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Effect of sulfonylurea tribenuron methyl herbicide on soil *Actinobacteria* growth and characterization of resistant strains

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ABSTRACT

Repeated application of pesticides disturbs microbial communities and cause dysfunctions on soil biological processes. Granstar® 75 DF is one of the most used sulfonylurea herbicides on cereal crops; it contains 75% of tribenuron-methyl (TBM). Assessing the changes on soil microbiota, particularly on the most abundant bacterial groups, will be a useful approach to determine the impact of Granstar® herbicide. For this purpose, we analyzed *Actinobacteria*, which are known for their diversity, abundance, and aptitude to resist to xenobiotic substances. Using a selective medium for *Actinobacteria*, 42 strains were isolated from both untreated and Granstar® treated soils. The number of isolates recovered from the treated agricultural soil was fewer than that isolated from the corresponding untreated soil, suggesting a negative effect of Granstar® herbicide on *Actinobacteria* community. Even so, the number of strains isolated from untreated and treated forest soil was quite similar. Among the isolates, resistant strains, tolerating high doses of Granstar® ranging from 0.3 to 0.6% (v/v), were obtained. The two most resistant strains (SRK12 and SRK17) were isolated from treated soils showing the importance of prior exposure to herbicides for bacterial adaptation. SRK12 and SRK17 strains showed different morphological features. The phylogenetic analysis, based on 16S rRNA gene sequencing, clustered the SRK12 strain with four *Streptomyces* type strains (*S. vinaceusdrappus*, *S. mutabilis*, *S. ghanaensis* and *S. enissocaesilis*), while SRK17

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strain was closely related to *Streptomyces africanus*. Both strains were unable to grow on TBM as unique source of carbon, despite its advanced dissipation. On the other hand, when glucose was added to TBM, the bacterial development was evident with even an improvement of the TBM degradation. In all cases, as TBM disappeared, two compounds were detected with increased concentrations. These by-products appeared to be persistent and were not degraded either chemically or by the studied strains. Based on these observations, we suggested that bacterial activity on carbon substrates could be directly involved in the partial breakdown of TBM, by generating the required acidity for the first step of the hydrolysis. Such a process would be interesting to consider in bioremediation of neutral and alkaline TBM-polluted soils.

Introduction

The soil ecosystem is the theater of complex linked biological processes, regulated by its microbiota. Changes, such as introducing xenobiotic substances, cause fluctuation on microbial quality and quantity, affecting then the soil balance. Therefore, the microbial population reflects environmental changes and may be taken as an efficient indicator to evaluate the impact of exogenous molecules.[1]

Sulfonylurea herbicides are widely used around the world to protect cereal crops. They could constitute a long-term environmental hazard,[2] with even a contamination risk of groundwater and surface waters, due to leaching processes.[3,4] Furthermore, they are considered as disturbers for the soil microbiota.[1,5] Granstar® 75 DF (DuPont de Nemours) is one of the most used herbicides in Algeria.[6] It is used under different commercial denominations, in North Africa, Europe, North and South America and Asia.[7–10] This herbicide contains 75% of tribenuron-methyl (TBM), a sulfonylurea molecule active against a large number of annual dicotyledons[11] and it is used in low but effective concentrations (12 g/300 L/ha). TBM acts by stopping cellular division of meristematic tissues of plants via inhibition of acetolactate synthetase; an enzyme present in higher plants, bacteria and fungi, but absent in humans and animals.[7,12,13]

Several studies reported effects of sulfonylurea herbicides on global microbial community and on specific groups like fungi.[1,5,13] However, the impact on soil *Actinobacteria* is still little explored, despite their abundance and importance in soil.[14] Actually, these Gram-positive bacteria have considerable potential for biotransformation and biodegradation of pesticides,[15,16] due to their ability to produce variable extracellular enzymes, degrading complex and recalcitrant pollutants[17] in various environments.[18]

The main objective of this study was to examine the effect of Granstar® herbicide on soil *Actinobacteria*. For that purpose, *Actinobacteria* strains were isolated from both untreated and Granstar® treated soils. The resistance of isolated strains to different concentrations of the herbicide was evaluated. The two most resistant strains were further characterized and the impact of TBM on their growth determined.

Materials and methods

Soil treatment and Actinobacteria isolation

Herbicide Granstar® 75 DF was obtained as a commercial powder from Du Pont de Nemours (Algeria). Three soils were selected for our study. Two agricultural soils, from two different eastern regions of Algeria: Ain Karma, Constantine (soil 1) and Ain Babouche, Oum El Bouaghi (soil 2). The third soil was from a forest, taken at Chaabet Ersas, Constantine (soil 3). Soils 2 and 3 were free of pesticides, while soil 1 was regularly treated with various herbicides, including Granstar®. Soil samples were collected at 10 cm depth. The soils 2 and 3 were incubated at laboratory with the herbicide as follows: Granstar® was added to 150 g of soil at 40 mg L^{-1}, which is the recommended field application dose. The incubation was performed in covered aerated two liters beakers at room temperature for three weeks. Hereafter, these treated soils are named treated soil 2 and treated soil 3.

For isolation of *Actinobacteria* strains, 10 g of each soil sample (soil 1, soil 2, soil 3, treated soil 2, treated soil 3) were suspended in 90 mL of physiological water (NaCl 9‰). For platting isolation, serial dilutions (10^{-2}, 10^{-3} and 10^{-4}) were prepared using physiological water. Isolation was carried out on Bennett agar, containing 10 g glucose, 2 g casamino acid, 1 g meat extract, 1 g yeast extract, 15 g agar per liter of distilled water, pH 7.3,[19] supplemented with 40 mg L^{-1} of Granstar®. This medium allows *Actinobacteria* growth[20] and is considered as a production medium for enzymes and bioactive substances.[21] After incubation at 30 °C for a week, *Actinobacteria* colonies were selected by a direct light microscopic observation (×10) based on their morphological characteristics (presence of short branching filaments). The isolated strains were preserved on 50% glycerol solution (v/v) at −80 °C and on starch-casein agar slant, after sporulation, at 4 °C.

Herbicide-resistance screening

Resistance of the isolated strains to various doses of Granstar® was verified by using Bennett agar medium supplemented with herbicide at different concentrations: 0.004, 0.04, 0.05,

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