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Biodegradation of the Phenoxy Herbicide MCPA by Microbial Consortia Isolated from a Rice Field

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The phenoxy herbicide 4-methyl-2-chlorophenoxyacetic acid (MCPA) is widely used as a broad leaf weed controller (Loos 1975; Que Hee and Sutherland 1981). MCPA is readily susceptible to biodegradation and MCPA-degrading bacteria are relatively ubiquitous in soils with histories of previous applications. The pathway of MCPA biodegradation has been elucidated in several bacteria, including *Pseudomonas* spp. (Gaunt and Evans 1971; Kilpi et al. 1980). Some species of *Alcaligenes* (Pieper et al. 1988) and *Flavobacterium* (Horvath et al. 1990) can also degrade MCPA as the sole source of carbon and energy. Enzymes involved in the degradative pathway of MCPA are commonly plasmid borne in *Pseudomonas* spp. (Fisher et al. 1978). MCPA degradation in soils has also been elucidated in previous studies (Soderquist and Crosby 1975; Smith and Hayden 1981; Smith and LaFond 1990).

In the present work, MCPA-degrading bacteria were derived from soil samples collected from a rice field. The enrichments were maintained as mixed cultures and were examined for their ability to degrade MCPA under various experimental conditions.

MATERIALS AND METHODS

The microbial consortia, designated as MA1 and MA2, were originally enriched from surface soil samples from a rice field near the Ye-dang reservoir, Chung-Nam Province, South Korea. The consortia were maintained in liquid medium which contained mineral salts (Oh and Tuovinen 1990) supplemented with up to 1,000 mg MCPA/liter. The pH of medium was adjusted to pH 6.5 with NaOH before autoclaving (121°C, 15 min). The enrichment cultures were grown at 22°C in shake flasks at 156 rpm. Growth was monitored by changes in optical density at 550 nm.

MCPA was analyzed by reverse-phase HPLC (equipped with a UV-detector set at 229 nm) and GC-MS. For HPLC analyses, a Lichrosorb ODS column (250 mm x 4.6 mm, particle size 10 μm) was eluted with a mobile phase which contained 40% (vol/vol) acetonitrile and 60%

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(vol/vol) phosphate buffer (6 g K_2HPO_4 and 3 ml concentrated H_3PO_4 per liter). The flow rate of the mobile phase was 1.5 ml/min. For GC-MS analyses, a centrifuged culture sample (8,000 x g, 20 min) was acidified to pH 3 with 6 M HCl, followed by extraction twice with equal volume of ethyl acetate. The solvent was removed under vacuum and the residue was redissolved in dichloromethane. MS data were obtained with a Hewlett-Packard 5970 mass selective detector equipped with a Hewlett-Packard gas chromatograph. A DB-1 capillary column (30 m by 0.25 mm) was used, and programmed from 80°C to 270°C at 10°C/min. The injector temperature was 250°C. The carrier gas was helium at 1.0 ml/min.

UV-spectrometry was also used to monitor the biodegradation of MCPA. Cultures were centrifuged at 6,000 x g for 15 min (4°C) followed by dilution of the supernatants with distilled water. The absorption spectra of centrifuged culture media were recorded from 310 nm to 230 nm with a Jasco spectrophotometer. The analytical methodology has been previously described in detail (Oh and Tuovinen 1991).

Technical and analytical grade MCPA, 2,4-D (2,4-dichlorophenoxyacetic acid) and 2,4-DP (2,4-dichlorophenoxypropionic acid) were obtained from Sigma Chemical Co. (St. Louis, MO); analytical grade CMP (4-chloro-2-methylphenol) and 2,4-DCP (2,4-dichlorophenol) from Aldrich Chemical Co. (Milwaukee, WI); HPLC-grade acetonitrile and water from Sigma Chemical Co.

RESULTS AND DISCUSSION

The degradation of MCPA was studied with two mixed cultures (designated as MA1 and MA2) which were initially enriched with this herbicide. The consortia previously grown with MCPA completely degraded this substrate. Changes in turbidity and protein concentration associated with the biodegradation of MCPA are shown in Figure 1 for the culture MA1. Complete depletion of MCPA was achieved in this experiment within 12 days. The turbidity of consortium MA1 was in good agreement with the pH change during the time course. The degradation of MCPA is an acid-yielding reaction due to the dechlorination step in the degradative pathway. The initial pH 6.5 decreased to pH 4.0 in the culture which was amended with 500 mg MCPA/liter. The consortium also grew at pH 5.5 and 7.5, but MCPA was only partially degraded. MCPA degradation was not observed above pH 8.5 in this study.

Culture MA1, which was initially enriched with MCPA, was transferred into culture media which contained either 2,4-D or 2,4-DP as the sole substrate. As shown in Figure 2, the culture was capable of utilizing 2,4-D as the substrate. The culture did not degrade 2,4-DP.

Growth of culture MA1 was also tested in media which were supplemented with 250, 500, 750 and 1,000 mg MCPA/liter as the sole

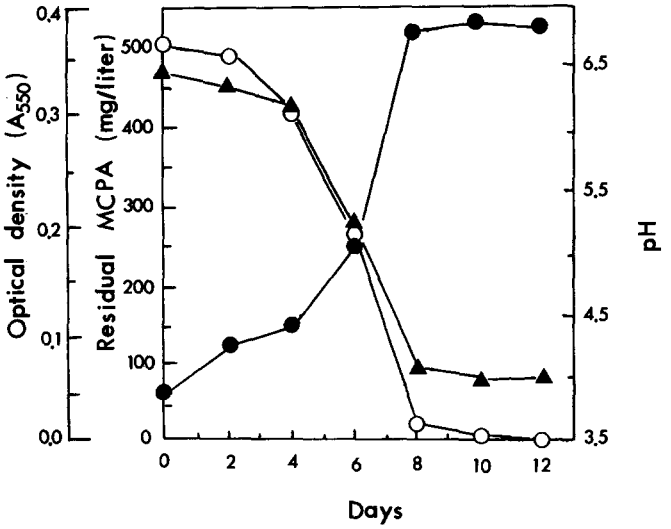

Figure 1. Degradation of MCPA (○) by culture MA1 and the associated changes in optical density (●) and pH (▲).

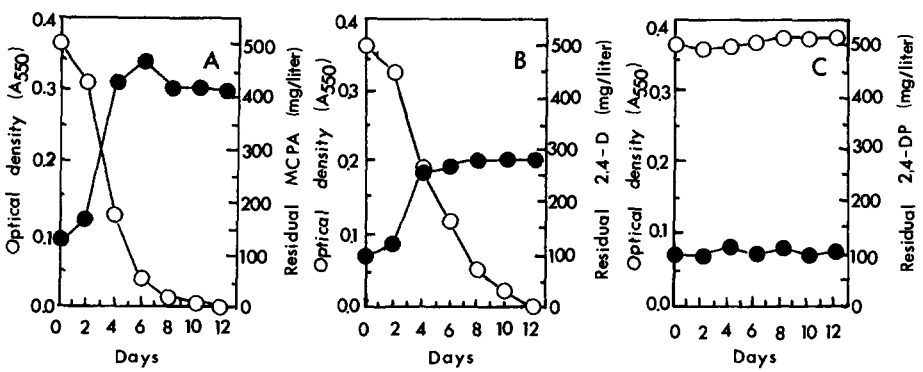

Figure 2. Time course of substrate degradation (○) and growth (●) of culture MA1 in media which contained MCPA (A), 2,4-D (B), or 2,4-DP (C) as the sole C source. The inoculum was previously grown with MCPA.

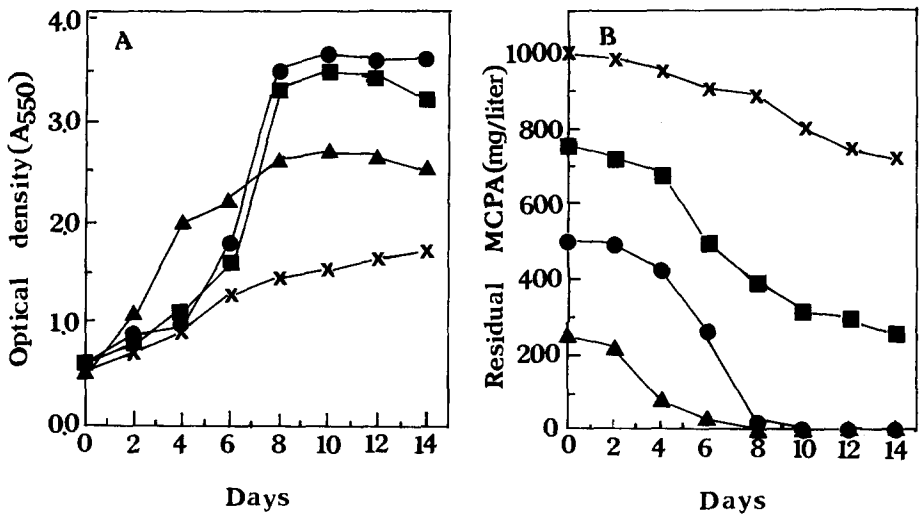

Figure 3. Time course of (A) growth and (B) degradation of MCPA by culture MA1. The media contained 250(▲), 500(●), 750(■), or 1,000 (X) mg MCPA/liter.

substrate. The degradation was slow and did not appear to proceed to completion with high MCPA concentrations (Figure 3).

Figure 4 shows the effect of yeast extract and glucose on MCPA degradation by culture MA1. Yeast extract (20 mg/liter) slightly enhanced the degradation of MCPA, whereas glucose (20 mg/liter) was inhibitory to the herbicide degradation although growth was not inhibited. Since this was a mixed culture, the growth with glucose may be attributed to the presence of bacteria which can utilize central metabolites but not the parent herbicide.

CMP was detected as an intermediate of MCPA degradation (Figure 5). This metabolite was successfully resolved from parent MCPA by the reverse-phase HPLC conditions employed in this study.

GC-MS data are shown in Figure 6 for a culture supernatant analyzed after 6 days of incubation. The total ion chromatogram (TIC) of this sample displayed two peaks with retention times 6.38 min for CMP and 12.12 min for MCPA. The fragmentation pattern of the peak and molecular ion for CMP (m/z=142) and MCPA (m/z=200) were consistent with those of the authentic standards.

UV spectrometry of the authentic MCPA showed a maximum peak of absorption at 278 nm. Peak shifts were not detected during the incubation. CMP could not be resolved in the spectra because its A_{max} (280 nm) is too close to the A_{max} (278 nm) of MCPA.

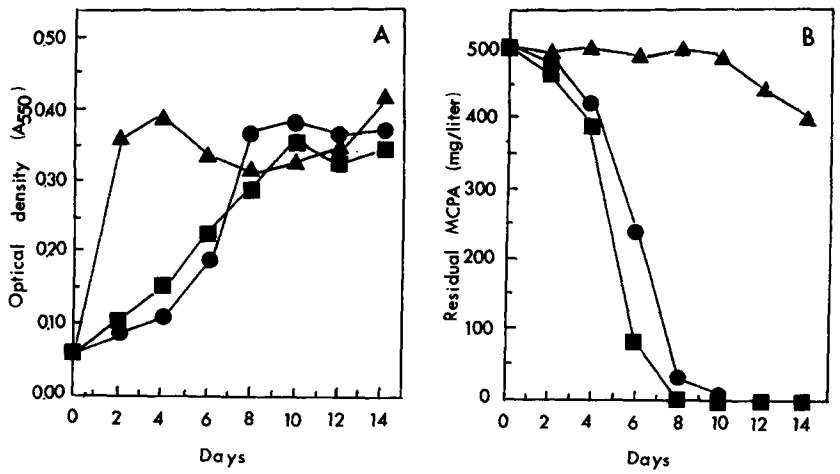

Figure 4. Time course of (A) growth and (B) degradation of MCPA by culture MA1. The media were supplemented with 20 mg yeast extract/liter (■), 20 mg glucose/liter (▲), or no additional nutrients (●)

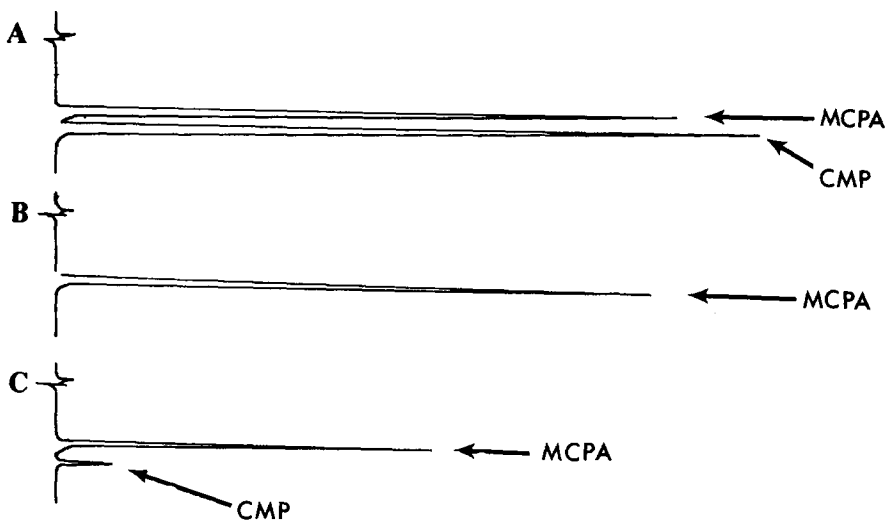

Figure 5. HPLC chromatograms of authentic standards of MCPA and CMP (4-chloro-2-methylphenol) (A), and supernatants of culture MA2 initially (B) and after 6 days of incubation (C). The retention times were 5.70 min for MCPA and 6.86 min for CMP.

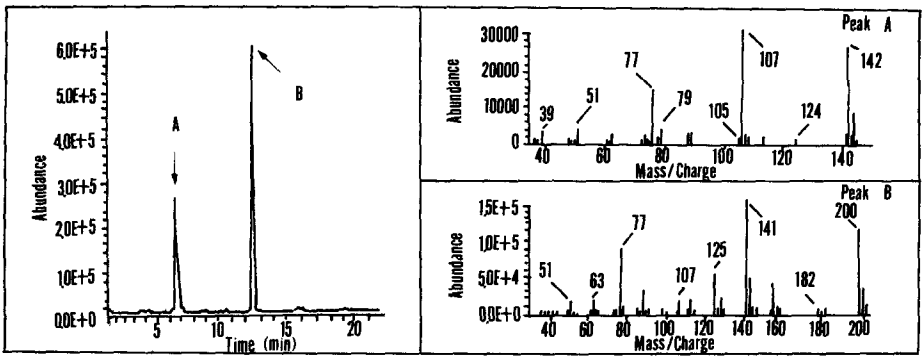

Figure 6. GC/MS data for a supernatant of culture MA2 after 6 days of incubation. MS fragmentation patterns of the compounds representing the two peaks (A and B) in the TIC are indicated.

Eight isolates from the microbial consortia used in this study were initially obtained as small colonies on plates containing solid media and MCPA. Microscopic examination of the MCPA-grown isolates revealed that all were Gram-negative and rod-shaped cells. Rapid API NFT tests of grown on trypticase soy agar isolates indicated that the bacteria could be assigned to *Pseudomonas, Flavobacterium*, and *Arthrobacter* spp. These pure cultures will be a valuable tool in characterizing the pathway and regulation of MCPA degradation. The mixed cultures will be relevant in environmental remediation situations where proper selection pressure and aseptic conditions cannot be maintained.

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