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Physiological aspects of glyphosate degradation in *Alcaligenes spec.* strain GL

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Abstract. *Alcaligenes spec.* strain GL (IMET 11314) is able to grow on glyphosate (N-[phosphonomethyl]glycine) and other phosphonates as sole source of phosphorus. Degradation of glyphosate to inorganic phosphate and sarcosine by this strain is subject to several regulatory principles. While uptake and dephosphonation of glyphosate are regulated by P_i starvation, the intensity of glyphosate degradation is also controlled by the cellular ability to utilize the C-skeleton derived from glyphosate. Depending on the external concentration of glyphosate, the liberated sarcosine is differentially metabolised. Utilization of the sarcosine moiety and complete incorporation of 3-[^{14}C]-label of glyphosate into cellular material occur only in cultures adapted to higher concentrations (5 mM) of the herbicide. At low concentrations of glyphosate (1 mM) only the P_i required by the growing cultures is utilized but not the sarcosine. Initially high rates of glyphosate uptake obtained after P_i-starvation decrease in the presence of low glyphosate concentrations. It is suggested that uptake and metabolism of glyphosate are connected with the expression of the sarcosine metabolizing capacity of the *Alcaligenes* cells.

Key words: *Alcaligenes spec. GL* – Phosphonate metabolism – Glyphosate – Sarcosine – Phosphate starvation

Cleavage of carbon to phosphorus bonds is a capacity of various microorganisms (Cook et al. 1978; Mastalerz et al. 1965), enabling them to grow on phosphonic and phosphinic acid derivatives as sole source of phosphorus. In the case of glyphosate[1] it was shown that only a minority of microbial strains possessing C–P cleaving activity are able to use this compound as an efficient P source (Moore et al. 1983; Balthazor and Hallas 1986; Pipke and Amrhein 1988; Wackett et al. 1987a). This restriction could be due to the occurrence of C–P lyases with different substrate requirements (Wackett et al. 1987a). Bacterial strains belonging to the genus *Flavobacterium* (Balthazor et al. 1986) decarboxymethylate and convert glyphosate to aminomethylphosphonic acid (AMPA), which may be used as a source of phosphorus by many other bacteria, including different strains of *Escherichia coli* (Cordeiro et al. 1986).

The C–P cleavage resulting in the formation of sarcosine or a one-carbon compound plus glycine has been reported for strains belonging to the genus *Pseudomonas* (Shinabarger et al. 1984), *Agrobacterium* (Wackett et al. 1987a), *Alcaligenes* (Talbot et al. 1984), and *Arthrobacter* (Pipke et al. 1987b), respectively. Also a *Pseudomonas* sp. strain LBr has been described, which is able to realize both pathways of glyphosate degradation (Jacob et al. 1988). In all of the strains described so far the induction of phosphonate metabolism seems to be at least partially regulated by phosphate starvation (Fitzgibbon and Braymer 1988; Pipke and Amrhein 1988). For *E. coli* a clear relation between the psiD locus and C–P lyase activity was demonstrated by transposon mutagenesis (Loo et al. 1988; Wackett et al. 1987b). Moreover, *E. coli* mutants defective in phosphonate utilization have been complemented by cosmid cloned wild type DNA (Loo et al. 1988), but a more detailed characterization of the gene structure is still lacking.

In this paper we provide evidence that glyphosate metabolism in a recently isolated *Alcaligenes* strain is controlled at different metabolic levels. Besides a continuously present low level of degradative activity, uptake of glyphosate and C–P lyase activity are inducible by P_i-starvation. The rate of glyphosate C-skeleton utilization as well as the primary degradation steps are shown to be strictly controlled by the exogenous amount of glyphosate.

Materials and methods

Chemicals. Glyphosate (free acid, 99.8% pure) was kindly provided by Dr. W. Mögelin (Section of Chemistry of the Martin-Luther-University Halle-Wittenberg). [^{14}C]-Methyl labelled N-(phosphonomethyl)glycine (3-[^{14}C]-glyphosate), specific activity = 47.4 MBq/mmol) was synthesized according to Issleib et al. (1980) by adding [^{14}C]-formaldehyde to N-(diphenylphosphonomethyl)methylglycinate and following hydrolysis to the free acid. By HPLC and TLC in different solvent systems the product seems to be radiochemically homogenous.

Selection and cultivation of bacteria

Alcaligenes spec. strain GL (IMET 11314) was isolated from non-axenic cultures of the cyanobacterium *Anacystis*

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Abbreviation: AMPA, aminomethylphosphonic acid

[1] Glyphosate (N-[phosphonomethyl]glycine) is the active component of a commercially used herbicide (Roundup®) from Monsanto Agricultural Products Co., St. Louis, MO, USA

nidulans, which were tested for resistance against the herbicide Roundup®. After several cycles of ampicillin and kanamycin selection during growth on glyphosate as the only P source the isolated strain was tentatively identified as an *Alcaligenes sp.* The bacteria were grown at 28°C on a modified *Escherichia coli* M9 medium with glucose as carbon source. Inorganic phosphate was excluded and substituted by 50 mM Tris-HCl (pH 7.5), phosphates or phosphonates were added as indicated. For maintenance the strain was kept on solidified medium (1.5% Bacto-agar, Difco) containing 10 mM glyphosate and 0.8 mM kanamycin. Growth was further improved by addition of 1 ml/l trace element solution (Rippka et al. 1979).

Uptake and labelling experiments

Bacteria were grown in modified M9 medium for 48 h (late log-phase). After pelleting (5000 rpm) and washing with P-free medium they were resuspended in fresh nutrient solution. Radioactive glyphosate was added as described in the legends of the illustrations. Incubated aliquots, usually 1.5 ml, were sedimented by centrifugation in an Eppendorf centrifuge for 1.5 min at maximum speed and washed three times in modified mineral salt solution to remove extracellular radioactivity.

Extraction of bacteria

After resuspending the bacteria pellet in a small volume of extraction mixture (water:methanol:acetic acid:chloroform = 60:30:10:0.5) the extracts were centrifuged to remove insoluble material and were evaporated to dryness in vacuo. The dry residues were dissolved in 10% NH_4OH and used for thin-layer chromatography. Cellular debris, after two times of washing in extraction mixture, were dried and solubilized in NaOH/SDS (1 M NaOH, 1% SDS) and used for liquid scintillation counting of the radioactivity incorporated into high molecular weight material. Partition of radioactivity has been calculated from cell homogenates after treatment with phenol/chloroform (1:1) followed by phase separation. Nucleic acids were precipitated from the aqueous phase by ethanol (2.5 vol.), proteins from the interphase and the organic phase by acetone (5 vol). Non-precipitable material was counted for water-soluble and lipophilic low molecular weight compounds, respectively.

TLC analysis

Thin-layer chromatography of the soluble radioactive material was performed on HPTLC-Silica plates (Merck, Darmstadt) developed successively in the solvent systems iso-propanol:NH_4OH (15%):TCA (100% w/w):H_2O = 60:20:2:20, and ethanol:NH_4OH (15%):TCA (100% w/w):H_2O = 40:20:5:30, respectively. For control purposes and to verify the chromatography of labelled spots with pure marker substances, several extracts were separated on cellulose sheets (Eastman Kodak Company) with either acetone:n-butanol:acetic acid:H_2O = 34:34:10:22 or chloroform: methanol:NH_4OH (15%) = 40:45:20 as solvent systems.

Results

Phosphonate utilization of Alcaligenes spec. strain GL

The phosphonate degrading GL strain was isolated under high selective pressure (100 mM glyphosate) and is able to grow in the presence of glyphosate as its sole source of phosphorus at concentrations of up to 100 mM. Depending on the concentration of applied glyphosate about 50 to 80% of the carbon-bound phosphorus disappear from the nutrient solution during cultivation and are mobilized for P utilization during growth. Accumulation of radioactivity derived from ^{14}C-glyphosate, i.e. metabolism of the sarcosine moiety occurs, to a major extent only in the late logarithmic or early stationary phase (Fig. 1). Continuous growth in the presence of ^{14}C-glyphosate, as well as pulse experiments in which unlabelled glyphosate was immediately replaced by radioactive glyphosate result in high incorporation of radioactivity into cellular material (Fig. 1). Incorporation of radioactivity during continuous growth in the presence of P_i plus glyphosate is relatively low compared with the amount of glyphosate taken up by the cells. Replacement of P_i, or P_i plus glyphosate by radioactive glyphosate alone seems to increase the accumulation of soluble cellular radioactivity but does not affect the incorporation of ^{14}C-label into high molecular weight cell material.

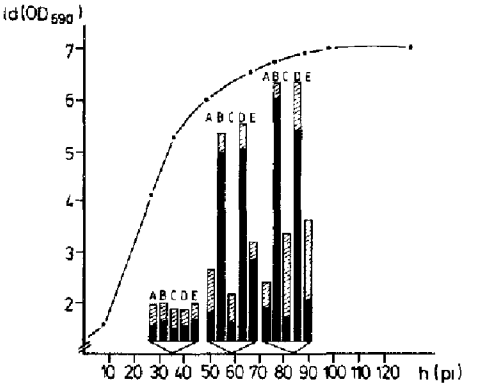

Fig. 1. Utilization of 3-[^{14}C] glyphosate by *Alcaligenes spec.* GL at different growth stages. Cell density (OD 590 nm) of a culture grown in modified M9 medium with 10 mM glyphosate as the only P source is drawn in logarithmic scale (ld: logarithmus dualis). Columns along the time axis represent accumulation of cellular radioactivity (max. accumulation 5.5×10^4 cpm/ml culture) from inoculation (p.i.: post inoculationem) up to the time indicated (*A, B*) or during 12 h of replacement (*C, D, E*). Filled part of columns correspond to the amount of radioactivity incorporated into protein and nucleic acid fractions. Cells grown on 5 mM glyphosate (0.2 mCi/mmol) plus 5 mM inorganic phosphate (A) or only 10 mM (0.1 mCi/mmol) glyphosate (B) show nearly identical gowth. For replacement experiments aliquots of the cultures grown previously either in 10 mM inorganic phosphate (*C*), 10 mM glyphosate (*D*) or 5 mM inorganic phosphate + 5 mM glyphosate (*E*) were harvested by centrifugation at the times indicated, and the washed bacteria were resuspended in modified M9 containing 1 mM 3-[^{14}C]-glyphosate (1 mCi/mmol) for 12 h

When bacteria are grown on 3-[^{14}C]-labelled glyphosate, after equilibration of label, the radioactivity is incorporated without loss into protein (57%), the nucleic acid fraction (11%) and into lipophilic material (32%). The soluble radioactivity consists mainly of intracellular glyphosate, sarcosine, small amounts of AMPA, and labelled material with chromatographic properties similar to amino acids biosynthesized via C_1-metabolism. The labelled compounds marked by an asterix (Fig. 4) have not yet been identified. It is unclear whether they are degradation products or, for instance, conjugates of glyphosate. Their relative amount is influenced by the different experimental conditions and

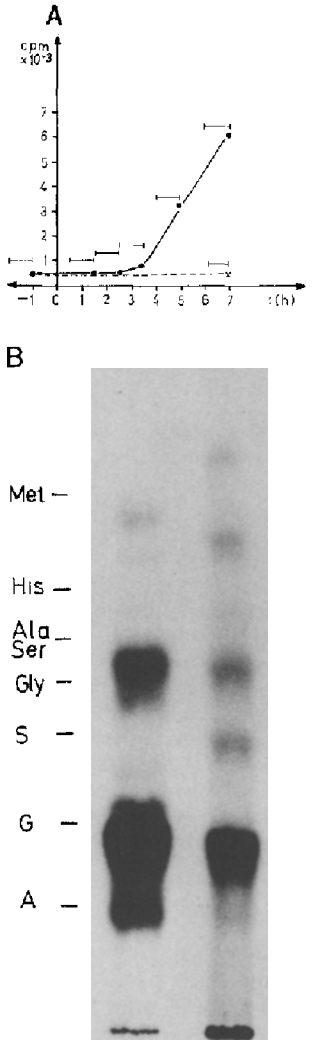

Fig. 2 A, B Induction of glyphosate uptake by P_i-starvation. **A** Glyphosate uptake during 1 h incubation periods in different times after onset of starvation. **B** Autoradiogram of a TLC of soluble cellular radioactivity extracted from bacteria labelled for 1 h after 6 h of phosphate starvation (left) and for 7 h beginning at zero time of phosphate starvation (right). A 48 h old culture grown on 5 mM inorganic phosphate (modified M9) was harvested, washed and resuspended in fresh phosphate free medium (0 h). At the times indicated by bars (1 h) aliquots of the culture (1 ml) were incubated with 0.5 mM labelled glyphosate (1 mCi/mmol). Soluble cellular radioactivity was extracted (cf. Materials and methods) and processed for TLC and radioactivity determination. The "−1" value and the 7 h (*broken line*) are those from corresponding unstarved cultures labelled by glyphosate in the presence of P_i. *S*, *G* and *A* indicate the TLC position of sarcosine, glyphosate and AMPA, respectively

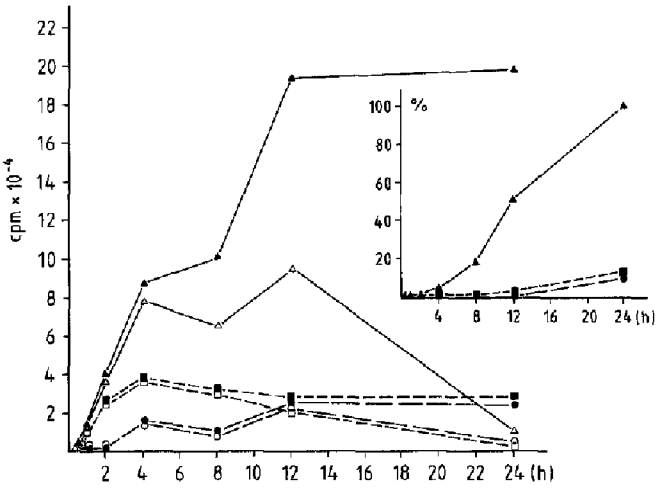

Fig. 3. Glyphosate utilization by differentially adapted cultures. Accumulation of 3-[^{14}C]-glyphosate derived radioactivity during 24 h replacement of cultures precultivated on 1 mM glyphosate (*circles*), 5 mM glyphosate (*triangles*) and 5 mM inorganic phosphate (*squares*). Open symbols: soluble cellular radioactivity, closed symbols: total activity accumulated. The insert shows the relative amount of radioactivity incorporated into high molecular weight material. Bacteria from liquid maintainance medium were used for inoculation of modified M9 containing glyphosate or P_i as indicated. After 48 h of growth the cultures were harvested by centrifugation, washed, adjusted to equal cell densities and starved for phosphorus for 24 h in modified M9. The starvation solution was replaced by incubation medium (modified M9 containing 1 mM 3-[^{14}C]-labelled glyphosate (1 mCi/mmol). Samples were taken at the times indicated and processed as described (Material and methods)

seems to be independent on the endogenous amount of glyphosate. Also, their appearance already after a relative short incubation period (less than 10 min) should exclude labelling by recycling of the 3-[^{14}C]-label from glyphosate.

P_i-starvation induced glyphosate uptake

Complete removal of inorganic phosphate causes a drastic stimulation of glyphosate uptake (Fig. 2A), but the pattern of radioactive metabolites after short periods of starvation differs from that obtained under conditions of active glyphosate metabolism. After TLC of soluble extracts most of the labelled material has comigrated with glyphosate and AMPA, only minor amounts of 3-[^{14}C]-label appear in the amino acid fractions. Detection of labelled sarcosine requires either a prolonged incubation of cells in the presence of 3-[^{14}C]-glyphosate immediately after removal of P_i (Fig. 2B), or labelling experiments with cultures already adapted to glyphosate degradation (Fig. 4). Likewise, extended P_i-starvation (12 to 24 h) allows detection of radioactive sarcosine even after very short labelling periods. However, these cultures are not able to incorporate glyphosate-derived ^{14}C into high molecular weight material (Fig. 3).

Metabolic adaptation to glyphosate degradation

Bacteria precultivated on sufficiently high concentration of glyphosate (5 mM) as sole source of phosphorus after starvation show a biphasic uptake of radioactivity from the nutrient solution within 12 h (Fig. 3). The amount of cellular soluble radioactivity increases up to 4 h, maintains a maximum level between 4 and 12 h and then decreases. The 3-[^{14}C]-label from glyphosate is incorporated without loss into the protein and nuclei acid fractions. It was evident from another type of feeding experiments (not shown) that incorporation of ^{14}C radioactivity from glyphosate into high molecular weight material even in adapted cells (5 mM glyphosate) is still limited by the metabolic capacity of the bacteria. Bacteria precultivated in 5 mM glyphosate were starved for phosphorus and resuspended in nutrient solutions containing different amounts of glyphosate (60 µM up to 1.5 mM). The incorporation of radioactivity from glyphosate into high molecular weight material, i.e. utilization of sarcosine during the first 5 h after resuspension, seems to be saturated at external concentrations of 0.5 mM glyphosate. Similar to the results shown in Fig. 3, after 5 to 8 h of incubation, both the amount of radioactivity taken up and the rate of incorporation increases continuously, and saturating conditions were no longer observed.

Bacteria adapted to 1 mM glyphosate show a different behaviour after starvation (Fig. 3). The total uptake of glyphosate from the nutrient solution and the incorporation of 3-[^{14}C]-label into high molecular weight material is only about 10 to 20% compared with cultures adapted to 5 mM glyphosate. Most of the soluble radioactivity is recovered in the sarcosine fraction, only trace amounts are found in other amino acids. Glyphosate itself accumulates relatively late and slowly.

Bacteria precultivated 5 mM P_i show within the first 4 h after 24 h of P_i-starvation (Fig. 3) an uptake of radioactivity

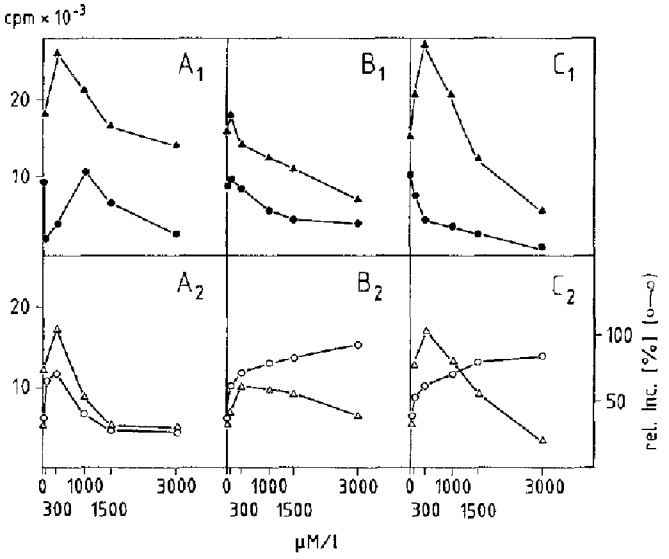

Fig. 4A–C. Competition effects of different putative degradation products on glyphosate uptake and incorporation. In a replacement experiment (cf. Fig. 3 for details) starved cultures precultivated on 5 mM glyphosate were resuspended in modified M9 supplemented with different concentrations of sarcosine (**A**), AMPA (**B**) and inorganic phosphate (**C**), respectively. After 30 min of incubation 0.5 mM 3-[^{14}C]-labelled glyphosate was added to each of the cultures. $\mathbf{A_1}$, $\mathbf{B_1}$, $\mathbf{C_1}$ show the total amount of radioactivity taken up during 1 h (*circles*) and 7 h (*triangles*) after addition of glyphosate. $\mathbf{A_2}$, $\mathbf{B_2}$, $\mathbf{C_2}$ document the incorporation of radioactivity into high molecular weight material (*triangles*) and the relative incorporation rate (*circles*), i.e. incorporated radioactivity divided by total amount of accumulated radioactivity

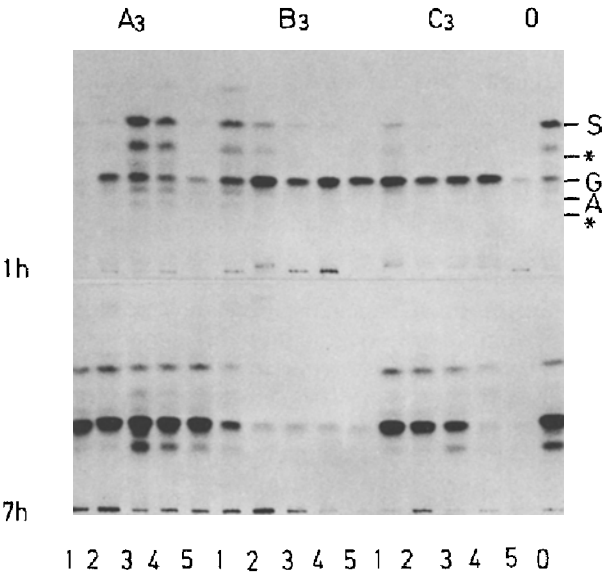

Fig. 5. Competition effects of different putative degradation products of glyphosate metabolism. Autoradiographs of TLC-separated soluble material of all samples (cf. Fig. 4) after 1 h (*upper part*) and 7 h (*lower part*) of incubation. Lanes one to five indicate increasing concentrations (0.06, 0.3, 1.0, 1.5 and 3.0 mM) of sarcosine (A_3), AMPA (B_3) and P_i (C_3), respectively. Control (0) means an extract obtained from bacteria incubated only with 0.5 mM/l 3-[^{14}C] glyphosate (1 mCi/mmol) in modified M9. *S*, sarcosine; *G*, glyphosate; *A*, AMPA. * Not yet identified. Details as in Fig. 4

similar to cultures grown on 5 mM glyphosate. During continued incubation in the presence of 1 mM labelled glyphosate the accumulation of radioactivity ceases and the remaining cellular label is slowly incorporated into cellular material in a manner similar to cultures previously grown on 1 mM glyphosate. The initially high rate of glyphosate uptake and the transiently increased internal concentration of sarcosine are apparently not able to promote an intensive metabolism of both compounds in these cultures.

Glyphosate metabolism in adapted cultures

Glyphosate utilization in adapted cultures can be influenced differentially by the putative intermediates of glyphosate degradation. Additional feeding of unlabelled sarcosine immediately reduces accumulation of radioactive material from glyphosate by the cells (Figs. 4A, $5A_3$). It also acts as a cellular trapping agent (Kishore and Jacob 1987) for glyphosate derived 3-[^{14}C]-sarcosine, which is apparently not further metabolized. Incubation for 7 h in the presence of sarcosine overcomes the inhibition of uptake. The amount of cellular radioactive material after this time is comparable to that of the control. Incorporation of 3-[^{14}C] from glyphosate into high molecular weight material is still influenced by the competing effect of inactive sarcosine. Unlabelled sarcosine in excess of 300 µM reduces the incorporation of radioactivity from 3-[^{14}C]-labelled glyphosate at a nearly theoretical dilution rate; only at low sarcosine concentrations incorporation of 3-[^{14}C]-label is increased.

AMPA as another possible metabolite of glyphosate degradation and as an alternative source of phosphorus decreases the accumulation of radioactivity from glyphosate by 50% compared with the control (Figs. 4B, $5B_3$). A direct inhibition of glyphosate uptake is not observed, as indicated by TLC-analysis of the soluble material. The pool of soluble radioactivity after 7 h of incubation is relatively small; most of the accumulated 3-[^{14}C] from glyphosate is incorporated into the high molecular weight fraction. Glyphosate is detectable only in trace amounts except at very low concentrations of AMPA.

Similar to AMPA, inorganic phosphate inhibits accumulation of radioactivity up to 90% during the first hour of incubation without a drastic reduction of glyphosate uptake (Figs. 4C, $5C_3$). During long-term incubation at low concentrations of P_i uptake and conversion of glyphosate are stimulated, only higher concentrations of P_i inhibit accumulation and absolute incorporation of glyphosate derived 3-[^{14}C]. At any concentrations of P_i used the radioactivity from cellular glyphosate is efficiently incorporated, i.e. relative incorporation (incorporated radioactivity divided by total amount of accumulated radioactivity) is enhanced with increasing concentrations of inorganic phosphate.

Discussion

Alcaligenes spec. strain GL possesses different modes of adaptation to P-nutrition by glyphosate. Always present is a base level of glyphosate degrading activity, which is indicated by a low incorporation of 3-[^{14}C] from glyphosate into cellular material also during growth in the presence of inorganic phosphate or in complex media.

P_i-starvation causes an induction of glyphosate uptake without an immediate increase of C–P cleaving activity. Cleavage of the C–P bond and subsequent accumulation

of glyphosate derived sarcosine is detectable after prolonged P_i-starvation. At this time C–P lyase activity is enhanced relative to the sarcosine utilising capacity. An inhibition of sarcosine dehydrogenase by acetate, as reported for *Arthrobacter* (Pipke and Amrhein 1988), is not required for the demonstration of sarcosine formation. Transient accumulation of sarcosine and complete incorporation of 3-[^{14}C]-label of glyphosate in adapted cultures, without any loss of radioactivity, should be due to a sarcosine degradation typically for *Alcaligenes* (Talbot et al. 1984). The additional expression of this sarcosine metabolism is a strong requirement for efficient degradation of glyphosate and seems to be stimulated by low concentrations of exogenous sarcosine or a continuous presence of higher concentrations of glyphosate. A non-metabolized excess of sarcosine apparently acts back on glyphosate uptake either directly or via inhibition of glyphosate C–P cleavage.

In contrast to *Arthrobacter* (Pipke et al. 1987a), the addition of sources of phosphorus other than glyphosate to adapted cultures (AMPA, inorganic phosphate) initially seems to effect glyphosate conversion more than glyphosate uptake. The transiently increased cellular content of unconverted glyphosate in the presence of AMPA and P_i could be due to the saturation of C–P lyase by AMPA as a better available substrate. Inhibition of the enzyme by liberated and exogenously added phosphate is a possible explanation to be proven by in situ and in vitro studies. The increased incorporation of 3-[^{14}C]-label from glyphosate caused by low concentrations of P_i, during long term incubation, results probably from a "general" activation of cellular metabolism and an efficient uptake of glyphosate. During prolonged incubation in the presence of AMPA or inorganic phosphate the cellular glyphosate is metabolised efficiently, as indicated by the increasing relative incorporation rate. Total accumulation of radioactivity is reduced, however, i.e. increasing amounts of AMPA and P_i influence the uptake of glyphosate when present over longer periods. A competition between AMPA and the glyphosate-derived radioactivity can be excluded because of the increasing relative incorporation rate in the presence of AMPA. Therefore, the observed degradation of glyphosate via AMPA in *Alcaligenes spec.* strain GL is either a metabolic bypath or the reaction is not well expressed under the chosen experimental conditions.

These results strengthen the assumption of different regulatory aspects for the control of glyphosate degradation in *Alcaligenes spec.* strain GL: 1) low level phosphonate degradation active also in the presence of P_i; 2) P_i-starvation induced glyphosate uptake and cleavage in order to cover the phosphorus requirements of the growing cells, and 3) mass degradation of glyphosate indicated by the complete utilisation of the glyphosate-derived 3-[^{14}C]-label.

References

Balthazor TM, Hallas LE (1986) Glyphosate degrading microorganisms from industrial activated sludge. Appl Environ Microbiol 51:432–434

Cook AM, Daughton CG, Alexander M (1978) Phosphonate utilization by bacteria. J Bacteriol 133:85–90

Cordeiro ML, Pompliano DL, Frost JW (1986) Degradation and detoxification of organophosphonates: cleavage of the carbon to phosphorus bond. J Am Chem Soc 108:332–334

Fitzgibbon I, Braymer HD (1988) Phosphate starvation induces uptake of glyphosate by *Pseudomonas* sp. strain PG 2982. Appl Environ Microbiol 54:1886–1888

Issleib K, Balszuweit A, Mueller K, Moegelin WZ (1980) N-alkylaminomethanphosphonsaure-dialkylester via hexahydro-s-triazine. Anorg Allgem Chem U69:109–115

Kishore GM, Jacob GS (1987) Degradation of glyphosate via sarcosine intermediate. J Biol Chem 262:12164–12168

Jacob GS, Garbow JR, Hallas LE, Kimack NM, Kishore GM, Schaefer I (1988) Metabolism of glyphosate in *Pseudomonas* sp. strain LBr. Appl Environ Microbiol 54:2953–2958

Loo SH, Peters NK, Frost JW (1988) Genetic characterization of an *Escherichia coli* mutant deficient in organophosphonate biodegradation. Biochem Biophys Res Commun 148:148–152

Mastalerz PZ, Wieczorek Z, Kochmann M (1965) Utilization of carbon-bound phosphorus by microorganisms. Acta Biochim Pol 12:151–156

Moore IK, Braymer HD, Larson AD (1983) Isolation of a *Pseudomonas* sp. which utilizes the phosphonate herbicide glyphosate. Appl Environm Microbiol 46:316–320

Pipke R, Amrhein N (1988) Carbon-phosphorus lyase activity in permeabilized cells of *Arthrobacter sp.* GLP-1. FEBS Lett 236:148–152

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

Pipke R, Amrhein N, Jacob GS, Schaefer J, Kishore GM (1987b) Metabolism of glyphosate in an *Arthrobacter sp.* GLP-1. Eur J Biochem 165:267–273

Rippka R, Deruelles J, Waterbury JB, Herdman M, Stanier RY (1979) Generic assignments, strain histories and properties of pure cultures of cyanobacteria. J Gen Microbiol 111:1–61

Shinabarger PL, Schmitt EK, Braymer HD, Larson AD (1984) Phosphonate utilization by the glyphosate-degrading *Pseudomonas* sp. strain PG 2982. Appl Environ Microbiol 4:1050

Talbot HW, Lohnson LM, Munnecke DM (1984) Glyphosate utilization by *Pseudomonas sp.* and *Alcaligenes sp.* isolated from environmental sources. Curr Microbiol 10:255–260

Wackett LP, Shanes SpL, Venditti ChP, Walsh ChT (1987a) Bacterial carbon-phosphorus lyase: products, rates, and regulation of phosphonic and phosphinic acid metabolism. J Bacteriol 169:710–717

Wackett LP, Wanner BL, Venditti ChP, Walsh ChT (1987b) Involvement of the phosphate regulon and the psiD locus in carbon-phosphorus lyase activity of *Escherichia coli* K-12. J Bacteriol 169:1753–1756

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