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Glyphosate degradation by *Agrobacterium radiobacter* isolated from activated sludge

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SUMMARY

Two species of bacteria capable of growth on *N*-phosphonomethylglycine (glyphosate) were isolated from a bench scale sequencing batch reactor degrading a waste stream containing glyphosate. The enrichment and isolation medium contained defined salts and glyphosate as the sole carbon and energy source. Glyphosate was stoichiometrically degraded to aminomethylphosphonic acid (AMPA). The bacteria have been identified as *Agrobacterium radiobacter* and *Achromobacter* Group V D.

INTRODUCTION

The biodegradation of glyphosate in the environment has been known for a decade [10]. Yet, it has only been in recent years that microorganisms with glyphosate degrading activity (GDA) have been isolated and characterized. Studies of these microbes have found glyphosate mineralization with either aminomethylphosphonic acid (AMPA) or sarcosine as intermediates. In the AMPA pathway, glyphosate can be metabolized in the presence or absence of inorganic phosphate. For example, a *Flavobacterium* sp. GD1, initially degraded glyphosate to AMPA and in the absence of inorganic phosphate mineralized AMPA to PO_4^{3-} [1]. Two bacteria, a *Pseudomonas* species strain PG2982 [8,11] and an *Arthrobacter* sp. GLP-1 [9] cleave the carbon-phosphorus bond as the first step of glyphosate degradation utilizing the sarcosine pathway. In addition, an *Agrobacterium radiobacter* cleaved the carbon-phosphorus bond of glyphosate and other C-P compounds [15]. In these three isolates, GDA was inhibited by inorganic phosphate [7,9,15]. Finally, a *Pseudomonas* sp. strain $LB4_1$ uses both the AMPA and sarcosine pathways. This bacteria could remove 20 mM glyphosate from growth medium converting it to AMPA. The sarcosine pathway served as a source of phosphate during phosphate deprivation [5].

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The isolation of microorganisms which utilize glyphosate as a sole carbon source has not been reported. Many investigators have assumed that the dominant process in glyphosate degradation under conditions where phosphate is available is co-metabolism, because microorganisms which utilize glyphosate as a sole carbon and energy source have not been isolated [2]. The purpose of this study was to isolate from a bench scale sequencing batch reactor (SBR), the bacteria responsible for the degradation of glyphosate to AMPA in the presence of inorganic phosphate. The bacteria were tested for the ability to utilize glyphosate as a sole source of carbon in the presence of phosphate.

MATERIALS AND METHODS

Media and culture conditions. Enrichment culture broth used to isolate bacteria from a sequencing batch reactor [4] consisted of basal salts medium: K_2HPO_4, 5.8 g/l; KH_2PO_4, 4.5 g/l; $(NH_4)_2SO_4$, 2 g/l; $MgCl_2 \cdot 2H_2O$, 0.16 g/l; $CaCl_2$, 20 mg/l; $NaMoO_4$, 2 mg/l; $FeSO_4 \cdot 7H_2O$, 1 mg/l; $MnCl_2$, 1 mg/l; and glyphosate (analytical grade: 99.9% by assay; courtesy of Monsanto Co., St. Louis, MO) as the sole carbon source (0.1% (w/v)). Each flask was inoculated with a 1/10 dilution of settled sludge and shaken at 28°C for 30 days. Ten ml of the original enrichments were subcultured into Stanier's medium [12] and 0.1% glyphosate (w/v) and shaken an additional 30 days. These cultures were streaked on solid media using purified agar (Difco Laboratories, Detroit, MI) in

Stanier's medium and glyphosate as a sole carbon source and the resulting colonies were restreaked several times to obtain pure cultures.

Microbial characterizations. Two bacterial strains were isolated from plates with glyphosate as a sole carbon source. The first isolate grew well with large white colonies (LW9). It was a Gram-negative rod. The second isolate (SW9) was discovered on the original isolation plate as pinpoint white colonies. It was also Gram-negative and grew very slowly, taking 3 weeks to form colonies on Stanier's medium with glyphosate. This isolate also grew slowly on a complex medium (Trypticase Soy Agar, BBL). API Rapid NFT strips (Analytab Products, Sherwood Medical, Plainview, NY.) were used to identify the pure isolates (Table 1). LW9 was identified as *Achromobacter* Group V D and SW9 was identified as *Agrobacterium radiobacter* [6].

Glyphosate degradation by *Agrobacterium radiobacter* was determined by a shake flask assay. Shake flasks with Stanier's medium were autoclaved and a sterile solution of glyphosate was added aseptically (0.1% w/v). The glyphosate solution was prepared by adding analytical glyphosate to double distilled water, adjusting the pH to 7.0, and sterilizing through 0.45 μm filters. The shake flasks were inoculated with one colony of each isolate. As turbidity increased, samples were taken, filtered, and analyzed for glyphosate and AMPA by a modification of a high pressure liquid chromatographic method used for the examination of industrial effluent [14].

The diluted samples were injected on an ion-exchange column (Brownlee AX-300). The mobile phase was sodium hydroxide with the pH lowered to 2.1 with trifluoroacetic acid. The components (glyphosate, aminomethylphosphonic acid, phosphite and phosphate) are separated by the column. These separated components are oxidized postcolumn releasing phosphate, which in turn is reacted with molybdate to form a blue complex which is detected at 660 nm with a tungsten lamp.

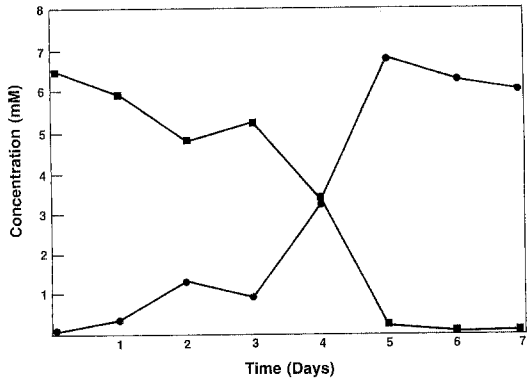

Fig. 1. SW9 glyphosate shaker flask assay. Initial glyphosate concentration 6.5 mmol (1099 mg/l). Glyphosate (■) and AMPA-produced (●). Samples were taken the same time every 24 h until glyphosate was completely absent.

TABLE 1

Identification of LW9 and SW9 with API Rapid NFT strips

	LW9	SW9
Nitrite production	+	+
Denitrification	−	+
Tryptophanase	−	−
Glucose fermentation	−	−
Arginine dihydrolase	−	−
Urease	+	−
Esculin hydrolysis	−	+
Gelatin liquefaction	−	−
β-Galactosidase	−	+
Sole carbon sources		
Glyphosate	+	+
Glucose	+	+
L-Arabinose	+	+
D-mannose	+	+
Mannitol	−	+
N-acetyl-D-glucosamine	+	+
Maltose	+	+
L-Malate	+	−
Citrate	−	−
Oxidase	+	+

Glyphosate metabolism. Strains SW9 and LW9 converted glyphosate to AMPA in the presence of inorganic phosphate (40 mM) with a small amount of degradation of AMPA. Fig. 1 shows the conversion of glyphosate to AMPA by SW9. At day 7, the turbidity of the bacterial suspension was 0.17 OD at 425 nm. SW9 and LW9 seem to be unique in their ability to utilize glyphosate as a sole carbon source in the presence of inorganic phosphate. In shake flasks experiments, glyphosate was catabolized to AMPA. SW9 also was observed to degrade small amounts of AMPA in the presence of inorganic phosphate. Other pure isolates that catabolize glyphosate have been isolated with enrichment cultures which were free of inorganic phosphorus and contained glyphosate as a sole phosphorus source. These isolates required additional sources of carbon and/or were inhibited by inorganic phosphates [1,5,7,9,15]. *Pseudomonas* sp. Strain PG2982 [7], *Agrobacterium radiobacter* [15] and *Arthrobacter* GLP-1 [9] which cleave the carbon-phosphorus bond as the initial reaction (sarcosine pathway) were inhibited by inorganic phosphorus. Presumably, glyphosate was not catabolized unless there was a need for phosphorus for growth. The *Flavobacterium* sp. GD1 [1] metabolized glyphosate to AMPA (AMPA pathway) in the presence of PO_4^{-3} and was dependent on alternative sources of carbon (pyruvate and gluconate). *Pseudomonas* sp. strain LBr

[5], utilizes both the AMPA and sarcosine pathways, with glyphosate as a sole source of phosphorus. It was inhibited by inorganic phosphate, requiring severe phosphorus starvation to induce glyphosate degradation. Additional sources of carbon were also required.

The operational strategy of a sequencing batch reactor can impose strong selective pressures on the microbial population [4]. Selective pressure was applied in this study by extending the aeration phase which would provide an opportunity for growth of organisms able to utilize glyphosate as a carbon and energy source. This strategy resulted in the isolation of the organisms reported in this study that are able to degrade glyphosate in the presence of inorganic phosphate.

The enzyme(s) responsible for the breaking of the carbon-nitrogen bond to form AMPA and a C_2 fragment have not been isolated. Rueppel et al. [10] and Jacob et al. [5] have speculated that the C_2 fragment is glyoxylate because of the cellular incorporation of the carboxymethyl moiety (methylene carbon). The fragment would be processed through the glyoxylate shunt of the TCA cycle. Alternatively, the observations in this paper could be explained by a tetrahydrofolate-dependent cleavage of the carbon-nitrogen bond of glyphosate. This would result in the transfer of the methylene carbon of glyphosate to tetrahydrofolate forming a C_1 unit and producing CO_2 and AMPA. A similar reaction occurs in the glycine cleavage enzyme system with glycine reacting with tetrahydrofolate (THF) yielding $HOCH_2$-THF, $CO_2$and NH_3 [13]. This would explain how *A. radiobacter* and *Achromobacter* sp. described here could utilize glyphosate as a sole source of carbon and energy.

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REFERENCES

1 Balthazor, T.M., and L.E. Hallas. 1986. Glyphosate-degrading microorganisms for industrial activated sludge. Appl. Environ. Microbiol. 51 (2): 432–434.

2 Grossbard, E. and D. Atkinson. 1984. The Herbicide Glyphosate, pp. 159–182, Butterworths, Boston.

3 Hallas, L.E., E.M. Hahn and C. Korndorfer. 1988. Characterization of microbial traits associated with glyphosate biodegradation in industrial activated sludge. J. Ind. Microbiol. 3: 377–385.

4 Irvine, R.L., and A.W. Busch 1979. Sequencing batch biological reactors – an overview. J. Water Poll. Cont. Fed. 51: 235–243.

5 Jacob, G.S., J.R. Garbow, L.E. Hallas, N.M. Kimack, G.M. Kishore and J. Schaefer. 1988. Metabolism of glyphosate in *Pseudomonas* sp. strain LBr. Appl. Environ. Microbiol. 54 (12): 2953–2958.

6 Kersters, K., and J. De Ley. 1984. Genus *Agrobacterium*. In Bergey's Manual of Systematic Bacteriology, Vol. 1, (Krieg N.R. and J.G. Holt, eds.), pp. 244–254. The Williams and Wilkins Co., Baltimore.

7 Kishore, G.M. and G.S. Jacob. 1987. Degradation of Glyphosate by *Pseudomonas* sp. PG2982 via a Sarcosine Intermediate. J. Biol. Chem. 262 (25): 12164–12167.

8 Moore, J.K., H.D. Braymer, and A.D. Larson. 1983. Isolation of a *Pseudomonas* sp. which utilizes the phosphonate herbicide glyphosate. Appl. Environ. Microbiol. 46: 316–320.

9 Pipke, R., A. Schulz, and N. Amrhein. 1987. Uptake of glyphosate by an *Arthrobacter* sp. Appl. Environ. Microbiol. 53 (5): 974–978.

10 Rueppel, M.L., B.B. Brightwell, J. Schaefer, and J.T. Marvel. 1977. Metabolism and degradation of glyphosate in soil and water. J. Agric. Food Chem. 25 (3): 517–522.

11 Shinabarger, D.L., and H.D. Braymer. 1986. Glyphosate catabolism by *Pseudomonas* sp. strain PG2982. J. Bacteriol. 168: 702–707.

12 Stanier, R.Y., N.J. Palleroni, and M. Doudoroff. 1986. The aerobic *Pseudomonads*: a taxonomic study. J. Gen. Microbiol. 43: 159–271.

13 Umbarger, H.E. 1978. Amino acid biosynthesis and its regulation. Ann. Rev. Biochem. 47: 533.

14 U.S. Environmental Protection Agency. 1983. Methods for nonconventional pesticide analysis of industrial and municipal wastewater: Method 127–determination of glyphosate in wastewater. U.S. Environmental Protection Agency Publication No. 440/1-83/079-C, p. 1-10/U.S. Environmental Protection Agency, Washington, D.C.

15 Wackett, L.P., S.L. Shames, C.P. Venditti and C.T. Walsh. 1987. Bacterial carbon-phosphorus lyase: products, rates, and regulation of phosphonic and phosphininc acid metabolism. J. Bacteriol. 169 (2): 710–717.