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Biodegradation of Methyl Parathion by Soil Isolates of Microalgae and Cyanobacteria

M. Megharaj,[1] D. R. Madhavi,[2] C. Sreenivasulu,[2] A. Umamaheswari,[2] K. Venkateswarlu[2]

[1]Department of Botany, Nagarjuna University, Nagarjunanagar 522510, India
[2]Department of Microbiology, Sri Krishnadevaraya University, Anantapur 515003, India

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Methyl parathion (*O,O*-dimethyl *O*-*p*-nitrophenyl phosphorothioate), a substitute for its more toxic, now banned analogue, parathion, is extensively used in rice cultivation to control various insect pests (Adhya et al. 1981). The nature and extent of microbial degradation of organophosphorus insecticides by bacteria and fungi have been well documented (Ware and Roan 1970; Barik 1984). Microalgae and cyanobacteria have also been implicated in the metabolism of certain organophosphates such as phorate (Ahmed and Casida 1958), malathion (Christie 1969), parathion (Zuckerman et al. 1970), and monocrotophos and quinalphos (Megharaj et al. 1987). A perusal of the available literature indicates that there are no studies on the metabolism of methyl parathion by soil isolates of microalgae and cyanobacteria (Venkateswarlu 1993). The present study was therefore aimed at determining the role of two green microalgae and four cyanobacterial species, all isolated from soil enrichments, in degradation of methyl parathion.

MATERIALS AND METHODS

Enrichment cultures were developed by adding 1 ml of 1000 ppm aqueous solution from the commercial formulation of methyl parathion (Metacid 50 EC from Bayer (India) Ltd., Bombay) to 20-g portions of the soil samples, maintained at 50% water-holding capacity. After five additions of the insecticide at 10-day intervals, the soil samples were withdrawn for isolation of predominant microalgae and cyanobacteria following the most-probable number (MPN) method (Muralikrishna and Venkateswarlu 1984). Two species of microalgae, *Chlorella vulgaris* and *Scenedesmus bijugatus,* and four of cyanobacteria, *Nostoc linckia, N. muscorum, Oscillatoria animalis* and *Phormidium foveolarum*, were isolated from the enrichments, and raised to axenic cultures. The two diazotrophs, *N. linckia* and *N. muscorum,* were grown in Allen's nitrogen-free medium while others were maintained in Bold's basal medium as described earlier (Megharaj et al. 1991a).

The ability of selected algal and cyanobacterial species to utilize the insecticide as a source of phosphorus and nitrogen was tested by streaking the cultures on agarized growth medium without N and P, but with methyl parathion. The plates were observed for visual growth of the cultures over a period of 3 weeks.

Correspondence to: K. Venkateswarlu

The capability of the soil isolates in the degradation of methyl parathion was tested as follows. From the stock solution of technical grade insecticide (obtained from Bayer AG, Leverkusen, Germany), prepared in analytical reagent grade acetone, 1000 ug methyl parathion was added to sterilized 150 ml Erlenmeyer flasks. After evaporation of the solvent, 50 ml portions of steam-sterilized Bold's basal medium or Allen's N-free medium (in case of *N. linckia* and *N. muscorum*) were added to the flasks containing the insecticide residues. The residues were then equilibrated for a day to obtain aqueous solutions of the insecticide. The flasks were inoculated with exponentially growing algal or cyanobacterial cultures (Megharaj et al. 1987). Uninoculated media with the insecticide served as controls. All the culture flasks including controls were incubated under constant fluorescent illumination (200 uE m^{-2} s^{-1} PPFD) at room temperature (28 $\pm$ 4°C). Triplicate samples were withdrawn after 5, 10, 20 and 30 days of incubation for solvent extraction and estimation of the insecticide residues. Before solvent extraction, 10 ml portions of the culture medium from each sample were used to determine the content of nitrite (Adhya et al. 1981). In another experiment, the culture media, supplemented with PNP, were inoculated with *C. vulgaris* and *N. muscorum* to know the rate of nitrite formation from PNP until 15 days.

The residues of the parent compound from the media were extracted three times with chloroform-diethyl ether (1:1) mixture. The solvent fractions were pooled and after evaporation of the solvent at room temperature, the residues were redissolved in 2 ml methanol for further analysis by thin-layer chromatography (TLC). The residues, in methanol, were spotted alongwith authentic samples on chromatoplates coated with silica gel G, 300 um thick. The plates were developed for a distance of 15 cm with hexane-chloroform-methanol (7:2:1, v/v), as employed for other organophosphates (Adhya et al. 1981), and air-dried. The standards were located by spraying with acidic solution of palladium chloride (0.5% in 2N HCl) followed by 0.1N NaOH. The silica gel areas of the samples corresponding to the standards were scrapped from the chromatoplates into test tubes. PNP was eluted from silica gel directly into ethanolic 0.1N NaOH, centrifuged and assayed spectrophotometrically at 410 nm. Methyl parathion in the silica gel was treated with alkaline hydroxylamine hydrochloride, centrifuged and the resulting PNP was assayed spectrophotometrically at 410 nm (Ramakrishna and Ramachandran 1976).

The data were subjected to analysis of variance, and the means were compared by Duncan's new multiple range (DMR) test at the 5% level.

RESULTS AND DISCUSSION

A microscopic examination was made for the qualitative occurrence of microalgae and cyanobacteria in insecticide-treated soil enrichments. A total of 10 species including 3 unicellular green algae and 7 filamentous cyanobacteria were recovered (Table 1). The most abundant species were *Lyngbya gracilis, Nostoc punctiforme, Oscillatoria animalis* and *Phormidium foveolarum.*

Initially, in order to know whether the isolates could utilize the insecticide as a source of phosphorus and nitrogen, the cultures were streaked on agarized growth medium, impregnated with methyl parathion as a source P and N, and were incubated at 37°C for a period of 3 weeks. Occasionally, the cultures were checked for contamination by streaking on bacteriological medium. All the four cyanobac-

teria, but not the green algae, tested grew very well, indicating their ability in utilizing the insecticide.

Table 1. Qualitative occurrence of microalgae and cyanobacteria in soil enriched with methyl parathion

Organism	Occurrence
Chlorococcum sp.	+
Chlorella vulgaris	+
Scenedesmus bijugatus	+
Anabaena variabilis	+
Lyngbya gracilis	+ + +
Nostoc linckia	+
N. muscorum	+ +
N. punctiforme	+ + +
Oscillatoria animalis	+ + +
Phormidium foveolarum	+ + +

+, abundant; + +, more abundant; + + +, most abundant

The role of microalgae and cyanobacteria in biodegradation of methyl parathion was tested by measuring the rate of disappearance of the insecticide and its major hydrolysis product, *p*-nitrophenol (PNP), from the culture media. There was a significant decrease in the concentration, of methyl parathion even in uninoculated culture medium (Table 2). Thus, about 22% of the insecticide was lost from the uninoculated controls during the 30-day incubation period. Such an instability of the insecticide even in uninoculated media with pH 6.8 could be expected since the organophosphates are highly susceptible to hydrolysis and are inherently unstable, decomposing slowly at normal temperatures (Brown et al. 1966).

All the six species hydrolyzed the insecticide at an appreciable rate as evidenced by the appearance of PNP in the culture medium. *N. muscorum, O. animalis* and *P. foveolarum* hydrolyzed the insecticide to undetectable levels by 20 days while *N. linckia, C. vulgaris* and *S. bijugatus* effected complete hydrolysis by 30 days. According to an earlier report (Megharaj et al. 1987), both microalgae and cyanobacteria were equally potential in the degradation of two other organophosphates, monocrotophos and quinalphos. Further degradation of PNP, resulting in the accumulation of nitrite, was rapid only by cyanobacteria. Thus, about 80-110 ug of NO_2^- accumulated in the culture medium of cyanobacteria as against to only 18-45 ug nitrite in algal medium by the end of 30 days. Again, in a repeat experiment, the liberation of nitrite was 5% of PNP supplemented to the culture medium of *C. vulgaris* after 15 days while the corresponding value for *N. muscorum* was 14% (Table 3). Our results support the generalization that nitrogroup oxidation of PNP by the release of nitrite is common in prokaryotes (Barik 1984). Only traces of p-aminophenol were detected in the medium of all cultures, suggesting that the nitrogroup reduction is another means of PNP degradation. This observation also confirms the fact that reduction of nitrite to amino group is not the main pathway for PNP degradation by microorganisms (Sethunathan et al. 1977). In general, the ability of the selected microalgae and cyanobacteria decreased in the following order: *P. foveolarum* > *O. animalis* > *N. muscorum* > *N. linckia* > *S. bijugatus* > *C. vulgaris.*

Table 2. Degradation of methyl parathion (MP) by microalgae and cyanobacteria

Organism	μg compound recovered, after incubation							
	5 days		10 days		20 days		30 days	
	MP	PNP	MP	PNP	MP	PNP	MP	PNP
Uninoculated	912a	26f	826a	65c	789a	79g	760	90d
Chlorella vulgaris	512c	180d	318c	270a	88b	380b	n.d.	365a
Scenedesmus bijugatus	534b	171e	340b	258b	96b	362a	n.d.	356a
Nostoc linckia	485d	198c	258d	271a	32c	325c	n.d.	189b
N. muscorum	438e	210b	238e	262b	n.d	295d	n.d.	120c
Oscillatoria animalis	435e	240a	212f	278a	n.d	250e	n.d.	n.d.
Phormidium foveolarum	405f	248a	180g	261b	n.d	216f	n.d.	n.d.

1000μg methyl parathion was added to 50 ml medium
0-day recovery of methyl parathion was 970 μg
Means (n = 3) in each column followed by the same letter are not significantly different ($P < 0.05$) from each other according to Duncan's multiple range (DMR) test
n.d. = not detected

Table 3. Nitrogroup oxidation of PNP by *Chlorella vulgaris* and *Nostoc muscorum*

Incubation (days)	Recovery of compound (μg/25 ml)			
	C. vulgaris		*N. muscorum*	
	PNP	Nitrite	PNP	Nitrite
2	595	0	350	7
4	500	4	295	14
6	460	8	0	84
8	420	12	0	85
10	380	14	0	89
12	365	18	0	90
15	190	31	0	90

626 μg PNP was added to 25 ml culture medium

The effectiveness of nonheterocystous filamentous cyanobacteria over the heterocystous forms in degrading the insecticide merits further consideration. Also, it is unknown why the algal species could not further degrade PNP efficiently compared to cyanobacteria. The wide differences among the cultures tested in metabolizing PNP could be related, in part, to the expression and activity of the PNP-degrading catabolic enzymes.

Although methyl parathion is a less persistent insecticide, the hydrolysis product, PNP, is more toxic than its parent compound (Barik 1984). It is of great environmental concern since PNP is considered to be a priority pollutant (Keith and Telliard 1979; Barik and Sethunathan 1978). For instance, PNP is highly toxic to aquatic microorganisms (Bruhn et al. 1987), even at and above 5 ug/ml being the concentration toxic to the growth and metabolism of algae and cyanobacteria (Megharaj et al. 1986,1991a,1991b,1992). Also, PNP was found accumulating at a level of 42 ug/g particles in aerosols and in precipitation under smog conditions (Nojima et al. 1983). Thus, from the stand point of environmental contamination of pesticides, the present observation of algal and cyanobacterial degradation of methyl parathion, probably new to the literature, may be gainfully exploited further in biotechnology for effective detoxification and bioremediation of toxicant-polluted systems.

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