ScienceAsia 27 (2001) : **261-270**

Degradation of Methyl Parathion in an Aqueous Medium by Soil Bacteria

Charoensri Keprasertsup[a], E Suchart Upatham[b,c], Nouwarath Sukhapanth[b] and Pairote Prempree[d]

[a] Department of Environmental Engineering, Faculty of Engineering and Technology, Rangsit University, Bangkok, Thailand.
[b] Department of Biology, Faculty of Science, Mahidol University, Bangkok, Thailand.
[c] Department of Medical Science, Faculty of Science, Burapha University, Chonburi, Thailand.
[d] Department of Chemistry, Faculty of Science, Mahidol University, Bangkok, Thailand.
* Corresponding author, E-mail: charoensrik@hotmail.com

Received 4 Aug 2000
Accepted 9 Jul 2001

ABSTRACT Agricultural soil was inoculated in a basal mineral medium (BMM) containing methyl parathion (MP) with glucose (0.5 mg/ml) and without glucose. Mixed bacterial cultures and four pure bacterial cultures, isolated from soil, were able to degrade MP (50 µg/ml) in BMM. *Bacillus* sp. and two unknowns, isolated from the mixed culture in the BMM with glucose, were able to degrade only the commercial grade MP in the BMM with glucose, but were able to degrade the analytical grade MP in the BMM without glucose. *Burkholderia cepacia* was the best MP-degrading bacterium. It was isolated from the mixed culture in the BMM without glucose. The bacterium was able to grow in the BMM at all pH ranges (pH 4-pH8) and salinity levels (0-3.5% NaCl). The addition of glucose (0.5 mg/ml) did not enhance the MP-degrading ability of *Burkholderia cepacia. Burkholderia cepacia* at $6x10^7$ cells/ml degraded MP at 50, 100, and 150 µg/ml in standard BMM rapidly ($t_{1/2}$ = 0.55, 0.58, and 0.87 hour). Furthermore, *p*-nitrophenol (PNP), a hydrolysis product of MP, was completely degraded by *Burkholderia cepacia*. MP and PNP were carbon sources for growth of *Burkholderia cepacia.*

KEYWORDS: methyl parathion, *p*-nitrophenol, *Burkholderia cepacia*, biodegradation.

INTRODUCTION

Methyl parathion (*o,o*-dimethyl *o*-*p*-nitrophenol phosphorothioate, MP) is an important broad-spectrum organophosphorus insecticide. It is used increasingly in agriculture and public health as an effective replacement of its ethyl analogue, parathion (*o,o*-diethyl *o*-*p*-nitrophenyl phosphorothioate), which has been banned in many countries because of its higher mammalian toxicity. In 1987, Thailand imported about 1,600 metric tons of MP, its importation is increasing every year and it is the most highly used insecticide in Thailand. Although MP is considered a relatively non-persistent insecticide in the environment because it can be degraded in natural soil and water by microorganisms in a short period of time, its residue can be found in soil, water and fish due to extensive and excessive applications of MP in agriculture. Furthermore, MP can be potentially hazardous as a result of accidental spills and discharges from pesticide containers and wastes. Its concentrations in these discharges are very high, and its half-life in natural water is about one month, which is long enough to be toxic to many living organisms in the environment.[1-5]

The detoxification procedure of MP is the use of strong alkali (1 N NaOH) which is slow, a large amount of salt is required, and the final pH of the treated solution is above 13.[6] Therefore, the microbial application for MP detoxification would represent a significant improvement in waste disposal technology.

In the past, many scientists studied the biodegradation of MP and its metabolites in soil, sediment, and water, and found that microorganisms can degrade MP into dimethyl thiophosphoric acid and *p*-nitrophenol (PNP) by hydrolysis.[7-11] *P*-nitrophenol is the major metabolite of MP and it has moderate toxicity after single oral or dermal exposure.[12] It is important to note that PNP is very persistent, toxic, and inhibits microbial growth in natural aquatic systems.[13,14] So, we have to deal with the toxicity of PNP besides having to cope with the toxicity of residual MP.

In 1977, Laveglia and Dahm[15], and Lewis et al[16] reported that *Bacillus subtilis* and *Flavobacterium* sp. degraded MP in nutrient broth, respectively. In 1989, Ou and Sharma[17] isolated a *Pseudomonas* sp. and a *Bacillus* sp. from soil treated with MP. The *Pseudomonas* sp. had a capacity to degrade PNP but could not degrade MP, while the *Bacillus* sp. was able

to degrade both MP and PNP. In 1990, Heitkamp et al[18] used a mixed culture of all three PNP-degrading *Pseudomonas* (*P. fluorescens, P. putida,* and *P. mendocina*) to remove PNP from aqueous waste streams. In 1994, Rani and Lalithakumari[11] reported that *Pseudomonas putida* was able to degrade both MP and PNP.

In this report, MP-degrading bacteria were isolated from agricultural sites in Thailand and were identified. The best degrading bacteria for both MP and PNP were selected for studying MP degradation rates in liquid media at different conditions, such as inoculum sizes of bacteria, with and without glucose, pH, salinity, and concentrations of MP. In addition, the metabolism of PNP was also investigated.

Materials and Methods

Chemicals

Commercial grade methyl parathion (MP), Folidol-E 605 M50 (emulsifiable concentrate), used in enrichment and MP degradation studies was purchased from Bayer Thai Co, Ltd, Bangkok, Thailand. Pure MP was provided from the same company.

Media and enrichment of methyl parathion (MP) degrading microorganisms

Basal mineral medium (BMM) contained the following ingredients (in grams per liter): K_2HPO_4, 4.8; KH_2PO_4, 1.2; NH_4NO_3, 1.0; $MgSO_4.7H_2O$, 0.25; $CaCl_2$, 0.04; and $FeSO_4.7H_2O$, 0.005.[9,17] The BMM was either supplemented with glucose (0.5 mg/ml) or without. The pH values were adjusted to 4 to 7 with 3 N HCl and to 8 with 1N NaOH, and then the medium was autoclaved. The BMM which was added with MP (50 μg/ml) without glucose and NaCl, at pH 7, was used as a control in this study.

Soil samples were collected from MP-treated agricultural sites at Talingchan, Bangkoknoi, Bangkok, Thailand. The enrichment of MP-degrading microorganisms was done as follows: 10 g soil samples were suspended in 30 ml of sterile BMM containing 0.3 ml of commercial grade MP (1 mg/ml) in 250 ml Erlenmeyer flasks, with glucose (0.5 mg/ml) or without glucose. The flasks were incubated on a rotary shaker (200 rpm) at room temperature (26° ± 2°C). Every two weeks, an additional amount of MP (1 mg/ml) was applied into the flasks at increasing volumes of 0.6, 0.9, 1.2, and 1.5 ml. After 2.5 months of incubation, a 10 ml sample of each flask was transferred to 90 ml of sterile BMM containing 5 mg of MP in 250 ml Erlenmeyer flasks, with or without glucose (0.5 mg/ml), and were shaken at room temperature. Once every two weeks, 10 ml samples of each flask were transferred to fresh media similar to those described above. After three consecutive transfers, mixed bacterial cultures were collected, tested for MP-degrading ability, and were isolated to pure bacterial cultures.[9, 10] The mixed bacterial culture isolated from the standard MP-BMM with glucose (0.5 mg/ml) was labeled Mixed Culture I, that isolated from the standard MP-BMM without glucose was labeled Mixed Culture II.

Methyl parathion (MP) degrading ability test (screening test)

Each mixed culture was inoculated into 30 ml of the standard MP-BMM without glucose and shaken at room temperature. Non-inoculated media served as controls. Both 1 ml and 5 ml samples were collected from each flask and bacterial populations were counted using the plate count technique. The residual concentration of MP and its metabolites (*p*-nitrophenol and *p*-aminophenol) at various time intervals (0, 6, 12, 15 and 24 hours) were analyzed by a high performance liquid chromatography (HPLC).

All pure bacterial cultures were inoculated into the standard MP-BMM (30 ml) with or without glucose (0.5 mg/ml) to give an initial cellular density of $4x10^6$ cells/ml and shaken at room temperature. Non-inoculated media served as controls. Two days later, 5-ml samples were collected from each flask and were analyzed for the residual MP concentration.

Pure bacterial cultures having MP-degrading ability were selected for studying MP degradation ability at different conditions. They were identified by using biochemical test and were confirmed by CSIRO, Division of Soils, Waite Campus, South Australia, using the MIDI automated microbial identification system (MIS; an identification of bacteria by a gas chromatography of cellular fatty acids).

The ability to utilize methyl parathion as a sole source of carbon for growth

The pure bacterial cultures with the MP-degrading ability (5 ml of inoculum) were inoculated into the standard BMM (95 ml) with 200 μg/ml analytical grade MP (crystal) to give an initial cellular density of $4x10^6$ cells/ml, while the control flasks were not inoculated. The experiment was done in three replicates. The colors of the media and the presence of crystal of MP in the media were observed every six hours for two days.

The ability to degrade methyl parathion (MP) at different conditions

The best degrading bacteria for both MP and PNP were studied for their MP degradation rates at different conditions. The different parameters were inoculum sizes (10^6 and 10^7 cells/ml) with or without glucose (0.5 mg/ml), pH (4, 5, 6, 7, and 8), salinity (0, 0.1, 0.5, 1.0, 2.0, and 3.5% NaCl), and concentrations of MP (50, 100, and 150 μg/ml). Non-inoculated media served as controls for every condition. The experiment was done in three replicates. Both 1 ml and 5 ml samples were collected from each flask and bacterial populations were counted by plate count. Then, the residual concentrations of MP and its metabolites (*p*-nitrophenol and *p*-aminophenol) were analyzed by HPLC at various time intervals.

The ability to degrade *p*-nitrophenol (PNP) at different conditions

Five ml of 2-day-old inoculums of *Burkholderia cepacia,* giving an initial cellular density of $6x10^7$ cells/ml, were inoculated into 95 ml standard BMM with PNP (50 μg/ml) and 2% NaCl, into 95 ml standard BMM plus PNP only, and into 95 ml standard BMM with glucose (0.5 mg/ml) and 2%NaCl. The residual concentrations of PNP and growth of bacteria were similarly measured as aforementioned section.

Analysis of residues by HPLC

Analytical grade methylene chloride (CH_2Cl_2) was used to extract MP from culture fluids.[19-20] A waters HPLC system, consisting of Model 510 pumps, a 20 μl loop injector, an automated gradient controller, and a Model 484 tunable absorbance UV detector, was used for the analysis of residual MP and its metabolites (Waters Associates Milford, MA, USA). A reverse-phase 5-μm Spherisorb ODS-2 stainless-steel (15 cmx4.6 mm ID) analytical column and a 25-μm Prepex silica guard column (3 cmx4.6 mm ID) were used. Both columns were obtained from Phenomenex (CA, USA).

The solution of mobile phase was methanol-water (70:30, v/v) at a flow rate of 1.0 ml/min Chromatograms were recorded at a wavelength of 220 nm and chart speed of 1.0 cm/min and peak areas were used for quantification.[21-23] The percentages of residual MP were calculated as the MP degradation rate and half-life values ($t_{1/2}$).[24-25] The data of *p*-nitrophenol residues and *p*-aminophenol residues were presented as concentrations.

RESULTS

1. Methyl parathion (MP) degradation by mixed bacterial cultures

Each 10 ml of 2-day-old inoculums of mixed bacterial cultures was inoculated into the standard MP-BMM without glucose to give an initial cellular density of $6x10^7$ cells/ml. Both concentrations of MP and its residues were detected by HPLC.

MP at 50 μg/ml in the BMM was degraded completely by both Mixed Cultures I and Mixed Cultures II within 24 hours, as indicated by MP disappearance and the appearance-disappearance of PNP in the media. Mixed Culture I degraded MP slower than Mixed Culture II, but Mixed Culture I released less PNP than Mixed Culture II (Table 1).

2. Methyl parathion (MP) degradation by pure bacterial cultures

After Mixed Cultures I and II were isolated into 11 and 15 pure bacterial cultures, respectively, all of the pure bacterial cultures were tested for the MP-degrading ability.

Only one pure bacterial culture, isolated from Mixed Culture I, possessed MP-degrading ability. It

Table 1. Degradation of methyl parathion (MP) by mixed bacterial cultures ($6x10^7$ cells/ml) in the standard MP-BMM[\a].

Incubation period (hours)	Mixed Culture I[\b]	Mixed Culture II[\c]	Control
Percentage of residual MP (μg/ml) (X ± SE)			
0	100	100	100
6	89.31±5.84	51.66±1.08	96.30±0.66
12	61.09±4.09	ND[\d]	95.00±1.02
15	27.29±2.32	ND[\d]	94.70±1.04
18	9.86±1.21	ND[\d]	93.70±2.93
24	ND[\d]	ND[\d]	92.30±2.75
Half-life values ($t_{1/2}$) of MP			
	9.63	5.25	
Concentration of *p-n*itrophenol (μg/ml) (X ± SE)			
0	ND[\d]	ND[\d]	0.30±0.02
6	ND[\d]	5.02±0.08	0.32±0.02
12	ND[\d]	ND[\d]	0.44±0.03
15	ND[\d]	ND[\d]	0.52±0.03
18	ND[\d]	ND[\d]	0.61±0.02
24	ND[\d]	ND[\d]	0.64±0.03

\a : Basal mineral media, with 50 μg/ml MP, but without glucose and sodium chloride, pH7.
\b : Mixed culture, isolated from soil sample which was inoculated into standard MP-BMM.
\c : Mixed culture, isolated from soil sample which was inoculated into standard MP-BMM with glucose.
\d : ND = Not detected.

was a gram-negative rod and its colonies were not visible on a standard BMM-agar plate. They were opaque white, convex and punctiform colonies, and were identified as *Burkholderia cepacia* by using MIDI automated microbial identification system. Upon inoculation with *B. cepacia*, the faint lemon-juice color of the medium (the color of commercial MP) disappeared within 12 hours and turbidity of the medium developed within 24 hours. At an initial cellular density of $4x10^6$ cells/ml, *Burkholderia cepacia* was able to degrade MP (50 μg/ml) in BMM with glucose (50 mg/ml) within 12 hours (Table 2, Fig 1).

Three pure bacterial cultures, *Bacillus* sp., and two unknowns, isolated from Mixed Culture II, were able to degrade MP in standard BMM, but their ability was less than that of *Burkholderia cepacia* (Table 2). The turbidity of media inoculated with *Bacillus* sp. and two unknowns also developed within 24 hours but the faint lemon-juice color of the media still appeared. Tables 1, 2, and 3 showed half-life values ($t_{1/2}$) for the MP-degrading ability of two mixed cultures and four pure bacterial cultures, which indicated that *Burkholderia cepacia* possessed the best MP-degrading ability.

Methyl parathion (MP) as a sole source of carbon for growth of pure bacterial cultures

Analytical grade MP is a white crystalline compound, which hardly dissolved in water at room temperature (25°C). Analytical grade MP was added to 200 μg/ml into 95 ml standard BMM without glucose, which was then inoculated with pure bacterial cultures ($4x10^6$ cells/ml), while the control

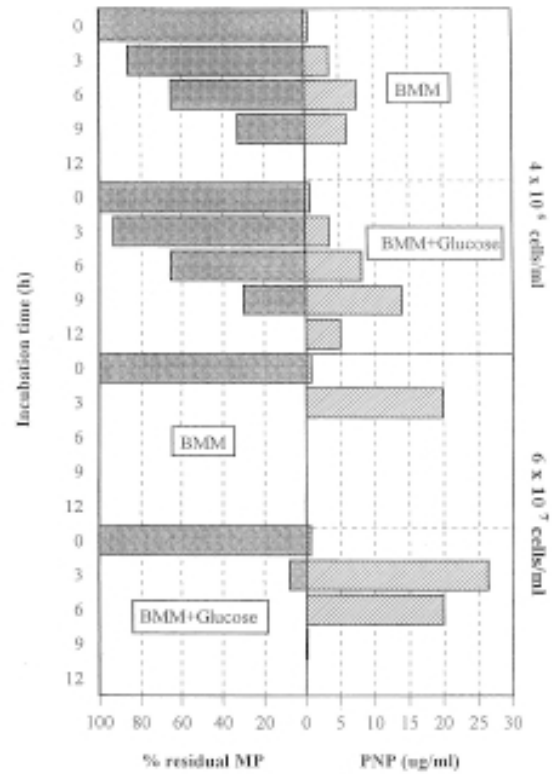

Fig 1. Degradation of methyl parathion (MP) by *Burkholderia cepacia* in the standard MP-BMM, comparison between inoculum size with and without glucose (0.5 mg/ml).

Table 2. Degradation of methyl parathion (MP) by pure bacterial cultures ($4x10^6$ cells/ml) isolated from soil in the standard MP-BMM\a with glucose (0.5 mg/ml).

Days	*Burkholderia cepacia*	*Bacillus* sp.	Unknown 1	Unknown 2	Control
		Methyl parathion (% residue)			
0	100	100	100	100	100
1	ND\b	35.09±0.6	40.96±1.1	21.34±1.6	97.76±1.3
2	ND\b	28.20±1.2	33.67±0.7	16.33±0.7	96.46±1.2
3	ND\b	11.68±0.3	24.36±0.5	11.35±1.1	90.58±2.3
4	ND\b	11.40±0.2	20.75±1.1	10.42±0.9	84.80±1.2
		***p*-nitrophenol (μg/ml)**			
0	0.63±0.03	0.69±0.02	0.66±0.03	0.74±0.04	0.54±0.02
1	ND\b	1.53±0.36	2.11±0.10	2.14±0.05	1.06±0.01
2	ND\b	2.07±0.10	2.24±0.05	2.73±0.17	1.25±0.03
3	ND\b	2.43±0.84	2.48±0.03	2.96±0.05	1.45±0.08
4	ND\b	2.24±0.42	2.28±0.03	2.74±0.05	1.67±0.13
		Half-life values ($t_{1/2}$) of methyl parathion			
	6.95\c	21.93	31.35	13.83	

\a : Basal mineral media, with 50 μg/ml MP but without glucose and sodium chloride, pH7.
\b : ND = Not detected.
\c : Calculated from data shown in Fig 1.

was not inoculated. Three hours later, the media inoculated with *Burkholderia cepacia* became a faint yellow color, and turbidity developed within 24 hours. The chemical associated with the yellow color was identified by HPLC to be *p*-nitrophenol (PNP). Furthermore, the crystals of MP in the media disappeared within 24 hours. Media inoculated with other pure bacterial cultures and control flasks were colorless, turbidity did not develop, and the crystals of MP remained in the media.

3. Methyl parathion (MP) degradation by *Burkholderia cepacia* at different conditions

This study consisted of five parameters, namely, inoculum size ($4x10^6$ and $6x10^7$ cells/ml), presence or absence of glucose (0.5 mg/ml), pH (4, 5, 6, 7, and 8), salinity (0, 0.1, 0.5, 1.0, 2.0, and 3.5% NaCl), and concentration of MP (50, 100, and 150 μg/ml).

B. cepacia at $6x10^7$ cells/ml was able to degrade MP (50 μg/ml), in MP-BMM both with and without glucose (50 mg/ml), faster than that at $4x10^6$ cells/ml. The concentrations of PNP in both MP-BMM inoculated with *B. cepacia* at $6x10^7$ cells/ml increased more than in the BMM inoculated with *B. cepacia* at $4x10^6$ cells/ml (Fig 1).

The half-life values ($t_{1/2}$) of MP (0.55 and 0.71 hours) in both BMM (BMM with and without glucose) inoculated with *B. cepacia* ($6x10^7$ cells/ml) were much lower than that in both BMM (7.04 and 6.95 hours) inoculated with *B. cepacia* ($4x10^6$ cells/ml) (Table 3). These results indicated that the initial inoculum size of bacteria affected the degradation of MP and PNP.

MP (50 μg/ml) in the BMM with glucose (0.5 mg/ml) was degraded by *B. cepacia* ($6x10^7$ cells/ml) completely within six hours, but MP in the BMM without glucose was degraded completely within three hours. PNP concentrations in the BMM with glucose increased more than in the BMM without glucose, and PNP in the BMM with glucose was degraded slower than in the BMM without glucose at both inoculum sizes (Fig 1).

The rate of MP degradation by *B. cepacia* ($4x10^6$ cells/ml) in the BMM with glucose (0.5 mg/ml) did not differ significantly ($p > 0.05$) from that in the BMM without glucose.

Five ml of 2-day-old inoculums of *B. cepacia*, grown in the standard MP-BMM, was inoculated into the standard MP-BMM (pH values from 4-8) to give an initial average cell density of $4x10^6$ cells/ml. *B. cepacia* degraded MP (50 μg/ml) in the standard BMM at all pH levels completely within 12 hours. At 9-h incubation, the residual MP in the alkali media remained less than that in the acid media and the concentrations of PNP in the alkali media increased more than that in the acid media (Fig 2). The half-life value ($t_{1/2}$) of MP in the standard MP-BMM inoculated with *B. cepacia* at pH 4 was the highest, while that of MP at pH 8 was the lowest (Table 3). These results indicated that the MP degradation by *B. cepacia* in the alkali media was better than that in the acid media.

The inoculum preparation and the test for MP-degrading ability of *B. cepacia* at different salinity levels were done similar to the above section. MP at 50 μg/ml in the BMM with NaCl (0.1% and 3.5%) was degraded by *B. cepacia* completely within 24 hours. At 12-h incubation, residual MP in the BMM at 0, 0.1, 0.5, and 1.0% NaCl had disappeared, but residual MP remained in the media with 2% and 3.5% NaCl (Fig 3). The half-life value ($t_{1/2}$) of MP in the standard MP-BMM with 1.0% NaCl inoculated with *B. cepacia* was the lowest, and that of MP with 3.5% NaCl was the highest. MP in the BMM with low salinity (1.0% NaCl) was degraded by *B. cepacia* better than in the BMM without salinity and with high salinity (2.0% and 3.5% NaCl) ($p \leq 0.05$) (Table 3).

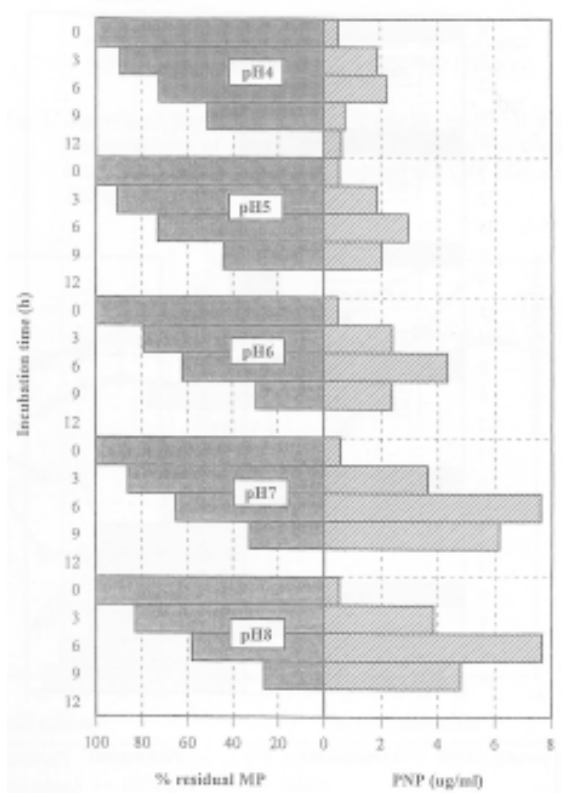

Fig 2. Degradation of methyl parathion (MP) by *Burkholderia cepacia* ($4x10^6$ cells/ml) in the standard MP-BMM at different pH.

Table 3. First order rate constants (k) and half-life values ($t_{1/2}$) for methyl parathion (MP) degradation by *Burkholderia cepacia* in the BMM at different conditions.

Basal mineral medium					
MP (μg/ml)	**Glucose (0.5 mg/ml)**	**pH**	**%NaCl**	**k (h^{-1})**	**$t_{1/2}$ (h)**
		4 x 10^6 cells/ml			
50	-	**4**	0	6.72×10^{-2}	10.31
50	-	**5**	0	7.56×10^{-2}	9.17
50	-	**6**	0	1.07×10^{-1}	6.46
50	-	**7**	0	9.84×10^{-2}	7.04
50	-	**8**	0	1.15×10^{-2}	6.00
50	-	7	**0.1**	1.21×10^{-2}	5.71
50	-	7	**0.5**	1.29×10^{-2}	5.36
50	-	7	**1.0**	1.34×10^{-2}	5.18
50	-	7	**2.0**	9.27×10^{-1}	7.48
50	-	7	**3.5**	8.78×10^{-1}	7.89
50	**+**	7	0	9.97×10^{-1}	6.95
		6 x 10^7 cells/ml			
50	-	7	0	1.26	0.55
50	**+**	7	0	9.68×10^{-1}	0.71
100	-	7	0	1.20	0.58
150	-	7	0	7.93×10^{-1}	0.87

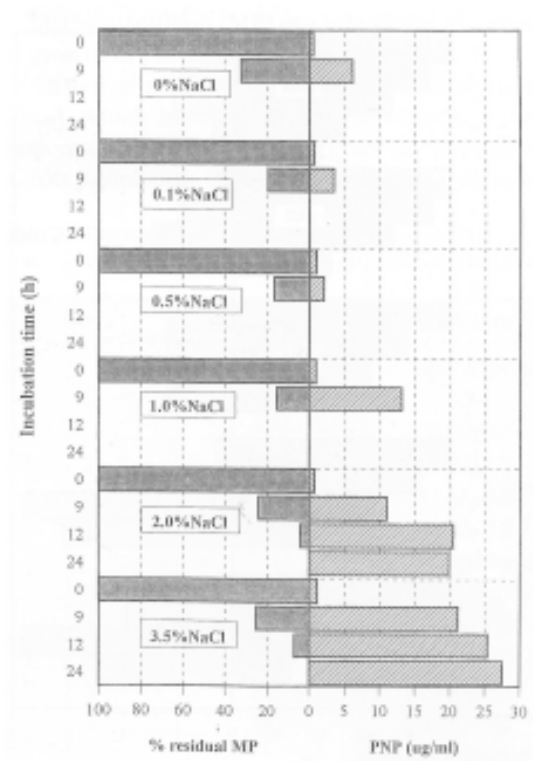

Fig 3. Degradation of methyl parathion (MP) by *Burkholderia cepacia* (4x10^6 cells/ml) in standard MP-BMM at different salinity levels.

Throughout the incubation time, concentrations of PNP in the BMM, with 2.0% and 3.5% NaCl, increased a great deal and remained in the BMM. The PNP in the BMM containing low salt concentrations (0%, 0.1%, 0.5%, and 1.0% NaCl) was degraded by *B. cepacia* (Fig 3).

The study for the MP-degrading ability of *B. cepacia* in the standard BMM, with various initial MP concentrations, was done with an initial cell density of *B. cepacia* at 6x10^7 cells/ml. Figure 4 shows that the residual MP in the BMM with initial concentrations of MP at 50, 100, and 150 μg/ml were degraded completely within 3, 4, and 5 hours, respectively. The residual PNP in the BMM with MP at 50 and 100 μg/ml was degraded completely within 12 and 28 hours, respectively. When the initial MP concentration in the BMM increased, the PNP concentration in the BMM also increased (Fig 4). When the PNP concentrations in the BMM increased, the populations of *B. cepacia* in the BMM also decreased. Thus, the populations increased after the PNP concentrations decreased (Fig 5).

4. Growth of *B. cepacia* in methyl parathion-basal mineral medium (MP-BMM) at different conditions

Growth of *B. cepacia* in the MP-BMM at different pH is shown in Fig 6-A. *B. cepacia* (4x10^6 cells/ml) could grow in the MP-BMM at pH values ranging from 4 to 8; their growths differed slightly. The growth

curve in MP-BMM at pH 4 was lower than that at other pH values. *B. cepacia* ($4x10^6$ cells/ml) was able to grow in the MP-BMM with 0.1%, 0.5%, and 1.0% NaCl, but the population decreased rapidly in the MP-BMM with 2.0% and 3.5% NaCl as the residual concentrations of PNP in the MP-BMM increased (Fig 6-B).

Growth of *Burkholderia cepacia* at inoculum size of $6x10^7$ cells/ml in the MP-BMM with and without

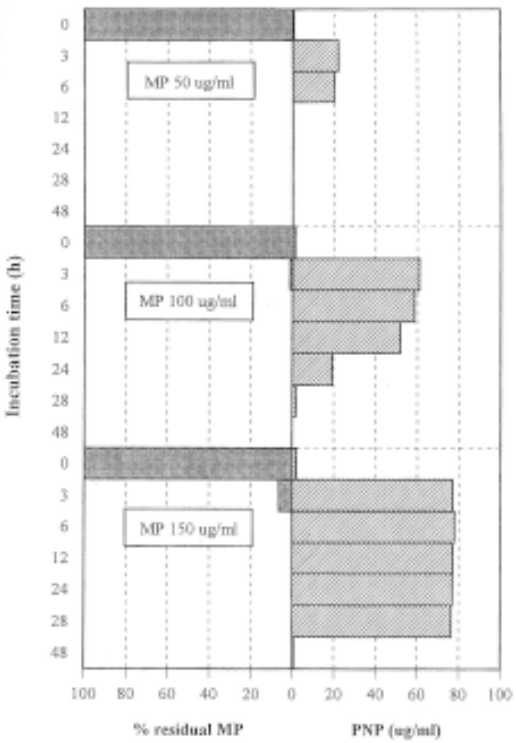

Fig 4. Degradation of methyl parathion (MP) by *Burkholderia cepacia* ($6x10^7$ cells/ml) in standard BMM at different MP concentrations.

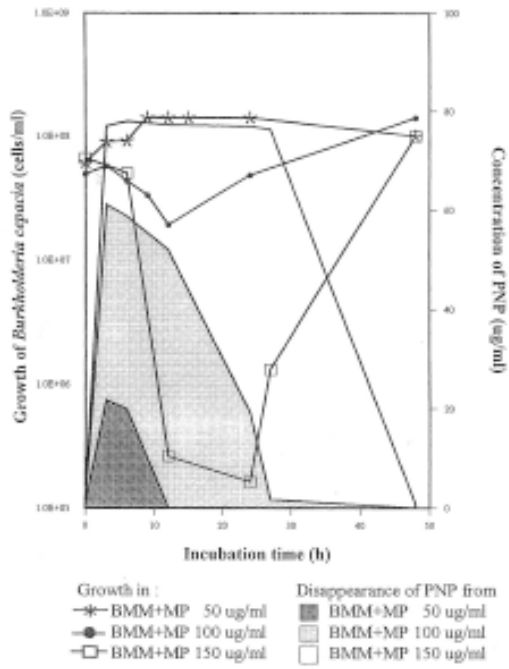

Fig 5. Growth of *Burkholderia cepacia* ($6x10^7$ cells/ml) and disappearance of p-nitrophenol (PNP) from the BMM containing different concentrations of MP (50, 100, and 150 ug/ml).

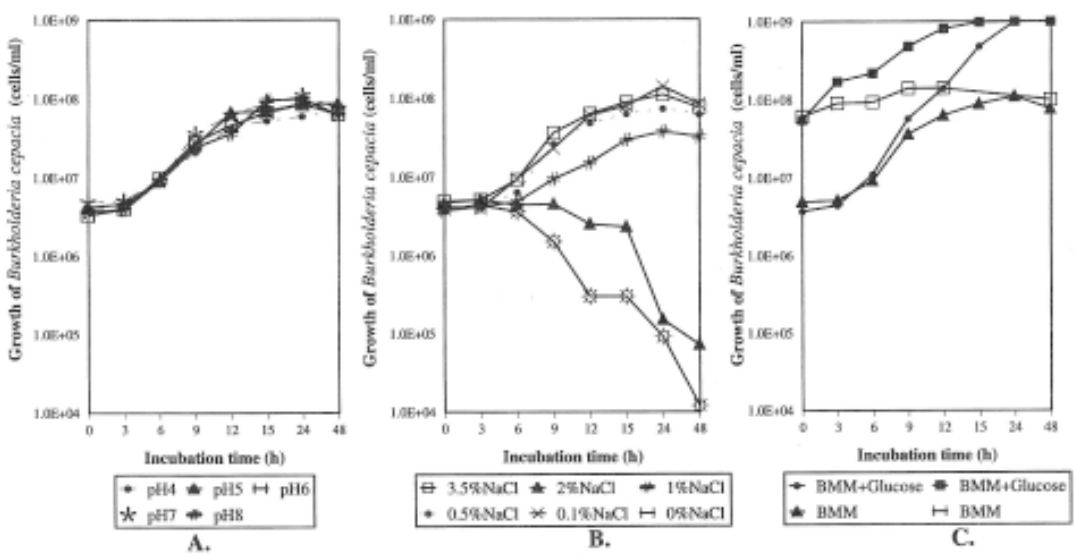

Fig 6. Growth of *Burkholderia cepacia* in the standard MP-BMM at different conditions. A, different pH; B, different salinity; C, comparison between the BMM containing glucose (0.5 mg/ml) and without glucose.

glucose increased less than that at inoculum size of $4x10^6$ cells/ml. Growth of *Burkholderia cepacia* at both inoculum sizes in the MP-BMM with glucose (0.5 mg/ml) was better than that in the MP-BMM without glucose (Fig 6-C).

5. Degradation of *p*-nitrophenol (PNP) (50 μg/ml) by *Burkholderia cepacia* in the basal mineral medium (BMM) at different conditions

PNP at 50 μg/ml in the BMM without NaCl was degraded completely by *B. cepacia* within 24 hours, but it was only partially degraded in BMM with 2% NaCl (Table 4). *B. cepacia* at $6x10^7$ cells/ml was able to grow in the BMM with glucose and 2% NaCl and in the BMM with 50 μg/ml PNP, but it was unable to grow in BMM with 2% NaCl and 50 μg/ml PNP (Fig 7).

Discussion

This study has isolated *Burkholderia cepacia* which has a potential methyl parathion (MP)-degrading ability from agricultural soil. Furthermore, it is also able to degrade *p*-nitrophenol (PNP), a metabolite of MP, in the basal mineral medium (BMM). In HPLC analysis, *p*-aminophenol was not found in any media, but the residual MP and PNP were detected in the media. *P*-aminophenol is a metabolite of MP from reductive reaction and PNP is a metabolite of MP from hydrolytic reaction. The results indicate that MP in the BMM was hydrolyzed to PNP by *B. cepacia*.

When *Burkholderia cepacia* was inoculated into the standard BMM without glucose, to which was added analytical grade MP, *B. cepacia* was able to hydrolyze the analytical grade MP and the residual PNP. This is not the first study to find that pure bacterial cultures degraded MP, but this is the first study to show that MP and PNP were degraded completely by *B. cepacia*, and that it did not require glucose or another carbon source for growth. MP and PNP were used as sole sources of carbon for growth of *B. cepacia*.

The other pure bacteria isolated from soil, *Bacillus* sp. and two unknowns, were able to degrade commercial grade MP but not analytical grade MP. They did not grow in the medium containing only analytical grade MP. The commercial grade MP was degraded by these bacteria cultures because they utilized MP not as a carbon source but as a co-metabolite. Commercial grade MP is composed of many organic compounds which may be a carbon source for the bacteria, so they degraded commercial grade MP in the standard BMM but did not utilize MP as a sole carbon source.

Mixed Bacterial Culture II degraded MP better than the Mixed Bacterial Culture I, although *B. cepacia* was isolated from the Mixed Bacterial Culture I. Because both mixed bacterial cultures from soil are composed of many bacterial strains, MP degrada-

Table 4. Degradation of *p*-nitrophenol (PNP) at a concentration of 50 μg/ml by *Burkholderia cepacia* ($6x10^7$ cells/ml) in the standard BMM\a with and without 2% sodium chloride.

Incubation (hours)	Recovery concentrations of PNP (μg/ml)		
	BMM\a	BMM + 2% NaCl\b	Control
0	50.00±0.23	50.00±0.08	50.00±0.81
12	41.12±1.15	45.7±0.10	49.93±0.86
24	ND\c	45.5±0.26	49.83±0.22
48	ND\c	45.4±0.17	49.73±0.99
72	ND\c	45.4±0.16	49.79±1.13

\a : Basal mineral media, without glucose and sodium chloride, pH7.
\b : Basal mineral media, without glucose but with 2% sodium chloride, pH7.
\c : ND = Not detected.

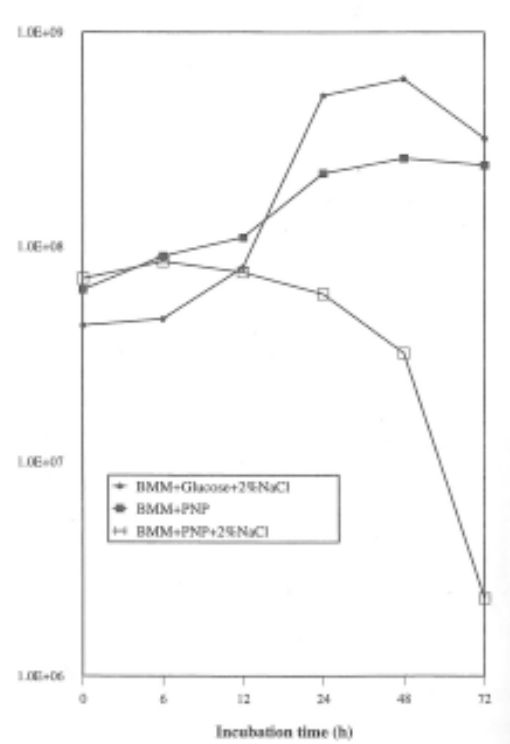

Fig 7. Growth of *Burkholderia cepacia* in standard BMM with glucose (0.5 mg/ml) and 2%NaCl, in standard BMM with PNP only, and in the standard BMM with 50 μg/ml PNP and 2%NaCl.

tion in both mixed bacterial cultures is a concerted effort of many bacteria, and *B. cepacia* in the Mixed Bacterial Culture I may be depressed by other bacteria.

The results of the MP degradation by *B. cepacia* in BMM at different pH values showed that MP degradation by *B. cepacia* in alkali BMM was better than that in acidic BMM. High acidic BMM (pH4) may not be appropriate for *B. cepacia* growth, and that MP is more stable in acidic BMM than in alkali BMM.

In our study, it was shown that the pure culture of *B. cepacia* was able to grow in BMM with salt and to degrade MP, but MP in high salinity was degraded slowly because of the decrease of the bacterial population. Salinity in the media does not affect the growth of *B. cepacia* but does affect its PNP degradation. The toxicity of PNP causes the population to decrease.[26,27] A pure culture of *B. cepacia* was also able to degrade PNP at low salinity (0.1% to 1.0% NaCl) but not in high salinity (2.0% and 3.5% NaCl). Similarly, Spain et al (1980) indicated that mixed bacteria from salt marsch cannot adapt themselves to degrade MP and PNP.[28] The reason for this is not known, hence further investigation is needed.

Comparison between inoculum size of *B. cepacia* indicated that when the inoculum size was increased, MP was degraded more rapidly and the concentration of PNP was also be increased. Therefore, in the detoxification of MP by *B. cepacia* should be recognized that metabolite (PNP) increases when the initial inoculum size increases, because PNP is also toxic to living organisms.

The comparison of the MP degradation by *B. cepacia* with and without glucose showed that *B. cepacia* prefers to utilize glucose as a carbon source more than MP and PNP. The addition of supplements, which were other carbon sources, was not effective in increasing the MP-degrading ability of *B. cepacia*. In fact, its MP-degrading ability was decreased. *B. cepacia* is able to degrade MP rapidly even at high concentrations. But at high MP concentrations, PNP in the BMM increased and the population decreased rapidly. When the PNP in the BMM was degraded completely, cell density increased.

B. cepacia degrades MP rapidly and degrades PNP moderately. Furthermore, *B. cepacia* can adapt to a wide range of pH (pH4 to pH8) and salinity (0.1% to 3.5% NaCl). Unfortunately, when the concentration of PNP increased, the population decreased due to the toxicity of PNP, and thus it may be unable to degrade PNP.

The present study was conducted under laboratory conditions. Therefore, in future studies *B. cepacia* should be tested with industrial waste and in natural environments, because micro-organisms which are able to degrade organic pollutants in culture may sometimes fail to function when they are inoculated into a sludge system of industry and natural environments. They may lose their degrading ability because they may be susceptible to toxins or predators in the environment, they may use other organic compounds in preference to MP, or they may be unable to move through soil to sites containing MP. Genetic engineering of *B. cepacia* may be conducted in order to improve biodegradation. Furthermore, *B. cepacia* should be tested for its pathogenicity to aquatic organisms, such as fish, prawn, crab, etc, before being applied for the detoxification of pesticides in the environment.

Acknowledgements

We thank Bayer Thai Co, Ltd, for providing pure methyl parathion and the Division of Clinical Pathology, Department of Medical Science, Ministry of Public Health, and CSIRO, South Australia for help with identification of microorganisms. This work was supported in part by a research grant from the Faculty of Science, Mahidol University, Bangkok, Thailand.

References

1. Prithard PH et al (1987) Biotic and abiotic degradation rates of methyl parathion in freshwater and estuarine water and sediment samples. *Chemosphere* 16, 1509-20.
2. Buerger TT et al (1994) Metabolism and acute toxicology of methyl parathion in pen-reared and wild northern bobwhites. *Environ Toxicol Chem* 13(7), 1139-43.
3. Mathew G et al (1992) Methyl parathion induced sperm shape abnormality in mouse. *Mutat Res* 280, 169-73.
4. Reddy RR et al (1994) Effect of sublethal concentration of methyl parathion on aspects of nitrogen metabolism in oziotelphusa-senex-senex. *Fresenius Environ Bull* 3(1), 55-62.
5. Ortiz D et al (1995) Acute toxicological effects in rats treated with a mixture of commercially formulated products containing methyl parathion and permethrin. *Ecotoxicol Environ Saf* 32(2), 154-8.
6. Lawless EW et al (1975) EPA Tech Ser Rep EPA 670/2-75-057. Washington DC.
7. Ou LT et al (1983) Methyl parathion degradation in soil: influence of soil-water tension. *Soil Biol Biochem* 15, 211-5.
8. Ou L T (1985) Methyl parathion degradation and metabolism in soil: influence of high soil-water contents. *Soil Biol Biochem* 17, 241-3.
9. Chaudhry GR et al (1988) Isolation of a methyl parathion-degrading *Pseudomonas* sp. that possesses DNA homologous to the *opd* gene from a *Flavobacterium* sp. *Appl Environ Microbiol* 54, 288-93.

10. Megharaj M et al (1994) Biodegradation of methyl parathion by soil isolates of microalgae and cyanobacteria. *Bull Environ Contam Toxicol* 53(2), 292-7.
11. Rani NL and Lalithakumari D (1994) Degradation of methyl parathion by *Pseudomonas putida*. *Can J Microbiol* 40(12), 1000-6.
12. BG Chemie (1993) Toxicological Evaluation 6: Potential Health Hazards of Existing Chemicals, 105 pp. Employment Accident Insurance Fund of the Chemical Industry, Germany.
13. Ghisalba O (1983) Chemical wastes and their biodegradation-an overview. In Reviews: Microbial degradation of environmental pollutants. *Experientia* 39, 1247-57.
14. Megharaj M et al (1993) Influence of rice straw amendment on persistence and algal toxicity of *p*-nitrophenol in soil. *Soil Biol Biochem* 25, 1185-8.
15. Laveglia J and Dahm PA (1977) Degradation of organophosphorus and carbamate insecticides in the soil and by soil microorganisms. *Ann Rev Entomol* 22, 483-513.
16. Lewis DL et al (1985) Multiphasic kinetics for transformation of methyl parathion by *Flavobacterium* species. *Appl Environ Microbiol* 50, 553-7.
17. Ou LT and Sharma A (1989) Degradation of methyl parathion by a mixed bacterial culture and a *Bacillus* sp. isolated from different soils. *J Agr Food Chem* 37, 1514-8.
18. Heitkamp MA et al (1990) Biodegradation of *p*-nitrophenol in an aqueous waste steam by immobilized bacteria. *Appl Environ Microbiol* 56, 2967-73.
19. Yamagata N and Ohkita T (1972) Pesticides, Analytical Method of Environmental Pollutants, Volume 10. Dainippon Tosho, Tokyo, Japan.
20. McGarvey BD (1993) High-performance liquid chromatographic methods for the determination of N-methylcarbamate pesticides in water, soil, plants and air. *J Chromatogr* 642, 89-105.
21. Merck E (1986) Application Manual HPLC / TLC / HPTLC, 188 pp. Germany.
22. Martinez RC et al (1992) Sensitive method of the determination of organophosphorus pesticides in fruits and surface waters by high-performance liquid chromatography with ultraviolet detection. *J Chromatogr* 607, 37-45.
23. Lindsay S (1991) High Performance Liquid Chromatography: Analytical Chemistry by Open Learning, 244 pp. John Wiley & Sons, Singapore.
24. Munnecke DM (1980) Enzymatic detoxification of waste organophosphate pesticides. *J Agr Food Chem* 28, 105-11.
25. Van Veld PA and Spain JC (1983) Degradation of selected xenobiotic compounds in three types of aquatic test systems. *Chemosphere* 12, 1291-305.
26. Munnecke DM and Hsieh DPH (1974) Microbial decontamination of parathion and *p*-nitrophenol in aqueous media. *Appl Microbiol* 28, 212-7.
27. Spain JC and Gibson DT (1991) Pathway for biodegradation of *p*-nitrophenol in a *Moraxella* sp. *Appl Environ Microbiol* 57, 812-9.
28. Spain JC et al (1980) Effects of adaptation on biodegradation rates in sediment/water cores from estuarine and freshwater environments. *Appl Environ Microbiol* 40, 726-34.