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Biodegradation of Methylparathion by microbial consortium

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ABSTRACT

In the present study degradation of methyl parathion was tested using a bacterial consortium obtained by selective enrichment from highly contaminated soils. Microorganisms identified in the consortium were Brevibacillus agri and Pseudomonas sp, in culture medium enriched with methyl parathion, the consortium was able to degrade 500 mg/l of methyl parathion in 4 days. During the degradation intermediate metabolite p-nitrophenol was identified by HPLC method. Finally p-nitrophenol was completely disappeared within 5 days. Results of this present investigation confirmed the complete mineralization methyl parathion in the liquid medium. This consortium is used to remediate the methyl parathion contaminated sites.

Keywords: Methylparathion, *Pseudomonas* sp, *Brevibacillus agri*, Consortium, Biodegradation.

INTRODUCTION

Methyl parathion (O, O-Dimethyl-O-(4-nitrophenyl) phosphorothioate) is a broad-spectrum non-systemic pesticide, used to control insects such as aphids, mealybugs and mites on a wide variety of crops, including cereals, fruits, vegetables, ornamentals and cotton. Methyl parathion (MP) has neurotoxicological properties that suppress the activity of acetylcholinesterase, causing irreversible phosphorylation of esterases in the central nervous system of insects and mammals [1].

Microbial degradation is considered to be a major factor determining the fate of organophosphorus insecticides in the environment. Studies of microbial degradation are useful in the development of strategies for the detoxification of the insecticides by micro-organisms therefore, many strains with the ability to degrade methyl parathion have been isolated worldwide, such as *Pseudomonas* sp. A3, *Plesiomonas* sp. M6, *Pseudomonas* sp. strain WBC-3, *Ochrobactrum* sp. B2, *Serratia* sp. strain DS001, *Stenotrophomonas* sp. SMSP-1 [2-7]. *p*-Nitrophenol (PNP) is one of the major degradation product of the OP pesticides, which is considered as a priority pollutant by the Environmental Protection Agency (EPA) of USA [8-10]. PNP concentrations in natural waters generally are less than 10 mg l^{-1}[11,12], and its fate in the environment has been studied extensively [13,14].Mineralization of PNP by microorganisms have been reported previously [15,16]. Several PNP degrading bacteria also have been isolated from different geographical regions, including *Arthrobacter* sp. Y1, *Serratia* sp. DS001, *Rhodococcus* sp. CN6, *Moraxella* sp. and *Bacillus sphaericus* [15,17,18,12]. It was reported that MP or PNP was hardly

degradable and persistently toxic in the environment [19] and isolates that can simultaneously degrade both compounds are scarce [10,2,4].

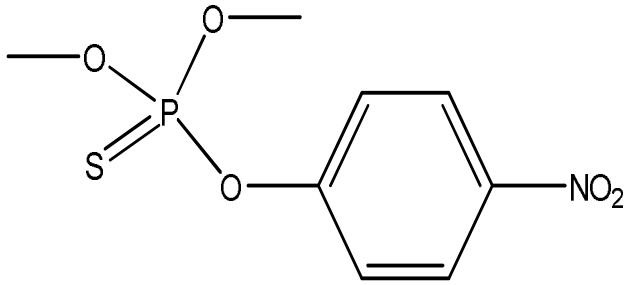

Figure 1: Structure of methyl parathion

The aim of this study was to evaluate the ability of an enriched bacterial consortium obtained from a highly polluted area, to degrade methyl parathion in liquid culture medium.

MATERIALS AND METHODS

Chemicals: Technical grade methylparathion 97% purity was purchased from Sigma Aldrich Pvt Ltd Bangalore, India. All other chemicals are analytical grade purchased from Hi- Media Pvt Ltd Mumbai.

Sample collection: Soil sample was collected from pesticide contaminated agricultural field of Cuddalore district, Tamilnadu, India where methylparathion is sprayed extensively. Soil sample was stored at 20°C for further use.

Enrichment isolation: Soil enrichment was carried out in Minimal Salt Medium (pH 7.0) containing (g L^{-1}) K_2HPO_4, 1.5; KH_2PO_4, 0.5; $(NH_4)_2SO_4$, 0.5; NaCl, 0.5; $MgSO_4$, 0.2; $CaCl_2$, 0.05; $FeSO_4$, 0.02. 1 g of soil was added to an Erlenmeyer flask (250 mL) containing 100 mL MSM supplemented with methylparathion (200mgL^{-1}) as the sole carbon source and incubated at 37°C on a rotary shaker at 150 rpm for 7 days. After 7 days 5 mL culture was recovered from each replicate and transferred to fresh MSM containing methylparathion as the only carbon source and incubated for 7 days. One week following the last transfer, 10 fold dilutions of cultures was prepared and 100µL of each dilution was spreaded on nutrient agar plates. Isolated colonies were purified by repeated streaking. After purification all isolates were tested for growth and methylparathion utilization by inoculating them in MSM containing methylparathion (500mg L^{-1}) as sole carbon and energy source.

Biodegradation of methylparathion: Shake flask studies were carried to work out the methylparathion degrading capacity of the isolated strains. Seed culture of each isolated strains were grown in nutrient broth containing methylparathion (300 mg L^{-1}). Following 24 h of incubation, 1% inoculum of the cultures were inoculated in MSM (200 mL) containing 500 mg L^{-1}methylparathion and incubated at 37°C and 180 rpm on a rotary shaker. MSM flask without inoculum was kept as control.

Extraction efficiency: Samples were recovered from culture flasks at respective time intervals (0,2,5 and 6 days) and centrifuged at 10000 rpm for 10 min to obtain cell free medium. The supernatant thus obtained was added to the separating funnel and methylparathion residues were extracted from supernatant using equal volume of dichloromethane. After partitioning, organic layer of dichloromethane was evaporated by solvent evaporator to obtain a powdery residue of the organic compound.

HPLC- Analysis

Table1: Instrumental condition for HPLC analysis

Sl. No	Condition	Parameter
1	Mobile Phase	Acetonitrile and water 15:85 (v/v)
2	Column	C18 column
3	Wave length	225 nm
4	Column temperature	25ºC
5	Flow rate	1.0ml/min
6	Sample volume	20µl
7	Running time	10 min

RESULTS AND DISCUSSION

Several bacterial strains with the ability of degrading MP were isolated from the soil samples. Two strains were more efficient and designated as MPD1 and MPD2 capable of utilizing MP and PNP as sole carbon, energy sources for their growth. The strain MPD1 was Gram positive spore forming aerobic, motile, and rod shaped bacterium. The strain MPD1 showed the positive result for starch hydrolysis, catalase and negative for gelatinase test. MPD2 was Gram negative, non spore forming positive result for catalase, gelatinase and negative for starch hydrolysis, based on the above biochemical characterization the two strains were identified as *Pseudomonas* sp and *Brevibacillus agri* according to Bergey's manual of systemic bacteriology [20].These two bacterial strains were selected and developed in to a consortium for further study. The first attempts to isolate microorganisms capable of degrading pesticides in soil and water were focused on identifying individual species. However, studies report the efficacy of using microbial consortia [21] instead of single species. This is because in the degradation of toxic compounds there are groups of microorganisms involved that are responsible for co-metabolizing these compounds, a process that does not occur efficiently if using a single microbial species [22]. Consequently, in this work a consortium of microorganisms was isolated from contaminated soil. After the acclimatization period in liquid culture to isolate soil microorganisms capable of degrading methyl parathion, a microbial consortium was isolated composed of the following bacteria: *Brevibacillus agri* and *Pseudomonas* sp.

Biodegradation of methyl parathion: The degradation patterns of methylparathion microbial consortium were studied in MSM media with methyl parathion as the sole carbon energy source (fig 2.). About 98% of degradation was observed in 4 days the degradation of methyl parathion accompanied by bacterial growth and transient accumulation of *p*- nitrophenol. Then *p* – nitrophenol was degraded rapidly and disappeared finally within 6 days, moreover this consortium completely degraded the methylparathion and their degradation product *p*-nitrophenol. Biodegradation occurring in an environment exposed to the chemical pollutant is a complex process in which many different metabolically active microbial communities take part. Several co-cultivated or mixed cultures have been reported for efficient mineralization of resistant or complex contaminants [23,24,25]. The interaction between different microbial species on biodegradation of methylparathion is an important consideration in developing bioremediation strategies for methylparathion removal.

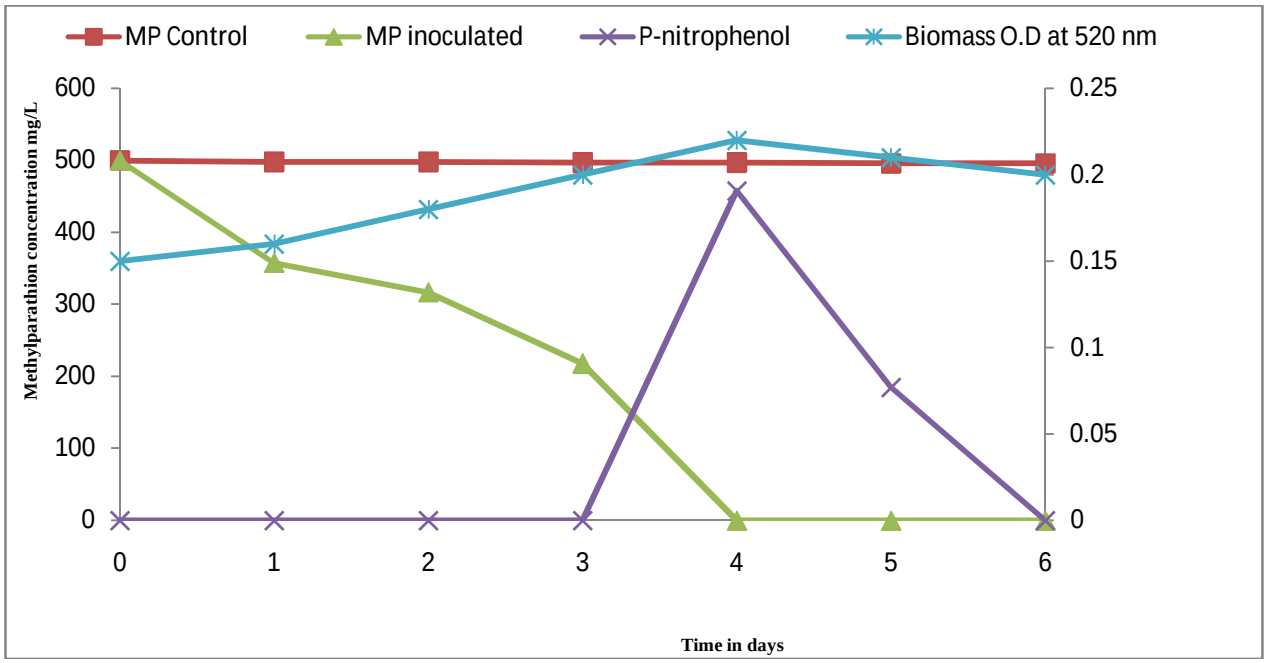

Figure 2. Degradation of methylparathion by microbial consortium (*Brevibacillus agri* + *Pseudomonas* sp) and bacterial growth monitored by measuring O.D at 520 nm, valus are mean ± SD of three replicates.

HPLC analysis of methyl parathion: The HPLC analysis showed that the consortium was able to efficiently degrade methyl parathion, which suggests that the bacteria used these compounds as carbon and energy sources for growth. In the various transfers of cultures enriched with a pesticide, the consortium was able to completely degrade methyl parathion and their metabolites. The peak observed with the retention time of 3.2 for the parent compound (fig 3a.). After two days the parent peak was disappeared completely and new peak was observed with retention of 4.2 (fig 3b).Its indicates the methyl parathion hydrolytic product *p*-nitrophenol, finally this peak was completely disappeared (fig 3c.). The result shows that there was no inhibition of the degradation of methylparathion and its metabolites. Some reports indicate that the presence of a contaminant can inhibit the degradation of a second compound [26].

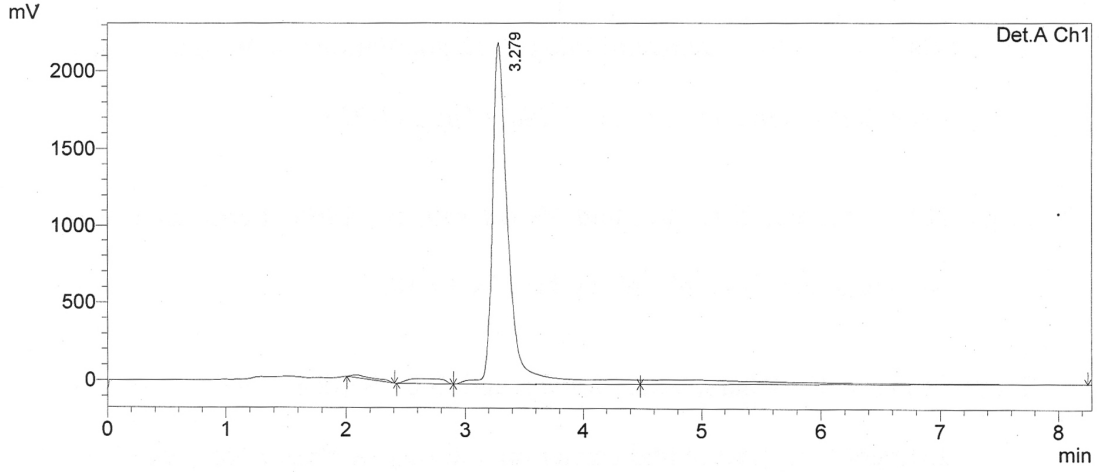

Figure 3a. HPLC analysis of the standard methyl parathion.

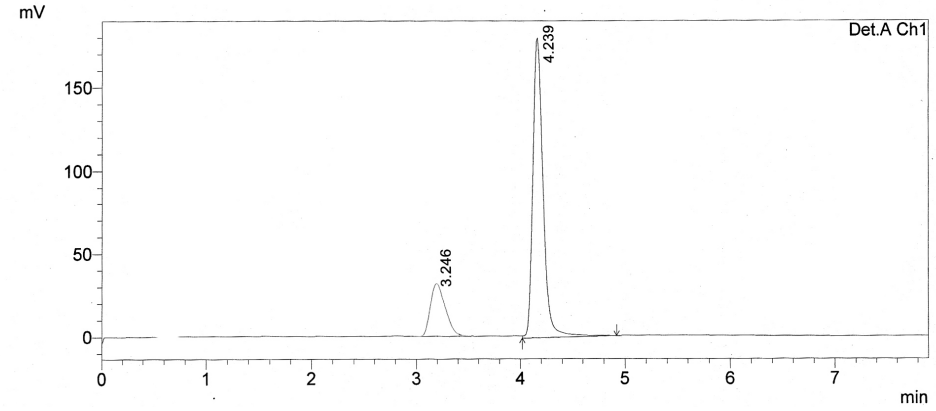

Figure 3 b. HPLC analysis of the degradation product *p*-nitrophenol

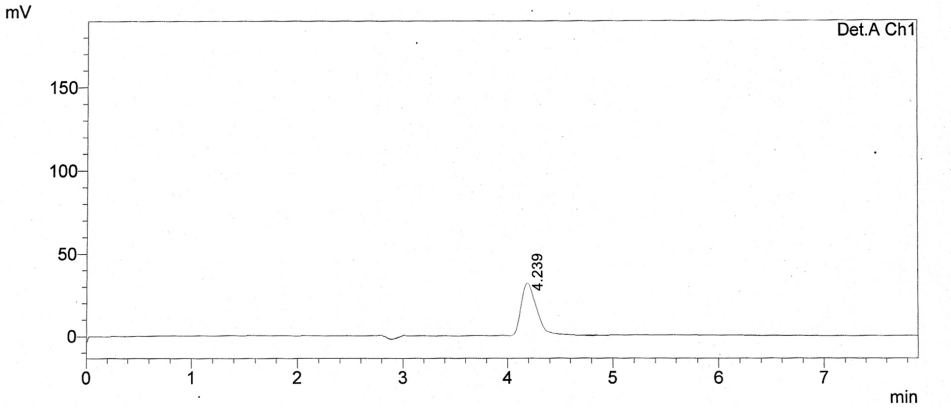

Figure 3c: complete degradation of methyl parathion and *p*-nitrophenol

APPLICATIONS

Thus, effective microbial consortium for bioremediation of various soil and water heavily contaminated by organophosphorus and methylparathion pesticides.

CONCLUSIONS

A microbial consortium composed of *Brevibacillu agri* and *Pseudomonas* sp was isolated and confirmed the ability to degrade methyl parathion in culture medium. The microbial consortium degraded 98 % of Methylparathion. This consortium was used to degrade methylparathion in contaminated sites.

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