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Efficiency of potassium and phosphate solubilizing Actinobacteria in wheat plant growth promotion

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Abstract: Soil fertility and plant nutrition require an adequate management of essential macronutrients such as potassium (K) and phosphorus (P), which are mandatory for plant development. Bioleaching of K and P bearing minerals improves their chemical weathering and increases the performance of the biofertilization strategies. In this study, in vitro and greenhouse experiments were carried out to investigate P and K solubilization traits of nine Actinobacteria (P13, P14, P15, P16, P17, P18, BC3, BC10, and BC11) under fertilization with rock phosphate (RP). K and P solubilization were evaluated on Alexandrov and NBRIP media containing mica and six RP samples, respectively. The actinobacterial strains were able to solubilize K in Alexandrov medium supplemented with RP. However, when soluble P was used instead of RP, only four strains of Actinobacteria (P18–BC3–BC10 and BC11) solubilized K. The solubilization values of K ranged from 2.6 to 41.45 mg/L while those of P varied from 0.1 to 32 mg/L. Moreover, all strains were able to produce IAA, siderophore, HCN, and ammonia and significantly improved the germination rate and the vigor index of wheat. The pot experiments revealed that four strains (*Streptomyces alboviridis* P18, *Streptomyces griseorubens* BC3, *Streptomyces griseorubens* BC10, and *Nocardiopsis alba* BC11) significantly improved the growth parameters of wheat, namely root length (1.75–23.84%), root volume (41.57–71.46%), root dry weight (46.89–162.41%), shoot length (8.92–23.56%), and shoot dry weight (2.56–65.68%) compared to the uninoculated control. These findings showed that *Streptomyces griseorubens* BC10 and *Nocardiopsis alba* BC11 are promising candidates for the implementation of efficient biofertilization strategies to improve soil fertility and plant yield under rock P and rock K fertilization.

Keywords: Actinobacteria; rock phosphate; potassium; solubilization; biofertilization; PGP traits; wheat germination

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1. Introduction

With roughly 83 million people being added to the world's population every year, it is estimated that the total demand for food will increase by 40% by 2030 and 70% by 2050 [1]. Therefore, achieving such goal will require the implementation of efficient and sustainable fertilization approaches to improve bioavailability of essential nutrients such as phosphorus (P) and potassium (K). Nonetheless, the low solubility of P and K is a major drawback to their direct application, notably in non-acidic soils [2]. Currently, converting the insoluble portion of P into soluble fraction is a key objective in sustainable agriculture [3]. In this context, soil application of phosphate solubilizing microorganisms (PSM) based

inoculum is a promising approach which take advantage of the capacity of PSM to assimilate phosphorus for their own requirement, while making it available for plant uptake [4]. Actinobacteria or Actinomycetes are considered as plant growth-promoting rhizobacteria (PGPR) and their P solubilization capacity has been well documented [5][6]. These filamentous bacteria have the ability to persist in very difficult and competitive environments by producing spores which adhere to soil particles [7]. Moreover**,** several interesting properties of these microorganisms have been evidenced such as the production of metabolites that improve plant growth and tolerance to biotic and abiotic stresses [8] which make them suitable candidates for the production of highly versatile biofertilizers [9]. Unfortunately, until now Actinobacteria have been scarcely investigated compared to other plant growth promoting rhizobacteria such as Proteobacteria and firmicutes [10]. Consequently, the current study focused on the evaluation of the ability of Actinobacteria strains isolated from contrasting environment to solubilize rock potassium RK and different rock phosphates RPs, their ability to produce PGP related compounds such as IAA, siderophore, HCN and ammonia as well as their effect on wheat plant development.

2. Methods

2.1. Screening for rock phosphate solubilization

Nine Actinobacteria, isolated from two different sites of Morocco (Desert) were used in this study. Their taxonomic position showed that they belong to *Streptomyces* and *Nocardiopsis* genera. The strains were labeled as follows: P13 - P14 – P15 – P16 – P17 – P18 – BC3 – BC10 and BC11. The phosphate solubilizing activities of each Actinobacteria strain was evaluated on NBRIP agar plates and on liquid medium containing 5g of RP as a sole source of phosphorus [11]. After 3, 7 and 11 days of incubation, 1 ml of each culture was collected in three replicates and centrifuged at 10.000 rpm for 10 min. Non-inoculated sterilized broth was used as the control. Soluble P was determined according to Nagul *et al* [12] and pH variation was recorded at T0, T3, T7 and T11.

2.2. Screening for rock potassium solubilization

K solubilization was evaluated on Alexandrov agar medium containing 5 g of mica as an insoluble source of potassium [8]. The quantitative estimation of potassium solubilization rate was carried out on Alexandrov liquid media supplemented with mica, through adding 1 mL of a 48h culture of each strain to 100 mL of Alexandrov broth incubated at 28°C. After 11 days, K release in inoculated and non-inoculated treatments was evaluated by atomic absorption spectrometry according to Othman et al [12].

2.3. *Dual solubilization of Potassium and phosphate*

To assess the ability of Actinobacteria strains to simultaneously solubilize K and P, Alexandrov broth containing RP and mica as the sole source of P and K were used. 10 µl of each pure culture were suspended in 100 ml of broth. The cultures were incubated at 28°C for 11 days. The available K and P in the supernatant were evaluated using the previously described protocol.

2.4. In-vitro evaluation of other actinobacteria PGP traits (Indole Acetic Acid (IAA), Hydrogen cyanide (HCN), ammonia and siderophore production)

The production of IAA was determined colorimetrically according to Sachdev *et al***.** [8]**.** On the other hand, the ability of Actinobacteria to produce HCN was tested for each strain in overnight LB broth supplemented with glycine (4.4 g/L). The qualitative test was determined by putting underneath each Petri dish lids, a Whatman filter paper flooded for 1 min by a solution of 0.5% picric acid in 2% sodium carbonate and incubated for 1 week at 28°C. A change from yellow to orange / red color on the Whatman paper indicates a positive production [12]. Furthermore, ammonia production by the selected strains was evaluated by inoculating them into 1 ml of peptone water and incubated at 28 °C, 120 rpm

for 11 days. Nessler's reagent (0.5 ml) was then added in each tube and the ammonia production was detected by the development of a yellow-brown color. Finally, for siderophore production, the Actinobacteria strains were inoculated on Chrome Azurol S (CAS) reagent [13]. Each Actinobacteria strain was spotted on each plate and incubated at 28 °C for 11 days. An un-inoculated plate was used as control. After incubation, the formation of an orange zone around colonies was reported as positive.

2.5. Effect of Actinobacteria on wheat plant: Greenhouse experiment

Based on the in vitro assays results, the top four performing Actinobacteria (P18-BC3- BC10 and BC11) were selected to evaluate their capacity to release, under greenhouse conditions, P and K form RP and mica respectively. The experiment was conducted at the experimental farm of the Mohamed VI Polytechnic University in Benguerir, Morocco from September to November 2020 by using sterilized Wheat (*Triticum aestivum* variety *Vitron)*. The seeds were grown in PVC pots containing 1.5 Kg of sterilized mixture of sand (with low P) and perlite (3:1 w/w). Pots (4 seeds per pot) were arranged according to a completely randomized block design with five replicates and six treatments: **(1)** (C-) Negative control (without bacterial inoculation, Mica nor RP fertilization) ; **(2)** C+ (TSP) positive control containing triple superphosphate (containing 46 % soluble P_2O_5); **(3)** treatment N3 fertilized with Mica ; (4) Treatment N4 containing RP (32.5 % total P_2O_5); (5) treatments N5 containing with Mica + RP and (6) Treatment N6 with four co-inoculation containing RP + Mica each with the strains (P18 –BC3 –BC10 and BC11). Plants were grown for 60 days and watered 2 times per week with 1/2 modified Hoagland solution [12] without any sources of P and K. At the end of the experiment, plants were carefully taken out of the pots and washed with distilled water, then root traits were determined using WinRHIZO image analyzing system (Regent Instructions, Quebec, Canada). Shoot and root dry weight was determined after over-drying at 68°C for three days. Thereafter, the estimation of the plant response to inoculation with the Actinobacteria strains in terms of biomass enhancement was calculated using the following formula:

$$\text{GE (\%)} = \frac{(\text{Shoot dry weight of treated plants} - \text{shoot dry weight of control plants})}{\text{Shoot dry weight of control plants}} \times 100$$

Where GE: Growth enhancement

2.6. Statistical analysis

All the results were statistically analyzed using IBM SPSS Statistics 20 Software. Comparison between treatments were performed using one-way analysis of variance (ANOVA) with least significant difference (LSD). Tukey's comparison test was performed at $p=0.05$ in case of significant impact by factor.

3. Results

In this study, the Actinobacteria strains showed a broad RP solubilization spectrum. In general, all strains were able to dissolve at least one RP type. The solubilization rate of RP was directly correlated to RP P_2O_5 content and their capacities to solubilize the RPs ranged from 0.1 to 32 mg /L. These results are in agreement with findings of Hamdali *et al* [8] who reported high amount of phosphate solubilizing activity by *Streptomyces griseus* and *Streptomyces cavourensis* with 29.67 and 21.43 mg/L, respectively. Several mechanisms can be involved in microbial P solubilization with the most common one being via media acidification as it was evidenced by our experiment. In the literature, several reports suggest that the solubilization of mineral phosphate by microorganisms might be due to the production of siderophores [14]. Interestingly all tested Actinobacteria strains were able to produce siderophores, which may suggest that a dual solubilization process may be involved, implying both media acidification and siderophore secretion. On the other hand, K solubilization assays revealed that among the selected strains, only *Streptomyces alboviridis* P18, *Streptomyces griseorubens* BC3, *Streptomyces griseorubens* BC10, and *Nocardiopsis alba* BC11 were able to solubilize mica at 3, 11, 12.75 and 17.8 mg /L, respectively. However, in this study K solubilization under phosphorus deficiency showed some

relevant results since all the actinobacteria strains were able to solubilize the potassium source. These results showed that the deficiency in P stimulates the solubilization of K. For instance, K solubilization value of *Nocardiopsis alba* BC11 was 41.5 mg /L under P deficiency and 17.8 mg/L under P (KH_2PO_4) sufficiency. Such difference could be explained by the fact that molecules involved in P solubilization may also solubilize the insoluble source of K (Mica) and, therefore, trigger more efficient K solubilization. Similar results were found by Abou-el-Seoud and Abdel-Megeed [15] who reported that co-inoculation of P and K dissolving bacteria under RP fertilization increased P and K availability and uptake. Beside their capacity to solubilize RP and RK, the Actinobacteria strains were screened for plant growth promoting (PGP) factors which are considered as an effective tool in the investigation of microorganisms that can be used as biofertilizers. Interestingly, all tested strains showed notable PGP activities such IAA, HCN and ammonia production. These results were in agreement with those reported by Doumbou *et al* [16]. Moreover, most of the evaluated strains had positive effect on germination and growth traits of wheat seedling (Table 1), which is in agreement with previous works of Sharma *et al* [17] who demonstrated that the use of PSB inoculants (*P.fluorescens* and *B.megaterium*) improved the percent of radicle and plumule lengths by 59.7 and 56.4%, respectively compared to non-inoculated treatments. Under greenhouse conditions, the selected Actinobacteria strains (*Streptomyces alboviridis* P18 – *Streptomyces griseorubens* BC3 – *Streptomyces griseorubens* BC10 and *Nocardiopsis alba* BC11) significantly enhanced several wheat growth parameters including root lengths (1.75 – 23.84%), shoot lengths (8.92 – 23.56%), root volume (41.57 – 71.46%), root dry weight (46.89 – 162.41%) and shoot dry weight (2.56 – 65.68%); over the un-inoculated control (Table 2). The maximum root length was recorded with the treatments inoculated with *Streptomyces griseorubens* BC10 and *Nocardiopsis alba* BC11 (19.35 and 23.84% respectively). Those results support our *in-vitro* evaluation of the tested Actinobacteria strains in which high amount of IAA was positively correlated with the improvement of plant growth parameters. Our findings are also in concordance with the investigation of Sreevidya *et al* [18] who reported an increase of chickpea root length (17%) and shoot length (3%) following *streptomyces* inoculation.

Table 1. Effect of RP-phosphate-solubilzing actinobacteria co-inoculation on wheat growth.

Treatments	**Effect of RP and Actinobacteria inoculation**		
	Plumule increasment	Root increasment	Vigor index increasment
Negative control			
Control (RP)			
P13 +RP	+ 7.14%	+ 51%	11.66% **(+2.13)**
P14+ RP	+ 15.38%	+ 37.09%	12.05% **(+2.29)**
P15 +RP	+ 42.85%	+ 52.94%	16.17% **(+ 7.07)**
P16 +RP	+ 34.61%	+ 90.47%	9.44% **(+ 2.78)**
P17 +RP	+ 20.53%	+ 14.86%	12.64% **(+1.7)**
P18 + RP	+ 31.94%	+ 15.38%	17% **(+3.3)**
BC3 + RP	+ 86.44%	+ 92.3%	16% **(+7.5)**
BC10 + RP	+ 72.72%	+ 101.21%	17.75% **(+8.15)**
BC11 + RP	+ 66.66%	+ 70.21%	18.5% **(+7.7)**

Table 2. Effect of Actinobacteria inoculation on biomass yield and root traits of wheat.

Treatments	**Shoot length (cm)**	**Root length (cm)**	**Shoot dry weight (g /plant)**	**Root dry weight (g /plant)**	**Root volume (cm^3)**
C-	23.85 ± 0.96 d	46.15 ± 2.57 c	0.23 ± 0.03 d	0.27 ± 0.04 c	1.12 ± 0.12 e
C+ (TSP)	60 ± 2.96 a	48.12 ± 6.12 bc	3.22 ± 0.35 a	1.15 ± 0.31 a	2.93 ± 0.42 ab
Mica	55.95 ± 1.95 a	49.56 ± 6.24 bc	2.51 ± 0.381 b	1.72± 0.41 a	3.13 ± 0.06 a
C (Mica + RP)	32.77 ± 1.77 c	56.32 ±7.41 ab	0.51 ± 0.08 cd	0.29 ± 0.45 bc	1.33 ± 0.25 cde
RP	32.75 ± 3.15 c	50.59 ± 7.70 bc	0.40 ± 0.08 cd	0.28 ± 0.06 bc	1.33 ± 0.18 de
P18	36.55 ± 2.60 bc	57.31 ± 5.06 ab	0.52 ± 0.03 cd	0.42 ± 0.06 bc	1.94 ± 0.2 bcde
BC3	35.70 ± 2.74 bc	68 ± 8.79 a	0.57 ± 0.08 cd	0.45 ± 0.05 bc	2.29 ± 0.68 abcd

BC10	40.50 ± 6.08 b	67.22 ± 9.15 a	0.82 ± 0.12 c	0.76 ± 0.15 b	2.19 ± 0.42 abc
BC11	39.45 ± 3.09 b	69.75 ± 1.68 a	0.84 ± 0.06 c	0.53 ± 0.05 bc	1.89 ± 0.30 cde

Data are mean values ± SD of five replicates. Different letters in the same column indicate the existence of significant differences according to Tukey (p=0.05).

4. Conclusion

The evidence obtained through this study indicates that among the nine strains of Actinobacteria, *Streptomyces alboviridis* P18, *Streptomyces griseorubens* BC3, *Streptomyces griseorubens* BC10 and *Nocardiopsis alba* BC11 exhibited significant capacity to solubilize mica and RPs under *in-vitro* condition. While, in greenhouse conditions, two of these four strains, *Streptomyces griseorubens* BC10 and *Nocardiopsis alba* BC11 showed a coarse root architecture and highest performance of wheat shoots and roots growth. The ability of these strains to solubilize mica and RPs and to promote wheat growth are probably related to organic acids, IAA, siderophore and ammonia released by these strains.

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