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Degradation of Malathion by Microorganisms Isolated from Industrial Effluents

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Malathion, S - (1,2-dicarbethoxyethyl) - o, o - dimethyl dithiophosphate, is a widely used insecticide of the organophosphorous group of pesticides. Like most organophosphates, malathion is degraded in soil (Walker and Stojanovic, 1974), in aquatic systems (Lewis et al., 1975), in terrestrial plants and animals (Bourke et al., 1968). Despite the studies on the fate of malathion in soil and salt-marsh environments, little information is available about the degradation of this chemical by microorganisms present in industrial effluents. Further, the microorganisms isolated so far have not been found to degrade high concentrations of malathion.

Therefore, in the present study attempts were made to isolate microorganism capable of readily degrading high concentrations of malathion from industrial effluents using an enrichment technique. Attempts were also made to isolate and identify the major metabolites of malathion formed during biodegradation of the pesticide.

MATERIALS AND METHODS

Malathion was obtained from American Cyanamid, Co., Princeton, N.J.

Malathion degrading microorganisms were isolated from industrial (paper mill) effluents by an enrichment culture technique. Malathion (analytical grade) dissolved in ethanol was added to one liter of mineral salts medium (Singh et al., 1986), pH 6.8, in a two liter incubation flask. The final concentration of malathion was 100 mg/liter and that of ethanol was 0.5% (v/v). Papermill effluent (50 ml) was added to the incubation flask and the contents were incubated at 30 ± 1 °C on a rotary shaker for one month. Malathion at a

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rate of 10 mg/liter was added after every 7 days and the samples were finally plated out on nutrient and noble agar plates containing 100 ppm of malathion. Colonies picked from these plates were used to test the ability of microorganism to degrade malathion.

Bacterial strain selected from enrichment culture was tested for their ability to degrade malathion. Aliquots (2 ml) of an 18 hours culture grown in nutrient broth were inoculated into 100 ml of mineral salts medium containing malathion concentrations ranging from 100 to 200 mg/liter with and without ethanol (1.0% v/v) and incubated for 5 days at 30°C on a rotary shaker. Control flasks of equal volume of salts medium and malathion without any bacterial population were run in parallel. Aliquots (5 ml) were removed at 8 hour intervals and mixed with 50 ml of carbon tetrachloride in a separatory funnel. The contents were shaken for 30 minutes to extract malathion. Malathion contents were measured as described previously (Report by the Malathion Panel, 1960).

To determine the malathion metabolite, the bacterium was incubated in 1 liter of mineral salts medium containing malathion (200 ppm) and ethanol (1% v/v) for 5 days at 30 °C on rotary shaker. After incubation, the cells were filtered (using 0.2 micron Millipore membrane filter). The aqueous cell free filtrate was evaporated to dryness under vacuum at 50°C. The salts residue was extracted with approximately 100 ml nanograde acetone. The acetone extract was concentrated to 3 to 5 ml with a stream of dry air. About 20 to 30 µl aliquots of the concentrated acetone extract were spotted on a thin layer glass plate coated with silica gel G absorbent. The plate was developed in a solvent mixture of benzene and acetic acid (4:1), air dried and sprayed with freshly prepared 0.5% (w/v) N-2, 6-trichloro-p-benzoquinoneimine (Eastman Kodak Co., Rochester N.Y.) in nanograde acetone and then kept at 110°C for 10 minutes (Jaglan and Gunther, 1970). Spots representing malathion and its metabolite appeared as a dark reddish-pink color on a light background. R_f values were determined and compared with the reported standard values (Walker et al., 1974). The metabolite for infrared spectral analysis was isolated by streaking 1 to 2 ml of the concentrated acetone extract on a TLC plate and developed as above. Area corresponding to metabolite band was carefully scraped from the plate and extracted with 50 ml of acetone. This extract was concentrated to 3-5 ml under vacuum at 35 °C and a suitable portion was added to KBr for analysis on a Perkin-Elmer 337 Grating Infrared Spectrophotometer.

Spectral tracing of the metabolite was compared with the reported infrared spectral tracing of malathion metabolites (Walker et al., 1974).

RESULTS AND DISCUSSION

A bacterial strain, M-3, was selected as a malathion degrading microorganism. The taxonomical characteristics of the strain are listed in Table 1.

Table 1. Characteristics of Malathion Degrading Strain (M-3)

Tests	Strain M-3
Shape and size	Rods, measuring 0.6 by 1.2 μm
Growth on nutrient agar	Round, smooth and convex
Motility	Motile
Gram stain	Gram negative
Gelatin liquefaction	Positive
Starch hydrolysis	Positive
Nitrate reduction	Positive
Indole production	Negative
Hydrogen sulfide formation	Negative
Oxidase and catalase	Positive
Ammonia production from peptone	Positive
Arginine and casein hydrolysis	Positive
MR and VP reactions	Negative
Pigment formation	Positive (Greenish pigment)
Citrate utilization	Positive
Acid without gas from glucose, fructose, sucrose, mannose, ribose, xylose, mannitol and lactose	Positive

According to "Bergey's Manual of Determinative Bacteriology" 8th ed., the strain was classified in the genus Pseudomonas and tentatively named Pseudomonas sp. M-3

Figure 1 shows the degradation of malathion by M-3 strain in the presence and absence of an additional carbon source e.g. ethanol under aerobic conditions. No degradation of malathion was observed in the absence of ethanol. The bacterium was found to degrade malathion only in the presence of ethanol (1.0% v/v) as a cosubstrate. Up to 150 ppm of malathion was degraded in a period of 28 hours. The rate of degradation of

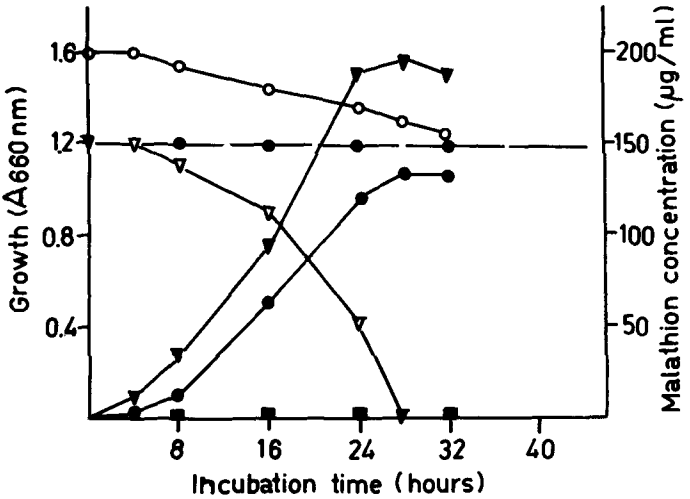

Figure 1. Degradation of malathion by M-3 strain in the presence and absence of ethanol (1% v/v) under aerobic conditions.
▽—▽ Degradation of malathion (150 ppm) in the presence of ethanol, o—o Degradation of malathion (200 ppm) in the presence of ethanol, ●—● Degradation of malathion (150 ppm) in the absence of ethanol, ▼—▼ Growth on malathion (150 ppm) in the presence of ethanol, ■—■ Growth on malathion (150 ppm) in the absence of ethanol, ●—● Growth on malathion (200 ppm) in the presence of ethanol.

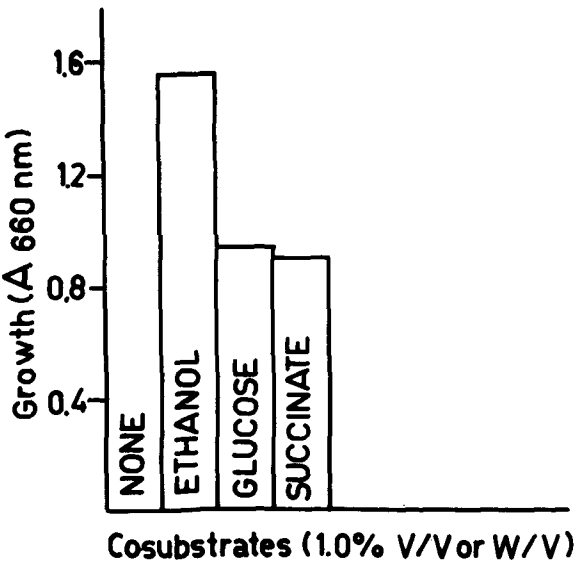

Figure 2. Growth of M-3 strain on various cosubstrates in the presence of malathion (150 ppm) under aerobic conditions.

malathion was found to be low at higher concentrations of malathion (200 ppm). Under anaerobic conditions there was none or very insignificant degradation of malathion (Data not shown).

Figure 2 shows the growth of M-3 strain on the cosubstrates like glucose, ethanol and succinate under aerobic conditions. Among these cosubstrates, ethanol was found to be the best in supporting the growth of the strain. The maximum growth of the strain was observed when the concentration of ethanol in the medium was 1.0% (v/v). Under anaerobic conditions very little growth of the bacterium was observed (Data not shown).

Figure 3 shows the degradation of malathion by M-3 strain in the presence of cosubstrates like glucose, ethanol and succinate under aerobic conditions. As observed in case of the growth of the strain, ethanol was also found to be the best cosubstrate for the degradation of malathion.

Figure 4 shows the effect of temperature on the growth of M-3 strain on ethanol (1.0% v/v). the bacterium was able to grow between 20 ^{o}C and 45 ^{o}C with optimum at 30 ^{o}C.

The effect of pH on the growth of M-3 strain on ethanol (1% v/v) is shown in Figure 5. The bacterium was able to grow between pH 5.5 to 9.5 with the optimum around 7.0

The single additional band found on TLC plates using benzene: acetic acid (4:1) solvent mixture was identified to be malathion monocarboxylic acid from its R_f value of 0.71 which was close to that of malathion monocarboxylic acid reported by Walker and Stojanovic, (1974). This was further confirmed by infrared spectral analysis. The infrared spectral tracing of the metabolite isolated on TLC showed major bands at 665, 1380, 1730 and reduced absorption at 1100 and 1170 cm^{-1} (Figure 6). These characteristics were identical with those of malathion monocarboxylic acid (Walker and Stojanovic, 1974). Accordingly, the metabolite was identified as malathion monocarboxylic acid.

Amongst the various alcohols tested for the growth of M-3 strain, only ethanol was found to be utilized as carbon and energy source while the other alcohols like methanol, iso-propanol, n-butanol, tert-butanol, iso-amylalcohol did not support the growth of the bacterium.

The present study indicates that the papermill effluents contains microorganisms capable of readily degrading malathion cometabolically upto a concentration of 150 ppm in the presence of ethanol as a cosubstrate. A

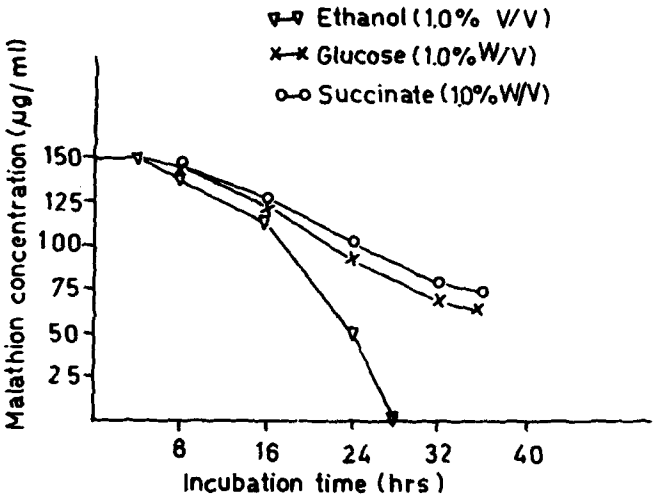

Figure 3. Degradation of malathion by M-3 strain in the presence of various cosubstrates (1% v/v or w/v) under aerobic conditions.

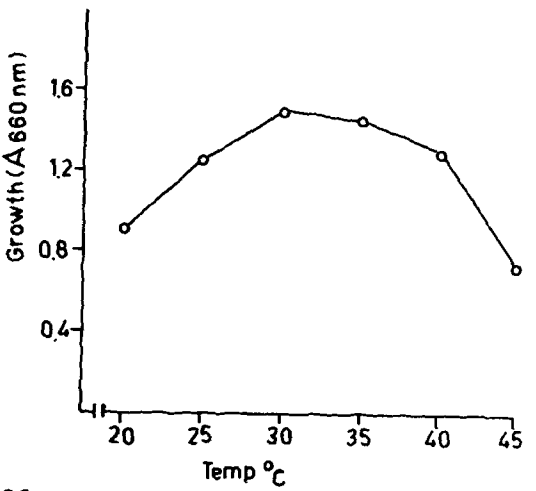

Figure 4. Effect of temperature on the growth of M-3 strain on ethanol (1.0% v/v).

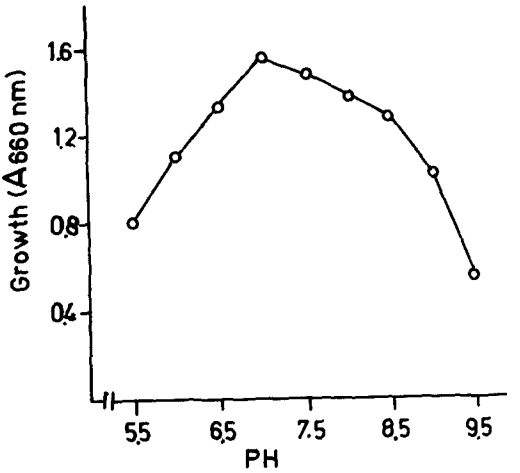

Figure 5. The effect of pH on the growth of M-3 strain on ethanol (1% v/v).

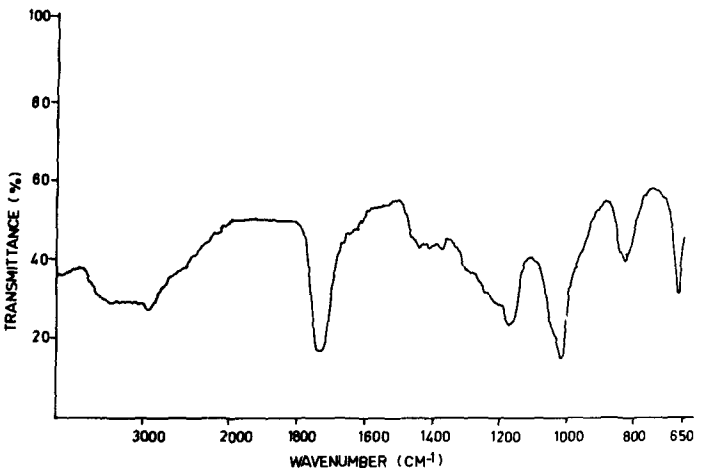

Figure 6. Infrared spectral tracing of the metabolic product of malathion.

decrease in the growth of the microorganism at a concentration of 200 ppm of malathion indicates that the pesticide is toxic to the microorganism at higher concentration. The M-3 strain seems to be different from the microorganisms isolated from soil and salt-marsh environment (Walker and Stojanovic 1974; Bourguin, 1977), since it degraded malathion only to malathion monocarboxylic acid while the latter degraded it to malathion mono and dicarboxylic acids.

The isolation of the strain M-3 capable of readily degrading high concentrations of malathion, provides hope for utilization of this strain in biodegradation of malathion in industrial effluents. However, further studies are needed to confirm this.

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