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Glyphosate Catabolism by Pseudomonas Sp. Pg2982.

Dean Lee Shinabarger
Louisiana State University and Agricultural & Mechanical College

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GLYPHOSATE CATABOLISM BY PSEUDOMONAS SP. PG2982

The Louisiana State University and Agricultural and Mechanical Col. Ph.D. 1986

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GLYPHOSATE CATABOLISM
BY PSEUDOMONAS SP. PG2982

A Dissertation

Submitted to the Graduate Faculty of the
Louisiana State University
Agricultural and Mechanical College
in partial fulfillment of the
requirements for the degree of
Doctor of Philosophy

in

The Department of Microbiology

by

Dean Lee Shinabarger
B. S., Emporia State University, 1982
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# TABLE OF CONTENTS

<table>
<thead>
<tr>
<th>Section</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>ACKNOWLEDGEMENT</td>
<td>ii</td>
</tr>
<tr>
<td>TABLE OF CONTENTS</td>
<td>iv</td>
</tr>
<tr>
<td>LIST OF TABLES.</td>
<td>v</td>
</tr>
<tr>
<td>LIST OF FIGURES</td>
<td>vii</td>
</tr>
<tr>
<td>ABSTRACT</td>
<td>x</td>
</tr>
<tr>
<td>LITERATURE REVIEW</td>
<td>1</td>
</tr>
<tr>
<td><strong>PART I. Determination of the pathway for glyphosate degradation in</strong></td>
<td></td>
</tr>
<tr>
<td><em>Pseudomonas</em> sp. PG2982</td>
<td>14</td>
</tr>
<tr>
<td>Introduction</td>
<td>15</td>
</tr>
<tr>
<td>Materials and Methods</td>
<td>18</td>
</tr>
<tr>
<td>Results</td>
<td>26</td>
</tr>
<tr>
<td>Discussion</td>
<td>47</td>
</tr>
<tr>
<td><strong>PART II. The degradation of phosphonates by</strong></td>
<td></td>
</tr>
<tr>
<td><em>Pseudomonas</em> sp. PG2982</td>
<td>60</td>
</tr>
<tr>
<td>Introduction</td>
<td>61</td>
</tr>
<tr>
<td>Materials and Methods</td>
<td>62</td>
</tr>
<tr>
<td>Results</td>
<td>66</td>
</tr>
<tr>
<td>Discussion</td>
<td>70</td>
</tr>
<tr>
<td>LITERATURE CITED</td>
<td>75</td>
</tr>
<tr>
<td>VITA</td>
<td>84</td>
</tr>
</tbody>
</table>

LIST OF TABLES

<table>
<thead>
<tr>
<th>TABLE</th>
<th>PART I.</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>Distribution of radioactivity in PG2982 cells after incubation with $^{14}$C-glyphosate and fractionation by the modified Roberts technique</td>
<td>34</td>
</tr>
<tr>
<td>2.</td>
<td>Distribution of radioactivity in nucleic acid bases from PG2982 cells incubated in the presence of $^{14}$C-glyphosate</td>
<td>36</td>
</tr>
<tr>
<td>3.</td>
<td>Incorporation of radioactivity from $^{14}$C-glyphosate into amino acids of PG2982</td>
<td>37</td>
</tr>
<tr>
<td>4.</td>
<td>Thin-layer chromatography on 100 um cellulose plates of radioactive peaks I and II obtained from PG2982 cells pulsed with $[3-^{14}]$C-glyphosate</td>
<td>39</td>
</tr>
<tr>
<td>5.</td>
<td>Loss of radioactivity from $^{14}$C-labeled metabolite after incubation with sarcosine dehydrogenase</td>
<td>40</td>
</tr>
</tbody>
</table>
6. Migration of 2,4-dinitrophenyl-hydrazine derivative of volatile
$^{14}$C-labeled compound on 500 um
silica gel plates .................. 43

7. Thin-layer chromatography on 100 um
cellulose plates of radioactive peaks
I and II obtained from PG2982 cells
pulsed with $[1,2-^{14}$C]glyphosate ... 45

8. Sarcosine oxidase activity in PG2982
cells grown with sarcosine as the
nitrogen source and glyphosate as
the phosphorus source ............... 49

9. Comparison of sarcosine oxidase
enzyme activities from PG2982 cells
grown in the presence of sarcosine,
glyphosate, or inorganic phosphate .. 50
<table>
<thead>
<tr>
<th>PART II.</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. Utilization of phosphonates by PG2982</td>
<td>67</td>
</tr>
<tr>
<td>2. Production of benzene and methane from phosphonates by PG2982</td>
<td>69</td>
</tr>
</tbody>
</table>
# List of Figures

<table>
<thead>
<tr>
<th>FIGURE</th>
<th>PAGE</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>PART I.</strong></td>
<td></td>
</tr>
<tr>
<td>1. Structure of glyphosate</td>
<td>16</td>
</tr>
<tr>
<td>2. Growth of PG2982 in glyphosate broth and the disappearance of glyphosate</td>
<td>27</td>
</tr>
<tr>
<td>3. $[3^{-14}C]$glyphosate uptake and CO$_2$ production by PG2982 cells grown in batch culture</td>
<td>28</td>
</tr>
<tr>
<td>4. $[1,2^{-14}C]$glyphosate uptake and CO$_2$ production by PG2982 cells grown in batch culture</td>
<td>30</td>
</tr>
<tr>
<td>5. Utilization of Pi and glyphosate during batch growth</td>
<td>31</td>
</tr>
<tr>
<td>6. The effect of Pi on $[3^{-14}C]$glyphosate transport and oxidation by PG2982 cells</td>
<td>33</td>
</tr>
</tbody>
</table>
LIST OF FIGURES (CONT'D)

<table>
<thead>
<tr>
<th>FIGURE</th>
<th>PAGE</th>
</tr>
</thead>
<tbody>
<tr>
<td>7. Fractionation of trichloroacetic acid extract from PG2982 cells incubated with [3-^{14}C]glyphosate for 1 hr</td>
<td>38</td>
</tr>
<tr>
<td>8. Fractionation of trichloroacetic acid extract from PG2982 cells incubated with [1,2-^{14}C]glyphosate for 1 hr</td>
<td>44</td>
</tr>
<tr>
<td>9. CO_2 production from ^{14}C-formate and ^{14}C-formaldehyde by PG2982 cells</td>
<td>46</td>
</tr>
<tr>
<td>10. Growth of PG2982 in media containing sarcosine as the sole nitrogen source and the disappearance of sarcosine</td>
<td>48</td>
</tr>
<tr>
<td>11. Proposed pathway for glyphosate degradation by <em>Pseudomonas</em> sp. PG2982</td>
<td>52</td>
</tr>
</tbody>
</table>

PART II.

1. Degradation of methylphosphonate and phenylphosphonate by PG2982 | 73 |
ABSTRACT

The pathway for the degradation of glyphosate (N-phosphonomethylglycine) by Pseudomonas sp. PG2982 has been determined using metabolic radiolabeling experiments. Radiorespirometry experiments utilizing [3-\(^{14}\)C]glyphosate revealed that approximately 50-59\% of the C3 carbon was oxidized to CO\(_2\). Fractionation of stationary phase cells labeled with [3-\(^{14}\)C]glyphosate revealed that from 45-47\% of the assimilated C3 carbon is distributed to proteins and that the amino acids methionine and serine are highly labeled. The nucleic acid bases adenine and guanine received 90\% of the C3 label that was incorporated into nucleic acids, and the only pyrimidine base labeled was thymine. These results indicated that C3 of glyphosate was at some point metabolized to a Cl compound whose ultimate fate could be both oxidation to CO\(_2\) and distribution to amino acids and nucleic acid bases that receive a Cl group from the Cl-donating coenzyme tetrahydrofolate. Pulse labeling of PG2982 cells with [3-\(^{14}\)C]glyphosate revealed that [3-\(^{14}\)C]sarcosine is an intermediate in glyphosate degradation. Examination of crude extracts prepared from PG2982 cells revealed the presence of an enzyme that oxidizes sarcosine to glycine and
formaldehyde. These results indicate that the first step in glyphosate degradation by PG2982 is cleavage of the carbon-phosphorus bond, resulting in the release of sarcosine and a phosphate group. The phosphate group is utilized as a source of phosphorus, and the sarcosine is degraded to glycine and formaldehyde. This pathway is supported by the results of [1,2-14C]glyphosate metabolism studies which showed that radioactivity in the proteins of labeled cells was found only in the glycine and serine residues. Inorganic phosphate inhibits glyphosate metabolism at the transport level but does not seem to have a direct effect on the oxidation of C3 of glyphosate to CO₂.

Phosphonate utilization by Pseudomonas sp. PG2982 was investigated. Each of the ten phosphonates tested were utilized as a sole source of phosphorus by PG2982. Representative compounds tested included alkylphosphonates, 1-amino-substituted alkylphosphonates, amino-terminal phosphonates, and an arylphosphonate. PG2982 cultures degraded phenylphosphonate to benzene and produced methane from methylphosphonate. The data indicate that PG2982 is capable of cleaving the carbon-phosphorus bond of several structurally different phosphonates.
LITERATURE REVIEW

Glyphosate is the active ingredient in the herbicide Roundup, which is manufactured by the Monsanto Chemical Company, St. Louis, Mo. The biological activity of this compound, also known as N-phosphonomethylglycine, was first reported by Baird et al. in 1971 (3). Initial field tests showed that Roundup was a very effective herbicide which could control a wide spectrum of weeds at application rates of 0.75 to 2.0 lb/acre. Roundup has the advantage of being non-herbicidal to fruit trees if foliar spraying is avoided, and is an effective postemergent herbicide for perennial and annual weeds. Roundup is generally used as a postemergent herbicide that is applied to fields with an implement that allows the herbicide to come into contact with weeds which are taller than the commercial crop. Another effective but limited method of Roundup use is as a pre-emergent herbicide which is sprayed onto fields during or soon after planting. The dormant seeds of the commercial crop are not affected but weeds are killed.

Glyphosate can become inactivated if it binds to soil. Early work by Sprankle et al. (51) indicated that glyphosate is bound to soil particles through the
phosphonic acid moiety, and that phosphate competes for binding sites. The presence of tri-valent cations such as $\text{Al}^{+++}$ and $\text{Fe}^{+++}$ in soil greatly enhances glyphosate adsorption, suggesting that an adsorbent-cation-herbicide complex may form with clay and organic matter (52). A 1985 study by Glass (18) has shown that glyphosate is an effective metal chelator which forms stable complexes with cadmium and cupric ions through interactions with the carboxylate, amino, and phosphono groups, and a water molecule.

In 1972, Jaworski (27) reported the first attempt to identify the mechanism of plant growth inhibition by glyphosate. Using the bacterium $\text{Rhizobium japonicum}$ and the aquatic plant duckweed, he found that growth inhibition caused by glyphosate could be reversed in both organisms through the simultaneous addition of the amino acids phenylalanine, tryptophan, and tyrosine. Similar results were obtained in studies with $\text{Escherichia coli}$ and the unicellular alga $\text{Chlamydomonas}$ (19, 55). The results of these studies suggested that glyphosate interfered with the biosynthesis of aromatic amino acids (shikimate pathway) in bacteria and plants.

The enzymatic step of the shikimate pathway
inhibited by glyphosate was first studied by Steinrucken and Amrhein using *Klebsiella pneumoniae* (55). They found that the enzyme 3-phosphoshikimate-1-carboxyvinyltransferase, (EC 2.5.1.19, EPSP synthase) was potently inhibited by glyphosate with a \( K_i \) of about 10,000 nM (55). EPSP synthase catalyzes a reaction in which an intact enolpyruvyl (carboxyvinyl) group is transferred from phosphoenolpyruvate (PEP) to the 5-hydroxyl group of shikimate-3-phosphate to form EPSP, as shown in the following reaction:

Recent enzymological analysis of EPSP synthase from suspension-cultured cells of bacteria (*Klebsiella pneumoniae*) and higher plants (*Nicotiana silvestris*) have shown that glyphosate inhibition of EPSP synthase is competitive with respect to PEP and that glyphosate competes with PEP for binding to an enzyme:shikimate-3-P complex (45,56). EPSP synthase molecules are then rendered inactive by the resulting
dead end enzyme:shikimate-3-P:glyphosate complex.

Molecular genetics studies have recently shown that EPSP synthase is an in vivo target of glyphosate in bacteria. Rogers et al. cloned the aroA gene (which codes for EPSP synthase) from E. coli onto a multicopy plasmid which overproduces the enzyme 5-17 fold (43). These investigators have shown that cells carrying this plasmid exhibit an 8-fold increase in the minimal inhibitory concentration of glyphosate. Smart et al. have recently reported that plant cell cultures of Corydalis sempervirens grown in the presence of 5 mM glyphosate overproduce EPSP synthase nearly 40-fold (49). The other enzymes of the shikimate pathway had the same level of activity as nonadapted cells. EPSP synthase purified from glyphosate-tolerant C. sempervirens cells had the same physical, kinetic, and immunological properties as glyphosate-sensitive cells, indicating that enzyme overproduction can increase glyphosate resistance in plants as well as in microorganisms. Shah et al. recently demonstrated that a glyphosate-tolerant Petunia hybrida cell line overproduces EPSP synthase messenger RNA 20-fold (47).

Comai et al. developed a glyphosate tolerant mutant of Salmonella typhimurium which contains an
EPSP synthase that is resistant to glyphosate (7). Sequencing of the aroA gene from this glyphosate tolerant mutant showed that there is a single amino acid change (proline to serine) in the mutant EPSP synthase (54). Comai et al. have recently introduced this mutant aroA gene into tobacco leaf cells using a Ti-plasmid based vector (8). EPSP synthase assays of the transformed cells revealed that enzyme levels were 4 times greater in one clone, indicating that the mutant allele was being expressed. When actively growing transformed plants were sprayed with glyphosate and examined 40 days later, they weighed 70% of the unsprayed controls. This study indicated that glyphosate resistance can also be conferred through the expression of a mutant aroA gene.

Glyphosate contains within its structure a carbon-phosphorus (C-P) bond, as shown below:

\[
\begin{array}{cccccc}
O & H & H & H & O \\
HO-P-C-N-C-C-OH \\
OH & H & H 
\end{array}
\]

The presence of a C-P bond places glyphosate into a unique class of organophosphorus compounds called
phosphonates. The C-P bond of unsubstituted phosphonates is thermally stable and can withstand autoclaving at 120°C and 15 lbs. pressure for 60 min. without undergoing any detectable decomposition. This is due to the high bond energy of the C-P bond, which has been calculated to be 62 Kcal as compared to other high energy bonds such as carbon-silicon (68 Kcal) and carbon-carbon (64 Kcal) bonds (17).

The C-P bond of phosphonic acids is also resistant to chemical attack. Phenylphosphonic acid can be nitrated with nitric acid at 100°C (17), and it is common practice to recrystallize phosphonic acids from hot 6 N hydrochloric acid. In the chemical synthesis of glyphosate under alkaline conditions (15), a sodium hydroxide concentration of 8 M is maintained while refluxing for 24 hrs.

The first naturally occurring phosphonate to be detected, aminoethylphosphonate (AEPn), was isolated by Horiguchi in 1959 from a mixed culture of rumen protozoa (25). AEPn has subsequently been found in many species of plants and animals as a free molecule in lipids and in macromolecular material (22,28,29,38). It has been suggested that the resistance of the C-P bond to enzymatic attack by phosphatases confers a biological advantage to organisms which have phosphonolipids in their membranes (23). Other
naturally occurring phosphonate molecules include the antibiotics fosfomycin and alafosfalin produced by bacteria of the genus *Streptomyces* (22).

In 1963, Zelenick et al. reported the first evidence for bacterial cleavage of a C-P bond by showing that *E. coli* could grow on either methylphosphonate or ethylphosphonate as a sole source of phosphorus (60). Subsequently, there have been several reports citing cleavage of the C-P bond by a variety of bacterial genera (6,9,11,20).

Cook et al. (9) isolated 14 species of phosphonate-utilizing bacteria from sewage sludge by growing each isolate with a given phosphonate as the sole source of phosphorus. Each organism generally utilized only the phosphonate it was enriched on and AEPn. The prevalence of AEPn in the environment presumably contributes to its ubiquitous degradation by microorganisms.

The most thorough study of phosphonate metabolism by a single microorganism was conducted by LaNauze and Rosenberg in the late 1960's. These two investigators isolated a strain of *Bacillus cereus* which utilized AEPn as a sole source of phosphorus (30). In a 1968 report (31), they showed that AEPn is deaminated to form phosphonoacetaldehyde as shown below:
In 1970, La Nauze et al. (32) described the only successful purification and characterization of an enzyme that cleaves the C-P bond. Their phosphonatase enzyme, phosphonoacetaldehyde hydrolase (EC 3.11.1.1), degraded only phosphonoacetaldehyde yielding acetaldehyde and inorganic phosphate by the following hydrolysis reaction:

\[ \text{H}_2\text{N}((\text{CH}_2)_2\text{PO}_3\text{H}_2) \rightarrow \text{H-C-CH}_2\text{PO}_3\text{H}_2 \]

2-AMINOETHYLPHOSPHONATE PHOSPHONOACETALDEHYDE

Several investigators have identified degradation products from phosphonate metabolism \((1,6,11,12)\). Cook et al. identified a strain of *Klebsiella pneumoniae* which produces benzene from the cleavage of methylphosphonate or phenylphosphonate \((11)\). Daughton et al. isolated a *Pseudomonas testosteroni* strain.
which cleaves the C-P bond of several alkylphosphonates (13). No oxidized organic products were found in these degradation studies. The cleavage of the C-P bond apparently occurred by a hydrolytic process in which the -OH of water is incorporated into phosphorus and the H to the carbon.

Glyphosate appears to be co-metabolized by microorganisms in the soil. An early study by Sprankle et al. in 1975 showed that the initial rate of CO₂ production from [3-¹⁴C]glyphosate was rapid during the first 2 days after application to soil (52). This rapid degradation rate was followed by a longer period of slow but continuous CO₂ production, suggesting that glyphosate was rapidly degraded by soil microflora to a metabolite whose degradation rate was slower. Confirmation of metabolite accumulation was provided by Rueppel et al. in 1977 when they isolated [¹⁴C]aminomethylphosphonate ([¹⁴C]AMPn) from [3-¹⁴C]glyphosate treated soil (46). [¹⁴C]AMPn levels in soil/water shake flasks rapidly increased during the initial glyphosate degradation period, then slowly decrease over a period of 10 days. Additional evidence for co-metabolism of glyphosate by soil microflora
has been provided by other investigators (40,41).

Despite the ability of the soil microflora to degrade glyphosate, very few of those microorganisms have been isolated. Talbot et al. reported in 1984 that a *Pseudomonas* sp. isolated from an aerobic sewage digester could utilize glyphosate as a sole source of phosphorus (57). This isolate decreased the glyphosate concentration of the growth medium from 240 uM to 190 uM in 70 hrs. Balthazor and Hallas (4) have recently reported that a strain of *Flavobacterium* isolated from a sludge pond used to treat glyphosate containing wastes utilized either glyphosate or AMPn as a source of phosphorus. This organism cleaves glyphosate to AMPn and either a single C₂ unit or two C₁ units, then breaks down the AMPn to obtain phosphorus for growth (4) as shown below:

\[
\begin{align*}
\text{GLYPHOSATE} & \quad \text{AMINOMETHYLPHOSPHONATE} \\
\text{AMINOMETHYLPHOSPHONATE}
\end{align*}
\]
Unlike other phosphonate degrading organisms, this *Flavobacterium* sp. can degrade glyphosate in the presence of inorganic phosphate. However, AMPn degradation is inhibited by inorganic phosphate (4).

The study of glyphosate metabolism by bacteria was greatly facilitated in 1983 when Moore et al. (39) isolated a *Pseudomonas* sp. which they designated as strain PG2982. This organism utilizes glyphosate as a sole source of phosphorus in a defined medium. PG2982 was fortuitously isolated from a *Pseudomonas aeruginosa* ATCC 9027 stock culture by Moore as he was routinely screening bacteria for the ability to degrade glyphosate. The *P. aeruginosa* culture appeared to be slowly degrading glyphosate over a period of 2 weeks. After streaking the culture out on a complex medium, Moore et al. found that there were both white and brown colonies present. When these two different colonies were isolated and studied individually, it was found that the white strain required the addition of yeast extract to a minimal medium in order to grow. The white colony could degrade glyphosate under these conditions, but the brown colony could not. Subsequent characterization studies showed that PG2982 (white colony) differed from the ATCC 9027 *P. aeruginosa* culture in several important characteristics (39). *P. aeruginosa* is motile by a polar monotrichous
flagellum, prototrophic, has a Mol% G+C of 66% in the DNA, and does not degrade glyphosate. PG2982 was found to be a nonmotile, thiamine auxotroph that has a Mol% G+C of 60% and the ability to degrade glyphosate. These data indicate that PG2982 is not a mutant of *P. aeruginosa* ATCC 9027. The origin of PG2982 remains a mystery to this day. Several other investigators have obtained *Pseudomonas aeruginosa* ATCC 9027 from the American Type Culture Collection and have unsuccessfully tried to isolate PG2982 using the techniques described by Moore et al.

The transport of glyphosate by a pure culture of bacteria was first studied by Moore in 1983 using PG2982. He found that glyphosate transport was immediately inhibited upon addition of inorganic phosphate to the growth medium, and that aminomethylphosphonate inhibited transport 82% (J. K. Moore, Masters thesis, Louisiana State University, Baton Rouge, Louisiana). This finding agreed well with other studies on phosphate inhibition of phosphonate metabolism (1,6,20). To date there has been no report of a bacterium which can cleave the C-P bond in the presence of inorganic phosphate.

Moore was the first to use radiotracer experiments to investigate glyphosate metabolism by a pure culture
of bacteria (J. K. Moore, Masters thesis, Louisiana State University, Baton Rouge, Louisiana). Using [3-$^{14}$C]glyphosate as a tracer, he showed that approximately 50% of the C-P bond carbon (C3) was oxidized to $^{14}$CO$_2$. This study showed for the first time that a pure culture of bacteria could cleave the C-P bond of glyphosate. Analysis of the cells showed that 30% of the assimilated radioactivity was in the form of trichloroacetic acid precipitable material, indicating that C3 of glyphosate was being used for the synthesis of cellular components. No radioactive metabolites other than CO$_2$ were found and therefore a catabolic pathway could not be constructed.

A second, somewhat more revealing study of glyphosate metabolism by PG2982 was conducted by Jacob et al. in 1985 using cells grown on $^{15}$N and $^{13}$C-labeled glyphosate (26). Solid-state NMR analysis of cells revealed that the glycine moiety of glyphosate could be found intact in proteins and purines. This suggested that at some point in glyphosate degradation glycine was released and subsequently used to synthesize proteins and nucleic acids. Jacob et al. claimed to have determined the complete route for glyphosate metabolism by PG2982—while admitting that they had failed to detect any intermediates. Despite the efforts of Moore and Jacob et al., the pathway for glyphosate degradation by PG2982 has not been determined.
Part I. Determination of the pathway for glyphosate degradation in *Pseudomonas* sp. PG2982.
INTRODUCTION

Glyphosate (N-phosphonomethylglycine) is the active ingredient in the herbicide Roundup (Figure 1). The primary mode of action of glyphosate is the inhibition of 3-phosphoshikimate-1-carboxyvinyl transferase (EPSP synthase), an enzyme of the shikimate pathway responsible for the biosynthesis of aromatic amino acids in bacteria and plants (27,55). Recent studies using glyphosate resistant *Salmonella typhimurium* mutants (7) and recombinant *Escherichia coli* strains containing plasmids that overproduce EPSP synthase (43) have confirmed that glyphosate is a potent inhibitor of this enzyme.

In several studies the mode of action of glyphosate and the development of glyphosate resistance in bacteria and plants has been investigated. In contrast, the study of glyphosate metabolism has been hampered by the lack of pure bacteria that can degrade glyphosate. In soil, the fate of glyphosate is complete degradation by soil microbes (41,46). However, these studies yielded little information about the pathway of glyphosate metabolism utilized by individual bacterial species.

Moore et al. (39) previously reported the isolation of a thiamine-requiring *Pseudomonas* sp.
Fig. 1. Structure of glyphosate
PG2982 from a stock culture of *Pseudomonas aeruginosa* ATCC 9027 that can utilize glyphosate as a sole phosphorus source. In batch culture this organism completely utilizes 1.0 mM glyphosate, yielding a cell density equal to that obtained with an equimolar concentration of inorganic phosphate (39). Recently, Jacob et al. (26) used solid-state $^{13}$C and $^{15}$N NMR experiments to study the metabolism of glyphosate by PG2982. They reported that glyphosate is cleaved directly to glycine, which is then utilized by PG2982 for protein and nucleic acid biosynthesis. However, their study did not indicate whether the organism breaks the carbon-phosphorus bond (C-P bond) of glyphosate before or after glycine is released in order to obtain phosphorus for growth.

In order to determine the pathway for glyphosate degradation by PG2982, we performed radiotracer experiments using $^{14}$C-labeled glyphosate. These experiments have resulted in the successful isolation of intermediates of glyphosate degradation in sufficient quantities for chromatographic and enzymatic analysis. Subsequent studies with these metabolites, and radiorespirometry experiments using [3-$^{14}$C]glyphosate, have led to the formulation of a pathway of glyphosate degradation in which cleavage of the C-P bond is the first step.
MATERIALS AND METHODS

Organism and culture conditions. PG2982 is a glyphosate degrading Pseudomonas sp. isolated in our laboratory (39). The medium used for the maintenance and growth of PG2982 has been described in a previous report (39). Cultures were routinely grown in 300 ml side-arm flasks at 32°C on an orbital shaker (250 rpm). Cultures for experiments were always started with a 1% log-phase inoculum.

Chemicals and radiochemicals. The free acid form of glyphosate (99.7% purity) and [3-14C]glyphosate (specific activity 9.3 mCi mmol\(^{-1}\)) were gifts from Monsanto Co., St. Louis, Mo. \([U-14C]glycine\) (specific activity 100 mCi mmol\(^{-1}\)), \([14C]formaldehyde\) (specific activity 40 mCi mmol\(^{-1}\)), and \([14C]formate\) (specific activity 44 mCi mmol\(^{-1}\)) were obtained from Research Products International Corp. (Mount Prospect, Ill.). Chloromethylphosphonic acid was purchased from Aldrich Chemical Co., Milwaukee, Wis. All other chemicals used in this study were of reagent grade.

Analytical methods. Culture turbidity was measured using a Klett-Summerson colorimeter and a red filter (660 nm). Bacterial dry weight measurements were made by filtering cells (using a 0.45 um membrane filter)
and washing them with 10 ml of Dworkin-Foster salts solution (39). The membrane filter was dried for 6 hr in an oven at 60°C and weighed. Glyphosate and other amine-containing metabolites were assayed on a Beckman Amino Acid Analyzer, model 120C according to the procedure of Moore et al. (39). Formaldehyde was determined using the method of Nash as described by Wood (59). Unless indicated otherwise, radioactivity was determined by adding a portion of the sample to 5 ml of Aquasol scintillation cocktail (New England Nuclear) and counting in a Beckman LS 6800 scintillation counter.

Radiorespirometry experiments. To determine whether [3-14C] glyphosate was oxidized to CO2 by PG2982, 300 ml side-arm flasks were fitted with a CO2 trap using the apparatus described by Dobrogosz (14). The cultures were incubated at 32°C on an orbital shaker (250 rpm) and allowed to reach stationary phase. During the course of the experiment, a 250 ul sample of each culture was removed using a sterile syringe fitted with a 10 cm (22 gauge) needle that had been inserted through a rubber septum in the culture flask. The culture samples were treated as follows: (i) duplicate 100 ul portions were filtered, washed, and dried as described above. These samples were weighed for bacterial dry weight measurements and the radioactivity
was counted in scintillation vials as assimilated $^{14}$C]glyphosate. (ii) duplicate 10 ul portions of the culture filtrate obtained above were counted to determine the radioactivity remaining in the extracellular medium. To determine whether $^{14}$C]formaldehyde or $^{14}$C]formate were oxidized to CO$_2$, mid-log phase PG2982 cells (10 ml) growing in glyphosate broth were harvested by centrifugation, washed once with Dworkin-Foster salts solution (39), and resuspended in 10 ml of glyphosate broth. Either 0.5 uCi of $^{14}$C]formaldehyde or $^{14}$C]formate was added to the culture and CO$_2$ was trapped as outlined above.

**Fractionation of labeled cells.** Mid-log phase PG2982 cells growing in medium containing either [3-$^{14}$C]glyphosate or [1,2-$^{14}$C]glyphosate were harvested and washed twice with Dworkin-Foster salts solution (39) before fractionation by a modified Roberts technique (14). Duplicate samples were counted for each fractionation step.

**Distribution of radioactivity in nucleic acid bases.** The nucleic acid fraction obtained from the modified Roberts procedure was precipitated with ethanol to obtain the nucleic acids. This precipitate was hydrolyzed with 12 N perchloric acid, and the bases were separated by thin-layer chromatography on
plastic-backed cellulose sheets (5). The bases were visualized under UV light and scraped into scintillation vials for counting.

**Distribution of radioactivity in proteins.** The protein fraction obtained from the modified Roberts procedure was precipitated and washed twice with 6 N HCL. This precipitate was then hydrolyzed in 6 N HCL at 110°C for 24 hr. An aliquot of this hydrolysate was injected into a Beckman 120C amino acid analyzer for quantitative determination of amino acid content, and a second aliquot was chromatographed by two-dimensional thin-layer chromatography on plastic backed cellulose plates (20 x 20 cm, 0.1 mm layer) using isopropanol-formic acid-water (20:1:5, v/v/v) in the first direction and butanol-acetone-diethylamine-water (30:30:6:15, v/v/v/v) in the second direction. Radioactive amino acids were located by scraping the spots on the plate where standard amino acids migrated and counting in liquid scintillation vials.

**Radiotracer experiments.** Mid-log phase cells (20 ml) grown in glyphosate broth were centrifuged at 12000 x g for 10 min at room temperature and washed once with Dworkin-Foster salts solution. The cell pellet was resuspended in 5 ml of Dworkin-Foster salts solution containing 10 uCi of [3-¹⁴C]glyphosate (specific
activity 9.3 mCi/m mole) and incubated at 32°C on an orbital shaker for 1 hr. The cells were harvested by centrifugation and extracted 2 times with 0.5 ml portions of 10% trichloroacetic acid (TCA). The TCA extracts were concentrated under reduced pressure and resuspended in 0.25 ml of 0.2 N sodium citrate buffer.

**Purification of 14C-labeled metabolites** Fractionation of glyphosate and intermediary metabolites was carried out using a Beckman 120C amino acid analyzer modified so that fractions could be collected directly from the ion-exchange column (W1 type resin) of the amino acid analyzer without passing through the ninhydrin mixing chamber. Fractions (1 ml) were collected and assayed by liquid scintillation as described above. Radioactive fractions were pooled and passed through a 1 x 15 cm column of Dowex 50W-X8 (20-50 mesh, H+ form, Bio-Rad Co.). The sodium citrate buffer from the amino acid analyzer and [14C]-glyphosate were eluted with 100 ml of water while bound metabolites were eluted with 25 ml of 2.0 N NH₄OH. The NH₄OH wash was concentrated under reduced pressure and assayed by thin-layer chromatography on cellulose sheets (20 x 20 cm, 100 um layer) using the following solvent systems: methanol-pyridine- hydrochloric acid-water (80:20:2:18 v/v/v/v), ethanol-water-17 N ammonium hydroxide-
trichloroacetic acid-15 N acetic acid (55:35:2.5:3.5:2 v/v/v/w/v), isopropanol-formic acid-water (20:1:5 v/v/v), sec-butanol-formic acid-water (70:10:20 v/v/v), and n-butanol-acetic acid-water (30:7.5:12.5 v/v/v).

Identification of sarcosine. The $^{14}$C-labeled metabolite identified as sarcosine by both ion-exchange and thin-layer chromatography was further analysed by treatment with the enzyme sarcosine oxidase (sarcosine:oxygen oxidoreductase(demethylating) EC 1.5.3.1). The assay system consisted of 3000 cpm of [3-$^{14}$C]sarcosine (60 ul), 1 ml of 50 mM potassium phosphate buffer (pH 7.5), and 1 unit of sarcosine oxidase. The reaction was carried out at 37°C in a 10 x 65 mm. glass tube sealed with a serum stopper. After a 30 min incubation period, 2 ml of a solution containing 0.2% 2,4-dinitrophenylhydrazine in 2 N HCL was injected through the serum stopper. Unlabeled formaldehyde (2.0 umoles) was then injected into the tube to act as a carrier for the precipitation of any $[^{14}$C]formaldehyde formed during the reaction. After 30 min of incubation at room temperature, the yellow precipitate formed in the tube was collected on a 0.45 um filter and washed with 10 ml of 2 N HCL. The yellow precipitate was dissolved in ethyl acetate and chromatographed on silica gel plates (20 x 20 cm, 100 um, AllTech)
Associates, Inc.) using three different solvent systems (61): carbon tetrachloride-acetone (9:1 v/v), benzene, and chloroform-diethyl ether (8:2 v/v). Radioactivity on these plates was located by cutting the thin-layer plate into 1.0 cm squares and dropping each square into a vial containing 5.0 ml of scintillation fluid. The migration of radioactive compounds on the plates was compared with the migration of the 2,4-dinitrophenylhydrazone derivatives of formaldehyde and other aldehydes.

**Synthesis and purification of glyphosate.** Glyphosate was synthesized using the procedure of Fredericks and Summers (16) for the synthesis of aminomethylphosphonic acids related to glyphosate. Glycine (0.2 moles) and 0.02 moles of chloromethylphosphonic acid were dissolved in 50 ml of water, adjusted to pH 10.5 with 8 M NaOH, and refluxed for 24 h. The crude product was precipitated from the reflux mixture with absolute ethanol, resuspended in 0.2 N sodium citrate buffer, and fractionated using a Beckman 120C amino acid analyzer as described above. For the synthesis of [1,2-\textsuperscript{14}C] glyphosate, 100 uCi of [U-\textsuperscript{14}C]glycine (specific activity 20 mCi mmol\textsuperscript{-1}) were refluxed with 4 umoles of chloromethylphosphonic acid. The entire reflux mixture was concentrated to 1 ml under reduced pressure and applied to the Beckman 120C amino acid
Liquid scintillation assay of the resultant fractions gave two radioactive peaks (I and II) eluting at fractions 5-6 and 17-19. Control experiments indicated that glyphosate eluted at the former volume. Fraction I was applied to a 30 x 1.5 cm column containing AG1-X8 (100-200 mesh, chloride form, Bio Rad Co.). The [1,2-\textsuperscript{14}C]glyphosate was eluted with 150 ml of water while the sodium citrate buffer was retained by the column. This water wash was concentrated under reduced pressure and chromatographed on cellulose plates using the method described by Sprankel et al. (53). Radioactivity on the plates was located by cutting the plates into 1 cm squares and counting by liquid scintillation assay. The yield of glyphosate under these conditions represented 25-30% of the theoretical yield. Using this purification scheme, no \textsuperscript{14}Cglycine was detected in the final product.

Enzyme assays. Crude cell-free extracts for enzyme assays were prepared from washed PG2982 cells grown under various conditions. In all cases the cells were pelleted at 12000 x g and washed twice with Dworkin-Foster salts solution (39) before resuspension in 50 mM Tris-HCL buffer (pH 7.0). The cells were broken by three 30-s periods of sonication in an ice bath. The extracts were centrifuged at 20000 x g (4°C) for 20 min to sediment unbroken cells and cell
wall material, and the supernatant was collected and kept on ice until the assays were run (always within 30 min.). The assay for sarcosine oxidase contained 0.2 ml of the crude extract, 0.6 ml of 50 mM Tris hydrochloride (pH 7.0), and 0.1 ml of 0.5 M sarcosine. The reagents were added in this order and incubated at 30°C for 40 min. The reaction was stopped by the addition of 0.2 ml trichloroacetic acid and centrifuged at 8,740 x g in a microfuge to sediment the protein. Portions of the supernatant were injected into a Beckman 120C amino acid analyzer for glycine determination.

RESULTS

Utilization of glyphosate by PG2982

Figure 2 shows that glyphosate rapidly disappears from the culture during batch growth. PG2982 cells began oxidizing the C3 carbon of glyphosate to CO₂ early in the logarithmic phase of growth (Figure 3). Data obtained from several independent experiments have shown that 50 to 59% of the C3 carbon of glyphosate is found in the CO₂ trap at the end of batch growth. Radioactivity was assimilated by whole cells (Figure 3) until mid-log phase, after which time the amount of label assimilated per milligram of bacterial dry weight
Fig. 2. Growth (●) of PG2982 in glyphosate broth and the disappearance of glyphosate (■).
Fig. 3. [3-\textsuperscript{14}C]glyphosate uptake (▲) and CO\textsubscript{2} production (■) by PG2982 cells grown in batch culture. The minimal medium contained 5 uCl of [3-\textsuperscript{14}C]glyphosate (0.5 mM final concentration) as the sole phosphorus source. Cell growth (○) was monitored with a Klett-Summerson colorimeter.
decreased dramatically.

There was no detectable CO₂ production from Cl or C2 of glyphosate (Figure 4). Instead there was a steady assimilation of radioactivity by PG2982 cells throughout the growth cycle until stationary phase was reached. Approximately 100% of the label from [1,2-¹⁴C]glyphosate was assimilated by whole cells while less than 1% of the original radioactivity remained in the culture supernatant.

Effect of inorganic phosphate on glyphosate utilization

Figure 5 shows that when inorganic phosphate was present in addition to glyphosate as an alternative source of phosphorus for PG2982, the cells utilized the inorganic phosphate first, with very little glyphosate degradation taking place during this time. No radioactivity was found in the CO₂ trap during this time of inorganic phosphate utilization. However, when the inorganic phosphate was exhausted from the medium, glyphosate was rapidly utilized as the sole phosphorus source. Concomitant with glyphosate utilization was the appearance of radioactivity in the CO₂ trap, indicating that the C3 carbon of glyphosate was being oxidized.

In order to further investigate the inhibition of glyphosate degradation by inorganic phosphate, cells
Fig. 4. [1,2-\(^{14}\)C]glyphosate uptake (▲) and CO\(_2\) production (■) by PG2982 cells grown in batch culture. The minimal medium contained 1 uCi of [1,2-\(^{14}\)C]glyphosate (0.5 mM final concentration) as the sole phosphorus source. Cell growth (○) was monitored with a Klett-Summerson colorimeter.
Fig. 5. Utilization of Pi (□) and glyphosate (▲) during batch growth. $^{14}$CO$_2$ production (○) was monitored throughout the growth cycle.
were resuspended in fresh 0.5 mM glyphosate broth containing 1 uCi of \([3-^{14}C]\)glyphosate and allowed to begin oxidizing C3 of glyphosate to CO\(_2\). When oxidation of C3 to CO\(_2\) was proceeding at a linear rate, inorganic phosphate was added to the culture such that the final inorganic phosphate concentration was 1 mM (41 min. on Figure 6). Continuous monitoring of radioactivity in the CO\(_2\) trap and in PG2982 cells revealed that transport of glyphosate stopped immediately after addition of inorganic phosphate, while CO\(_2\) production continued at a slower rate. The radioactivity present in PG2982 cells exposed to 1 mM inorganic phosphate decreased as C3 of glyphosate was oxidized to CO\(_2\). Control cultures which did not receive inorganic phosphate continued to transport glyphosate and oxidize C3 of glyphosate to CO\(_2\) at a rapid rate.

**Fractionation of labeled cells**

PG2982 cells labeled with \([3-^{14}C]\)glyphosate contained approximately 50% of the radioactivity in the protein and cell wall fraction, while the remainder of the assimilated label was evenly incorporated into the nucleic acid (24-26%) and lipid (20-23%) pools (Table 1). Only 3-5% of the assimilated radioactivity could be found in the amino acid-organic acid pool (metabolic pool).
Fig. 6. The effect of Pi on \([3^{-14}\text{C}]\text{glyphosate}\) transport and oxidation by PG2982 cells. Glyphosate grown cells were harvested and divided into equal portions before resuspending in fresh \([3^{-14}\text{C}]\text{glyphosate}\) broth. Pi (1 mM final concentration) was added to one culture at 41 min.
TABLE 1. Distribution of radioactivity in PG2982 cells after incubation with $^{14}C$glyphosate and fractionation by the modified Roberts technique

<table>
<thead>
<tr>
<th>Fraction</th>
<th>[3-$^{14}$C]glyphosate</th>
<th>[1,2-$^{14}$C]glyphosate</th>
</tr>
</thead>
<tbody>
<tr>
<td>Metabolic pool</td>
<td>3-5</td>
<td>1-2</td>
</tr>
<tr>
<td>Lipid</td>
<td>20-23</td>
<td>10-13</td>
</tr>
<tr>
<td>Nucleic acid</td>
<td>24-26</td>
<td>22-24</td>
</tr>
<tr>
<td>Protein</td>
<td>45-47</td>
<td>68-71</td>
</tr>
</tbody>
</table>
Fractionation of PG2982 cells after incubation with [1,2-\(^{14}\)C]glyphosate revealed that 1% of the label remained in the metabolic pools, 10-13% was incorporated into lipids, 24% was found in the nucleic acid fraction, and 68-71% was found in the protein and cell wall fraction (Table 1).

**Distribution of radioactivity in nucleic acid bases**

Analysis of the component nucleic acid bases from cells grown in the presence of [1,2-\(^{14}\)C]glyphosate revealed that all of the radioactivity was found in the purine bases adenine and guanine (Table 2). No detectable radioactivity was found in the pyrimidine bases of these cells. The nucleic acid bases adenine and guanine were extensively labeled in cells incubated with [3-\(^{14}\)C]glyphosate (Table 2). Only one of the pyrimidine bases analyzed from these cells, thymine, contained radioactivity.

**Distribution of radioactivity in proteins**

Acid hydrolysis and subsequent chromatographic analysis of the protein fraction from [\(^{14}\)C]glyphosate labeled cells is presented in Table 3. Glycine and serine were the only amino acids that showed detectable levels of radioactivity in PG2982 cells grown in the presence of [1,2-\(^{14}\)C]glyphosate. Analysis of the protein hydrolysate from [3-\(^{14}\)C]glyphosate labeled cells showed that methionine and serine contained the
TABLE 2. Distribution of radioactivity in nucleic acid bases from PG2982 cells incubated in the presence of $^{14}\text{C}\text{glyphosate}$

<table>
<thead>
<tr>
<th>Base</th>
<th>[3-$^{14}\text{C}\text{glyphosate}$]</th>
<th>[1,2-$^{14}\text{C}\text{glyphosate}$]</th>
</tr>
</thead>
<tbody>
<tr>
<td>Adenine</td>
<td>40</td>
<td>37</td>
</tr>
<tr>
<td>Guanine</td>
<td>51</td>
<td>63</td>
</tr>
<tr>
<td>Cytosine</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Thymine</td>
<td>9</td>
<td>0</td>
</tr>
<tr>
<td>Uracil</td>
<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>
### TABLE 3. Incorporation of radioactivity from [14C]glyphosate into amino acids of PG2982

<table>
<thead>
<tr>
<th>Amino acid</th>
<th>[3-14C]glyphosate (cpm/nmole)</th>
<th>[1,2-14C]glyphosate (cpm/nmole)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Methionine</td>
<td>17.6</td>
<td>0</td>
</tr>
<tr>
<td>Serine</td>
<td>6.7</td>
<td>2.4</td>
</tr>
<tr>
<td>Glycine</td>
<td>0</td>
<td>5.2</td>
</tr>
</tbody>
</table>

*PG2982 was grown to late log phase in 50 ml of glyphosate broth containing either 5 uCi of [3-14C]glyphosate or 1 uCi of [1,2-14C]glyphosate. The cells were fractionated by the modified Roberts technique and the protein fraction was hydrolyzed in 6 N HCL for 24 h.*
Fig. 7. Fractionation of trichloroacetic acid extract from PG2982 cells incubated with [3-\(^{14}\)C]glyphosate for 1 hr. The extract was injected into a Beckman 120C amino acid analyzer and fractions (1 ml) were collected directly from the ion-exchange column (W1 type resin) of the analyzer. The peak represented by fractions 5-6 is [3-\(^{14}\)C]glyphosate, and the later peak (fractions 14-16) is [3-\(^{14}\)C]sarcosine.
TABLE 4. Thin-layer chromatography on 100 μm cellulose plates of radioactive peaks I and II obtained from PG2982 cells pulsed with [3-\textsuperscript{14}C]glyphosate.

<table>
<thead>
<tr>
<th>sample</th>
<th>A</th>
<th>B</th>
<th>C</th>
<th>D</th>
<th>E</th>
</tr>
</thead>
<tbody>
<tr>
<td>Glyphosate</td>
<td>.31</td>
<td>.40</td>
<td>.20</td>
<td>.28</td>
<td>.26</td>
</tr>
<tr>
<td>Sarcosine</td>
<td>.53</td>
<td>.70</td>
<td>.47</td>
<td>.44</td>
<td>.41</td>
</tr>
<tr>
<td>Peak I</td>
<td>.30</td>
<td>.38</td>
<td>.19</td>
<td>.28</td>
<td>.25</td>
</tr>
<tr>
<td>Peak II</td>
<td>.51</td>
<td>.70</td>
<td>.45</td>
<td>.42</td>
<td>.39</td>
</tr>
</tbody>
</table>

\textsuperscript{a}A = methanol-pyridine-hydrochloric acid-water (80:20:2:18 v/v/v/v); B = ethanol-water-17 N ammonium hydroxide-trichloroacetic acid-15 N acetic acid (55:35:2.5:3.5:2 v/v/v/w/v); C = isopropanol-formic acid-water (20:1:5 v/v/v); D = sec-butanol-formic acid-water (70:10:20 v/v/v); E = n-butanol-acetic acid-water (30:7.5:12.5 v/v/v).
<table>
<thead>
<tr>
<th>assay conditions</th>
<th>beginning (cpm)</th>
<th>remaining (cpm)</th>
<th>nmoles glycine produced</th>
</tr>
</thead>
<tbody>
<tr>
<td>Enzyme, metabolite, DCPIP&lt;sup&gt;a&lt;/sup&gt;</td>
<td>1500</td>
<td>113</td>
<td>316</td>
</tr>
<tr>
<td>Enzyme, metabolite</td>
<td>1500</td>
<td>1431</td>
<td>0</td>
</tr>
<tr>
<td>Enzyme, DCPIP</td>
<td>1500</td>
<td>1317</td>
<td>0</td>
</tr>
</tbody>
</table>

<sup>a</sup>1500 cpm of $^{14}$C-labeled metabolite, 0.25 micromoles of dichlorophenolindophenol (DCPIP), 0.7 ml of 50 mM Tris-HCL buffer (pH 7.0), and 0.4 units of sarcosine dehydrogenase were incubated at 32°C for 30 min.
protein-associated radioactivity, with methionine receiving nearly three times as much label as serine.

Analysis of intermediates

Fractionation of the TCA extract from PG2982 cells incubated with [3-\(^{14}\)C]glyphosate gave two radioactive peaks (I and II) eluting at fractions 5-7 and 13-16, respectively (Figure 7). Control experiments indicated that [14C]glyphosate eluted at the former volume. Preliminary experiments indicated that the size of peak II increased greatly if the cells were incubated with [3-\(^{14}\)C]glyphosate in the presence of 50 mM sarcosine. Peak II was further purified by passage through a Dowex 50W-X4 column as outlined above, and the radioactive material that was eluted in the NH\(_4\)OH wash was analyzed by thin-layer chromatography. The radioactive compound purified by this procedure migrated with sarcosine in all five solvent systems tested (Table 4).

Incubation of the \(^{14}\)C-labeled metabolite with the enzyme sarcosine dehydrogenase resulted in a loss of radioactivity from the compound (Table 5). When this reaction was carried out in a closed tube, it was found that the volatile product produced during the reaction could be precipitated by 2,4-dinitrophenylhydrazine which was injected through a rubber septum. This product was identified as the
2,4-dinitrophenylhydrazone derivative of [\(^{14}\)C]formaldehyde by thin-layer chromatography using three different solvent systems (Table 6). Radioactivity associated with the precipitated product migrated with authentic 2,4-dinitrophenylhydrazone-formaldehyde on each silica gel plate.

Fractionation of the TCA extract obtained from chloramphenicol treated (100 ug ml\(^{-1}\)) PG2982 cells incubated with [1,2-\(^{14}\)C]glyphosate gave two radioactive peaks (I and II) eluting at fractions 5-7 and 16-18, respectively (Figure 8). Control experiments indicated that [\(^{14}\)C]glyphosate eluted at the former volume. Peak II was further purified by passage through a Dowex 50W-X4 column as outlined above and analyzed by thin-layer chromatography. The radioactive compound purified by this procedure migrated with glycine in all five solvent systems tested (Table 7).

**Utilization of formate and formaldehyde by PG2982**

Incubation of logarithmic phase PG2982 cells with either [\(^{14}\)C]formaldehyde or [\(^{14}\)C]formate resulted in the rapid production of CO\(_2\) after a 20 min. lag period (Figure 9). Approximately 66% of the formaldehyde and 100% of the formate added was oxidized to CO\(_2\) after 7 hrs. of incubation. There was no radioactivity in CO\(_2\) traps from uninoculated control flasks.
TABLE 6. Migration of 2,4-dinitrophenylhydrazone derivative of volatile $^{14}$C-labeled compound on 500 um silica gel plates.

<table>
<thead>
<tr>
<th>compound</th>
<th>A</th>
<th>B</th>
<th>C</th>
</tr>
</thead>
<tbody>
<tr>
<td>Volatile $^{14}$C-compound</td>
<td>.64</td>
<td>.60</td>
<td>.93</td>
</tr>
<tr>
<td>formaldehyde</td>
<td>.63</td>
<td>.61</td>
<td>.92</td>
</tr>
</tbody>
</table>

$^a$A= chloroform-acetone (9:1 v/v); B= benzene; 
C= chloroform diethyl ether (8:2 v/v).
Fig. 8. Fractionation of trichloroacetic acid extract from PG2982 cells incubated with [1,2-\textsuperscript{14}C]glyphosate for 1 hr. The extract was injected into a Beckman 120C amino acid analyzer and fractions (1 ml) were collected directly from the ion-exchange column (W1 type resin) of the analyzer. The peak represented by fractions 5-6 is [3-\textsuperscript{14}C]glyphosate, and the later peak (fractions 17-18) is [\textsuperscript{14}C]glycine.
TABLE 7. Thin-layer chromatography on 100 um cellulose plates of radioactive peaks I and II obtained from PG2982 cells pulsed with $[1,2^{-14}C]$glyphosate.

<table>
<thead>
<tr>
<th>sample</th>
<th>A</th>
<th>B</th>
<th>C</th>
<th>D</th>
<th>E</th>
</tr>
</thead>
<tbody>
<tr>
<td>Glyphosate</td>
<td>.33</td>
<td>.38</td>
<td>.20</td>
<td>.30</td>
<td>.27</td>
</tr>
<tr>
<td>Glycine</td>
<td>.38</td>
<td>.56</td>
<td>.37</td>
<td>.39</td>
<td>.34</td>
</tr>
<tr>
<td>Peak I</td>
<td>.31</td>
<td>.36</td>
<td>.20</td>
<td>.28</td>
<td>.27</td>
</tr>
<tr>
<td>Peak II</td>
<td>.37</td>
<td>.55</td>
<td>.36</td>
<td>.37</td>
<td>.32</td>
</tr>
</tbody>
</table>

*aA= methanol-pyridine-hydrochloric acid-water (80:20:2:18 v/v/v/v); B= ethanol-water-17 N ammonium hydroxide-trichloroacetic acid-15 N acetic acid (55:35:2.5:3.5:2 v/v/v/v/v); C= isopropanol-formic acid-water (20:1:5 v/v/v); D= sec-butanol-formic acid-water (70:10:20 v/v/v); E= n-butanol-acetic acid-water (30:7.5:12.5 v/v/v).
Fig. 9. $^{14}$CO₂ production from $[^{14}$C]formate (●) and $[^{14}$C]formaldehyde (■) by PG2982 cells. Mid-log phase PG2982 cells growing in glyphosate broth were harvested by centrifugation, washed, and assayed for the production of $^{14}$CO₂.
Sarcosine oxidation enzyme assays

PG2982 can utilize sarcosine as a sole source of nitrogen in a defined minimal medium (Figure 10), and there is a sarcosine degrading activity present in crude extracts prepared from cells grown on glyphosate as the sole phosphorus source (Table 8). Glycine and formaldehyde are the products of this reaction, with one mole of glycine produced per mole of sarcosine utilized. This enzyme remains active after dialysis overnight in 50 mM Tris-HCl buffer (pH 7.0) and does not require the addition of cofactors or coenzymes for activity. Table 9 shows that the sarcosine oxidizing activity is approximately 6 times greater in cells grown with glyphosate as a phosphorus source than in cells utilizing inorganic phosphate as a phosphorus source. PG2982 cells utilizing sarcosine as a sole source of nitrogen had the highest sarcosine oxidase activity.

DISCUSSION

The results of this report indicate that Pseudomonas sp. PG2982 cleaves the carbon-phosphorus
Fig. 10. Growth (●) of PG2982 in media containing sarcosine as the sole nitrogen source and the disappearance (■) of sarcosine.

Cell Turbidity (Klett Units) vs. Sarcosine (mM) vs. Time (h)
TABLE 8. Sarcosine oxidase activity in PG2982 cells grown with sarcosine as the nitrogen source and glyphosate as the phosphorus source.

<table>
<thead>
<tr>
<th>assay conditions</th>
<th>activity (nmoles min$^{-1}$ mg$^{-1}$ protein)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Enzyme</td>
<td>0</td>
</tr>
<tr>
<td>Enzyme, sarcosine</td>
<td>3.1</td>
</tr>
<tr>
<td>Enzyme (dialyzed),</td>
<td>3.6</td>
</tr>
<tr>
<td>sarcosine</td>
<td></td>
</tr>
</tbody>
</table>
TABLE 9. Comparison of sarcosine oxidase enzyme activities from PG2982 cells grown in the presence of sarcosine, glyphosate, or inorganic phosphate.

<table>
<thead>
<tr>
<th>Growth medium</th>
<th>Activity (nmol min$^{-1}$ mg$^{-1}$ protein)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sarcosine$^a$</td>
<td>4.88</td>
</tr>
<tr>
<td>Glyphosate$^b$</td>
<td>1.84</td>
</tr>
<tr>
<td>Inorganic phosphate$^c$</td>
<td>0.30</td>
</tr>
</tbody>
</table>

$^a$ 30 mM sarcosine as the nitrogen source and 1 mM glyphosate as the phosphorus source.

$^b$ 1 mM glyphosate as the phosphorus source and 30 mM \((NH_4)SO_4\) as the nitrogen source (glyphosate broth).

$^c$ 1 mM \(K_2HPO_4\) as the phosphorus source and 30 mM \((NH_4)SO_4\) as the nitrogen source.
(C-P) bond of glyphosate, resulting in the release of a phosphate group and a molecule of sarcosine (Figure 11). The sarcosine is cleaved by a sarcosine oxidizing enzyme present in PG2982 to glycine and formaldehyde, and this glycine is used for the biosynthesis of proteins and purine bases. Approximately 50% of the formaldehyde derived from glyphosate catabolism enters into tetrahydrofolate mediated reactions, while the other 50% is oxidized to CO₂.

Evidence for cleavage of the C-P bond in glyphosate by PG2982 initially came from radiorespirometry studies performed in this lab (J.K. Moore, Masters thesis, Louisiana State University, Baton Rouge, 1983). These studies, along with radiorespirometry data from this report, show that the C-P bond carbon of glyphosate is oxidized to CO₂ as the organism utilizes glyphosate as a sole source of phosphorus. However, the distribution of [3-¹⁴C]glyphosate into various molecular fractions of PG2982 indicate that the C3 carbon is not initially released from glyphosate as CO₂. In order for radioactivity from [3-¹⁴C]glyphosate to be distributed into all of the cellular fractions examined by the modified Robert's technique, the C3 carbon of glyphosate must somehow be converted to a form which can react with tetrahydrofolate. This coenzyme is
Fig. 11. Proposed pathway for glyphosate degradation by *Pseudomonas* sp. PG2982.
responsible for the transfer of single-carbon compounds in a variety of cellular reactions such as the addition of carbons 2 and 8 to the purine ring and biosynthesis of the amino acids methionine and serine. Formaldehyde and formate are single-carbon compounds which can react with tetrahydrofolate, and both of these compounds can be oxidized to CO$_2$ by PG2982. Our evidence indicates that C3 of glyphosate is released as a formaldehyde molecule when [3-$^{14}$C]sarcosine derived from [3-$^{14}$C]glyphosate is cleaved by the sarcosine oxidizing enzyme of PG2982. This formaldehyde can then react with tetrahydrofolate to be used for the biosynthesis of methionine and serine (Table 3) as well as adenine, guanine, and thymine (Table 2). Approximately 50% of this formaldeyde is oxidized to CO$_2$.

We do not know at this time whether formaldehyde is converted to formate before being oxidized to CO$_2$. However, if this were the case formate could still react with tetrahydrofolate and also be oxidized to CO$_2$. The distribution of radioactivity into molecular fractions of PG2982 would be similar under these conditions.

PG2982 did not oxidize C1 or C2 of glyphosate to CO$_2$. This was not the case for soil microorganisms as reported by Rueppel et al. (46). They found that
the microflora from a variety of soil types oxidized up
to 50% of C1 and C2 of glyphosate in 28 days. Although
no C1 and/or C2 containing intermediates were found in
these soil samples, it was suggested that glyphosate
was being degraded to glyoxylate. Glyoxylate can be
readily metabolized to CO₂ via the glyoxylate and
citric acid cycles (34).

The distribution of [1,2-¹⁴C]glyphosate in PG2982
cells after fractionation by the modified Robert's
technique was very similar to that found by Jacob et
al. (26) in their study using [1-¹³C]glyphosate. We
found that proteins and cell wall material received
approximately 70% of the label, and that the amino
acids glycine and serine contained most of the
radioactivity. Analysis of the nucleic acid bases
revealed that only the purines were labeled, which is
exactly what Jacob et al. (26) found in their study.
Glycine contributes to purine biosynthesis by providing
carbons 4 and 5 as well as the nitrogen at position 7
of the purine ring (35). Although we did not determine
the position of the label in the purine bases, the
absence of radioactivity in the pyrimidine bases along
with the extensive labeling of glycine and serine
residues in proteins of PG2982 supported a pathway of
glyphosate metabolism in which the glycine moiety is
released intact from the glyphosate molecule. This
pathway was confirmed by the isolation of $[^{14}\text{C}]$glycine from PG2982 cells which were metabolizing $[1,2-{^{13}\text{C}}]$glyphosate.

Jacob et al. have published the only other report in which glyphosate metabolism by PG2982 has been investigated (26). They performed solid-state NMR experiments in order to determine the ultimate fate of the carbon and nitrogen atoms of glyphosate in PG2982 cells. These investigators have proposed a pathway for glyphosate degradation in which glyphosate is cleaved directly to glycine. However, since no intermediates were found in their study, Jacob et al. could not determine the product of the first step in the metabolism of glyphosate. Based on the isolation of $[3-{^{14}\text{C}}]$sarcosine, the presence of a sarcosine oxidizing activity in crude extracts of PG2982, and the labeling patterns obtained by this lab, we propose that C-P bond cleavage is the first step in the degradation of glyphosate by PG2982. This results in cleavage of glyphosate directly to sarcosine, which is then degraded to formaldehyde and glycine. The ability of PG2982 to oxidize formaldehyde and formate to CO$_2$ lend support to our proposed pathway. Studies with soil and sewage microorganisms have shown that aminomethylphosphonic acid, not sarcosine, is the major metabolic intermediate of glyphosate degradation in
those environments (4, 46, 57). Therefore it appears that the glyphosate degradation pathway used by PG2982 is unique to this pure culture.

The successful isolation of $[3-^{14}\text{C}]$sarcosine as an intermediate in the degradation of glyphosate by PG2982 required the addition of unlabeled sarcosine to the resuspension medium. This technique is similar to the pulse chase experiments used to study metabolic pathways in which the concentration of intermediates is low. In our study, the high concentration of sarcosine in the resuspension medium served to increase the concentration of sarcosine in the cellular pool. This presumably resulted in an intracellular sarcosine concentration that was high enough to prevent the metabolism of all the $[3-^{14}\text{C}]$glyphosate derived from $[3-^{14}\text{C}]$sarcosine. Likewise, the detection of $^{14}\text{C}]$glycine as an intermediate required the presence of an inhibitor of protein synthesis, chloramphenicol, in order to allow $^{14}\text{C}]$glycine derived from $[1,2-^{14}\text{C}]$glyphosate metabolism to accumulate to detectable levels. Without chloramphenicol, protein synthesis decreased the intracellular pool of glycine below a detectable level.

Tsuru et al. found that sarcosine dehydrogenase (EC 1.5.99.1) activity in $P.$ putida was induced by sarcosine, betaine, or dimethylglycine (58). Sarcosine
oxidation by PG2982 appears to be regulated by sarcosine levels in the cell (Table 9). Cells growing on ammonium salts and inorganic phosphate have a very low level of sarcosine oxidase activity. But when glyphosate replaces inorganic phosphate as a phosphorus source, the sarcosine oxidase levels are increased over 6 fold. Glyphosate metabolism produces sarcosine as an intermediate, and this sarcosine serves to stimulate sarcosine oxidase activity. The high levels of sarcosine oxidase activity (Table 9) measured in PG2982 cells utilizing sarcosine as a nitrogen source and glyphosate as a phosphorus source support our hypothesis that sarcosine plays a role in regulating its own degradation.

We suggest that the sarcosine degrading activity in PG2982 is due to the enzyme sarcosine oxidase (sarcosine:oxygen oxidoreductase [demethylating]EC 1.5.3.1). The other sarcosine degrading enzyme, sarcosine dehydrogenase (EC 1.5.99.1), requires an electron acceptor for maximal activity (42). Table 8 shows that the sarcosine degrading activity from PG2982 does not require an electron acceptor and is not decreased after an overnight dialysis— a treatment that removes loosely bound cofactors with a molecular weight of less than 10,000.
PG2982 cells prefer using inorganic phosphate over glyphosate as a source of phosphorus (Figure 5). This preference for inorganic phosphate was also reported for a Bacillus cereus strain which was grown in a medium containing inorganic phosphate and aminoethylphosphonate (44). The metabolism of glyphosate by PG2982 is inhibited by inorganic phosphate at the transport level, a phenomenon that has been reported before in studies with PG2982 (J. K. Moore, Masters thesis, Louisiana State University, Baton Rouge, LA). However, the addition of inorganic phosphate does not immediately inhibit the oxidation of C3 of glyphosate to CO₂ (Figure 6). Instead, there is a decrease in CO₂ production as the cells oxidize intracellular glyphosate via the pathway described in Figure 11. Moore has suggested that inorganic phosphate inhibits glyphosate transport because both compounds are transported by the same phosphate uptake system (J. K. Moore, Masters thesis, Louisiana State University, Baton Rouge, LA). The transport system for aminoethylphosphonate (AEPn) is induced by AEPn but is not inhibited by inorganic phosphate (44). However, inorganic phosphate does suppress the induction of the AEPn transport system (44). Leifer et al. have reported that 3,4-dihydroxybutyl-1-phosphonate (DHPB), an analogue of glycerol-3-phosphate, is actively
transported by the sn-glycerol-3-phosphate transport system of *E. coli* strain 8 (36). A separate study by Holden et al. produced a mutant strain of *Streptococcus faecalis* whose inability to transport 2-amino-3-phosphonopropionic acid (APP) was linked to an ineffective dicarboxylic amino acid transport system (24). Additional studies with transport mutants of PG2982 are needed to determine which transport system the cell uses to take up glyphosate.
Part II. The degradation of phosphonates by

*Pseudomonas* sp. PG2982.

60
INTRODUCTION

Phosphonates are a class of organic phosphorus compounds which possess a carbon-phosphorus (C-P) bond. This C-P bond is resistant to chemical hydrolysis, thermal decomposition, and photolysis (17). The most prominent naturally occurring organophosphonate, 2-aminoethylphosphonate (2-AEPn), was originally identified in the membrane lipid of rumen protozoa (21).

Research involving the metabolism of synthetic phosphonates by microorganisms has intensified in recent years because of their extensive use as antibiotics, herbicides, and adhesives (21). Although these metabolic studies have shown that many bacteria are capable of utilizing phosphonates as sole phosphorus sources, La Nauze et al. (32) have provided the only report in which a "phosphonatase" enzyme capable of cleaving the C-P bond has been purified and characterized.

In a previous report, Moore et al. (39) characterized a strain of bacteria isolated from a stock culture of Pseudomonas aeruginosa ATCC 9027 which utilizes the phosphonate herbicide glyphosate [N-(phosphonomethyl)glycine] as a sole phosphorus source. In their report, Moore et al. (39) also showed
that this organism, designated PG2982, could utilize aminomethylphosphonate (AMPn) as a sole phosphorus source with a growth rate slightly slower than that obtained on glyphosate. Although glyphosate is completely degraded by microbes in the soil (41,46), PG2982 represents one of two pure cultures of bacteria that have been shown to degrade glyphosate. The other isolate, a Flavobacterium species, was recently described by Balthazor and Hallas (4).

The present study was designed to determine whether PG2982 could utilize phosphonate compounds other than glyphosate and AMPn as sole sources of phosphorus. The efficiency of utilization of 10 organophosphonate compounds was determined. In addition, degradation products from the metabolism of two phosphonates were identified. The data indicates that PG2982 can degrade a wider range of structurally different phosphonates than any organism described to date.

MATERIALS AND METHODS

Organism and culture conditions. The glyphosate degrading Pseudomonas sp. strain PG2982 used in the present study was isolated and partially characterized
in a previous report (39). The medium used for the maintenance and growth of PG2982 was also described in that report (39). For growth studies, phosphonates were added to a concentration of 0.5 mM as the sole phosphorus source, and all media were adjusted to pH 7.5 before autoclaving. The cultures (50 ml), contained in 300-ml side-arm flasks, were inoculated (1%, vol/vol) with a culture of PG2982 grown on the phosphonate compound to be tested and incubated at 30°C on an orbital shaker (300 rpm). After 48 h, samples were assayed for turbidity (Klett-Summerson colorimeter, red filter-660nm), protein (37), extracellular Pi, and total phosphorus. A culture with Pi as phosphorus source served as a control. Sterile controls assayed for Pi before autoclaving and at the end of the experiment confirmed that there was not any nonbiological breakdown to Pi.

For the degradation studies, cultures were grown in 120 ml rubber septum sealed serum bottles containing 5 ml of Dworkin-Foster salt solution (39) and various concentrations of sterile phosphonate. A 100 ul inoculum was provided by a PG2982 culture pregrown on either methylphosphonate or phenylphosphonate. These cultures were incubated for 3 days at 30°C on an orbital shaker (250 rpm).
Identification of phosphonate degradation products.
The headspace of each serum bottle was sampled with a
gas tight syringe (Precision Sampling Corp., Baton
Rouge, LA) and injected into a Perkin Elmer 3920B gas
chromatograph. Culture fluid was assayed by
centrifuging 0.1 ml at 8,740 x g for 10 min and
injecting 10 ul of the clear supernatant into the gas
chromatograph. Separation of phosphonate degradation
products was achieved on a stainless steel Porapak N
column (6'x 1/8", Teklab). For the detection of
benzene, the carrier gas was helium (35 ml/min) and the
oven temperature was set at 180°C. Standards were
prepared by adding a 1 ul of 100% benzene to a 120 ml
serum bottle, sealing it, and allowing the benzene to
volatilize into the headspace. For the detection of
methane, the Porapak N column was heated to 55°C using
the same carrier gas and flow rate for benzene
analysis. The methane standard was prepared by adding
100% methane gas to a 120 ml serum bottle with a gas
tight syringe.

Analytical methods. Glyphosate and other amine
containing phosphonates were measured with a Beckman
120C amino acid analyzer as described by Moore et al.
(39). Cell protein at zero time was calculated from
the corresponding values in the culture used for
inoculation by the method of Lowry et al. (37).
Inorganic phosphate (2) and total phosphorus (10) were assayed in the extracellular fluid obtained after centrifugation at 8,740 x g in a Beckman microfuge.

Enzyme assays. Crude cell-free extracts were prepared from washed PG2982 cells grown on either methylphosphonate, phenylphosphonate, or glyphosate as the sole phosphorus source. Two liters of cells were centrifuged at 12000 x g and washed twice with Dworkin-Foster salts solution (39) before resuspension in 50 mM Tris-HCl buffer (pH 7.0). The cells were broken by a single pass through a French Pressure cell under 20,000 lbs pressure and centrifuged at 20000 x g (4°C) for 20 min to sediment unbroken cells and cell wall material. The supernatant was collected and kept on ice until the assays were run (always within 30 min). The assay for either methylphosphonate or phenylphosphonate degradation was as follows: portions of the crude extract (0.1 to 5 ml contained in 60 ml serum bottles) were added to 50 mM Tris-HCl buffer (pH 7.0) and supplemented with a final phosphonate concentration ranging from 0.05 to 100 mM. The serum bottles were then sealed and incubated at 32°C for up to 24 hr. Samples of the headspace were removed periodically and subjected to gas chromatographic analysis as described above. The assay for glyphosate degradation was the same except that activity was
monitored by sampling the suspension for glyphosate dissappearance according to the method described by Moore et al. (39).

RESULTS

Utilization of phosphonates

Table 1 lists data showing the utilization efficiency of 10 different organophosphonate compounds by PG2982. PG2982 utilized 100% of the Pi and produced 1.40 kg of protein per mol of phosphorus consumed. Phosphonates which were 100% utilized after 2 days of incubation included glyphosate, aminomethylphosphonate, 2-aminoethylphosphonate, 3-aminopropylphosphonate, phosphonoacetate, and 2-carboxyethylphosphonate. This organism utilized glyphosate and AMPn as efficiently as Pi, whereas 2-AEPn, 3-aminopropylphosphonate, 2-carboxyethylphosphonate, phenylphosphonate, and phosphonoacetate utilization was more efficient. The final Pi and final phosphonate concentrations were about equal after 2 days of incubation.

Utilization of 1-aminobutylphosphonate was not as efficient as the other organophosphonates, resulting in the production of only 1.10 kg of protein per mol of phosphorus consumed. Longer incubation (4 days) resulted in complete utilization, but the efficiency
### Table 1. Utilization of phosphonates* by PZ2047

<table>
<thead>
<tr>
<th>Days of Incubation</th>
<th>Phosphonate Source</th>
<th>Phosphonate Phosphate Initial</th>
<th>Phosphonate Phosphate Final</th>
<th>Pi Initial</th>
<th>Pi Final</th>
<th>Increase in protein (μg/ml)</th>
<th>Increase in protein / A. phosphorolumn (μg/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>2</td>
<td>Pi</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>702</td>
<td>1.39</td>
</tr>
<tr>
<td>2</td>
<td>Glyphosate</td>
<td>502</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>702</td>
<td>1.40</td>
</tr>
<tr>
<td>2</td>
<td>AMPn</td>
<td>503</td>
<td>0</td>
<td>5</td>
<td>1</td>
<td>714</td>
<td>1.41</td>
</tr>
<tr>
<td>2</td>
<td>1-AEPn</td>
<td>475</td>
<td>393</td>
<td>10</td>
<td>0</td>
<td>40</td>
<td>0.43</td>
</tr>
<tr>
<td>4</td>
<td>1-AEPn</td>
<td>475</td>
<td>94</td>
<td>10</td>
<td>0</td>
<td>354</td>
<td>0.91</td>
</tr>
<tr>
<td>2</td>
<td>2-AEPn</td>
<td>512</td>
<td>0</td>
<td>2</td>
<td>0</td>
<td>740</td>
<td>1.44</td>
</tr>
<tr>
<td>2</td>
<td>3-AEPn</td>
<td>493</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>710</td>
<td>1.44</td>
</tr>
<tr>
<td>2</td>
<td>1-ABPn</td>
<td>509</td>
<td>71</td>
<td>4</td>
<td>6</td>
<td>482</td>
<td>1.11</td>
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<tr>
<td>4</td>
<td>1-ABPn</td>
<td>509</td>
<td>0</td>
<td>4</td>
<td>2</td>
<td>577</td>
<td>1.13</td>
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<tr>
<td>2</td>
<td>PnF</td>
<td>500</td>
<td>275</td>
<td>8</td>
<td>0</td>
<td>74</td>
<td>0.32</td>
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<tr>
<td>4</td>
<td>PnF</td>
<td>500</td>
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<td>8</td>
<td>0</td>
<td>482</td>
<td>0.95</td>
</tr>
<tr>
<td>2</td>
<td>PnAc</td>
<td>491</td>
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<td>5</td>
<td>2</td>
<td>744</td>
<td>1.51</td>
</tr>
<tr>
<td>2</td>
<td>2-CEPn</td>
<td>520</td>
<td>2</td>
<td>2</td>
<td>0</td>
<td>772</td>
<td>1.48</td>
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<tr>
<td>2</td>
<td>PhenylPn</td>
<td>512</td>
<td>4</td>
<td>1</td>
<td>8</td>
<td>772</td>
<td>1.54</td>
</tr>
</tbody>
</table>

* Abbreviations: Pi - inorganic phosphate; AMPn - aminomethylphosphonate; 1-AEPn - 1-aminomethylphosphonate; 2-AEPn - 2-aminomethylphosphonate; 3-APPn - 3-aminopropylphosphonate; 1-ABPn - 1-aminobutylphosphonate; PnF - phosphonoformate; PnAc - phosphonoacetate; 2-CEPn - 2-carboxyethylphosphonate; PhenylPn - phenylphosphonate.
did not increase. Phosphonoformate (PnF) and 1-AEPn did not support much growth, as shown by the low protein production in Table 1. After 2 days, only 45% of the PnF and 17% of the 1-AEPn had been utilized by PG2982, but if growth was allowed to continue for an additional 2 days, PnF was 100% utilized and 1-AEPn was 80% utilized. Although the utilization efficiency for PnF and 1-AEPn increased two to three-fold during the additional 2-day incubation time, their values never approached those of the other phosphonates (Table 1).

Degradation products from phosphonate metabolism

Gas chromatographic analysis of the headspace from a PG2982 culture utilizing phenylphosphonate as a sole phosphorus source yielded a peak which eluted from the column at 15 min. This compound comigrated with authentic benzene through the Porapak N column, leading to the identification of this volatile product of phenylphosphonate degradation as benzene.

Table 2 shows that nearly stoichiometric amounts of benzene are produced from phenylphosphonate degradation. Methane was detected in the headspace of PG2982 cultures degrading methylphosphonate (Table 2). Like benzene, methane was produced in nearly stoichiometric amounts.
Table 2. Production of benzene and methane from phosphonates by PG2982.

<table>
<thead>
<tr>
<th>phosphorus source</th>
<th>phosphorus supplied (umol)</th>
<th>product formed (umol)</th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>benzene</td>
<td>methane</td>
<td></td>
</tr>
<tr>
<td>Phenylphosphonate</td>
<td>1.0</td>
<td>0.92</td>
<td>0.0</td>
<td></td>
</tr>
<tr>
<td></td>
<td>0.5</td>
<td>0.49</td>
<td>0.0</td>
<td></td>
</tr>
<tr>
<td>Methylphosphonate</td>
<td>1.0</td>
<td>0.0</td>
<td>0.96</td>
<td></td>
</tr>
<tr>
<td></td>
<td>0.5</td>
<td>0.0</td>
<td>0.51</td>
<td></td>
</tr>
</tbody>
</table>
Enzyme assay for C-P bond cleavage. No enzyme activity capable of cleaving the C-P bond was found in extracts prepared from either methylphosphonate, phenylphosphonate, or glyphosate grown cells. The addition of various cofactors and coenzymes did not produce any activity either, and pH ranges from 4 to 10 were not helpful.

DISCUSSION

Moore et al. (39) showed that PG2982 can utilize AMPn as a phosphorus source with a growth rate similar to that obtained on glyphosate. The production of equivalent amounts of protein by PG2982 in this study after 48 h of growth on these two phosphonates correlates well with their findings. The phosphonates which were utilized more efficiently than glyphosate and Pi (2-AEPn, 3-APPn, 2-CEPn, phenylphosphonate, and phosphonoacetate) do not have much in common structurally. This makes it difficult to determine why these phosphonates are utilized more efficiently by PG2982. It is possible that the degradation products from each of these phosphonates could either inhibit or stimulate the growth of this organism. For example, if acetate were the product of phosphonoacetate
degradation, growth could be stimulated due to utilization of the acetate. The five phosphonates in Table 1 which were utilized more efficiently than Pi and glyphosate were completely utilized. In addition the final concentrations of Pi, glyphosate, and these five phosphonates were about equal after 2 days incubation. This result indicates that the five phosphonates were transported as efficiently as glyphosate and Pi.

It is significant that PG2982, like many other organisms, is capable of utilizing 2-AEPn as a phosphorus source. This compound is utilized by all bacteria that are capable of phosphonate degradation (23), and the prevalence of 2-AEPn in the environment presumably contributes to this ubiquitous degradation.

There has been a report by Cook et al. describing a P. putida strain which can utilize AEPn as a source of carbon, nitrogen, and phosphorus (9), and some organisms can use AEPn as a sole source of nitrogen and phosphorus (50). PG2982 failed to utilize any of the 10 phosphonates listed in Table 1 as a sole source of carbon or nitrogen. These compounds also failed to support growth when acting as a source of nitrogen and phosphorus. Since Pi inhibits the transport of glyphosate into PG2982 cells (J.K. Moore, M.S. thesis, Louisiana State University, Baton Rouge,
all of the phosphonates were tested as sole sources of carbon and phosphorus. No growth was observed in these experiments, and none of the nitrogen-containing phosphonates could serve as a sole source of nitrogen and phosphorus.

PG2982 can cleave the C-P bond of phenylphosphonate and methylphosphonate. The products of this reaction, benzene and methane, are easily detected in the headspace of serum bottle-grown cultures (Table 2). Cook et al. has reported that a Klebsiella pneumoniae isolate can produce benzene from C-P bond cleavage of ionic methyl phenylphosphonate (11). Methane production from methylphosphonate degradation has also been described for a Pseudomonas testosteroni strain which can utilize methyl, ethyl, or propylphosphonate as a sole source of phosphorus (12).

Figure 1 shows the degradation schemes for methyl and phenylphosphonate. The exact mechanism of the reaction is not known, but it is obvious that the C-P bond carbon is reduced in both compounds to yield methane and benzene. C-P bond cleavage has been widely reported in the literature for a variety of bacterial strains (1,4,6,11-13,48,50). It has been suggested that C-P bond cleavage is a hydrolysis reaction in which the -OH and H of water are incorporated into
Fig. 1. Degradation of methylphosphonate and phenylphosphonate by PG2982.
phosphate and carbon, respectively (50). La Nauze et al. have reported that the phosphonatase enzyme (EC 3.11.1.1) that cleaves the C-P bond of phosphonoacetaldehyde (releasing acetaldehyde and Pi) utilizes a molecule of water during catalysis (33). In the same report, these investigators provided evidence for imine formation between a lysine group on the enzyme and the carbonyl carbon of phosphonoacetaldehyde.

PG2982 is a unique organism in that it is capable of degrading a much wider range of phosphonates than any organism described to date. This study has shown that alkylphosphonates of various chain lengths, 1-amino-substituted alkylphosphonates, amino-terminal phosphonates, and an arylphosphonate can all serve as a sole source of phosphorus for PG2982. The enzyme responsible for C-P bond cleavage is either very unstable or requires a different environment for activity than provided in this study.
LITERATURE CITED


VITA

Dean Lee Shinabarger was born October 16, 1960 in Verdun, France. He lived in France and Germany for the first 4 years of his life before moving to North Carolina in 1964. His family then traveled across the country to California and then on to Kansas in 1967. In May of 1978, he graduated from Santa Fe Trail High School near Carbondale, Kansas. He was awarded a Bachelor of Science degree from Emporia State University, Emporia, Kansas in May, 1982. In August of 1982, he entered the Department of Microbiology at Louisiana State University in pursuance of a Ph.D. degree. He is presently a candidate for the Doctor of Philosophy degree in Microbiology in December, 1986.
DOCTORAL EXAMINATION AND DISSERTATION REPORT

Candidate: Dean Shinabarger

Major Field: Microbiology

Title of Dissertation: Glyphosate Catabolism by Pseudomonas sp. PG2982

Approved:

[Signatures]

Major Professor and Chairman

Dean of the Graduate School

EXAMINING COMMITTEE:

[Signatures]

Date of Examination:

Friday, September 5, 1986