pesticide application (Bardoli taluka; 21°21′52.74"N 73°20′40.11"E). The soil samples were transported in plastic bags to the laboratory and stored at 4 °C till further use.

Isolation and screening of pesticide degrading bacteria

The enrichment culture technique was used for the isolation of bacterial strains capable of utilizing dimethoate as a sole source of carbon and energy.

The soil samples were enriched with pesticide in flasks and incubated on shaker with 37 °C for 7 days. After the incubation period, enriched soil sample was taken and serial dilutions (up to 10^{-6}) were made using sterile water. One mL aliquots of 10^{-5} and 10^{-6} dilutions were made. From the final dilution, 50 µL were spread on MSM agar medium with glass spreader and incubated at 37° C for 3-5 days. After incubation a number of isolated colonies were picked with a sterile loop and transferred onto fresh MSM agar medium. The pure culture was maintained at 4 °C. Two isolates which showed higher resistance were selected for further studies.

Identification of Dimethoate degradation isolates

The potential dimethoate degrading strain isolated and identified on the basis of its morphological and biochemical characters with 16S rRNA gene sequence analysis conducted by Saffron Life Science Navasari, Gujarat. The comparison of the sequences obtained was done with the GenBank database using Basic Local Alignment Search Tool BLASTN program using National Centre of Biotechnology Information (NCBI) database.

Dimethoate degradation

Biodegradation study of dimethoate accomplished using MSM media in 250 mL Erlenmeyer flasks. A 100mL MSM media with 1% dimethoate inoculated with 5 mL over night grown bacterial culture. Flasks were incubated at 37° C on shaker at 120 RPM for 21 days. Control flask containing dimethoate without bacterial culture maintained under similar conditions to check the loss of dimethoate. After incubation sample were taken for GC-MS analysis.

Detection of Dimethoate metabolites

Samples were collected from 100 mL bacteria solution and extracted three times with n-hexane (100 mL, 75 mL and 50 mL) by vigorous shaking for 15-20 minute in a separatory funnel. The n-hexane layer was separated and evaporated. The dried residue was dissolved in 10 mL methanol. After gently vortexing and filtering through a 0.2 mm membrane filter, sample was used for GC-MS (Agilent GC MSD Systems) analysis.

RESULTS AND DISCUSSION

Isolation and screening of pesticide degrading bacteria

Ten bacterial strain isolated having capacity to utilize dimethoate as a sole carbon and energy source. The strain KC showed the highest tolerance to dimethoate among all isolates.

Identification of dimethoate degrading strain

The strain KC was a gram positive cocci which gives small orange pigment colonies on MSM medium. The nucleotide sequence of the 16S rRNA gene of strain KC has been identified as *Kocuria turfanensis* and deposited in Gen Bank under accession no. KU521338.1.

Detection of Dimethoate metabolites

After 21 days of incubation the sample was extracted and analyzed for degradation products of dimethoate by GC-MS. The comparison with NIST Mass Spectrometry Data Center 1998 confirmed the standard spectra of dimethoate (Figure 1). The result indicated that the *Kocuria* strain degraded 78.22% dimethoate after 21 days and the presence of dimethoate at retention time of 17.853 minutes. The metabolites of dimethoate degradation by isolated strain are enlisted in Table 1 according to their molecular weight and retention time (Figure 2 and 3). The presence of these metabolites of dimethoate indicated the degradation of dimethoate. Very few literature is available that shows the degradation of dimethoate with the help of *Kocuria turfanensis.*

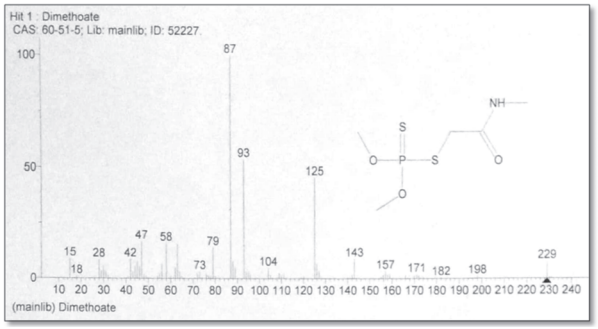

Fig. 1. GC-MS spectra of standard Dimethoate

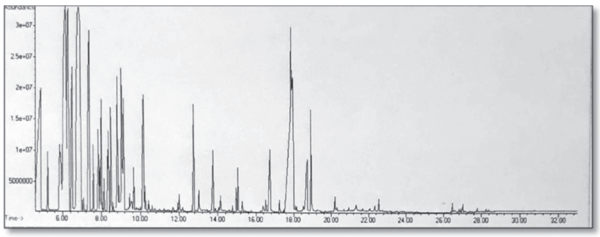

Fig. 2. GC-MS spectra of dimethoate degradation by *Kocuria turfanensis*

Other species of *Kocuria i.e. Kocuria kristinae* has been isolated and identified by Hamsavathani *et al.,*

Table 1. The metabolite compounds detected by GC-MS of cypermethrin degradation by *Kocuria turfanensis*

Code	Intermediate metabolites	Retention Time (Time)	Molecular weight (MW)
D1	Dimethoate	17.91	229
D2	3- Methoxy-5-nitrosalicylaldehyde	16.87	301
D3	4-hydroxy-3,5-dimethoxybenzoic acid	16.87	212
D4	N,N-Dimethyl-2-isopropoxyethylamine	13.79	131
D5	2- Methoxyethenyl-benzene	7.94	134
D6	Dimethylhexyloxydecyloxy-silane	16	316
D7	N-(3,4,5-Trimethoxybenzylidene)	16	301
D8	3,4-Dimethylbenezoic acid	11.97	264
D9	3,4-Dibromo-2-methyl-pentanoic acid	16.70	206
D10	6- Methoxy-3-pyridazinethiol	16.52	142
D11	5- Methoxy-benzotriazole	15.03	149
D12	5-Amino-6-piperidinofurazano	13.44	220

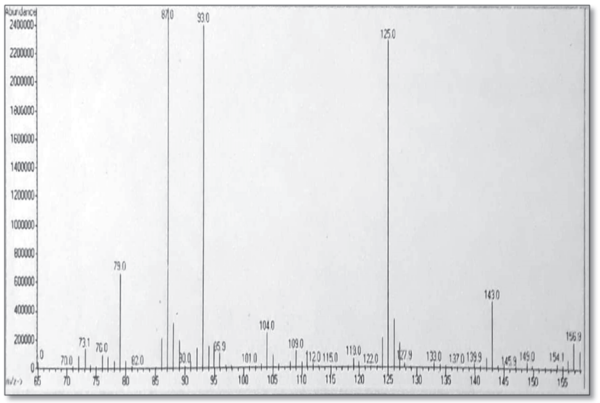

Fig. 3. GC-MS spectra of dimethoate metabolite products by *Kocuria turfanensis*

(2017) and studied its degradation potentials for chlorpyrifos. Degradation study by Reddy *et al.*, (2013) illustrates that *Kocuria rosea* utilizing about 82% of phenanthrene in 6 days. Dubey *et al.*, (2014) reported 89% degradation of monocrotophos after 7 days incubation by *Kocuria turfanesis* strain BS-J. Some researchers also reported the novel strain *Kocuria* sp. that can efficiently degrade the lindane pesticide (Kumar *et al.*, 2016, Abhilash *et al.*, 2011). Neti and Zakkula, (2013) suggested that *Kocuria* species was capable of degrading organophosphate pesticide (chlorpyrifos). They identify five different residues after the degradation. Parshetti *et al.*, (2006) used *Kocuria* sp. for biodegradation of Malachite Green and other textile dyes decolorizing by the same strain.

CONCLUSION

The present study reports the identification of a strain *Kocuria turfanensis*, which efficiently degrade the dimethoate by utilizing as a carbon and energy source. Therefore the strain can be used for bioremediation of pesticide contaminated soil, thus these compounds are removed from the environment. Microorganisms extensively used for the development of technologies that can be used for the environmental pollution control. In bioremediation technology living organisms are used for the removal of pollution from the environment because it is a cost effective and more eco - friendly approach for restoring environmental quality.

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REFERENCES

Abhilash, P. C., Srivastava, S. and Singh, N. 2011. Comparative bioremediation potential of four rhizospheric microbial species against lindane. *Chemosphere*. 82(1): 56-63.

Chen, J. Q., Wang, D., Zhu, M. X. and Gao, C. J. 2007. Photocatalytic degradation of dimethoate using nanosized TiO2 powder. *Desalination*. 207(1-3): 87-94.

DebMandal, M., Mandal, S., Pal, N. K. and Aich, A. 2008. Potential metabolites of dimethoate produced by bacterial degradation. *World Journal of Microbiology and Biotechnology.* 24(1) : 69-72.

Deshpande, N. M., Dhakephalkar, P. K. and Kanekar, P. P. 2001. Plasmid mediated dimethoate degradation in *Pseudomonas aeruginosa* MCMB427. *Letters in Applied Microbiology.* 33 (4) : 275-279.

Dubey, K. V. 2014. Bioaugmentation package of egg shell based biosurfactant formulation: an innovative approach for monocrotophos degradation in soil.

International Journal of Research in Biosciences, Agriculture and Technology. 2(2) : 797-815.

Hamsavathani, V., Aysha, O. S. and Ajith, A. R. 2017. Isolation and identification of chlorpyrifos degrading bacteria from agricultural soil. *International Journal of Advanced Research.* 5 (5) : 1209-1221.

Jiang, H., Yang, C., Qu, H., Liu, Z., Fu, Q. S. and Qiao, C. 2007. Cloning of a novel aldo-keto reductase gene from *Klebsiella* sp. strain F51-1-2 and its functional expression in *Escherichia coli. Applied and Environmental Microbiology*. 73 (15) : 4959-4965.

Karishma, B. and Hari, P.S. 2014. Isolation and characterization of organophosphorus pesticide-degrading bacterial isolates. *Achives of Applied Science Research*. 6 (5) : 144-149.

Kumar, D., Kumar, A. and Sharma, J. 2016. Degradation study of lindane by novel strains *Kocuria* sp. DAB-1Y and *Staphylococcus* sp. DAB-1W. *Bioresources and Bioprocessing.* 3(1) : 53: 1-16.

Liu, Y. H., Chung, Y. C. and Xiong, Y. 2001. Purification and characterization of a dimethoate-degrading enzyme of *Aspergillus niger* ZHY256, isolated from sewage. *Applied and Environmental Microbiology*. 67(8): 3746-3749.

Megeed, A. A. and El-Nakieb, F. A. 2008. Bioremediation of dimethoate by effective microorganisms in water. *Terrestrial and Aquatic Environmental Toxicology.* 2(1): 1-4.

Neti, N. and Zakkula, V. 2013. Analysis of chlorpyrifos degradation by *Kocuria* sp. using GC and FTIR. *Current Biotica.* 6 (4) : 466- 472.

Parshetti, G., Kalme, S., Saratale, G. and Govindwar, S. 2006. Biodegradation of Malachite Green by *Kocuria rosea* MTCC 1532. *Acta Chimica Slovenica*. 53(4): 492-498.

Reddy, M. S., Yusoff, F. M., Naresh, B. and Devi, P. 2013. Isolation and Characterization of Marine Beneficial Bacteria from Petroleum Contaminated Sites for Better Environment of Animal Life. *Asian Journal of Animal and Veterinary Advances.* 8 : 841-851.

Shinde, S. R., Bhailume, M. V., Patil, N. B., Patil, N. N. and Hamde, V. S. 2015. Screening, characterization and identification of soil isolates for degradation of organophosphorus group of pesticides (Dimethoate and Parathion). *International Journal of Current Microbiology and Applied Sciences.* 2: 240-244.

Van Scoy, A., Pennell, A. and Zhang, X. 2016. Environmental fate and toxicology of dimethoate. In: *Reviews of Environmental Contamination and Toxicology.* 237 : 53-70.