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Bacteria Mineralizing Phytate In The Bean Rhizosphere In An Algerian Agro-Ecosystem

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Abstract **–** Phosphorus (P) is an essential element for plant growth, but its low bioavailability in soil often limits agricultural production, particularly for common bean (*Phaseolus vulgaris* L.). This study aimed to investigate the presence of phytasic bacterial communities in soils with different levels of P deficiency, and their impact on the availability of P for P. vulgaris. The results showed that the phytasic bacterial communities carrying the BPP gene were present in both types of soils and were more abundant in the P-deficient soil and in the rhizosphere compared to the P-rich soil and bulk soil, respectively. This study highlights the importance of these bacteria in promoting plant growth and suggests that their density is influenced by both soil type and the presence of the plant.

Keywords – Cellular Beam, Circular Cut, Length, Openings, Load Location, Robot Structures, Euro-Code 3.

I. INTRODUCTION

Phosphorus, in its orthophosphate (Pi) form, is an indispensable nutrient for the growth and development of plants. Despite the high total P content in soil, the bioavailability of Pi is often limited due to its slow diffusion and high fixation in soil, making it a limiting factor for plant growth and productivity [1, 2].

The application of phosphate fertilizers can compensate for the low availability of Pi in cropping systems, but the excessive input of Pi can cause serious environmental problems such as eutrophication. Furthermore, the world's source of P-rock is non-renewable and rapidly depleting (3). Organic phosphorus (Po) is present in large quantities in soils, it can represent 25 to 80% of total phosphorus in soils [4, 5]. The dominant class of organic phosphorus, representing between 60 and 80% of (Po), is the phosphate inositol, better known as "Phytate". These phytates are synthesized in terrestrial ecosystems mainly by plants and are highly complexed in soils [6, 7].

Phytates can easily undergo physical and chemical reactions in soils, making them available to plants [8]. It is their mineralization that is likely to provide a source of available phosphorus for plants and microorganisms [9]. The hydrolysis of phytate in the soil is carried out by enzymes called “phytases” [8].

It has been proven by [10], that the activity of these enzymes increases considerably in the rhizosphere, the volume of soil surrounding the roots and interacting with them [11], and that the main source of this activity in the soil is of microbial origin [12].

Microorganisms are an integral part of the phosphorus (P) cycle in the soil and as such play an important role in mediating P availability to plants. There is growing evidence that a range of microorganisms possess the ability to utilize phytate in their environment [13], some bacterial genera, such as *Pseudomonas*, *Bacillus* and *Burkholderia* are already known to be effective phytase producers, but the importance and diversity

of phytase-producing strains in the rhizosphere is still largely unknown [9].

The objective of our study was to research the bacterial community that mineralizes phytate, by studying its density in two contrasting soils with respect to P deficiency, and this at the level of the Bulk soil and the rhizosphere, and by highlighting the presence of the BPP gene responsible for the synthesis of the enzyme phytase. We took as a model plant, the bean (*Phaseolus vulgaris* L.) because it is one of the most consumed legumes and which requires a high level of phosphorus for its development.

II. MATERIALS CHARACTERIZATION

A. Plant material and soil sampling

The experimentation was conducted on a line of beans (*Phaseolus vulgaris* L.) sensitive to phosphorus deficiency, known as P-inefficient [14]. This is the 147 line resulting from the crossing between BAT 477 and DOR 364. The soils used were previously collected for a thesis project as part of the "FabaTropimed" grand project. They come from an experimental agro-ecosystem located in the wilaya of Sétif. We selected 2 soils contrasting in P content, namely site 1 located in the locality of l'hamiat with 5.42 ppm of P and site 6 located in the locality of kasr el abtal with 21.53 ppm of P.

B. Sampling of rhizosphere soil and bulk soil

The two collected soils were each separated into two fractions. The first fraction, represented by the soil surrounding the root system, is called the "rhizosphere", and the second fraction is called the "bulk soil", defined as the soil outside the influence of the plant roots [15]. We separately collected the two fractions from the two soils, weighed them, and suspended them in a volume of Phosphate-Sodium Buffer (PBS) corresponding to: 1ml of PBS per gram of soil [9].

C. Enumeration and isolation of soil bacteria

The process involves counting the number of bacteria present or CFUs (Colony Forming Units) on a solid medium obtained from a dilution. For each suspension (rhizosphere soil and bulk soil from both soils), we performed successive dilutions at 1/10th (1ml of dilution solution in 9ml of 9% physiological saline.

20µl of the dilutions were taken and deposited onto a rich culture medium, the Luria-Bertani (LB) medium, using the "drop" technique where the plates are divided into four parts, each part being seeded once with a dilution.

D. Molecular analysis of soil bacteria isolated on specific Angle medium

To characterize the presence of the BPP gene, we performed a colony PCR, which is a PCR that is performed directly on a bacterial colony, allowing for a rapid and reliable screening of the target gene. Each isolated colony presenting a halo (a clear zone around the colony) was collected using a sterile cone and suspended in Eppendorf tubes containing the PCR Mix and amplified to detect bacterial colonies carrying the specific BPP gene.

The amplification of this gene was performed with a final reaction volume of 25µL containing 12.5µl of AmpliTaq Mix (containing Buffer, MgCl2, dNTP and Taq polymerase enzyme), 1µL for each primer, 1 bacterial colony, and 10.5µL of sterile water. The detection of the phytase-coding gene was performed by testing 2 specific primer pairs to compare the results obtained previously with degenerated BPP primers (Maougal unpublished data). The first primer pair from [16], called BPP.left 8 and BPP.right 856, and the second primer pair was designed at the eco and sol laboratory (Montpellier) and is denoted L_BPP.Phy 5'.

Table 1 primers used for the detection of the BPP gene

Primers	Primer sequence	Length (pb)
BPP.left 8	5'- CTGATGATCCTGCGATTTGG-3'	20
BPP.right 856	5'- CGTCCTGTGCGACAAAAATA-3'	21
L_BPPphy5'-941	5'- CTTCCCAGGCAAAGCATAAG-3'	21
R_BPPphy3'-941	5'- CGTCCTGTGCGACAAAAATA-3'	21

These two primer pairs were previously designed to target a 200 bp fragment. The PCR was conducted as follows [17): initial denaturation at 95°C for 4

minutes, followed by denaturation at 95°C for 30 seconds, a series of 8 cycles at 57°C for 30 seconds with an increase of 1°C per cycle, 72°C for 30 seconds, and 95°C for 30 seconds, followed by a series of 27 cycles at 48°C for 30 seconds, 72°C for 30 seconds, and a final synthesis at 72°C for 5 minutes.

We added 5µL of loading buffer (Proméga) to the obtained PCR products. From this total volume, 5µl were withdrawn to be deposited on a 1.5% agarose gel with a high molecular weight marker (1kpb DNA ladder and a low molecular weight marker (100 bp DNA ladder) by electrophoresis for 30 minutes at 135 Volts. Ethidium bromide (BET) staining was added to the agarose gel to visualize the obtained bands and determine the BPP gene.

III. ANALYSIS OF EXPERIMENTAL RESULTS

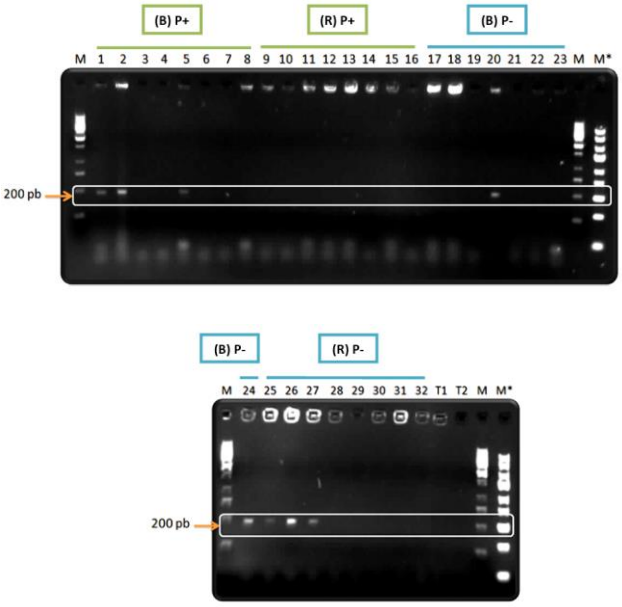

Fig. 1. An agarose gel of 1.5% is displaying the distinct electrophoretic profiles of PCR products (using Blair primers) from a total of 32 colonies.

In Figure 1, it is observed that for bacterial colonies 1/2/5/20/24/26/27, a single band of 200 bp was detected by PCR, corresponding to the size of the BPP gene. Therefore, it can be deduced that all these bacterial colonies carry the BPP gene.

When considering only the type of soil, it is noteworthy that the P- soil harbors a greater number of bacteria carrying the BPP gene compared to the P+ soil. Specifically, 31.25% of the bacterial colonies screened by PCR in the P- soil were positive for the BPP gene, whereas only 18.75% of the screened colonies in the P+ soil carried the gene.

In the P- soil, bacteria carrying the BPP gene were found in both the R and B soil fractions. A total of 25% of the bacteria in the Bulk soil and 37.5% of the bacteria in the rhizosphere were positive for the gene. These percentages clearly indicate that phytase bacteria are more abundant in the rhizosphere of *P. vulgaris* compared to the Bulk soil.

For the P+ soil, bacteria carrying the BPP gene were detected only in the Bulk soil fraction.

	P+		**P-**	
	Bulk	**Rhizosphère**	**Bulk**	**Rhizosphère**
Groupe BBP +	1/2/5		20/24	25/26/27
Groupe BPP -	3/4/6/7/8	9/10/11/12/13/ 14/15/16	17/18/19/21/22/ 23	28/29/30/31/32

Table. 2. Classification of bacteria based on the presence of the BPP gene using Blair primers.

As per the research conducted by [18], and [19], soil microorganisms play a vital role in mediating soil organic P mineralization, thereby contributing significantly to plant P availability. This elucidates the presence of bacteria carrying the BPP gene in bulk soils of both types.

According to the study carried out by [20], certain bacterial strains exhibit differential growth in the rhizosphere than in bulk soil. [21], suggest that plant root exudates facilitate the proliferation of specific microbial species, particularly those with high nutritional requirements and growth rates, such as *Pseudomonas*, in the rhizosphere. This explains the high density of phytasic bacteria in the rhizosphere.

[22], have established that the association between P-degrading bacteria and plant roots plays a crucial role in P nutrition in numerous agricultural ecosystems, particularly in P-deficient soils. This implies that phytate mineralizing bacteria, present in the soil-plant system, are favored near the roots and are involved in the recycling of phytate from the root.

IV. CONCLUSION

The experiments conducted aimed to evaluate the density of bacterial communities capable of mineralizing phytate through phytase secretion in the rhizosphere of Phaseolus vulgaris legumes, where they were isolated to determine their density in two contrasting soils with respect to P deficiency, and in their two fractions, the rhizospheric soil and the Bulk soil. The results obtained indicate that phytase bacteria carrying the BPP gene are ubiquitous in P. vulgaris soil, and their density is higher in P-deficient soil than in P-rich soil. These bacterial communities are more frequent in the rhizosphere than in the Bulk soil, indicating positive selection by the bean roots for strains possessing these capabilities. This study has revealed that the density of these bacteria is affected by the presence of the plant as well as the type of soil.

This study highlights the need for further research on these communities capable of mineralizing organic phosphorus to improve the performance of their use as biofertilizers. Complementary molecular analyses would be necessary to identify and define these isolated colonies through sequencing to establish a phylogenetic profile via DGGE (Denaturing Gradient Gel Electrophoresis).

The finite nature of natural resources and the negative impacts of excessive use of phosphate fertilizers, which pollute the environment, compel mankind to opt for sustainable agriculture. Therefore, the use of microorganisms to increase the availability of P in the soil is an interesting proposition to develop this type of agriculture. This is important for large-scale production systems in the developed world, as well as for developing countries where access to mineral fertilizers is limited.

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