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Isolation and Characterization of a Mutant of *Arthrobacter* sp. Strain GLP-1 Which Utilizes the Herbicide Glyphosate as Its Sole Source of Phosphorus and Nitrogen

RÜDIGER PIPKE† AND NIKOLAUS AMRHEIN‡*

Lehrstuhl für Pflanzenphysiologie, Ruhr-Universität Bochum, D-4630 Bochum, Federal Republic of Germany

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***Arthrobacter* sp. strain GLP-1, grown on glucose as a carbon source, utilizes the herbicide glyphosate [*N*-(phosphonomethyl)glycine] as its sole source of phosphorus as well as its sole source of nitrogen. The mutant strain GLP-1/Nit-1 utilizes glyphosate as its sole source of nitrogen as well. In strain GLP-1, P_i was a potent competitive inhibitor of glyphosate uptake (K_i, 24 μM), while the affinity of P_i for the uptake system of strain GLP-1/Nit-1 was reduced by 2 orders of magnitude (K_i, 2.3 mM). It is concluded that the inability of strain GLP-1 to utilize glyphosate as a source of nitrogen is due to the stringent control of glyphosate uptake by excess phosphate released during the degradation of the herbicide.**

The degradation of natural and synthetic organophosphonates by microorganisms is receiving considerable attention from environmental microbiologists and biochemists. Organophosphonates, possessing the stable carbon-to-phosphorus (C-P) bond, are increasingly released into the environment as pesticides, antibiotics, flame retardants, and the like (2, 5), necessitating investigations of their metabolic fate.

Alkyl, i.e., sarcosine, formation has been demonstrated to be involved in the degradation of the widely used organophosphonate herbicide glyphosate [*N*-(phosphonomethyl) glycine] by *Pseudomonas* sp. strain PG 2982 (8, 14) and *Arthrobacter* sp. strain GLP-1 (12) (Fig. 1, pathway I). An alternative pathway for glyphosate degradation, via the intermediate formation of aminomethylphosphonate, in which the C–P bond is still conserved, has been found in a *Flavobacterium* sp. (1) and in *Arthrobacter atrocyaneus* (11) (Fig. 1, pathway II). In all of the bacteria capable of C-P bond cleavage studied to date, C–P lyase activity appears to be expressed only during phosphate starvation (10, 12, 13, 16, 17). Repression of glyphosate degradation, as well as inhibition of glyphosate uptake by P_i (13), may explain the inability of *Pseudomonas* sp. strain PG 2982 and *Arthrobacter* sp. strain GLP-1 to utilize glyphosate as the sole source of nitrogen (12, 15), because utilization of the nitrogen of glyphosate for growth will result in the excess formation of P_i. The analysis of the mutant strain *Arthrobacter* sp. strain GLP-1/Nit-1 described here confirms this interpretation.

Isolation, growth, and maintenance of *Arthrobacter* sp. strain GLP-1 have been described previously (12). For the isolation of mutants capable of using glyphosate as the sole source of phosphorus and nitrogen, the previously used Tris hydrochloride buffer was replaced by 0.1 M maleate—NaOH, pH 6.5. In addition, NH_4Cl was omitted and 10 mM glyphosate was added to the medium. To ensure the complete absence of usable nitrogen in the solidified medium, 1.5% SeaPlaque agarose (FMC Corp., Marine Colloids Div., Rockland, Maine) was used. *N*-Nitro-*N'*-nitrosoguanidine was used in the chemical mutagenesis experiments (3). For the identification of glyphosate in cell extracts of *Arthrobacter* sp. strain GLP-1, 17 kBq of [3-^{14}C]glyphosate (final concentration, 40 μM) was added to 10^9 cells in the logarithmic growth phase in a total volume of 200 μl and the mixture was incubated for 5 min at 30°C. The bacteria were sedimented and washed five times with 0.5 ml of unlabeled glyphosate (25 mM) until no radioactivity was detectable in the medium. After disruption of the cells by sonication, acetone (final concentration, 80%) was added. Material which precipitated overnight was removed by centrifugation, and the supernatant was subjected to thin-layer chromatography (12) followed by autoradiography. All other procedures have been described previously (13).

Arthrobacter sp. strain GLP-1, which had been isolated by enrichment culture on a medium containing glyphosate as the sole source of phosphorus (11), was mutagenized with *N*-nitro-*N'*-nitrosoguanidine to obtain mutants capable of growth in medium containing glyphosate as the only nitrogenous compound. Two mutants, designated GLP-1/Nit-1 and GLP-1/Nit-2, were found to grow on solidified and in liquid media. The frequency of mutation was approximately 10^{-9}. Because of the less vigorous growth of strain GLP-1/Nit-2, all subsequent experiments were carried out with strain GLP-1/Nit-1. The growth of strain GLP-1/Nit-1 in medium containing 10 mM glyphosate as the sole source of phosphorus and nitrogen is shown in Fig. 2. The correlation between growth, disappearance of glyphosate from the medium, and appearance of P_i in the medium is evident. It can be seen that nearly 90% of the glyphosate phosphorus is released as P_i, resulting in a final concentration of almost 9 mM.

When strain GLP-1/Nit-1 was grown in increasing concentrations of either NH_4Cl or glyphosate as the source of nitrogen and 1 mM P_i as the source of phosphorus, equivalent cell densities were obtained with equimolar concentrations of the two compounds, indicating that both nitrogen sources were utilized to the same extent (Fig. 3). However, an extended lag phase and generation time were observed in the medium containing glyphosate.

* Corresponding author.

† Present address: Gesellschaft für Biotechnologische Forschung, Abteilung Mikrobiologie, D-3300 Braunschweig, Federal Republic of Germany.

‡ Present address: Institut für Pflanzenwissenschaften, Biochemie und Physiologie der Pflanzen, ETH Zürich, Sonneggstrasse 5, CH-8092 Zurich, Switzerland.

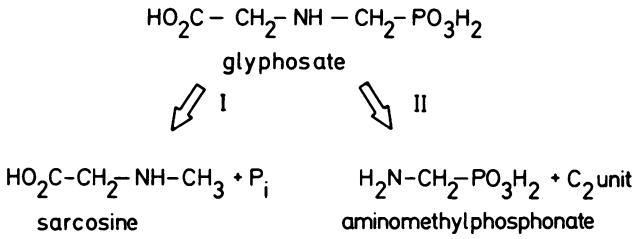

FIG. 1. Known pathways for the degradation of the phosphonate herbicide glyphosate. Pathway I is utilized by *Pseudomonas* sp. strain PG 2982 (8, 14) and *Arthrobacter* sp. GLP-1 (12), while a *Flavobacterium* sp. (1) and *A. atrocyaneus* (11) degrade glyphosate via pathway II.

Because glyphosate uptake is suppressed by P_i in *Arthrobacter* sp. strain GLP-1 (13), we examined the uptake of the herbicide by strain GLP-1/Nit-1 grown in medium containing either glyphosate or P_i as the source of phosphorus (Table 1). After growth on 10 mM glyphosate as a source of both nitrogen and phosphorus, GLP-1/Nit-1 took up glyphosate with a K_m of 120 μM and a V_{max} of 0.20 nmol · min^{-1} · 10^{-8} $cells^{-1}$. These data equal those previously obtained for strain GLP-1 grown under the same conditions except with NH_4Cl as the source of nitrogen (13). More than 95% of the radioactivity recovered from the extracts of *Arthrobacter* sp. strain GLP-1 cells, which had taken up labeled glyphosate and had been thoroughly washed prior to extraction, were found still to reside in unmetabolized glyphosate. Therefore, true uptake of glyphosate was being measured. However, whereas no uptake of glyphosate occurred when strain GLP-1 was grown in the presence of 5 mM P_i, strain GLP-1/Nit-1 took up the herbicide with a K_m of 105 μM and a V_{max} of 0.59 nmol · min^{-1} · 10^{-8} $cells^{-1}$, indicating a slight derepression of the glyphosate uptake system under these conditions. More important, the K_i for competitive inhibition of glyphosate uptake by P_i differed widely in the two strains (24 μM in strain GLP-1 and 2.3 mM in strain GLP-1/Nit-1; Table 1). Thus, the nearly 100-fold-reduced affinity of P_i for the glyphosate uptake system apparently enables the mutant to take up and utilize the herbicide even in the presence of millimolar concentrations of P_i.

While glyphosate uptake by the two strains was comparable (Table 1), P_i uptake differed considerably (Table 2). In strain GLP-1, the high-affinity phosphate-specific transport system is derepressed when cells grow in the presence of glyphosate as the source of phosphorus (V_{max}, 2.8 μmol · min^{-1} · 10^{-8} $cells^{-1}$) and repressed during growth in P_i (V_{max}, 0.17 nmol · min^{-1} · 10^{-8} $cells^{-1}$). In contrast, no effective regulation of P_i uptake by strain GLP-1/Nit-1 occurred (growth in P_i: V_{max}, 2.2 nmol · min^{-1} · 10^{-8} $cells^{-1}$; growth in glyphosate: V_{max}, 1.8 nmol · min^{-1} · 10^{-8} $cells^{-1}$). In addition, the affinities of the two strains for P_i differed by 2 orders of magnitude (Table 2). The reduced affinity of strain GLP-1/Nit-1 for P_i suggested that this strain utilizes P_i less efficiently than does the parent strain. In fact, whereas the growth characteristics of the two strains did not differ in a medium containing 1 mM P_i and 10 mM NH_4Cl as phosphorus and nitrogen sources, respectively (data not shown), at limiting concentrations of P_i (0.1 mM), the growth rate of the mutant strain was reduced (Fig. 4).

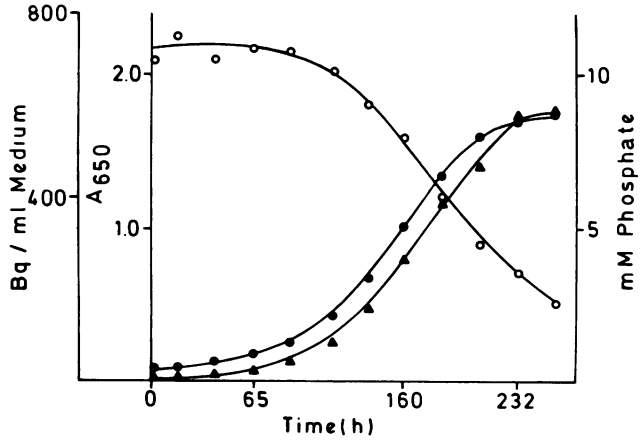

FIG. 2. Growth of *Arthrobacter* sp. strain GLP-1/Nit-1 in 10 mM ^{14}C-labeled glyphosate (specific activity, 8 kBq · $nmol^{-1}$) as the sole source of nitrogen and phosphorus. Growth (●), radioactivity (○), and P_i concentration (▲) in the medium were monitored.

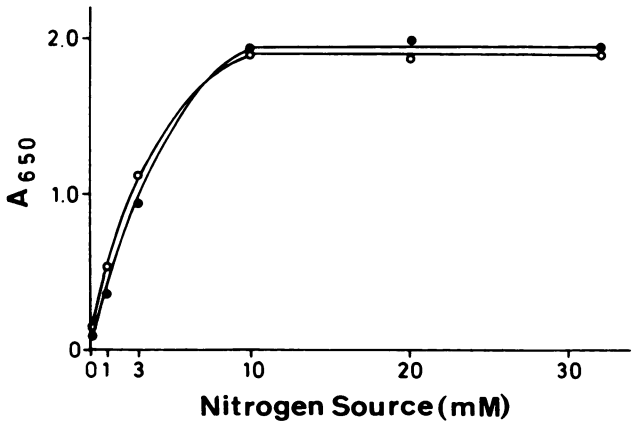

FIG. 3. Stationary-phase cell yields of *Arthrobacter* sp. strain GLP-1/Nit-1 as a function of the nitrogen concentration in the medium. The source of nitrogen was NH_4Cl (○) or glyphosate (●).

Whereas many bacteria are known to utilize aminophosphonates as the sole source of phosphorus (6, 7), the utilization of such compounds as a source of nitrogen is the exception. The naturally occurring 2-aminoethylphosphonate can serve as a source of both phosphorus and nitrogen for a strain of *Pseudomonas aeruginosa* (9) and as a carbon source as well for *Pseudomonas putida* (4). Comparable with the results obtained for the degradation of glyphosate by *Arthrobacter* sp. strain GLP-1/Nit-1 (Fig. 2), results with the *Pseudomonas* strains indicated that these bacteria release nearly all of the phosphonate phosphorus of 2-aminoethylphosphonate as P_i into the growth medium.

TABLE 1. Rates of glyphosate uptake by *Arthrobacter* sp. strains GLP-1 and GLP-1/Nit-1 as a function of the phosphorus source in the growth medium and competitive inhibition of glyphosate uptake by P_i

Strain	Phosphorus source	V_{max} (nmol · min^{-1} · 10^{-8} · $cells^{-1}$)	K_m (μM)	K_i Phosphate (μM)
GLP-1	Glyphosate[a]	0.14	125	24
	Phosphate	ND[b]	ND	
GLP-1/Nit-1	Glyphosate[c]	0.20	120	2,300
	Phosphate	0.59	105	

[a] Data for glyphosphate uptake by GLP-1 from Pipke et al. (13).
[b] ND, No glyphosate uptake detectable.
[c] Also source of nitrogen.

TABLE 2. Rates of phosphate uptake by *Arthrobacter* sp. strains GLP-1 and GLP-1/Nit-1 as a function of the phosphorus source in the growth medium

Strain	Phosphorus source	V_{max} (nmol · min^{-1} · 10^{-8} · $cells^{-1}$)	K_m (μM)
GLP-1	Glyphosate	2,800	1.6
	Phosphate	0.17	8.7
GLP-1/Nit-1	Glyphosate[a]	1.8	590
	Phosphate	2.2	830

[a] Also source of nitrogen.

As *Arthrobacter* sp. strain GLP-1 requires a P:N ratio of approximately 1:100 in the growth medium for balanced growth (R. Pipke, unpublished results), excretion of 90% of the phosphorus contained in glyphosate during growth of strain GLP-1/Nit-1 in glyphosate as the sole source of both nitrogen and phosphorus is plausible. In the parent strain, the liberation of excess P_i during glyphosate degradation will ultimately cut off the cells from their nitrogen supply due to repression and inhibition of glyphosate uptake (Table 1). Generally, bacteria can utilize organophosphonates as sources of phosphorus only in the absence of P_i and organophosphates from the growth medium (16). *Arthrobacter* sp. strain GLP-1 is no exception.

The results presented in this paper stress the importance of glyphosate uptake, and its regulation, in glyphosate degradation by *Arthrobacter* sp. strain GLP-1. Only owing to the constitutive expression of an uptake system with a greatly reduced (as compared with the parent strain) affinity for P_i, but not for glyphosate, is strain GLP-1/Nit-1 capable of using glyphosate as its sole source of nitrogen. The cost of this acquired capability is a reduced efficiency of P_i utilization (Fig. 4).

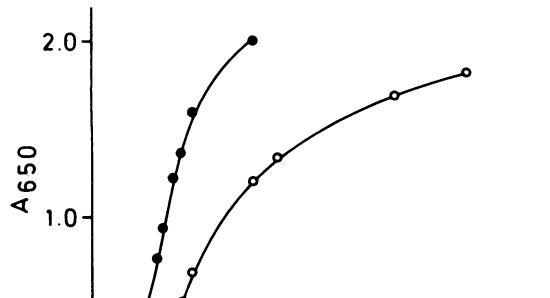

FIG. 4. Growth of *Arthrobacter* sp. strains GLP-1 (●) and GLP-1/Nit-1 (○) in a limiting (0.1 mM) concentration of P_i. The source of nitrogen was 10 mM NH_4Cl.

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