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THE ABILITY OF SELECTED BACTERIA TO GROW IN THE PRESENCE OF GLYPHOSATE

ZDOLNOŚĆ WYBRANYCH BAKTERII DO WZROSTU W OBECNOŚCI GLIFOZATU

Abstract: Glyphosate is an active substance in the Roundup herbicide. The key process affecting its decomposition in the soil is microbial biodegradation. Bacteria that are able to break the C-P bond use this substance as a source of phosphorus. The aim of the study was to investigate the ability of thirty strains of soil bacteria to grow in the presence of glyphosate which was the sole source of phosphorus. Morphologically and physiologically varied soil bacteria strains were the subject of the study. Their ability to grow in the presence of glyphosate being the only phosphorus source was examined using a modified Dworkin-Foster growth medium. The modification itself consisted in introducing to the medium $0.5 \text{ mM} \cdot \text{dm}^{-3}$ of glyphosate which was to serve as an alternative source of phosphorus. The control sample in the study was the bacterial growth in two Dworkin-Foster growth media: a complete one (unmodified) and a phosphorus-free one. The growth intensity of the analyzed strains was assessed by means of spectrophotometry ($\lambda = 490 \text{ nm}$). Substantial differences in the growth intensity of the analyzed bacterial strains were observed in the presence of glyphosate, which was the sole source of phosphorus. Only eight out of the analyzed strains showed growth similar to what was observed in the case of the unmodified Dworkin-Foster medium, whereas all the remaining ones grew at a much slower rate.

Keywords: indigenous soil bacteria, glyphosate, growth kinetics

Introduction

Plant protection products, and especially herbicides, are commonly used in modern agriculture. Herbicides compounds are highly toxic to living organisms and pose a potential threat to humans, animals and the environment [1, 2].

In spite of close supervision in the use of pesticides there is a serious risk that these agents are able to spread into the environment and contaminate water, soil, food, and feedstuffs. Recently, more and more studies have been focused on understanding the toxic mechanisms of herbicides actions.

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One of the most popular and commonly used herbicides that eradicate unwanted weed is Roundup introduced to agriculture in 1971 by a company called Monsanto. The herbicidal properties consist in inhibiting the biosynthesis of aromatic amino acids whereby the shikimic acid pathway in plants is blocked [3]. The inhibitor of 5-enol-pyruvylshikimate-3-phosphate synthase (one of the enzymes of shikimic acid) occurring in plants and microorganisms is glyphosate (N-(phosphonomethyl)glycine) [4].

Glyphosate, the active substance in the Roundup herbicide, belongs to organo-phosphorus compounds that contain in their structure a strong C-P bond that cannot be easily broken by physicochemical actions. Roundup is adsorbed and retained in the sorption complex soil and this affects the development of the environmental conditions of the soil which determines the development and activity of microorganisms. The biodegradation of glyphosate takes place mainly due to specialized strains of fungi and bacteria having the ability to break C-P bonds and use the resulting products mostly as a source of phosphorus, and, to a lesser extent, as sources of nitrogen and carbon [5–9]. The following genera of bacteria are known to possess the ability to break organo-phosphorus compounds: *Escherichia*, *Klebsiella*, *Shigella*, *Bacillus*, *Enterobacter*, *Pseudomonas Serratia*, *Pseudomonas Arthrobacter*, *Rhizobium* or *Streptomyces* [5–8, 10, 11]. Only a few strains have ability to use glyphosate as the sole source of nitrogen [6–9, 11, 12].

Degradation of N-(phosphonomethyl)glycine (PMG) proceeds primarily of involving specialized bacterial and fungal strains [8, 9, 13]. This ability results directly from the activity of adaptive and constitutive enzymes whose synthesis is induced in the presence of a xenobiotic. As a result of glyphosate biodegradation, sarcosine may be produced due to the activity of C-P – lyase or aminomethylphosphonic acid (AMPA), which is the product of glyphosate oxidoreductase (GOX) [14]. Rueppel et al [15] and Araujo et al [16] proved that AMPA could be either accumulated or biodegraded in the soil, albeit at a slower rate than glyphosate.

The time it takes glyphosate to decompose in the soil varies from several days to several years, depending on the physicochemical properties of the soil and the number of microbes. Depending on the strain, the C-P bonds may be broken into inorganic phosphorus and acetaldehyde [17] or methane [18] as the final products.

The specifics of glyphosate decomposition, as carried out by microbes and enzymes, are peculiar as it depends on the strain properties of the autochthonous microflora. In the case of microorganisms relying on the activity of glyphosate oxidoreductase (GOX), glyphosate-tolerance genes were detected, which allowed one to create Roundup Ready Crops (genetically engineered crops) [19].

The aim of the study was to investigate the ability of thirty strains of soil bacteria to grow in the presence of glyphosate, which was the sole source of phosphorus.

Materials and methods

The subject of the analysis was thirty bacterial strains isolated from the soil by means of microbial culture on Dworkin-Foster growth medium [20]. The study was conducted in July, after the process of rapeseed desiccation.

The predominant and macroscopically varied bacterial colonies underwent biochemical identification with the use of the API system: API 20 NE and ID32GN – Gram-negative bacilli, API 50 CHB – Gram-positive bacilli and ID32 STAPH – Gram-positive cocci.

The growth kinetics of the analyzed strains was examined in 250 cm³ Erlenmeyer flasks containing 100 cm³ of Dworkin-Foster (D) culture medium with 0.5 mM · dm⁻³ of glyphosate (PMG) as the only source of phosphorus (D-P + PMG).

Glyphosate, (N-phosphonomethylglycine) used in this study was obtained from commercial formulation Roundup1® (Monsanto).

The cultures were injected with inoculum of density $\zeta = 2$ at wavelength $\lambda = 460$ nm and incubated for 72 hours at a temperature of 25 °C. The relative control sample was the complete (unmodified) Dworkin-Foster (D) culture medium on which the strains grew. The absolute control sample was the phosphorus-free Dworkin-Foster culture medium (D-P).

The growth intensity of the analyzed strains was assessed on the basis of the extent to which the cultures became turbid after 4, 8, 24, 48 and 72 hours of incubation with the use of spectrometry ($\lambda = 490$ nm).

Results and discussion

After the process of rapeseed desiccation with the Roundup herbicide, the most frequently isolated bacterial genera from the soil were: *Bacillus*, *Arthrobacter*, *Pseudomonas*, *Corynebacterium*, *Micrococcus*, *Proteus* and *Sarcina* (Table 1).

Table 1

The bacterial strains isolated from the soil exposed to the Roundup herbicide on the complete Dworkin-Foster culture medium

Gram-positive	Gram-negative	
Bacilli	Rods	
<i>Bacillus subtilis</i> DII 2	<i>Pseudomonas</i> Ps13	<i>Alcalibacter denitryficans</i>
<i>Bacillus cereus</i> DII 4	<i>Pseudomonas fluorescens</i>	<i>Arthrobacter atrocyaneus</i>
<i>Bacillus</i> DII 5	<i>Pseudomonas stutzei</i>	<i>Proteus mirabilis</i>
<i>Bacillus</i> DI 3	<i>Pseudomonas putida</i>	<i>Serratia odorifera</i>
<i>Bacillus</i> DIII	<i>Commomonas testosteroni</i>	<i>Serratia fonticola</i>
Coccus	<i>Sphingomonas paucimobilis</i>	<i>Entrobacter agglomerans</i>
<i>Micrococcus lylae</i>	<i>Burkholderia cepacia</i>	<i>Entrobacter aerogenes</i>
<i>Staphylococcus warneri</i>	<i>Chryseomonas luteola</i>	<i>Ochrabactrum anthropi</i>
<i>Staphylococcus xylosus</i>	<i>Stenotrophomonas maltophila</i>	<i>Aeromonas caviae</i>
<i>Sarcina</i> sp.	<i>Flavobacterium</i> sp.	<i>Ralstonia pickettii</i>
	<i>Acinetobacter baumani/ hwoffii</i>	<i>Moraxella</i> spp.

Clear differences in the growth intensity of the analyzed strains were observed in the presence of glyphosate as the sole source of phosphorus. In the case of twenty-two analyzed strains, growth intensity comparable to the absolute control sample was

observed, *ie* similar to the growth in the phosphorus-free culture medium. None of the strains of *Bacillus* isolated from this environment were able to grow in the presence of glyphosate, albeit in many studies it was proven that these bacteria could use glyphosate as the source of both phosphorus and nitrogen [21].

However, the growth of the remaining eight bacterial strains differed in terms of the adaptation period and growth tendencies. The length of lag phase growth by bacteria strains was varied according to the species.

Out of these eight strains five showed growth that was inductive in nature, this having been proven by the presence of the adaptation phase. An eight-hour adaptation phase was present in the case of *Staphylococcus warneri*, *Proteus mirabilis* and *Serratia odorifera* (Fig. 1–3), whereas a twenty-four-hour phase in the case of *Ralstonia pickettii*

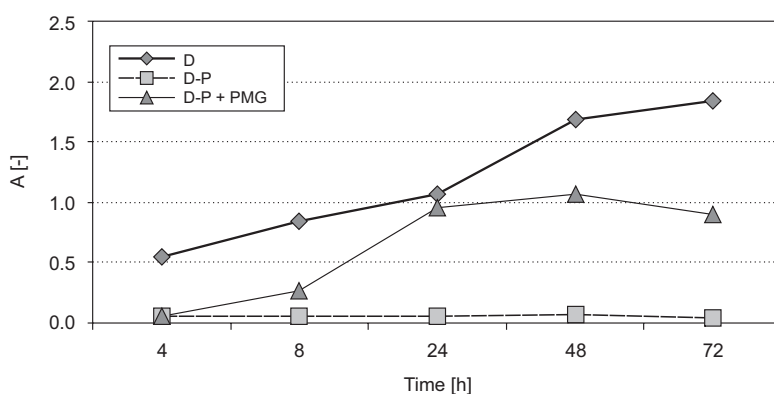


Fig. 1. Growth kinetics of *Staphylococcus warneri* in the presence of glyphosate (PMG) serving the function of the sole phosphorus source; D: complete mineral growth medium, D-P: mineral growth medium without phosphorus, D-P + PMG: mineral growth medium containing glyphosate as the alternative source of phosphorus

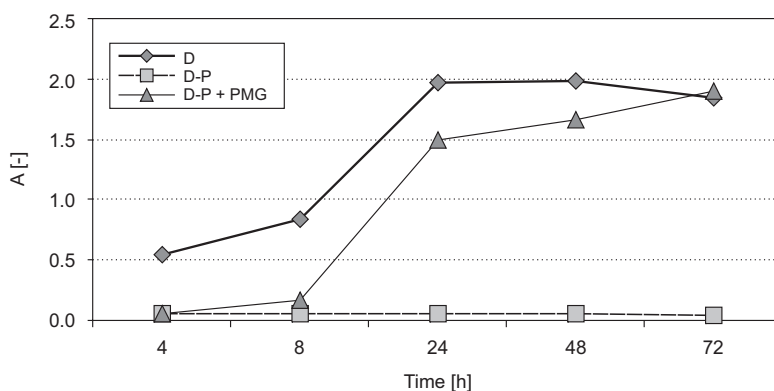


Fig. 2. Growth kinetics of *Proteus mirabilis* in the presence of glyphosate (PMG) serving the function of the sole phosphorus source; D: complete mineral growth medium, D-P: mineral growth medium without phosphorus, D-P + PMG: mineral growth medium containing glyphosate as the alternative source of phosphorus

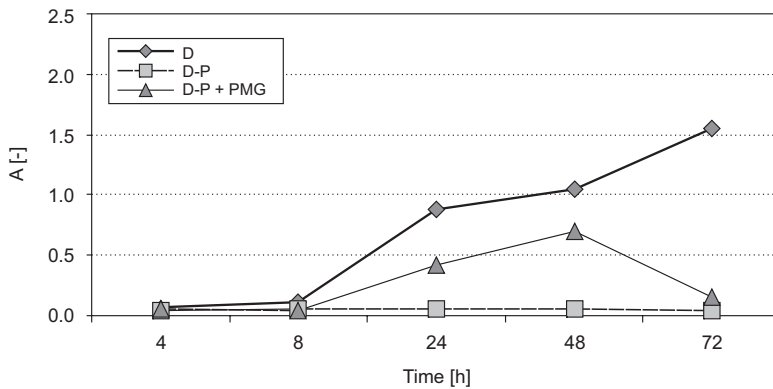


Fig. 3. Growth kinetics of *Serratia odorifera* in the presence of glyphosate (PMG) serving the function of the sole phosphorus source; D: complete mineral growth medium, D-P: mineral growth medium without phosphorus, D-P + PMG: mineral growth medium containing glyphosate as the alternative source of phosphorus

(Fig. 4). Differences between bacteria being able to use glyphosate were also observed by Dick and Quin as well as Araujo et al [16, 22].

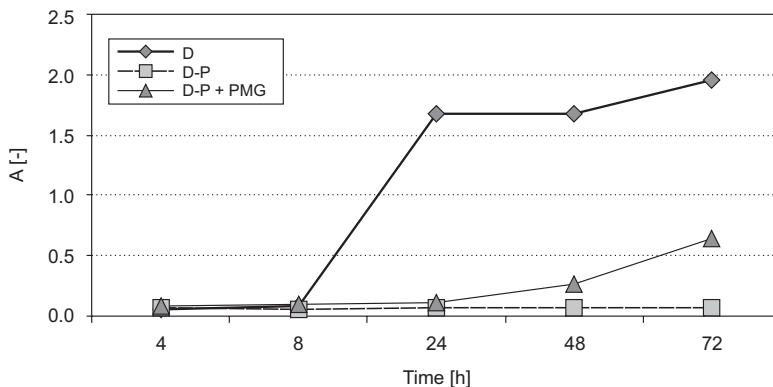


Fig. 4. Growth kinetics of *Ralstonia pickettii* in the presence of glyphosate (PMG) serving the function of the sole phosphorus source; D: complete mineral growth medium, D-P: mineral growth medium without phosphorus, D-P + PMG: mineral growth medium containing glyphosate as the alternative source of phosphorus

In contrast, in the case of *Sphingomonas paucimobilis* and *Pseudomonas fluorescens* strains, an intensive growth was observed without the adaptation phase (Fig. 5 and 6) which indicated that these Gram-negative bacilli were able to directly break the C-P bond. Other researchers, eg Gimsing, had obtained similar results [23].

The majority of the microbial strains isolated from the soil, under laboratory conditions, were able to use glyphosate as the source of phosphorus by breaking the C-P bond with the use of the C-P lyase [14]. Gimsing et al [23] claim that the Gram-positive bacteria conduct a biological decomposition of glyphosate by breaking

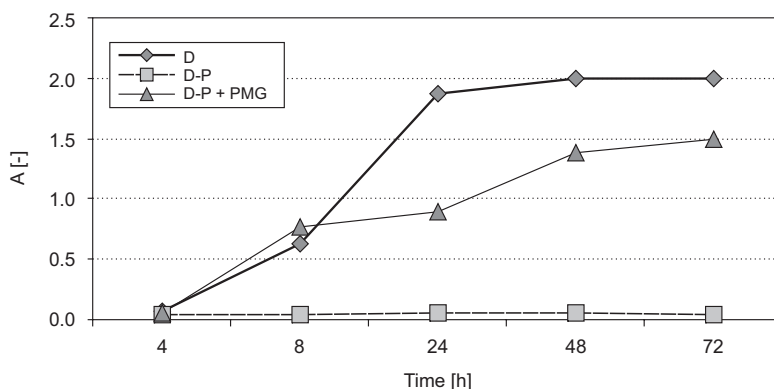


Fig. 5. Growth kinetics of *Spingomonas paucimobilis* in the presence of glyphosate (PMG) serving the function of the sole phosphorus source; D: complete mineral growth medium, D-P: mineral growth medium without phosphorus, D-P + PMG: mineral growth medium containing glyphosate as the alternative source of phosphorus

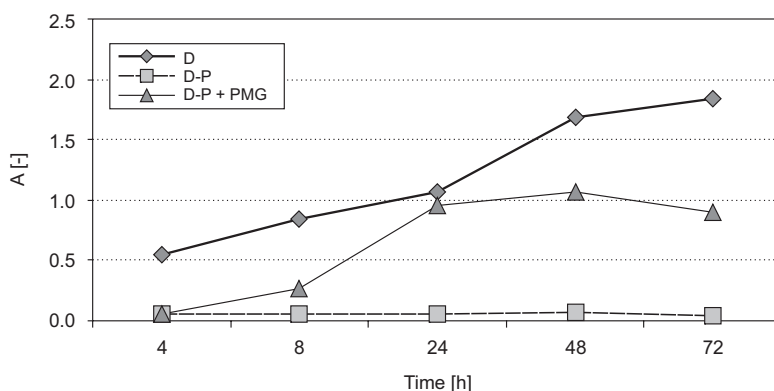


Fig. 6. Growth kinetics of *Pseudomonas fluorescens* in the presence of glyphosate (PMG) serving the function of the sole phosphorus source; D: complete mineral growth medium, D-P: mineral growth medium without phosphorus, D-P + PMG: mineral growth medium containing glyphosate as the alternative source of phosphorus

away phosphorus from the organophosphorus compounds, whereas the Gram-negative ones rely on breaking the C-P bond.

It is very likely that many bacterial enzymes involved in cleavage of the C-P bond are of the “C-P lyase” type; however, it has now been established that at least three other, Pi-insensitive, C-P cleavage enzymes do exist within microorganisms [17].

Some bacteria strains may rely on still other biochemical ways of glyphosate decomposition. *Arthrobacter sp.* GLP-1 uses glyphosate as the source of nitrogen [25], while strains of *Streptomyces* break glyphosate in the same way, using it as the source of either phosphorus or nitrogen or both nitrogen and phosphorus [7, 21]. Therefore, it may be assumed that bacterial activity in biodegradation of glyphosate is a property

related to particular strains [16, 24]. However, glyphosate degradation in soil is a co-metabolic process and decomposition rate depend on the general activity of soil bacterial and fungi, soil type and environmental conditions [25].

Conclusions

The effects of glyphosate degradation by microbes depended on the cell-substrate interaction. The influence of herbicides on the growth of microbes consisted in disrupting the cell's metabolism, albeit it was not always the case that the growth was inhibited. In the presence of glyphosate as the alternative source of phosphorus the bacterial growth depended on individual features. The inductive character of the growth was present in the case of four of the analysed bacterial strains – *Ralstonia pickettii*, *Serratia odorifera*, *Proteus mirabilis* and *Staphylococcus warneri*. Finally, *Sphingomonas paucimobilis* and *Pseudomonas fluorescens* grew without the adaptation phase.

All in all, when soil bacteria using glyphosate as the source of phosphorus are present in the soil, they will biodegrade this compound and, by implication, prevent it from accumulating in the environment.

Acknowledgments

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ZDOLNOŚĆ WYBRANYCH BAKTERII DO WZROSTU W OBECNOŚCI GLIFOZATU

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Abstrakt: Glifozat jest substancją czynną herbicydu Roundup. Kluczowym procesem wpływającym na jego rozkład w glebie jest degradacja mikrobiologiczna. Bakterie zdolne do rozkładu wiązania C-P wykorzystują go jako źródło fosforu. Celem badań była ocena zdolności do wzrostu trzydziestu szczepów bakterii glebowych w obecności glifozatu jako jedyne źródła fosforu. Materiał badawczy stanowiły zróżnicowane morfologicznie i fizjologicznie szczepy bakterii glebowych. Zdolność bakterii do wzrostu w obecności glifozatu (FMG) jako jedyne źródła fosforu oceniano w zmodyfikowanym podłożu Dworkin-Fostera. Modyfikacja polegała na wprowadzeniu 0,5 mM FMG jako alternatywnego źródła fosforu. Kontrolę stanowił rozwój bakterii w pełnym i pozbawionym źródła fosforu podłożu Dworkin-Fostera. Intensywność rozwoju

testowanych szczepów oceniano metoda spektrofotometryczną ($\lambda = 490$ nm). Zaobserwowano znaczne różnice w intensywności wzrostu testowanych szczepów w obecności glifozatu jako jedyne źródła fosforu. Tylko osiem spośród nich rosło podobnie jak w pełnym podłożu Dworkin-Fostera, a rozwój pozostałych był zdecydowanie słabszy.

Słowa kluczowe: autochtoniczne bakterie glebowe, glifozat, kinetyka wzrostu

