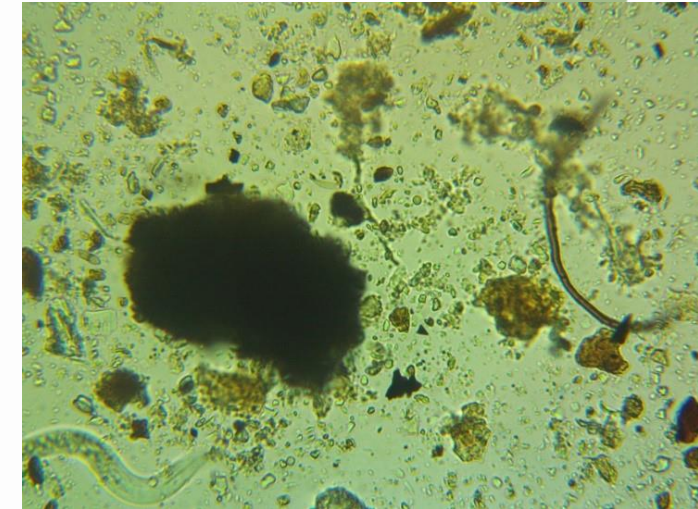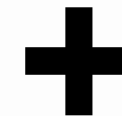# Microbes for Breaking Down Glyphosate

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SymSoil Biology

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Degradation of Glyphosate by Soil Microbes

Glyphosate (N-(phosphonome-thyl)glycine) was developed by Monsanto in the 1970s, and sold under the brand name Roundup. Today, glyphosate products are the most widespread herbicides.

Glyphosate does not breakdown in water, air or sunlight and can accumulate in natural environments. It is believed to be toxic not only to plants, but also for animals and bacteria.

While harmful to many microbes, there are a number of soil bacteria and fungi which break the chemical bonds of glyphosate.
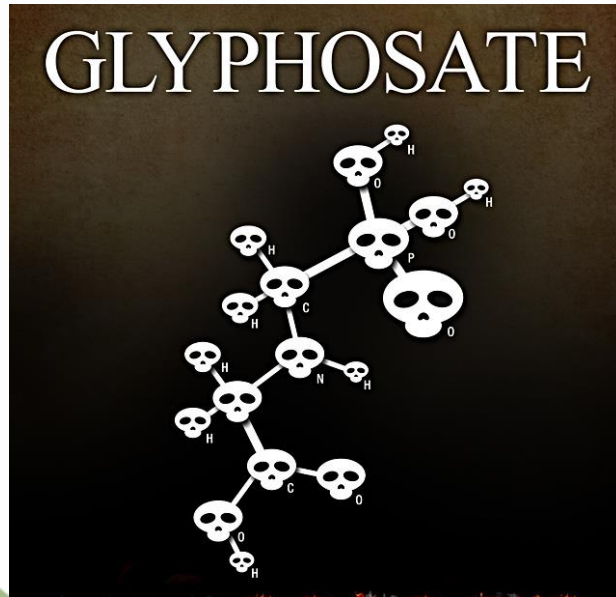
Degradation of Glyphosate Herbicides (Review)

SymSoil™ Robust Compost

SymSoil™ Robust Compost, with over 2,000 species, includes members of the Achromobacter, Flavisolibacter, Geobacillus, Pseudosprillum, Rhodobium and Streptomyces bacteria families, as well as Penicillum chrysogenum (a fungi)

GLYPHOSATE

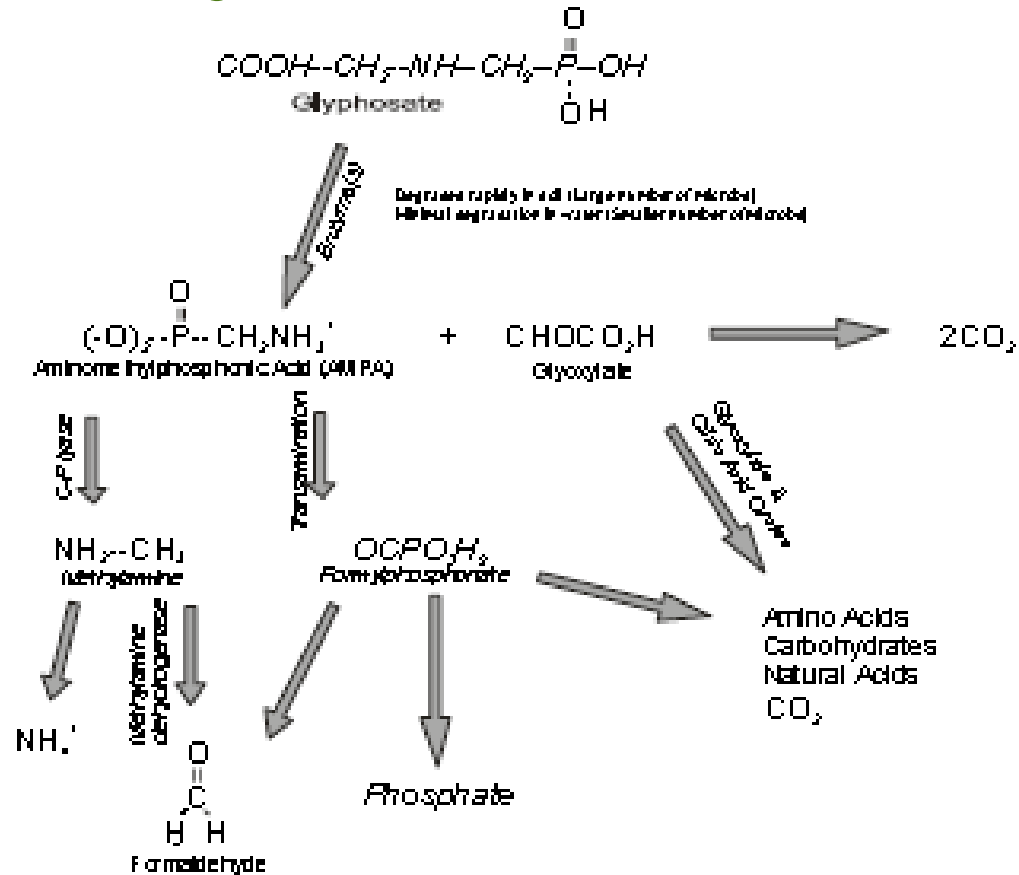**These have been shown to break glyphosate into safe compounds.**

This allows other parts of the soil biome to be reintroduced.

symsoi

Glyphosate Degradation Pathway
Published by Jeff Schuette, State of California
Department of Pesticide Regulation, November 1998

Microbial Degradation of Glyphosate Herbicides (Review)

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Abstract—This review analyzes the issues associated with biodegradation of glyphosate (N-(phosphonomethyl)glycine), one of the most widespread herbicides. Glyphosate can accumulate in natural environments and can be toxic not only for plants but also for animals and bacteria. Microbial transformation and mineralization of glyphosate, as the only means of its rapid degradation, are discussed in detail. The different pathways of glyphosate catabolism employed by the known destructing bacteria representing different taxonomic groups are described. The potential existence of alternative glyphosate degradation pathways, apart from those mediated by C—P lyase and glyphosate oxidoreductase, is considered. Since the problem of purifying glyphosate-contaminated soils and water bodies is a topical issue, the possibilities of applying glyphosate-degrading bacteria for their bioremediation are discussed.

Keywords: glyphosate, N-(phosphonomethyl)glycine, glyphosate metabolism, C—P lyase, glyphosate oxidoreductase, aminomethylphosphonic acid, bioremediation

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INTRODUCTION

A rapid growth in the diversity and production scales of synthetic phosphonates, stable compounds containing a direct carbon—phosphorus bond (C—P), has made them important environmental pollutants. The most widespread representatives of these xenobiotics are herbicides based on glyphosate (N-(phosphonomethyl)glycine, GP). GP is a unique inhibitor of 5-enolpyruvylshikimate-3-phosphate synthase (EPPS), the key enzyme of the shikimate pathway of aromatic compound biosynthesis in plants and some microorganisms. EPPS inhibition suppresses the synthesis of proteins and secondary metabolites, e.g., flavonoids, lignin, or coumarins, and deregulates energy metabolism [1, 2].

The expansion of GP-based herbicides was stimulated both by the appearance of GP-resistant transgenic varieties of the most significant agricultural species (e.g., soy, colza, maize, wheat, sugar beet, and cotton; altogether, nearly 90% of all transgenic plant cultures worldwide [1]), and by the concept of GP as a harmless substance, which was based on reports describing its rapid destruction (within 2 weeks) by aboriginal soil microbial communities [3, 4].

However, extensive use of GP resulted in its accumulation in soil and water environments [5, 6]. Moreover, negative effects of GP on animal metabolism have been reported, in particular, with respect to chronic and remote effects [7]. The main product of natural GP degradation, aminomethylphosphonic acid (AMPA), impairs the processes of DNA reparation and mRNA synthesis in both plants and animals [8—10]. At the same time, sublethal concentrations of both GP and AMPA were detected in fruits and sprouts of cultured plants [11, 12]. Finally, the initial data suggesting that GP influence on soil microbiota was insignificant [3, 13] were disproved by further studies showing that the physiological responses of soil microorganisms to GP were, in fact, highly variable [14]. For instance, microbial communities of GP-contaminated soils exhibited a higher resistance to its toxicity and differed significantly in their composition from the communities of normal soils [15].

GP biodegradation by aboriginal or introduced microorganisms can be an efficient means of its removal from soils and waters. However, the currently available data concerning the metabolic pathways of phosphonate catabolism in microorganisms, their regulation, and the properties of the key enzymes are severely limited.

Pathways of Glyphosate Catabolism

The processes of GP catabolism have mainly been studied in bacteria; the data for fungi, plants, and animals provide only preliminary information on the putative pathways of GP transformation.
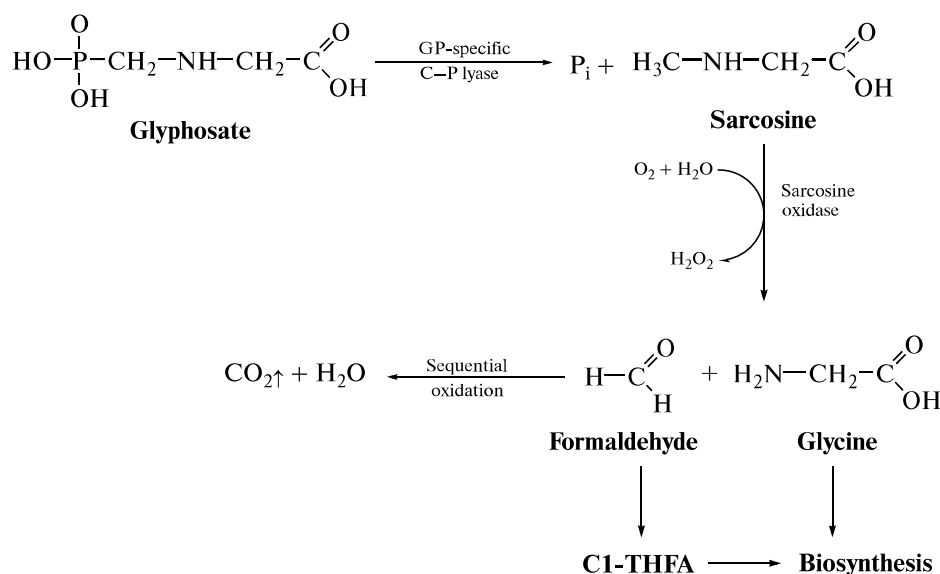
Fig. 1. C—P lyase-mediated GP metabolism [35, 51]. THFA—tetrahydrofolic acid.

Among the bacteria studied, a number of species utilize GP as a source of phosphorus, which implies that they possess enzymes cleaving the C—P bond. The few exceptions are the mutant *Arthrobacter* sp. GLP-1/Nit strain, which utilizes GP as a source of nitrogen [16], along with *Streptomyces* sp. StC [17] and *Achromobacter* sp. LW9 [18], in which GP can serve as a source of carbon. Two means of enzymatic GP digestion have been found in bacteria: direct cleavage of the C—P bond, yielding sarcosine and inorganic phosphorus (P_i) (Fig. 1), and cleavage of the C—N bond, yielding of glyoxylate and AMPA (Fig. 2). In the first case, the chemically inert C—P bond is destroyed by an intricate multienzyme complex known as C—P lyase. The activity of the best studied C—P lyase from *Escherichia coli* depends on the products of 14 genes organized into the *phn* operon. C—P lyases comprise the components responsible for phosphonate uptake by the cell, regulatory and auxiliary components, and the actual enzymes catalyzing the cleavage of the C—P bond. In fact, the latter form a metabolic pathway directing the transformation of intermediates before and, possibly, after cleavage of the C—P bond [19, 20]. Because of the extreme complexity of the C—P lyase complex and its irreversible inactivation in disintegrated cells, the mechanism of phosphonate degradation by C—P lyase remained enigmatic for nearly 30 years. It was not until recently that the first notions concerning its possible organization were formulated [21, 22]. The early concepts implied that C—P lyases were nonspecific, and the same multienzyme complex could cleave a whole range of alkyl- and aminoalkylphosphonates, including GP, to produce P_i and the corresponding (amino)carbon residue. However, it was noticed that destructor strains utilized GP via the C—P lyase pathway in a manner that was clearly different from the one observed with other

phosphonates. This fact invited a hypothesis suggesting the existence of two different C—P lyases, one that is similar to the C—P lyase from *E. coli* and specific to (amino)alkylphosphates other than GP and another that cleaves only the C—P bond of GP to produce sarcosine and P_i; the induction and functioning of the two systems had to be independent. This hypothesis was first proved in *Arthrobacter* sp. GLP-1 [23], and later the presence of a GP-specific C—P lyase that cleaves GP with production of sarcosine was demonstrated in *Achromobacter* sp. MPS 12A [24]. Apparently, bacteria possess more than two species of C—P lyases. The genome of *Pseudomonas stutzeri* was found to contain two different C—P lyase operons with different substrate specificities, and both C—P lyases were incapable of GP destruction [25].

The mechanism of GP degradation via the C—P lyase pathway is still insufficiently understood, both from the biochemical and the molecular biological point of view, and can currently be discussed only by analogy to the already known reactions of the C—P lyase pathway in *E. coli* [26]. In particular, the origin of the enzyme complex with such a narrow specificity to GP remains unclear. The ability to metabolize GP with sarcosine production was also detected in bacteria that had not previously been exposed to this compound (table), which suggests that there might exist some natural GP analogs that could serve as substrates for GP-specific C—P lyases.

Bacterial strains that possess GP-specific C—P lyases usually exhibit a very high efficiency in herbicide destruction under laboratory conditions when growing on mineral media with GP as a sole source of phosphorus. However, in natural ecosystems, their efficiency in GP degradation may drop significantly, since, in an overwhelming majority of cases, the
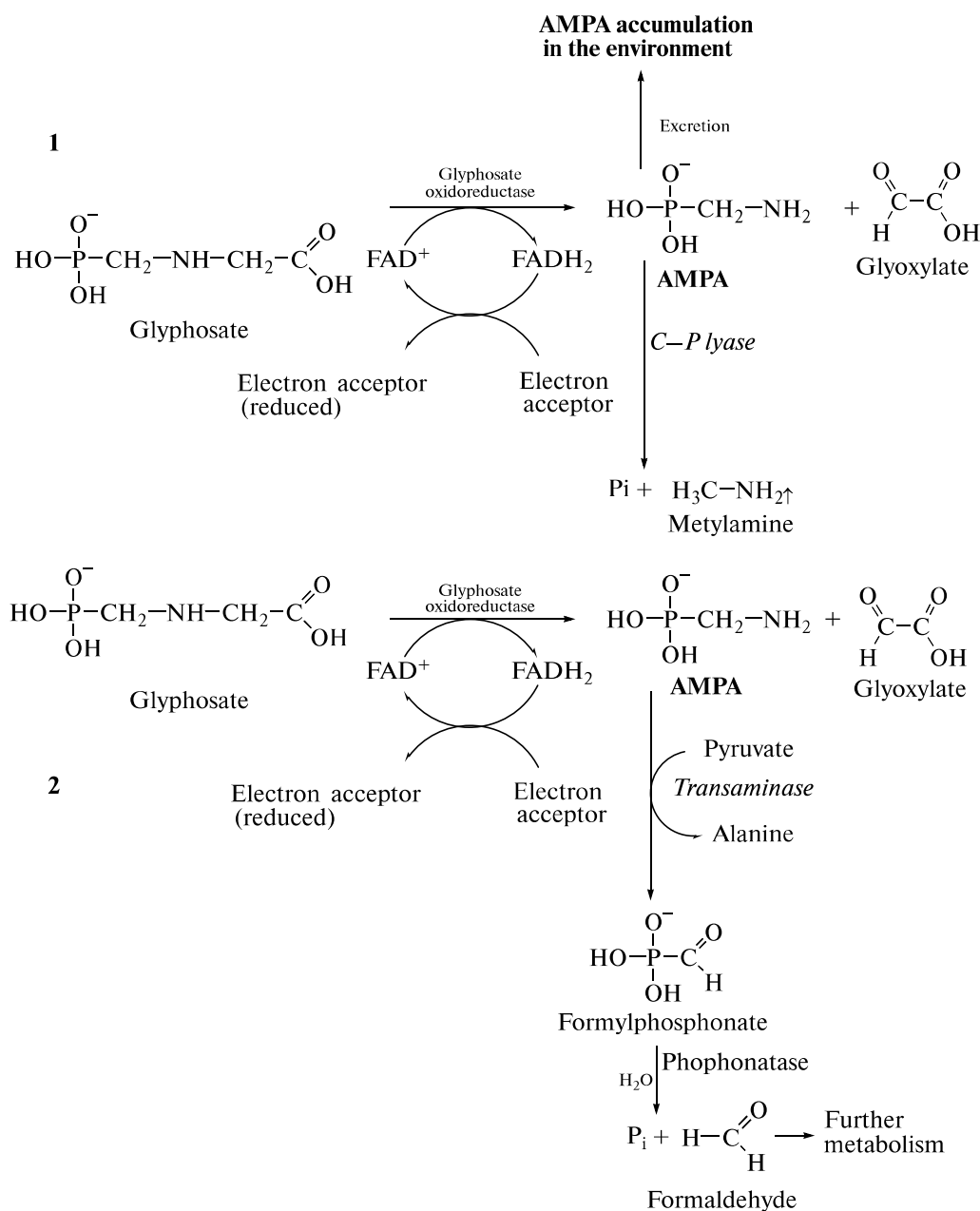
Fig. 2. GOR pathway of GP metabolism. 1, the pathway described in most known bacteria [33–36]; 2, a novel GP mineralization pathway described in *O. anthropi* GPK 3 [24, 30].

expression of the C—P lyase complex is activated only in response to intracellular P_i deficit and specific phosphorus deficiency, which is not typical for natural environments [15, 16, 27].

In the other widespread bacterial means of GP degradation, the herbicide molecule is first attacked by the enzyme known as glyphosate oxidoreductase (**GOR**) [28, 29]. Our group first described this enzyme, which cleaves the C—N bond in GP molecules, yielding stoichiometric quantities of AMPA and glyoxylate, in *Ochrobactrum anthropi* GPK 3; it was isolated in the electrophoretically pure quality,

and its catalytic properties were determined. GOR (EC 1.14.14.1) belongs to the superfamily of bacterial flavine monooxigenases and exhibits a relatively low affinity to GP ($K_M = 0.2$ mM). Its substrate specificity is relatively broad: apart from GP, the enzyme cleaved the C—N bond of iminodiacetate ($K_M = 0.1$ mM), phosphonomethyliminodiacetate ($K_M = 2.4$ mM), and, with a very low efficiency, in sarcosine, glycine, and D-alanine [30].

In most known GP-degrading bacteria, glyoxylate, as a convenient energy substrate, enters the glyoxylate bypass of the Krebs cycle [31], while AMPA is exported

Glyphosate–degrading microorganisms with known taxonomic classification

Microorganism	Source	Primary GP metabolite	Gram status
Bacteria			
Achromobacter sp. LW9 [18]	Activated sludge of waste treatment facilities	AMPA	−
Achromobacter sp. MPS 12A [24]	Alkylphosphonate-contaminated soil	Sarcosine	−
Agrobacterium radiobacter [60]	Activated sludge of waste treatment facilities	Sarcosine (putatively)	−
Alcaligenes sp. GL* [32]	Mixed culture with *Anacystis nidulans*, passage on a selective medium	Sarcosine (95%), AMPA (5%)	−
Arthrobacter atrocyaneus ATCC 13752* [16]	Collection of microorganisms and cell cultures, Germany	AMPA	+
Arthrobacter sp. GLP-1* [34]	Mixed culture with *Klebsiella pneumoniae*, passage on a selective medium	Sarcosine	+
Flavobacterium sp. GD1 [33]	Activated sludge of waste treatment facilities	AMPA	−
Geobacillus caldoxylosilyticus T20* [29]	Central heating system	AMPA	+
Ochrobactrum anthropi GDOS [61]	GP-contaminated soil	AMPA	−
O. anthropi GPK 3 [24]	GP-contaminated soil	AMPA	−
O. anthropi LBAA [29, 62]	Soil	AMPA	−
O. anthropi S5 [62]	Soil	AMPA	−
Pseudomonas pseudomallei 22 [63]	GP-contaminated soil	AMPA (putatively)	−
Pseudomonas sp. 4ASW [64]	GP-contaminated soil	Sarcosine	−
Pseudomonas sp. GLC11* [53]	Mixed culture with *P. aeruginosa* PAO1	Sarcosine	−
Pseudomonas sp. LBr [31]	Activated sludge of waste treatment facilities	AMPA (95%), sarcosine (5%)	−
Pseudomonas sp. PG2982* [50]	Mixed culture with *P. aeruginosa*, passage on a selective medium	Sarcosine	−
Rhizobium meliloti 1021 [52]	Spontaneous mutation of a wild-type strain	Sarcosine	−
Streptomyces sp. StC [17]	Activated sludge of waste treatment facilities	Sarcosine	+
Fungi			
Aspergillus niger [45]	Soil	AMPA	
Penicillium chrysogenum [65]	Soil	AMPA (putatively)	
Penicillium notanum [44]	Spontaneous growth on hydroxyfluorenyl-9-phosphate	AMPA	
Scopulariopsis sp. [45]	Soil	AMPA	
Trichoderma harzianum [45]	Soil	AMPA	

* Strains that had not been exposed to GP previously.

into the extracellular space [31—33]. There are only a few bacterial strains that can metabolize AMPA in a reaction catalyzed by an *E. coli*-type C—P lyase to yield methylamine and P_i [34—36]. In addition, several studies [18, 33, 37] provided some indirect evidence in favor of an alternative pathway of AMPA metabolism similar to the transformation of 2-aminoethylphosphonate (2-AEP), a natural phosphonate [19, 20], which includes transamination by a pyruvate-dependent aminotransferase and cleavage of the destabilized C—P bond by a specific hydrolase (Fig. 2). However, in light of the high substrate specificity of the known aminoethylphosphonate: pyruvate aminotransferases (EC 2.6.1.37) [38, 39] and phosphonohydrolases [40—42], it seems more likely that there exists a pathway of AMPA transformation different from that for 2-AEP. Indeed, investigation of the GP metabolism in *O. anthropi* GPK 3 led to identification of a novel AMPA-specific aminotransferase, the molecular and catalytic properties of which differed significantly from those of the two previously described 2-AEP-specific transaminases [38, 39]. It still remains to determine the specificity of the terminal hydrolase participating in AMPA transformation in *O. anthropi* GPK 3. When these bacteria were cultured using GP as a phosphorus source, phosphonoacetaldehyde hydrolase (EC 3.11.1.1) involved in degradation of 2-AEP [24] was induced.

A growing body of data suggests that alternative GP degradation pathways may exist along with the two major bacterial pathways. A recent study of a bacterial glycine oxidase (EC 1.4.3.19) showed that this enzyme could also cleave GP producing AMPA and glyoxylate, although the reaction mechanism was different from that of GOR from *O. anthropi* [43]. On the whole, it can be speculated that there exists a whole range of bacterial flavine oxidases differing in structure, substrate specificity, and the extent of mutual homology, which can be generically termed glyphosate oxidoreductases (referring to their ability to oxidize GP). In natural environments, these enzymes most probably participate in the metabolism of amino and imino acids. The facts that GOR-like activity was detected in strains occupying different taxonomic positions and habitats and that GOR affinity to iminodiacetate was higher than to GP speak in favor of this hypothesis [28, 29, 37].

Among eukaryotic organisms, GOR-like activity was observed in some fungi [44, 45], whose role in GP biotransformation into AMPA is probably comparable to that of bacteria [46]. High rates of GP transformation with AMPA production were observed in the presence of fungal ligninolytic laccases and Mn-peroxidases [47]. However, the fungal enzymes of GP degradation remain so far unstudied. The ability of certain plants to destroy GP yielding both AMPA and sarcosine is hardly understood; presumably, these processes involve an activity of associated microorganisms [8, 48]. GP transformation to AMPA was also described in animal cells: a novel oxidase isolated from

the gills of a Pacific oyster *Crassostrea gigas* catalyzed the reaction in which the cleavage of the GP C—N bond was coupled with four-electron oxygen reduction. In contrast to bacterial GORs, this enzyme could utilize not only FAD but also NAD as cofactors [10].

Microbial Degradation of Glyphosate

Most commonly, the first step of GP transformation both in natural environments (soil and water bodies) and in waste treatment facilities is cleavage of the C—N bond, yielding equimolar quantities of AMPA. Apparently, this process strongly depends on interactions between different groups of microorganisms, since the overwhelming majority of bacterial strains isolated from such communities were incapable of utilizing GP as a phosphorus source in pure cultures [3, 33, 49].

The first strain shown to utilize GP as a source of phosphorus was *Pseudomonas* sp. PG2982, which transformed GP into sarcosine and P_i [50, 51]. Similar metabolic pathways were later found in a number of other strains (table). Among the strains that convert GP to AMPA, there were also those that utilized both of these compounds as sources of phosphorus [34—36].

The currently available data on GP degradation by bacteria can be summarized as follows:

1. Most bacterial strains studied, both laboratory and wild-type strains, convert GP to AMPA, and this capability may be present even in bacteria that have never been exposed to GP previously [3, 15];

2. In most GP-degrading strains, AMPA is not mineralized but exported into the environment [31—33];

3. A number of bacterial strains can utilize AMPA as a source of phosphorus but cannot degrade the GP herbicide itself. This fact suggests that enzymes catalyzing GP conversion to AMPA originated and developed independently of those responsible for the metabolism of phosphorus [15];

4. The sarcosine pathway serves to utilize GP as a source of P_i [15, 51—53]. However, the cleavage of the C—P bond in the GP molecule strongly depends on the concentrations of exogenous and endogenous P_i and is therefore usually induced under conditions of a phosphorus deficiency, which rarely occurs in natural environments [15, 16, 23, 27].

All these facts illustrate a considerable diversity of pathways of enzymatic GP degradation and the mechanisms of their regulation existing in bacteria.

Bioremediation of Glyphosate-Contaminated Soils

Until recently, GP accumulation in the environment was not considered as a serious ecological threat, and, consequently, the problems of remediation of natural and anthropogenic environments polluted with GP and AMPA did not attract due attention. The few studies existing in this area were concerned with biopurification of industrial wastewaters and were sup-

ported by GP producers themselves [33, 49]. However, in view of the growing evidence of GP toxicity for living organisms, the problem of preventing its accumulation in natural environments (both soils and water bodies) and of its subsequent removal is becoming a topical issue. The only appropriate solution seems to be the use of microorganisms capable of degrading phosphonate xenobiotics into biologically safe compounds.

The required microorganisms must possess special physiological and biochemical properties: (1) low toxicity and lack of pathogenicity, (2) high viability immediately after the introduction into environment in combination with low long-term survival rates, (3) high efficiency of GP degradation independent of external conditions, and (4) ability to GP mineralization not associated with AMPA accumulation in the environment. It is an extremely rare case that all of these requirements should be fulfilled simultaneously. Nevertheless, such strains are much in demand, since rapid GP degradation after the treatment of the cropped land would prevent subsequent herbicide accumulation in the soil, as well as its migration along the soil profile into underground waters. Apart from that, bioremediation techniques may prove useful in emergency situations when the herbicide has to be rapidly removed following its leakage at the sites of production, storage, transportation, or application.

To a certain extent, GP can be destroyed by aboriginal microbial communities, but the degradation efficiency remains low and depends both on the conditions of this process and its duration and on the microorganisms' adaptation to GP [3, 54, 55].

GP bioaccessibility for degrading microorganisms may be reduced because of adsorption on the soil matrix [5, 6, 56]. Most herbicide accumulates in the upper horizon at the depths up to 10–15 cm, where it is readily accessible for degrading microorganisms; at the same time, its migration to the underlying horizons may result in herbicide accumulation due to the absence or a strong reduction in the microbial population [4, 57].

It was not until recently that researchers became interested in applying GP-degrading bacteria for bioremediation of polluted soils. The first attempt to apply the laboratory strain *Pseudomonas* sp. 4ASW, which is capable of cleaving GP with the production of sarcosine, was unsuccessful, because its C–P lyase was completely inactivated under field conditions [27]. Satisfactory results were achieved with *O. anthropi* GPK 3 and *Achromobacter* sp. Kg 16 isolated from soils heavily contaminated with GP [58]. As soon as a week after their introduction into soil, these strains were several times more efficient in GP degradation than the aboriginal microbial community. An important advantage of the introduced strains was their ability to utilize GP completely, making it possible to avoid an accumulation of toxic intermediates, such as AMPA. The successful soil bioremediation was verified toxicologically: the overall toxicity and phytotox-

icity of the soil diminished to levels corresponding to nonpolluted soils. At the same time, the activity of the soil biota was restored. The abundance of both strains was rapidly decreasing with GP consumption. Both GP-degrading strains were nonpathogenic for warm-blooded animals and did not exhibit any phytotoxic effects; therefore, they can be further freely utilized for the purposes of bioremediation [57, 59].

To sum up, we would like to note that the application of GP and its possible consequences is a complicated and controversial issue. On the one hand, the use of GP as a broad-spectrum herbicide became a major breakthrough in the agricultural technologies of the second half of the 20th century, making it possible to dramatically reduce weed-associated crop losses [1]. On the other hand, it is currently becoming ever more evident that our knowledge concerning the ecological safety of GP, its behavior in natural environments, the ways it interacts with living organisms, and the pathways of its degradation is insufficient. However, the research community has already developed an interest in the outlined problems, as illustrated by the rapidly growing number of publications concerning phosphonates in general and glyphosate in particular. For instance, after 30 years, C–P lyase activity was first observed in vitro, and the mechanism underlying the C–P bond cleavage with production of sarcosine was proposed [21, 26]. Novel approaches to the removal of accumulated GP from natural environments were suggested [59]; the existence of C–P lyase- and GOR-like enzymes in some multicellular eukaryotes was demonstrated [10, 48], and the pathways of mineralization of GP and its derivatives were identified [24]. This gives us hope that many questions concerning the behavior of this important xenobiotic compound in natural environments will be answered soon.

ACKNOWLEDGMENTS

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