Microbial Degradation of Quinoline and Methylquinolines

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Several bacterial cultures were isolated that are able to degrade quinoline and to transform or to degrade methylquinolines. The degradation of quinoline by strains of Pseudomonas aeruginosa QP and P. putida QP produced hydroxyquinolines, a transient pink compound, and other undetermined products. The quinoline-degrading strains of P. aeruginosa QP and P. putida QP hydroxylated a limited number of methylquinolines but could not degrade them, nor could they transform 2-methylquinoline, isoquinoline, or pyridine. Another pseudomonad, Pseudomonas sp. strain MQP, was isolated that could degrade 2-methylquinoline. P. aeruginosa QP was able to degrade or to transform quinoline and a few methylquinolines in a complex heterocyclic nitrogen-containing fraction of a shale oil. All of the quinoline- and methylquinoline-degrading strains have multiple plasmids including a common 250-kilobase plasmid. The 225-, 250-, and 320-kilobase plasmids of the P. aeruginosa QP strain all contained genes involved in quinoline metabolism.

Quinoline and its derivatives occur as contaminants in groundwater near creosote wood preservation and fossil fuel-processing facilities (17–19). Quinoline, methylquinolines, and other heterocyclic nitrogen compounds occur in shale oils (5). Several investigators have demonstrated microbial transformation or degradation of quinoline under both aerobic and anaerobic conditions (1–3, 7, 9, 13, 15, 17–20, 21, 23, 24). Most quinoline-degrading microorganisms have been identified as Pseudomonas species (3, 6, 7, 15, 20, 23), but quinoline-degrading strains of Rhodococcus (20), Nocardia (24, 26), Moraxella (1), and Desulfo bacterium (2) spp. have also been reported.

Quinoline degradation has been shown to involve hydroxylation at the position 2 (3, 13, 15, 17–19, 21–23). Shukla (24), in a study of four bacteria capable of utilizing quinoline as sole source of carbon and nitrogen, found two alternative pathways for quinoline catabolism. Both pathways formed 2-hydroxyquinoline; one pathway proceeded via 2,6-dihydroxyquinoline, whereas after 2-hydroxyquinoline formation the other pathway proceeded via 2,8-dihydroxyquinoline and 8-hydroxyxoumarin. Boyd et al. (6) also identified an alternate pathway carried out by a strain of Pseudomonas putida in which cis-dihydrodiols are formed.

In the current study, we examined the microbial utilization of various methylquinolines as well as unsubstituted quinoline. Unlike microbial degradation of quinoline, very few studies have examined the microbial metabolism of methylquinolines. Wang et al. (28) reported that methylquinoline was not biodegraded under anaerobic conditions in which quinoline and indole were attacked. Periera et al. (18, 19) reported that transformation of a limited amount of 2-methylquinoline occurred under anaerobic conditions when alternate sources of nutrients were provided, but that 2-methylquinoline was not attacked when it was the sole source of carbon; they also have reported the complete degradation of 4-methylquinoline under anaerobic conditions when nutrients and an alternate carbon source were provided, but when 4-methylquinoline was the only source of carbon, it was only partially degraded, with 95% being transformed to 4-methyl-2(1H)-quinoline.

MATERIALS AND METHODS

Enrichment cultures for quinoline and methylquinoline degraders. Enrichment cultures capable of transforming or degrading quinoline and methylquinolines were established in phosphate salts medium (23) containing either 1.2 mM quinoline (Aldrich Chemical Co., Inc.) or 1.2 mM 2-methylquinoline (Aldrich) as the sole source of carbon and 0.1% ammonium sulfate as the sole source of nitrogen; the 2-methylquinoline was emulsified by mixing 1.5 ml of 2-methylquinoline with 1 ml of Tween 80 (Fisher Scientific Co.) and 7.5 ml of distilled water and sonicating for 10 min. Oil- and creosote-contaminated soils were used as the sources of inocula. All enrichment cultures were incubated at 28°C on a rotary shaker at 200 rpm and maintained by regular transfer to fresh media. Degradation of quinoline and methylquinolines was monitored by measuring UV absorbance and by gas chromatographic (GC) analyses as described below.

Biodegradation of quinoline by pure bacterial cultures. Pure bacterial strains were isolated from quinoline enrichment cultures by plating onto 0.1% strength Trypticase soy broths (BBL Microbiology Systems) solidified with purified agar (Difco Laboratories). The isolated strains were identified by using Rapid NFT Strips (Analytab Products) and supplemental tests. These strains were tested for their abilities to use quinoline as a source of carbon and nitrogen by inoculating phosphate salts medium (23), containing 2.5 mM quinoline as the sole source of carbon and nitrogen, with approximately 10⁷ cells of a pure culture grown on quinoline medium. Cultures were incubated at 28°C with shaking at 200 rpm. Periodically, samples collected from the cultures were analyzed for quinoline and quinoline degradation products by UV absorbance and gas-liquid chromatography as described below. Sterile controls were also analyzed at each time interval.

One of the isolated strains was also tested for growth on a range of quinoline concentrations, using nitrogen-free phosphate salts medium amended with 2.5 to 40 mM quinoline. A 2% inoculum of an 18-h culture grown on 2.5 mM quinoline was used for these tests. After 7 days of incubation at 28°C...
with shaking at 200 rpm, quinoline degradation and biodegradation product formation were measured as described below.

Utilization of methylated quinolines and other compounds. Two quinoline-utilizing bacterial strains, one from creosote-contaminated soil and the other from oil-contaminated soil, were inoculated into replicate flasks of phosphate salts medium, each containing one of the following compounds at a concentration of 1.2 mM: 2-methylquinoline, 4-methylquinoline, 6-methylquinoline, 7-methylquinoline, 8-methylquinoline, 2,6-dimethylquinoline, indole, or isoquinoline; or pyridine at a concentration of 7.5 mM (Aldrich). To test the abilities of these bacterial strains to utilize each of these compounds as sole source of carbon, the medium was supplemented with 0.1% ammonium sulfate; ammonium sulfate was omitted from the medium to determine the abilities of these strains to utilize each of these compounds as sole source of carbon and nitrogen. Two percent inocula from 18-h cultures were used. Cultures were incubated for 7 days at 28°C.

Samples from the cultures, as well as from sterile controls, were collected and analyzed for growth by determining the protein concentration by the method of Lowry et al. (12). Samples were further analyzed by using scanning UV spectroscopy to detect parent aromatic compounds and biotransformation products. Also, cultures were extracted with methylene chloride and the extracts were analyzed by GC and GC-mass spectrometry (GC-MS) as described below.

A 2-methylquinoline-utilizing bacterial strain, *Pseudomonas* sp. strain MPQ, isolated from a 2-methylquinoline creosote-contaminated soil enrichment culture was inoculated into replicate flasks of phosphate salts medium containing 2.5 mM 2-methylquinoline as the sole source of carbon and nitrogen. The inoculum was approximately 10⁷ cells of a pure culture grown on 2-methylquinoline medium. The cultures were incubated at 28°C with shaking at 200 rpm. Periodically, samples were collected and analyzed for 2-methylquinoline and 2-methylquinoline degradation products by gas-liquid chromatography as described below. Sterile controls were also analyzed at each time interval.

Utilization of quinolines in a shale oil mixture. To test its ability to degrade quinoline and other heterocyclic nitrogen compounds in a complex shale oil mixture, the quinoline-degrading strain of *P. aeruginosa* QP, grown on quinoline to early log phase, was inoculated into phosphate salts broth containing either 1% raw shale oil or 0.2% heterocyclic nitrogen compounds extracted from the middle cut of Stuart shale oil as the sole source of nitrogen and 0.1% succinate as an alternate carbon source. Cultures were incubated for 7 days at 28°C with shaking at 200 rpm, and the residual heterocyclic nitrogen compounds were then extracted with methylene chloride at pH 7. Prior to extraction, 7.8-benzoquinoline was added as an internal standard. Shale oil and the extracted heterocyclic fraction were also incubated under sterile conditions as controls to detect any abiotic changes and to serve as a reference for determining the extent of biodegradation by *P. aeruginosa* QP.

Analytical methods. To detect degradation of quinoline, methylquinolines, and other heterocyclic nitrogen compounds, broth samples from the cultures, diluted in distilled water, were scanned from 200 to 400 nm by using a Perkin-Elmer Lambda 3 spectrophotometer to detect qualitative changes in the absorption spectra when compared with uninoculated controls. Specific absorbances of individual compounds were examined to determine the disappearance of the parent compound or appearance of biodegradation products or both. Absorbances of the substrates tested and of available hydroxylated quinolines (Aldrich) were measured as reference standards.

GC and GC-MS analyses were also performed to quantitate loss of quinolines and to quantify and identify biotransformation products. For these analyses, samples were extracted two times with 50-ml aliquots of methylene chloride. The samples were concentrated under vacuum, using a rotary evaporator. GC analyses were performed by using an SPB-5 coated fused silica column (30 m by 0.25 mm; Supelco) and a Hewlett-Packard model 5840 GC with a flame ionization detector operated at the following temperatures: injection port, 240°C; detector, 320°C; column temperature, 50°C isothermal for 5 min and then 5°C/min to 230°C. GC-MS analyses were performed by using the same GC operational parameters with a Hewlett-Packard model 5992 GC-MS. Results obtained with extracts from active cultures were compared with sterile controls. Standard hydroxylquinolines (Aldrich) were also analyzed as reference compounds.

Extracts of the shale oil and the heterocyclic nitrogen fraction recovered from the *P. aeruginosa* QP exposed and the reference sterile controls were concentrated to 1 ml under nitrogen and analyzed by capillary GC, using an SPB-5 coated fused silica column (30 m by 0.25 mm) and a Hewlett-Packard 5840 GC with a nitrogen-specific detector. The operational parameters were as follows: injection port, 240°C; detector, 320°C; column temperature, 50°C isothermal for 5 min, 5°C/min to 230°C, and isothermal at 250°C for 20 min; helium as carrier and makeup gas. GC-MS analyses were performed using the operational GC parameters with a Hewlett-Packard model 5992 GC-MS. Kjeldahl nitrogen analyses were performed on the extracted oils by Galbraith Laboratories, Knoxville, Tenn.

Plasmid DNA analyses. Plasmid DNA was isolated from quinoline-degrading bacterial strains by using a modification of the procedure of Casse et al. (8) (J. Foght, personal communication). Cells from 10 ml of 12-h cultures were centrifuged at 12,000 × g for 10 min at 4°C. The cells were washed once in TE buffer (50 mM Tris hydrochloride, 20 mM EDTA, pH 8.0), and approximately 80 to 100 mg of the wet cell pellet was suspended in 1.0 ml of TE buffer. A 9-ml portion of lysis solution (4% sodium dodecyl sulfate [SDS] in TE, pH 12.4) was added, and the mixture was mixed gently by inversion. To complete the lysis, the cells in the lysis solution were incubated at 55°C for 10 min and the solution was then neutralized by the addition of 2 M Tris hydrochloride, pH 7.0 (pH of the final solution, approximately 8.0). To minimize shearing forces, the samples were mixed gently by inverting the tube; when the viscosity of the solution began to decrease, the mixing was completed with 1-s pulses, using a Vortex mixer at minimal speed. The resulting solution was homogeneous and relatively nonviscous.

Solid NaCl was added to achieve a final concentration of 3% to precipitate the chromosomal DNA. The salt was dissolved by slow inversion of the tube containing the sample and incubated at 22°C for 30 min. The sample was purified twice, using phenol saturated with TE buffer. The upper phase was transferred into a fresh tube, and 0.11 volume of 3 M sodium acetate (pH 8.0) and 2 volumes of 95% ethanol were added. The sample was kept at −20°C for 12 h, after which the DNA pellet was collected by centrifugation at 12,000 × g for 10 min at −14°C; the pellet was dried under vacuum. The dried DNA pellet was suspended in 200 ml of TE buffer, and 20 to 40 ml of this solution was used for gel electrophoresis.
Also, a modified procedure of Eckhardt’s rapid identification of large plasmids (11) was used to determine the number and molecular weights of the plasmids from the quinoline-degrading pseudomonads. A 100-ml amount of a 1-ml overnight culture was centrifuged in a microcentrifuge (Eppendorf) for 2 min, and the supernatant was removed by aspiration. The cell pellet was suspended in 1 ml of 0.1% (wt/vol) sodium lauryl sarcosinate (Sarkosyl) in TE buffer. The cell suspension was centrifuged for 2 min, the supernatant was discarded, and the cells were washed once in TE buffer. A 40-ml portion of lysozyme mixture containing 20% (wt/vol) Ficoll in borate buffer (89 mM boric acid, 89 mM Tris base, 8.9 mM disodium EDTA, pH 8.3), 50 mg of DNase-free RNase per ml, 0.05% (wt/vol) bromophenol blue, and 1 mg of lysozyme per ml were added to the cells and mixed quickly with a micropipette tip, and the sample was loaded immediately into the well of a 0.8% (wt/vol) agarose gel. After 10 min, 40 to 50 ml of SDS mixture (2% [wt/vol] SDS in 10% [wt/vol] Ficoll dissolved in borate buffer) was carefully overlaid onto the sample. The two layers in the gel were not mixed. The wells of the gel were capped with 0.9% agarose. All gels were run in borate buffer at 35°C, initially at 35 V (approximately 5 mA) for 40 to 50 min and then at 120 V (approximately 30 mA) until the blue tracking dye moved two-thirds of the gel. The gel was stained with 3 × 10−4% (vol/vol) ethidium bromide solution and photographed. The sizes of the plasmids of quinoline-degrading pseudomonads were determined by using plasmids of Rhizobium leguminosarum 3718 (10) as size standards.

To determine which plasmids were associated with quinoline degradation, the quinoline-degrading strain from the oil-contaminated soil was cured of one or more of its plasmids by treatment with SDS by using the approach of Tomoeda et al. (27). In this procedure, approximately 2 × 108 cells of the quinoline-degrading strain P. aeruginosa QP were transferred into 100 ml of Trypticase soy broth containing 0.03 to 0.06% SDS and the cultures were grown at 30°C for 24 h while shaking, after which they were transferred to fresh SDS-containing Trypticase soy broth. After three to five transfers, aliquots were plated onto 10% strength Trypticase soy agar. After incubation at 30°C for 24 h, 300 colonies were selected at random and subjected to DNA analysis, using Eckhardt’s rapid plasmid preparation procedure. The presence and sizes of the plasmids in these strains were determined by extracting the plasmid DNA and analyzing by gel electrophoresis as described above. Colonies that showed different patterns of plasmid loss (plasmid curing) were examined to determine whether they could metabolize quinoline. Quinoline degradation and transformation capability by these strains were determined by inoculating minimal phosphate broth containing 1.2 mM quinoline, incubating for 24 to 48 h at 30°C, extracting the broth with methylene chloride, and analyzing the extract by using gas-liquid chromatography for the disappearance of quinoline and appearance of quinoline degradation products as described earlier.

RESULTS AND DISCUSSION

Biodegradation of quinoline. Growth, accompanied by pigment production, was readily observed in enrichment cultures when quinoline was supplied as the sole source of carbon and nitrogen. Many different-colored pigments were detected, from apricot-pink through green-dark brown. Two enrichment cultures were selected for further study, one from an oil-contaminated soil and the other from a creosote-contaminated soil. Based on UV absorbance measurements, quinoline was completely degraded within 24 h by the mixed bacterial populations in these cultures. No degradation was observed in sterile controls.

No problem was encountered in isolating a pure culture of a quinoline degrader from creosote-contaminated soil enrichment cultures, but isolation of a pure bacterial culture capable of degrading quinoline from the enrichment cultures derived from oil-contaminated soil proved to be difficult. A similar problem was experienced by Grant and El-Najjar (13), who reported that the quinoline-degrading microbe they isolated rapidly lost viability at the end of the logarithmic phase of growth and underwent gradual lysis over a period of several days.

The bacterium isolated from the oil-contaminated soil culture was identified as a strain of P. aeruginosa, QP; it produces a fluorescent green pigment and is able to grow at 42°C. The quinoline-degrading isolate from the creosote-contaminated soil culture was identified as a strain of P. putida, QP; it produces an orange-brown pigment and does not grow at 42°C. Quinoline degradation by these organisms occurred only under aerobic conditions, and no quinoline degradation by these organisms was detected under denitrifying conditions.

When phosphate salts medium containing 2.5 mM quinoline was inoculated with P. aeruginosa QP, quinoline degradation was detected within a few hours and 2-hydroxyquinoline was recovered from the medium by extraction with methylene chloride (Fig. 1). No quinoline degradation or production of products was observed in sterile controls. The 2-hydroxyquinoline was identified by GC and GC-MS analyses. Based on A232 measurements, 2-hydroxyquinoline first appeared after 9 h of growth, reached a maximal concentra-
P. putida QP also degraded quinoline with a rapid disappearance of quinoline within a few hours of inoculation, with 2-hydroxyquinoline and an unidentified nonaromatic intermediate accumulating between 3 and 21 h (Fig. 2). The unidentified compound gave a single major peak in mass spectral analysis with m/e = 43; nuclear magnetic resonance analyses did not yield additional information that could identify this compound. Complete disappearance of quinoline occurred by 12 h, and the hydroxylated quinoline and unidentified compound subsequently disappeared by 21 h (Fig. 2).

Biodegradation of methylquinolines and other heterocyclic nitrogen compounds. Neither P. aeruginosa QP nor P. putida QP was able to grow on any of the methylquinolines tested as sole sources of carbon and energy as evidenced by a lack of turbidity and lack of increase in protein concentration when the methylquinolines were supplied as the sole sources of carbon or of carbon and nitrogen. However, both of these strains were able to hydroxylate some methylquinolines. P. aeruginosa QP was able to hydroxylate 6-methylquinoline, 7-methylquinoline, and 8-methylquinoline, but not 2-methylquinoline, 4-methylquinoline, or 2,6-dimethylquinoline. P. putida QP was able to hydroxylate only 8-methylquinoline. Emulsification of methylquinolines had no effect on the ability of these pseudomonads to transform these compounds. No hydroxylation of methylquinolines was observed in sterile controls.

GC-MS analyses identified monohydroxymethylquinolines as the transformation products, but due to the lack of authentic standards we were unable to identify the specific sites of hydroxylation. GC-MS analyses showed no evidence of dihydroxymethylquinoline formation from any of the methylquinoline substrates. The lack of further degradation of methylquinolines, especially 2-methylquinoline, is not surprising because, as described above, quinoline degradation by these strains involves hydroxylation at the number 2 position so that methylation at that position would likely block the formation of the hydroxyquinoline intermediates in this quinoline degradation pathway.

Although neither P. aeruginosa QP nor P. putida QP could attack 2-methylquinoline, we were able to establish additional enrichment cultures from a site known to be contaminated with creosote and subsequently to isolate Pseudomonas sp. strain MPQ, which is capable of using 2-methylquinoline as the sole source of carbon. This strain also could degrade quinoline. The degradation of 2-methylquinoline by this culture proceeds more slowly than was.
Microbial degradation of quinoline and methylquinolines

FIG. 4. Biodegradation of quinolines in the heterocyclic nitrogen fraction of a shale oil by P. aeruginosa QP. (A) Capillary GC analysis of Stuart raw shale oil after 7 days of incubation under sterile conditions. Peaks analyzed by GC-MS included in this fraction and their retention times (in minutes) given in parentheses are as follows: A, quinoline (12.88); B, isoquinoline (13.54); C, 2-methylquinoline (15.01); D, 8-methylquinoline (15.29); E, 1-methylisoquinoline (15.96); F, 6-methylquinoline/7-methylquinoline (which coelute) (16.32); G, unidentified (16.50); H, unidentified (16.92); I, 4-methylquinoline (17.09); J, unidentified (17.41); K, 2,6-dimethylquinoline (18.27); L, unidentified (18.47); BQ, 7,8-benzoquinoline internal standard (27.88). (B) The same fraction after 7 days of incubation with the quinoline-degrading strain of P. aeruginosa QP. Analyses were performed with a nitrogen-specific detector. The peaks removed due to biodegradation were as follows: A, quinoline; D, 8-methylquinoline; F, 6-methylquinoline/7-methylquinoline (which coelute); G, unidentified; H, unidentified; BQ, 7,8-benzoquinoline internal standard.

found for the quinoline-degrading pseudomonads (Fig. 3), with degradation occurring over days rather than hours. Significant degradation with detectable products did not occur until after 3 days; by 7 days, the quinoline and the hydroxylated and other intermediary products disappeared. As determined by GC-MS analyses, this organism produced hydroxylated methylquinolines and other unidentified intermediary metabolites from 2-methylquinoline, including the same unidentified nonaromatic compound produced by P. putida QP growing on quinoline.

Even though quinoline and isoquinoline have very similar chemical structures, neither P. aeruginosa QP, P. putida QP, nor Pseudomonas sp. strain MPQ was able to degrade isoquinoline when quinoline-grown cells were provided with isoquinoline as the sole source of carbon and nitrogen, sole source of carbon, or sole source of nitrogen or when succinate and ammonium sulfate were supplied as alternate sources of carbon and nitrogen. Similarly, these Pseudomonas strains could not degrade the heterocyclic nitrogen compound pyridine or indole. Shukla (23) and Brockman et al. (7) also found that their quinoline-degrading strains could not attack pyridine.
Utilization of quinolines in a shale oil mixture. *P. aeruginosa* QP was able to degrade five compounds from the heterocyclic nitrogen fraction of the middle cut of the shale oil (Fig. 4). This organism degraded the same compounds when raw shale oil was supplied as the source of heterocyclic nitrogen compounds and when the extracted heterocyclic fraction was used as the substrate. The compounds that were biodegraded were quinoline, 6-methyl/7-methylquinoline, 8-methylquinoline, and two unidentified compounds. *P. aeruginosa* QP did not remove isoquinoline, 2-methylquinoline, 1-methylisoquinoline, 4-methylquinoline, or 2,6-dimethylquinoline from the shale oil. There was an 80 to 90% reduction in the concentrations of unsubstituted quinoline, 6-methyl/7-methylquinoline, and 8-methylquinoline in the shale oil after exposure to *P. aeruginosa* QP. The concentrations of isoquinoline, methylisoquinolines, and 2-methylquinoline were the same in the heterocyclic nitrogen fractions recovered from the sterile controls and the *P. aeruginosa* QP-exposed cultures, indicating no biodegradation of these compounds by *P. aeruginosa* QP. Also, no degradation of aliphatic hydrocarbons, aromatic hydrocarbons, or aliphatic nitriles was detected when shale oil was provided as the nitrogen source for *P. aeruginosa* QP.

Although *P. aeruginosa* QP selectively removed heterocyclic nitrogen compounds, it did not significantly reduce the nitrogen content of the oil. The total nitrogen (percent Kjeldahl-N) content of residual raw shale oil that had been incubated for 7 days with *P. aeruginosa* QP was 0.75 ± 0.07% compared with 0.88 ± 0.11% for the untreated oil. The failure to reduce the total nitrogen content is not surprising as the quinolines that were degraded represent only a small portion of the total nitrogen-containing compounds in this oil: the oil contains aliphatic nitriles and amines as well as heterocyclic nitrogen compounds. The ability of the *P. aeruginosa* QP to degrade or to transform quinolines has potential biotechnological applications for the upgrading of shale oils as well as for the cleanup of complex mixtures of environmental pollutants.

**Plasmids involved in quinoline and methylquinoline degradation.** *P. aeruginosa* QP contained four plasmids of 250, 320, 225, and 180 kilobases (kb) (Fig. 5). *P. putida* QP and *Pseudomonas* sp. strain MPQ also contained a 250-kb plasmid. *P. putida* QP had two additional plasmids of 123 and 90 kb. *Pseudomonas* sp. strain MPQ had one additional plasmid of 225 kb. Brockman et al. (7) also found multiple plasmids of 50, 100, 320, and 440 kb in a quinoline degrader. They found that the 320-kb plasmid, which is the same size as one of the plasmids found in *P. aeruginosa* QP, as well as the 50-kb plasmid are involved in quinoline degradation (Hicks et al., Abstr. Annu. Meet. Am. Soc. Microbiol. 1988).

Curbing *P. aeruginosa* QP of all four plasmids to produce *P. aeruginosa* Q resulted in the complete loss of the ability to transform quinoline (Table 1). On the other hand, curbing *P. aeruginosa* QP of only the 180-kb plasmid had no effect on the ability of the strain formed (*P. aeruginosa* QP-21) to degrade quinoline or on the pattern of product formation, indicating that it is not involved in quinoline metabolism (Table 1). The 225-, 250-, and 320-kb plasmids, on the other hand, all appear to be involved with quinoline metabolism. Strains containing both the 225- and 250-kb plasmids (*P. aeruginosa* QP-6, QP-21, and Q) completely degraded quinoline and did not accumulate significant quantities of hydroxylated quinoline metabolites. In contrast, strains with the 320-kb plasmid alone (*P. aeruginosa* QP-30) or in combination with either only the 225 (*P. aeruginosa* QP-15) or only the 250 (*P. aeruginosa* QP-16) kb plasmid transformed quinoline with the accumulation of significant amounts of hydroxyquinoline metabolites (Table 1). Thus, the combined activities of genes contained on the 225- and 250-kb plasmids appear to be involved in the degradation of hydroxyquinolines by these quinoline-degrading *P. aeruginosa* strains. Interestingly, *Pseudomonas* sp. strain MPQ, which

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**TABLE 1. Quinoline and 2-methylquinoline degradation activities of strains of *Pseudomonas* spp. containing various plasmids**

<table>
<thead>
<tr>
<th>Strain</th>
<th>Plasmid(s) present (kb)</th>
<th>Degradation of quinoline</th>
<th>Accumulation of hydroxylated quinoline</th>
<th>Degradation of 2-methylquinoline</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>P. aeruginosa</em> QP</td>
<td>180, 225, 250, 320</td>
<td>+</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td><em>P. aeruginosa</em> Q</td>
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<td></td>
<td></td>
<td></td>
</tr>
<tr>
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<td>225, 250</td>
<td>+</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td><em>P. aeruginosa</em> QP-30</td>
<td>320</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
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<td>250, 320</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td><em>P. aeruginosa</em> QP-15</td>
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<td></td>
<td></td>
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<tr>
<td><em>P. aeruginosa</em> QP-21</td>
<td>225, 250, 320</td>
<td>-</td>
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</tr>
<tr>
<td><em>P. putida</em> QP</td>
<td>90, 123, 250</td>
<td>+</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td><em>Pseudomonas</em> sp. strain MPQ</td>
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<td>+</td>
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</tbody>
</table>

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**FIG. 5. Visualization of plasmids in an agarose gel (1%).** Lane 1, Plasmids isolated from the quinoline-degrading *P. aeruginosa* QP; four DNA bands are visible corresponding to plasmids with estimated sizes of 180, 225, 250, and 320 kb. Lane 2, Plasmids isolated from *R. leguminosarum* 3718 which were used as size standards; the approximate sizes of these plasmids are 235, 242, 250, 288, 295 (total, 2), and 330 kb (10).
has the novel 2-methylquinoline-degrading capacity, contained both a 225- and a 250-kb plasmid.

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LITERATURE CITED