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Study of the potential of Rhodococcus strains for degradation of the pesticide Glyphosate (Roundup)

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Abstract The experiments were aimed at studying the tolerance of the studied strains of *Rhodococcus* sp. M1 and *Rhodococcus* sp. D1 to the presence of Glyphosate (N-(phosphonomethyl) glycine) in the culture medium. A rich organic medium (YEPD), a mineral medium (VD) with 1% glucose and a mineral medium without a carbon source were used for cultivation. Each of the media was supplemented with Glyphosate, up to 0.3 g/l. The obtained data showed that the tested strains grew well and were tolerant to the presence of Glyphosate in the media used.

The purpose of the following experiments was to determine the ability of both strains to degrade glyphosate. By GC-MS analyses, it was found that a strain of *Rhodococcus* sp. M1 utilized glyphosate as the only carbon source, degrading 36% of it within 120 hours. The other strain, over the same time period, degraded 40% of the pesticide tested.

Glyphosate is one of the most widely used organophosphate herbicides in modern agriculture. The presented studies on the potential of new strains of *Rhodococcus* for its biodegradation contribute to the complementarity of the known techniques for bioremediation of polluted environment.

Keywords: Biodegradation, Bacteria, Glyphosate, GC-MS

1. Introduction

During the second half of the last century, continued at the beginning of the new century, glyphosate has increasingly used as an herbicide in agriculture, maintenance of parks, and home gardens (Malik et al., 1989; Bai and Ogbourne, 2016). Respectively, its production is growing, which, according to trends, should exceed 34 billion tons in the near future. Its intensive application has long provoked discussion in the scientific community about its impact on the development of living organisms, and the environment (Rueppel et al., 1977; Mertens et al., 2018; Ayele and Assefa, 2017). Serious studies have been published concerning the involvement of microorganisms in the partial or complete degradation of glyphosate (Obojska and Lejczak, 2003; Doublet et al., 2009; Zhan et al., 2018; Elarabi et al., 2020). The existence of various metabolic chains described contributing to its elimination, as well as the occurrence of intermediates of decomposition has been studied (Huang et al., 2005; Sviridov et al., 2015; Manav et al, 2018; Elarabi et al., 2020).

To establish the degradation ability of soil microorganisms towards glyphosate as a single source of phosphorus, nitrogen, and/or carbon many research has been made. So far, representatives of *Ochrobactrum, Burkholderia, Flavobacterium*, and other bacteria with the potential to participate in bioremediation processes have been described (Balthazor and Hallas, 1986; Hadi et al., 2013; Manogaran et al., 2018;) There are not many data on the use of the herbicide as the only source of carbon (*Streptomyces, Achromobacter, Stenotrophomonas, Providencia*) and its complete removal from contaminated habitats (Sviridov et al., 2015; Nourouzi et al., 2017).

Studies have shown that strains of *Rhodococcus* sp. D1 and *Rhodococcus* sp. M1 possess tolerance to the presence of glyphosate in the environment, and were able to utilize it, as the only source of carbon and energy. Research on the ability of microorganisms other than those described so far to utilize and remove herbicides, including glyphosate, remains important. They can contribute to a better understanding of the possibilities for biotransformation, biodegradation, and the impact on biodiversity in various areas of the environment affected by such pollution.

The strains studied in this work have been obtained from polluted soils due to oil production of Kumkol, Kyzylorda istrict in Kazakhstan.
In our studies, we have shown that strains of *Rhodococcus* sp. D1 and *Rhodococcus* sp. M1 possessed tolerance to the presence of glyphosate in the environment. Both of them were able to utilize most of it as the only source of carbon and energy.

2. Materials and methods

2.1. Microbial cultivation

Strains *Rhodococcus* sp. M1 and *Rhodococcus* sp. D1 were cultivated according to the purpose of experiments in the following media:

Liquid complete medium Yeast Extract Peptone Dextrose (YEPD), contained 10 g/l Yeast extract (Sigma-Aldrch Co., USA); 20 g/l Peptone (Sigma-Aldrch Co., USA), 10 g/l Dextrose (glucose).

Liquid mineral medium (VD): 1 g/l NH_4NO3, 1 g/l K2HPO4,1 g/l KH2PO4, 0.2 g/l MgSO4, 0.02 g/l CaCl2x6H2O, 10 g/l NaCl, 0.01 g/l $FeCl_3$, with 0.3 mg/ml glyphosate (Sigma-Aldrch Co., USA), and 0.1% glucose; pH=7.0-7.2 (Spankulova et al., 2018).

Liquid VD medium complemented with 0.3 mg/ml glyphosate as a carbon source.
The strains were grown at aeration and at 30°C.

2.2. GC-MS analyses

The GC-MS analyses were performed on a Hewlett Packard 7890 instrument coupled with MSD 5975 equipment (Hewlett Packard, Palo Alto, CA, USA) operating in EI mode at 70 eV.

3. Results and Discussion

The first group of experiments had the purpose to find out whether the studied bacterial strains were tolerant to the presence of glyphosate. Both of them were cultivated under favorable nutritional and temperature conditions. The cultivation experiments were performed in a rich organic medium (YEPD) supplemented with glyphosate. Data on the tolerance of the studied strains to the pesticide presence showed that each of them had the capability to grow in the presence of glyphosate, included in different concentrations (from 0.1 g/l to 0.6 g/l). Later on, the microbial growth in the presence of glyphosate was monitored in VD medium, including 0.3 g/l glyphosate in the presence and absence of low glucose concentration (0.1%).
The growth of both strains is comparable in the presence of glucose in the medium. Better growth of *Rhodococcus* sp. D1 compared to that of *Rhodococcus* sp. M1 strain in the medium with glyphosate as the only carbon source was noticed (Fig.1).

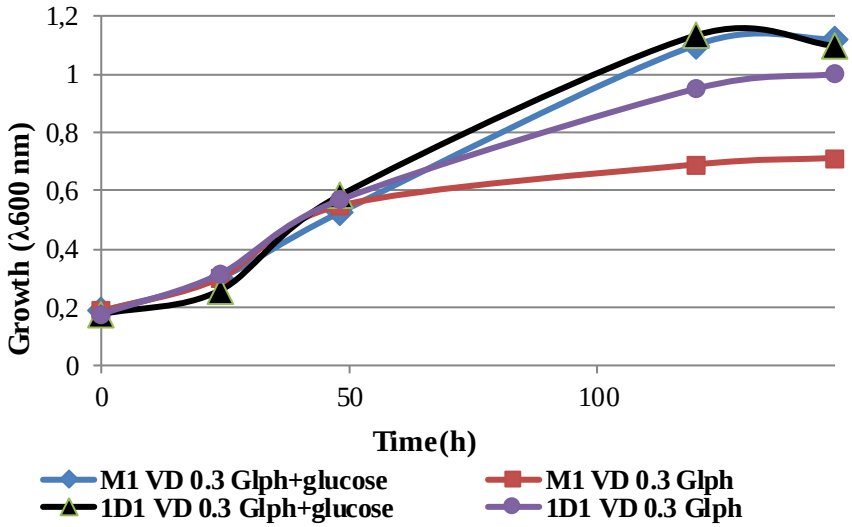

Figure 1. Growth curves of strains of *Rhodococcus* sp. D1 and *Rhodococcus* sp. M1 in mineral medium VD, supplemented with glyphosate

The gradual reduction of glyphosate concentrations included in the medium as sole carbon sources has following by gas chromatography-mass spectrometry GC-MS). The concentrations detected at 120th hour cultivation were 19.67µg/ml for *Rhodococcus* sp. M1, and 18.26 µg/ml for *Rhodococcus* sp. D1 (Fig.2).

a plant of the family Fabaceae, degradation of 97% glyphosate has been observed after 20 days. (Massot et al., 2021)

The obtained results convincingly show the resistance of the studied strains to the presence of glyphosate in the medium, as well as the ability to

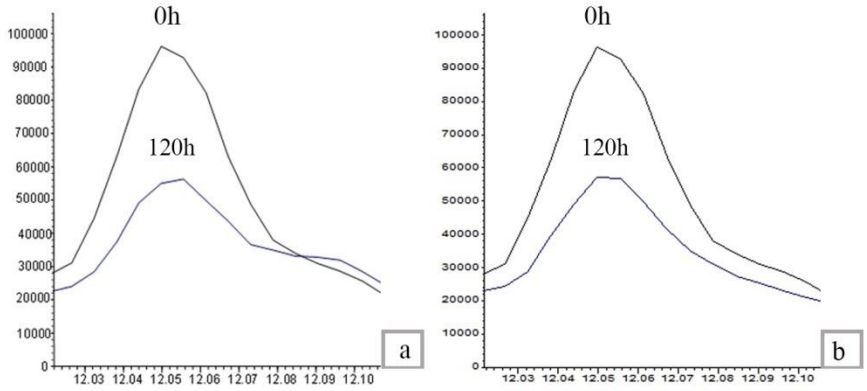

Fig. 2. GC-MS analysis for biodegradation of glyphosate by starin:
a - *Rhodococcus* sp. M1 and **b**- *Rhodococcus* sp. D1

To date, a large number of promising bacterial strains capable of participaton in bioremediation of chronically contaminated with glyphosate soils and waters have been brought to the scientific community's attention. A strain of *Burkholderia vietnamiensis* has been described, which is tolerant to glyphosate up to a concentration of 500 ppm, and 92.32% of the herbicide at a concentration of 100 ppm has degraded under optimal conditions for its development. (Manogaran et al., 2018). A strain of *Bacillus aryabhattai* FACU isolated from agro-industrial soil has shown tolerance to high glyphosate concentrations in a rich medium (Bacto-LB broth). The strain also showed good growth in mineral salt medium containing 50 mg/ml and more glyphosate. (Elarabi et al., 2020) The matching of the strains *Ochrobactrum haematophilum* P6BS-III and *Rhizobium* sp. P44RR-XXIV at *in vitro* experiments has resulted in the metabolism of almost half of 50 mg l^{-1} glyphosate in 9 days. In the combination of *O. haematophilum* P6BS-III and *Lotus corniculatus* L – use it for their development. It should be pointed that the mineral medium (VD) used for its cultivation included compounds providing nitrogen and phosphorus, but only glyphosate ensured the presence of carbon. By the present study, members of the genus *Rhodococcus* are included in the growing list of bacteria capable of using glyphosate as the sole carbon source.

To fully elucidate the mechanisms of glyphosate biodegradation in nature, many questions remain to be answered. The data accumulation on the processes taking place in newly isolated representatives of different bacterial species would contribute to its solution and the possibility of its practical application in bioremediation.

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