Genetic and Phenotypic Diversity of Parathion-Degrading Bacteria Isolated from Rice Paddy Soils

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Three parathion-degrading bacteria and eight pairs of bacteria showing syntrophic metabolism of parathion were isolated from rice field soils, and their genetic and phenotypic characteristics were investigated. The three isolates and eight syntrophic pairs were able to utilize parathion as a sole source of carbon and energy, producing p-nitrophenol as the intermediate metabolite during the complete degradation of parathion. Analysis of the 16S rRNA gene sequence indicated that the isolates were related to members of the genera Burkholderia, Arthrobacter, Pseudomonas, Variovorax, and Ensifer. The chromosomal DNA patterns of the isolates obtained by polymerase-chain-reaction (PCR) amplification of repetitive extragenic palindromic (REP) sequences were distinct from one another. Ten of the isolates had plasmids. All of the isolates and syntrophic pairs were able to degrade parathion-related compounds such as EPN, p-nitrophenol, fenitrothion, and methyl parathion. When analyzed with PCR amplification and dot-blotting hybridization using various primers targeted for the organophosphorus pesticide hydrolase genes of previously reported isolates, most of the isolates did not show positive signals, suggesting that their parathion hydrolase genes had no significant sequence homology with those of the previously reported organophosphatase pesticide-degrading isolates.

Keywords: Parathion-degrading bacteria, parathion, diversity, organophosphorus insecticide, syntrophic metabolism

Organophosphate insecticides have received widespread use to control a variety of pests throughout the world [34]. These pesticides, such as parathion \((O,O\text{-diethyl}-O-p\text{-nitrophenyl phosphorothioate})\), EPN \((O\text{-ethyl-}p\text{-nitrophenyl phenylphosphonothioate})\), fenitrothion \((O,O\text{-dimethyl-}O-[p\text{-nitro-}m\text{-tolyl}]\text{ phosphorothioate})\), and methyl-parathion \((O,O\text{-dimethyl-}O-p\text{-nitrophenyl phosphorothioate})\), inhibit the normal activity of the acetylcholine esterase, resulting in accumulation of acetylcholine at the synapses. This inhibition causes convulsion, paralysis, and finally death for insects and mammals [29]. Among organophosphorus compounds, parathion is one of the most toxic insecticides registered in the U.S. Environmental Protection Agency [7]. Thus, excessive use of parathion may result in hazardous effects in non-target organisms and impact human health, owing to extreme toxicity with ease of exposure [21]. Therefore, many studies have focused on its persistence and fate in the natural environment.

Microorganisms play a major role in the degradation of organophosphate insecticides [3, 9, 10, 28, 30, 31, 34]. The growth of the degrading microbial populations was substantially stimulated proportionally with increasing concentrations of the organophosphate in soils [24]. In fact, many bacterial strains capable of degrading completely or partially organophosphorus pesticides have been isolated from soils [10, 19, 28, 30, 35]. The microbial enzymatic hydrolysis of the phosphoester bond of the organophosphate insecticide substantially reduced its toxicity, and in the case of parathion, a 100-fold reduction in toxicity was reported [31].

In Korea, 75,662 kg of parathion was produced for rice farming and horticulture in 2004 [17] and thus it can be hazardous to wild animals in the area of application. Therefore, the detoxification of parathion is needed for better agricultural environment and very important for non-target organisms in terrestrial and aquatic environments exposed to parathion. Several microorganisms able to degrade parathion have been isolated from the environments, and their degradation pathways and degradative enzymes
were studied [4, 6, 22–24]. However, most of the previous parathion-degrading isolates were obtained from upland field soils [32], whereas a substantial amount of parathion has been applied in rice fields. Unlike upland fields, the wet rice fields are characterized by submergence during part or all of the cropping period, thus leading to development of diverse microorganisms quite different from upland fields. The investigation on parathion-degrading microorganisms of rice fields would provide new aspects on the genetic and physiological diversity of microbial populations with parathion-catabolic function.

In this study, we report, in addition to three parathion-degrading bacteria, eight pairs of bacteria showing syntrophic metabolism of parathion, which has not been reported in previous studies. We investigated species diversity by 16S rRNA gene sequence analysis and REP–PCR patterns of chromosomes, and describe the physiological and genetic properties of the rice field isolates on parathion biodegradation.

**Materials and Methods**

**Media and Culture Condition**

All isolates were maintained on mineral medium [26] containing parathion at a concentration of 100 ppm (µg/ml). Peptone-tryptone-yeast extract-glucose (PTYG) medium containing (per liter) 0.25 g of peptone (Difco Laboratories, Detroit, U.S.A), 0.25 g of tryptone (Difco), 0.5 g of yeast extract (Difco), 0.5 g of glucose, 0.03 g of magnesium sulfate, and 0.003 g of calcium chloride was used for strain isolation and colony production for the repetitive extragenic palindromic PCR (REP–PCR). All cultures were incubated at 28°C and liquid cultures were aerated by shaking at 150 rpm on a rotary shaker (Vision Co., Bucheon, Korea).

**Chemicals**

Analytical grade parathion (O,O-diethyl-O-p-nitrophenyl phosphorothioate), methyl parathion (O,O-dimethyl-O-p-nitrophenyl phosphorothioate), fenitrothion (O,O-dimethyl-O-[p-nitro-m-tolyl] phosphorothioate), 3-methyl-4-nitrophenol, EPN (O-ethyl O-p-nitrophenyl phenylphosphonothioate), p-nitrophenol, malathion (S-[1,2-dicarboxethyl]-O,O-dimethyl dithiophosphate), and chlorpyrifos (O,O-diethyl-O-(3,5,6-trichloro-2-pyridyl) phosphorothioate) were obtained from Sigma Chemical Co., St. Louis, MO, U.S.A.

**Isolation of Bacterial Strains**

Rice paddy soil samples were taken from various rice fields of a western area in South Korea. Samples from the top 15 cm of soil were taken, sieved through a 2-mm-pore-size sieve, and kept at 4°C prior to use. A 20-g amount of each soil sample was transferred to each 50-ml sterile beaker, treated with parathion dissolved in dichloromethane to a final concentration of 100 µg/g soil, and thoroughly mixed. The treated soil was incubated with periodic shaking at 150 rpm on a rotary shaker (Vision Co., Bucheon, Korea). Samples (0.1 ml) of appropriate 10-fold dilutions were inoculated into test tubes containing 3 ml of parathion medium (100 µg/ml) [27]. The tubes were incubated at 28°C for 4 weeks, and degradation of parathion was analyzed by spectrophotometry and reverse-phase HPLC on a µBondapack C18 column (3.9 by 300 mm; Waters, Milford, MA, U.S.A.) and a UV detector set at 283 nm; methanol 0.1% phosphoric acid (60:40) was used as the eluant. The culture of the terminal positive tube, showing substantial cell growth and less than 20% of the parathion remaining, was enriched by two additional transfers into fresh medium. Each enriched culture was streaked onto PTYG agar medium, and single colonies were then tested for parathion degradation in fresh parathion medium before strain isolation.

**Identification by 16S rRNA Gene Sequence Analysis**

Total genomic DNA was extracted from the isolates and PCR amplification of 16S rRNA genes was performed with 27mfd and 1492r as previously described [14, 16, 18]. The amplified 16S rRNA genes were sequenced using an ABI Prism BigDye Terminator Cycle Sequencing Ready Kit according to the manufacturer’s instruction (Perkin-Elmer) with the sequencing primers 27mfd and 519r [1, 18]. Unambiguous 746–1,465 nucleotide positions were used for comparison to the data in GenBank using the Basic Local Alignment Search Tool (BLAST) [2]. Sequences from nearest relatives were identified from the Ribosome Database Project (RDP) using the SIMILARITY-RANK program of the RDP [20].

**Colony REP–PCR**

Colony REP–PCR was performed using BOXA1R as a primer, as described previously [5]. Each isolate was grown on the PTYG agar medium for 24 to 48 h, and a small amount of cells was resuspended in 25 µl of PCR mixture. The cycle used was as follows: 1 cycle at 95°C for 7 min; 35 cycles at 92°C for 1 min, at 52°C for 1 min, and at 65°C for 48 h; 1 cycle at 65°C for 16 min; and a final soak at 4°C. After the reactions, PCR products were separated by electrophoresis on 1.2% agarose gels. After electrophoresis, the image was photographed with UV transillumination (306 nm).

**Degradation Phenotype Analysis**

Each strain was grown in PTYG medium. Cells were then harvested by centrifugation at 10,000 x g for 10 min at 4°C, washed, and resuspended in 0.85% NaCl solution. Aliquots of suspended cells were inoculated into 15-ml culture tubes, each of which contained 3 ml of mineral medium supplemented with one of the structural analogs at a concentration of 100 µg/ml. The tubes were cultured by reciprocal shaking at 150 rpm at 28°C for 4 weeks, after which the optical density at 600 nm was determined. To determine the degradation of organophosphorous insecticides, 3 ml of acetonitrile was added to a 3-ml culture of the tube, mixed thoroughly, and filtered by Minisart SRP 25. After filtration, the culture was used for the measurement of optical densities at 270 nm (fenitrothion), 274 nm (EPN), 275 nm (methyl parathion), 283 nm (parathion), and 410 nm (p-nitrophenol).

**Axenic Culture Experiment**

After growth in PTYG medium, cells were harvested, washed, and resuspended in mineral medium. Aliquots of cells were inoculated into 15-ml tubes containing 3 ml of mineral medium supplemented with parathion (100 µg/ml) as the sole carbon source at a final density of OD600=0.006. All cultures were incubated at 28°C in the dark on a rotary shaker (150 rpm) for 4 weeks. At specific intervals, two replicate tubes were taken out and used to determine cell
growth and the concentrations of parathion and p-nitrophenol. Cell growths were determined at optical density at 600 nm. For the quantification of parathion and p-nitrophenol, 3 ml of acetonitrile was added to a 3-ml culture of the tube, mixed thoroughly, and filtered by Minisart SRP 25. After filtration, the culture was used for the measurement of optical densities at 283 nm (for parathion) and at 410 nm (for p-nitrophenol) using spectrophotometry and reverse-phase HPLC. The concentrations of parathion and p-nitrophenol were calculated using standard curves prepared from the known concentrations of parathion and p-nitrophenol in the same medium.

Plasmid Detection
For detection of plasmid DNA, cells were lysed using the procedure described by Hynes et al. [12]. Curing of plasmid of the isolates was performed using the sodium dodecyl sulfate (SDS) plate and elevated incubation temperature method [8]. In brief, a portion of overnight parathion-degrading bacterial culture in PTYG was subcultured twice into PTYG medium supplemented with SDS and grown at 37°C for 3 days. The culture was appropriately diluted and spread onto PTYG plates. Single colonies were checked for parathion degradation activity.

PCR Amplification of the Organophosphate Hydrolysis Gene
The partial gene sequences specific to the parathion degradation pathway were amplified by PCR with specific primers targeted for the opd [31, 33], oph [25], and opdB [15] genes. The primers for gene opd were designed based on the conserved gene sequence found in parathion hydrolysis genes of P. diminuta [31] and Flavobacterium sp. [33]: opd-f primer, 5'-GTCGATGAGCGCCTTGT-3’; and opd-r primer, 5'-GATCGTCTGCAAGGCTG-3’; and opdB primer, 5'-GATCGCACCGCGATTCCAA-3’. The primer sequences for the opdB gene have been reported in Burkholderia sp. JBA3 [15]: BP/f-1 primer, 5’-CAGCTCTGTGCTTACCGGC-3’; and BP/r-3 primer, 5’-TGCACGCTGACACTAGCTGT-3’. The amplification of the opd, opdA, oph, and opdB genes with the corresponding primers is expected to produce a 641 bp, 1,000 bp, 998 bp, and 944 bp DNA fragment, respectively.

DNA Labeling and Dot-Blot Hybridization
The parathion hydrolyase gene probe was PCR amplified with the previously reported primers (BP/f-1 and BP/r-1) from Burkholderia sp. JBA3 [15] and with primers for the afr gene cloned from Burkholderia sp. Y113, which were able to degrade fenitrothion and parathion [13]: OFH/f-1 primer, 5’-GATGCTCGTGGCGGCATTCCG-3’; and OFH/r-1 primer, 5’-CCGTAGCGGAAACGACAG-3’. The gene probe was labeled with DIG using DIG High-Prime (Roche Diagnostics, Mannheim, Germany) according to the manufacturer’s instructions. For making of denatured DNA, 1 µg of genomic DNA was denatured in 0.4 M NaOH and 10 mM EDTA for 10 min at 100°C and immediately chilled on ice for 5 min. Then, it was neutralized by addition of an equal volume of cold 2 M ammonium acetate. The denatured DNA fragments were spotted onto a positive charged nylon membrane (BIO-RAD, Hercules, U.S.A.) that had been prewetted with triple distilled water at 10 min. The DNA on the membrane was blotted by a Bio-DOT Microfiltration apparatus (BIO-RAD) according to the supplier’s instructions. Prehybridization, hybridization, and posthybridization washes were performed as described in the supplier’s manual (DIG High Prime DNA Labeling and Detection Starter Kit 1).

Table 1. Nearest relatives of the parathion-degrading isolates based upon 16S rRNA gene sequences.

<table>
<thead>
<tr>
<th>Isolate</th>
<th>GenBank Accession No.</th>
<th>Sampling site</th>
<th>Nearest type strain</th>
<th>Similarity (%) (No. of bases compared)</th>
</tr>
</thead>
<tbody>
<tr>
<td>CN2a</td>
<td>GQ332343</td>
<td>Yesan, Chungchongnam-do</td>
<td>Arthrobacter defluvii 4C1-a</td>
<td>98(836)</td>
</tr>
<tr>
<td>CN2b</td>
<td>GQ332344</td>
<td>Yesan, Chungchongnam-do</td>
<td>Pseudomonas knackmussi B13</td>
<td>99(771)</td>
</tr>
<tr>
<td>CN3a</td>
<td>GQ214323</td>
<td>Yesan, Chungchongnam-do</td>
<td>Arthrobacter defluvii 4C1-a</td>
<td>98(746)</td>
</tr>
<tr>
<td>CN3b</td>
<td>GQ332345</td>
<td>Yesan, Chungchongnam-do</td>
<td>Variovorax paradoxus IAM 12373</td>
<td>99(795)</td>
</tr>
<tr>
<td>GG6a</td>
<td>GQ337854</td>
<td>Anjoong, Gyeonggi-do</td>
<td>Arthrobacter defluvii 4C1-a</td>
<td>98(1,454)</td>
</tr>
<tr>
<td>GG6b</td>
<td>GQ337855</td>
<td>Anjoong, Gyeonggi-do</td>
<td>Variovorax boronicumulans BAM-48</td>
<td>100(1,465)</td>
</tr>
<tr>
<td>GG7a</td>
<td>GQ332346</td>
<td>Anjoong, Gyeonggi-do</td>
<td>Arthrobacter defluvii 4C1-a</td>
<td>100(795)</td>
</tr>
<tr>
<td>GG7b</td>
<td>GQ332347</td>
<td>Anjoong, Gyeonggi-do</td>
<td>Variovorax boronicumulans BAM-48</td>
<td>100(792)</td>
</tr>
<tr>
<td>GG8a</td>
<td>GQ143226</td>
<td>Anjoong, Gyeonggi-do</td>
<td>Arthrobacter nitroguajacolicus G2-1</td>
<td>99(963)</td>
</tr>
<tr>
<td>GG8b</td>
<td>GQ143227</td>
<td>Anjoong, Gyeonggi-do</td>
<td>Pseudomonas moorei RW10</td>
<td>100(994)</td>
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<tr>
<td>GG9a</td>
<td>GQ332348</td>
<td>Anjoong, Gyeonggi-do</td>
<td>Arthrobacter defluvii 4C1-a</td>
<td>100(801)</td>
</tr>
<tr>
<td>GG9b</td>
<td>GQ332349</td>
<td>Anjoong, Gyeonggi-do</td>
<td>Variovorax boronicumulans BAM-48</td>
<td>100(813)</td>
</tr>
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<td>GG14a</td>
<td>GQ332350</td>
<td>Kunpo, Gyeonggi-do</td>
<td>Arthrobacter defluvii 4C1-a</td>
<td>100(766)</td>
</tr>
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<td>GG14b</td>
<td>GQ337856</td>
<td>Kunpo, Gyeonggi-do</td>
<td>Arthrobacter niagatensis LC4</td>
<td>99(1021)</td>
</tr>
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<td>CNB8</td>
<td>GQ214324</td>
<td>Hampojeong, Chollanam-do</td>
<td>Arthrobacter defluvii 4C1-a</td>
<td>100(783)</td>
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<td>CNBb</td>
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<td>100(826)</td>
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<td>JNA3</td>
<td>GQ332351</td>
<td>Hampojeong, Chollanam-do</td>
<td>Burkholderia caledonica LMG 19076</td>
<td>99(791)</td>
</tr>
<tr>
<td>JNB1</td>
<td>GQ214328</td>
<td>Hampojeong, Chollanam-do</td>
<td>Burkholderia caledonica LMG 19076</td>
<td>98(874)</td>
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<td>Gochang, Chollabuk-do</td>
<td>Burkholderia glathei ATCC 29195</td>
<td>98(1,453)</td>
</tr>
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</table>
The dot-blot hybridization signals were visualized using the DIG hybridization system (Roche Diagnostics).

**RESULTS**

Isolation of Parathion-Degrading Bacteria

Parathion-degrading bacteria and syntrophic pairs were isolated through enrichment processes from different rice field soils (Table 1). Among 63 rice field soil samples tested, 52 samples apparently did not show any detectable degradation of parathion during 5 weeks of the enrichment incubation, but 11 soils showed positive degradation of the pesticide. Three parathion-degrading bacteria were isolated and purified from three different soils (Table 1). However, some enriched cultures failed to produce purified strains able to degrade parathion. When the different colony types from each of these cultures were combined on parathion medium, some mixed cultures were able to grow and mineralize parathion. From these mixed cultures, eight pairs of presumably syntrophic bacteria (denoted by “a” and “b”) capable of degrading parathion were isolated from eight different rice soils (Table 1).

**16S rRNA Gene Sequence and REP–PCR Analyses**

When analyzed by 16S rDNA sequences, the isolates were found to be related to the genera *Burkholderia*, *Arthrobacter*, *Pseudomonas*, *Variovorax*, and *Ensifer* (Table 1). Many of the isolates were Gram-negative and belonged to the alpha, beta, and gamma subgroups of the Proteobacteria. However, nine strains were identified as *Arthrobacter* species, which were Gram-positive and belonged to the Actinobacteria. Although the isolates were isolated from different rice soils, some isolates were closely related to the same species, such as *Arthrobacter defluvii* 4C1-a and *Variovorax boronicumulans* BAM-48, which have not been reported as parathion-degrading bacteria in previous studies [23, 24]. To investigate the genomic relatedness among the closely related isolates by 16S rRNA gene sequence analysis, REP–PCR experiment was performed by PCR amplification with the BOXA1R primer [5]. The REP–PCR profiles of the 19 isolates were observed to be distinct from one another (Fig. 1), suggesting that each isolate was a different strain.

Growth Pattern and Degradation Phenotype of the Isolates on Parathion

To understand the growth patterns of the parathion-degrading isolates, each independent parathion-degrader and syntrophic pairs were grown on PTYG and inoculated into parathion minimal medium. The growth and parathion degradation curves of representative strains are shown in Fig. 2 and 3. For the syntrophic bacteria, each pair was able to degrade parathion completely, but each single strain...
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alone could not mineralize the pesticide (Fig. 2). Among the syntrophic bacteria, the strains that were able to hydrolyze parathion to p-nitrophenol were denoted by “a.” These strains could not attack p-nitrophenol. On the other hand, the syntrophic strains denoted by “b” were observed to be unable to hydrolyze parathion but degraded p-nitrophenol completely.

The isolates were classified into three groups according to their growth characteristics on parathion. Group A contained strains JNB1 and JNA3, which degraded and grew rapidly with intermittent accumulation of p-nitrophenol during degradation of parathion (Fig. 3A). Group B contained all of the 8 syntrophic pairs of bacteria, which also could degrade and grow on parathion rapidly, but these syntrophic pairs did not show any accumulation of p-nitrophenol during degradation of parathion (Fig. 3B). It took about 120 h for the Groups A and B strains to degrade 100 ppm of parathion completely. Group C contained strain JBA3, which also could degrade parathion rapidly but showed a substantial lag period before its active growth (Fig. 3C). During the lag period of JBA3 growth, most of the parathion was converted to p-nitrophenol, which was a yellow hydrolysis product of parathion. When most of the parathion residues were hydrolyzed, the accumulated p-nitrophenol began to be utilized by the isolate JBA3 with concomitant rapid cell growth (Fig. 3C). At about 70 h, p-nitrophenol had been completely utilized and cell density started to decrease.

Degradative Diversity Analysis

The isolates were grown on PTYG medium, and then examined for their ability to degrade other organophosphates structurally related to parathion. The substrate utilization abilities of the isolates are shown in Table 2. The three isolates capable of degrading parathion independently and the eight syntrophic pairs were able to degrade EPN, p-nitrophenol, fenitrothion, and methyl parathion, in addition to parathion, but none of them could degrade malathion. Among the syntrophic isolates, each bacterium denoted by b was able to degrade p-nitrophenol, but no single organism alone could degrade any of the other compounds listed in Table 2.

Plasmid Detection and Its Relationship to Parathion Degradation Phenotype.

When the isolates were subjected to the Hynes plasmid detection procedure [12], all of the isolates degrading parathion independently exhibited multiple plasmid bands ranging from two to eight in number (Fig. 4A). Among the syntrophic isolates, half of them showed one to three plasmid bands (Fig. 4B). Interestingly, the six strains CN2a, CN3a, GG6a, GG7a, GG9a, and CNB9a, which were identified as the same species Arthrobacter defluvii 4C1-a, exhibited distinct plasmid profiles (Fig. 4B). The plasmids detected in the isolates were stably maintained in cells cultivated for two months with repeated transfers into Luria–Bertani medium [13].

To investigate whether the parathion degradative genes are on the plasmid, the isolates containing plasmid were subjected to plasmid curing procedures. Using the SDS and sublethal temperature methods, we were able to obtain cured strains from three strains, CN3a, GG7a, and GG9a, among the isolates (Fig. 5). Three different types of cured strains were obtained from the strain CN3a, which contained
two plasmids (Fig. 5). The cured strain CN3a-C1, which lost the second plasmid, still could degrade parathion when combined with the syntrophic partner strain CN3b. By contrast, the cured strains CN3a-C2 and CN3a-C3 of the strain CN3a, which commonly lost the first plasmid, were not able to degrade the pesticides even when combined with strain CN3b. This result suggested that the organophosphate hydrolase gene was contained in the first plasmid of strain CN3a. In the case of strain GG7a, its cured strain GG7a-C was not able to degrade parathion completely when combined with its syntrophic partner GG7b. On the other hand, the cured strain GG9a-C was still able to degrade parathion with its syntrophic partner GG9b.

**Genetic Diversity Analysis by PCR Amplification and Dot-Blotting**

Since the three strains and the syntrophic bacteria denoted by “a” could hydrolyze other organophosphorous pesticides structurally related to parathion, we investigated whether these isolates had any sequence homology with organophosphorus hydrolase genes that were previously reported in other bacterial strains. When PCR amplification was performed with the primers targeting for the opdB gene of *Burkholderia* sp. JBA3 [15], only strain JNB1 showed positive DNA bands, but none of the other strains showed any positive DNA bands (data not shown). Moreover, PCR amplification with the primers specific for the *opd* [31, 33], *opdA* [11], and *oph* [25] genes did not produce any positive signals from all of the isolates. The absence of homology of our isolates with the previously reported parathion hydrolase genes was confirmed by dot-blotting experiments. When hybridized with the gene probe of strain *Burkholderia* sp. JBA3 [15], only strain JNB1 showed a positive signal.

### Table 2. Substrate utilization patterns by the parathion-degrading isolates.

<table>
<thead>
<tr>
<th>Isolates</th>
<th>Substrates</th>
<th>Parathion</th>
<th>EPN</th>
<th>p-Nitrophenol</th>
<th>Fenithion</th>
<th>Methylparathion</th>
<th>Malathion</th>
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<td>CN2ab</td>
<td>++</td>
<td>+</td>
<td>++</td>
<td>+</td>
<td>+</td>
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<td>+</td>
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<td>+</td>
<td>-</td>
</tr>
</tbody>
</table>

*The isolates were grown on PTYG before the test of substrate utilization.

*++, Over 95% reduction in peak height as determined by UV scanning and substantial growth (OD600>0.05); +, over 80% reduction in peak height as determined by UV scanning and substantial growth (OD600>0.025); -, below 10% reduction in peak height and scant growth (OD600<0.01).
PARATHION-Degrading Bacteria Isolated From Rice Soils

Fig. 5. Plasmid profiles of the representative isolates and their cured strains.
Lanes: 1, CN3a; 2, cured strain of CN3a (CN3a-C1); 3, cured strain of CN3a (CN3a-C2); 4, cured strain of CN3a (CN3a-C3); 5, GG7a; 6, cured strain of GG7a (GG7a-C); 7, GG9a; 8, cured strain of GG9a (GG9a-C); M, plasmid size marker.

The results suggested that the hydrolase genes involved in parathion degradation in most of our isolates might be different from those of the previously reported bacterial strains able to degrade the organophosphate pesticides.

DISCUSSION

We isolated dominant parathion-degrading bacteria from various rice paddy soils and analyzed their diversity and properties on parathion degradation by using phylogenetic, phenotypic, and genotypic analyses. Species identification by 16S rRNA gene sequence analyses revealed that our isolates were related to members of the genera Burkholderia, Arthrobacter, Pseudomonas, Variovorax, and Ensifer (Table 1). Although several bacterial strains have been previously isolated as parathion-degrading microorganisms [3, 23, 24, 31], syntrophic metabolism of parathion by multi-membered bacterial communities has not been reported in previous studies. Specifically, in rice field soils, syntrophic metabolism of parathion appeared to be dominant over independent degradation of parathion by pure culture. This seems to be because, unlike upland fields, flooding of rice fields affects soil pH, redox state, and nutrient availability, and thus forms diverse microhabitats that can support the growth of metabolically diverse microorganisms. Interestingly, Arthrobacter species were the most frequent isolates involved in syntrophic metabolism of parathion as the syntrophic member hydrolyzing parathion at the first step of its biodegradation.

The parathion-degrading isolates were classified into three groups based on their degradation and growth characteristics (Fig. 1). The strains JNA3 and JNB1 of Group A, which belonged to the genus Burkholderia, degraded parathion rapidly and showed intermittent accumulation of the intermediate metabolite p-nitrophenol during their growth on the pesticide. The Group A strains contained several different plasmid DNAs. These strains did not show any homology with the opd [31, 33], opdA [11], and oph [25] genes of the previously reported organophosphorus pesticide-degrading bacteria, but strain JNB1 showed a positive signal with the opdB [15] gene of Burkholderia sp. JBA3 of the Group C. The eight pairs of syntrophic bacteria, which belonged to the genera Arthrobacter, Pseudomonas, Variovorax, and Ensifer, constituted the Group B. These two-membered syntrophic communities also degraded parathion rapidly, but the metabolite p-nitrophenol was not accumulated at all during degradation of parathion. Half of the Group B strains contained plasmid DNAs, but none of the Group B strains exhibited homology with the organophosphate hydrolase genes previously reported, suggesting that these organisms had quite different types of organophosphorus hydrolase genes. The Group C strain JBA3, which belonged to the genus Burkholderia, degraded parathion the most rapidly among the isolates, but it showed a lag period before its vigorous growth on parathion. The strain JBA3 first hydrolyzed most of the parathion to p-nitrophenol during its lag period, and then it began to grow rapidly utilizing the intermediate p-nitrophenol as the carbon and energy source. Strain JBA3 harbored eight different plasmids, and its organophosphorus hydrolase gene (ophB) has been cloned from its fifth plasmid as a new type of hydrolase gene in our previous study [15].

The three isolates and the eight syntrophic bacteria denoted by “a” had parathion hydrolase activities, which converted parathion to p-nitrophenol, a yellow intermediate produced during degradation of parathion by the isolates. However, as shown by PCR amplification and dot-blotting experiments, most of these isolates did not exhibit any significant homology with the previously reported bacterial strains able to degrade various organophosphorus insecticides. The results suggested that our isolates possibly have new types of organophosphorus hydrolase genes. Further investigation on the new hydrolase genes would reveal the
divergence of sequences of organophosphorus hydrolase genes among the parathion-degrading bacteria.

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References


