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Research Article

PHOSPHATE SOLUBILIZATION AND ORGANIC ACIDS PRODUCTION BY FLUORESCENT PSEUDOMONADS ASSOCIATED WITH *POPULUS NIGRA* RHIZOSPHERE

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ABSTRACT. Application of phosphobacteria (PSB) has been noticed as the best eco-friendly method for plant P nutrition. For this purpose, PSB isolates were initially screened from the rhizosphere of *Populus nigra* by Sperber medium. Out of 138 isolates, 25 were found to belong to fluorescent pseudomonads based on screening with King's B^{+++} medium. Three superior isolates, FRPF4, FRPF6, and FRPF12, were evaluated for their potential to solubilize tricalcium phosphate (TCP) and hydroxyapatite (HAP), quantitatively and qualitatively. Maximum solubilized TCP (454.6±12.6µg/mL) and HAP (126.0±3.6µg/mL) belonged to FRPF4 on the 8th day of incubation ($p \leq 0.05$). Phosphate solubilization was also accompanied by a gradual decrease in Pikovskaya's broth acidity from 7 to 4.2 during the incubation. Aspartic acid along with three (fumaric, citric, and maleic acids) out of eight tested organic acids were detected in all the bacterial filtrates through HPLC analysis. However, nicotinic acid was only found in FRPF4 and FRPF6 filtrates. So, the possible P solubilization mechanisms of the isolates were to decreasing the media acidity as well as producing various organic acids. Finally, further studies under *in vivo* conditions should be conducted to test the feasible use of such beneficial rhizobacteria as biofertilizer in black poplar cultivation.

Keywords: *Bacterium, hydroxyapatite, poplar, rhizosphere, tricalcium phosphate.*

INTRODUCTION

The average annual growth rate of fast-growing trees like *Populus nigra* L. (black poplar), in optimum conditions is 40 m^3 per hectare. However, under unfavorable conditions, this amount may be reduced to 10 m^3 per hectare. Black poplar growth may be affected by several factors such as susceptibility to plant pests and diseases, soil acidity, and the availability of nutrient elements, especially phosphorus (P) in the soil [1]. Phosphorus is known as the second most critical macro-element for plants after nitrogen (N). It plays a vital role in most plant metabolic functions such as photosynthesis, respiration, energy transformations, macromolecular biosynthesis, and signal transduction [2]. Although total P content in the soil ranges from 0.01 to 0.2% (w/w), only 0.1% of soil P can be utilized by plants [3, 4].

Available phosphate forms to plants HPO_4^{2-} and $H_2PO_4^-$, are highly reactive and interact rapidly with cations like Fe^{3+}, Ca^{2+}, and Al^{3+} in the soil to form insoluble phosphate salt complexes which makes them easily inaccessible to the plants [5]. Accordingly, finding ways to increase the mobility and solubility of fixed forms of phosphate in the rhizosphere are noticed as an important subject of research for years. In

this regard, a group of beneficial soil microorganisms known as phosphate solubilizing bacteria (PSB) plays a key role in the natural P cycle and subsequent availability of phosphate to plants [6].

Commonly reported genera of PSB included *Achromobacter*, *Aereobacter*, *Agrobacterium*, *Azotobacter*, *Bacillus*, *Burkholderia*, *Erwinia*, *Flavobacterium*, *Micrococcus*, *Pseudomonas*, and *Rhizobium* [7, 8]. These rhizobacteria can increase plant access to soil inorganic P through a wide range of mechanisms such as lowering rhizosphere pH by the release of protons or the production of various organic acids [9, 10], and chelation of cations by the secretion of chelating agents to compete with phosphate for soil sorption sites [2].

The current study, therefore, aimed to screen and identify black poplar-associated PSB belonging to the fluorescent pseudomonads (FP) and to evaluate their efficiency in solubility hydroxyapatite (HAP) and tricalcium phosphate (TCP) quantitatively and qualitatively. Besides, acidification of the media and organic acid production by selected highly efficient isolates were investigated.

MATERIALS AND METHODS

Sampling

Samples consisted of black poplar trees rhizosphere soil along with pieces of the roots, taken at depths of 20-40 cm. They were gathered from three different regions of Firuzkuh (55° 77′ 24″ N, 35° 75′ 50″ E), Tehran, Iran, in October 2019.

Physicochemical analysis of soil samples

The collected soil samples were air-dried, ground into a fine powder, passed through a 3mm sieve, and stored in plastic bags at 4ºC. The soil samples were used for determining the electrical conductivity (EC), texture, pH, available phosphorus, organic carbon (OC), nitrogen (N), available potassium, saturated water content (SWC), available calcium, etc. in three replications [11]. The above-mentioned soil characteristics are shown in Table 1.

Table 1. *Physicochemical properties of the soil samples collected from the P. nigra rhizosphere at Firuzkuh, Iran in 2019.*

Site	Texture	pH	Available P (ppm)	EC (dS/m)	SWC (%)	OC (%)	Total N (%)	Available K (ppm)	Available Ca (mEq/L)	Total Fe (mg/Kg)	Soluble Na (mEq/L)
Firuzkuh	Sandy loam	7.7	2.0	1.77	27.83	1.15	0.15	28	1.66	3.6	37.33

Screening and isolation of PSB

Sperber medium (glucose 10g, $CaCl_2$ 0.14g, yeast extract 0.5g, $Ca_3(PO_4)_2$ (TCP) 2.5g, $MgSO_4.7H_2O$ 0.32g, and agar 15g per liter at pH: 7.2) was used for screening and isolation of rhizobacteria capable of solubilizing inorganic phosphate [12].

Each soil sample (1g) was suspended in the autoclaved 0.1 M $MgSO_4.7H_2O$ solution (9mL) and diluted serially. 100µl from each diluted (10^{-3}, 10^{-4}, and 10^{-5}) sample were cultured on Sperber medium and incubated at 27±2°C for a week. The formation of a

clear halo around the growing bacterial colony indicated that the isolate can convert the insoluble form of TCP in the Sperber medium to soluble forms. Therefore, these colonies were screened as PSB. The screened bacteria were sub-cultured by striking methods at the same conditions till the pure isolates were obtained.

Screening and isolation of fluorescent pseudomonads (FP)

King's B medium (Merck, Germany) amended with ampicillin (200µg/mL), cycloheximide (100 µg/mL), and chloramphenicol (10 µg/mL) (King's B^{+++}) was employed for screening FP isolates [13]. For this purpose, 100µl of each PSB isolate was cultured on the selective medium of King's B^{+++}. All the cultures were sealed with Parafilm and incubated at 27±2°C for 48hr. Only those bacteria that produce a fluorescent pigment under UV light will be isolated.

Assessment of phosphate solubilizing activity

The quantitative, as well as the qualitative determination of P solubilization potential of the three selected bacterial isolates (FRPF4, FRPF6, and FRPF12), was performed using broth culture and plate screening methods, respectively.

Qualitative analysis

The selected isolates were spot inoculated on the Pikovskaya's (PVK) (dextrose 10g/L, $(NH_4)_2SO_4$ 0.5g/L, yeast extract 0.5g/L, $Ca_3(PO_4)_2$/hydroxyapatite 5g/L, $MgSO_4 \cdot 7H_2O$ 0.1g/L, KCl 0.2g/L, $MnSO_4 \cdot H_2O$ 0.0001g/L, NaCl 0.2g/L, $FeSO_4 \cdot 7H_2O$ 0.0001g/L, and agar 15g/L) plates [14]. The initial acidity of PVK was adjusted to 7.0 before autoclaving. The plates were sealed with Parafilm and incubated at 27±2°C for 7 days. The halo zone around a growing isolate was considered as TCP or HAP solubilization index (SI) which was calculated according to the following formula:

$$PSI = \text{Clear zone diameter} + \text{Colony diameter} / \text{Colony diameter}$$

Quantitative analysis

PVK broth containing 5000 µg/mL TCP/HAP was employed to evaluate the P-solubilizing activity of the selected isolates. The bacterial cultures (with the initial population of 2×10^8 CFU/mL) were incubated at 27±2°C in a shaker incubator at 150 rpm for 8 days. The cultures were centrifuged at 8000 rpm at 5°C for 15min to obtain the supernatant on the 2nd, 4th, 6th, and 8th day after inoculation. To remove bacterial cells, the supernatant was passed through a 0.2µm PES syringe filter (Roth, Germany). The determination of the released soluble phosphate amount in the filtrate was conducted based on the Vanadate-molybdate yellow method using a spectrophotometer (UV–VIS Spectrophotometer, BioQuest, CE2502, Cecil, Cambridge, UK) at 430nm [14]. The pH value of the cultures was also recorded at the same interval using a digital pH meter (WTW, inoLab, Germany).

Analysis of organic acids

For organic acid determination, all the three selected isolates were cultured in PVK liquid medium containing TCP as described above. The isolates grown in PVK broth lacking TCP served as controls. On the 8th day, the supernatant was taken by the

centrifugation of the cultures at 8000 rpm at 5°C for 15 min and then filtered through a 0.2µm PES syringe filter (Roth, Germany). HPLC-UV (Knauer, Germany) was used to detect eight organic acids (maleic, nicotinic, benzoic, acetic, propionic, citric, fumaric, and succinic acids) and also aspartic acid in each bacterial filtrate. The system was equipped with an HPLC pump (Knauer K-1001) and a K-2301 refractive index (RI) detector.

Approximately, 20µl of each filtrate was injected into an HPLC column (length × ID: 250×4.6mm with precolumn, packing material: Eurospher 100-5C18). Buffer phosphate (pH=3) and methanol (85/15, v/v) were used as mobile phase at a constant flow rate of 1mL min^{-1} and the column was operated at 25°C. The retention time (RT) of each signal was recorded at a wavelength of 210nm [14].

HPLC chromatograms of organic acids produced by the isolates were assessed by comparison with the profiles of the nine selected organic acid standards (Merck, Germany) and their peak areas. HPLC analyses were performed using Agilent Chem Station software.

Statistical Analysis

Analysis of variance (ANOVA) was processed using IBM SPSS 22 Statistics Program (Chicago, USA). Means were compared by Duncan's multiple range test (DMRT) ($p \leq 0.05$). All the experiments were conducted based on a completely randomized design (CRD) with three replications per treatment.

RESULTS AND DISCUSSION

According to the results of the initial screening, out of 138 PSB isolates screened from the rhizosphere of black poplar in Firuzkuh, 25 isolates were belonged to the FP group and exhibited the potential of both solubilizing P and producing fluorescent pigment in the selective culture media. Amongst the FP isolates, three isolates (FRPF4, FRPF6, and FRPF12) were selected based on their greater efficiency in solubilizing TCP or HAP in a solid Sperber medium. Jha et al. [15] also reported that three strains (BFPB9, FP12, and FP13) of 80 screened FP strains were able to solubilize TCP.

Qualitative analysis

In the qualitative test, the phosphate solubilizing capacity of the bacterial isolates was tested not only with TCP but also with HAP. All the three superior FP isolates showed better performance in solubilizing TCP than HAP in a solid Sperber medium. Maximum TCP and HA solubility indices were observed in FRPF4 isolate with 3.5±0.32 and 2.03±0.06, respectively. Although FRPF6 showed higher potential for the dissolution of TCP compared to FRPF6, the HAP dissolution rate for FRPF12 was significantly higher than that in FRPF6 ($p \leq 0.05$) (Fig. 1). Similarly, Safari et al. [16] reported that both *Pseudomonas putida* (PP20) and *Pseudomonas kilonensis* (PK11) were able to dissolve TCP more than HAP.

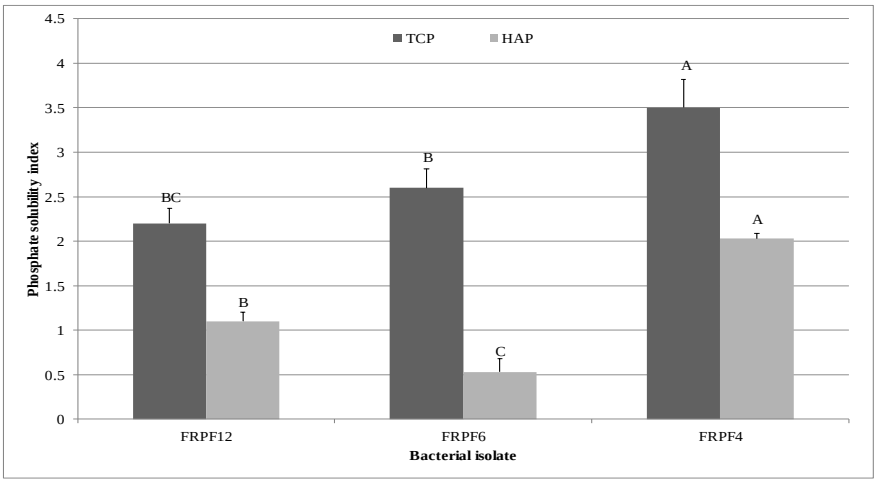

Fig. 1. *Tricalcium phosphate (TCP) and hydroxyapatite (HAP) solubility indices of the selected FP isolates associated with Populus nigra from Firuzkuh, Iran. Values marked with the same letters are not statistically different according to DMRT (n=3) (p≤0.05). Bars represent standard deviation.*

Quantitative analysis

During the eight days of incubation, gradual increases in the soluble P concentrations from two different sources of TCP and HAP, in PVK broth by all the three selected isolates were observed. The solubilization of TCP and HAP by the selected FP isolates ranged from 131.7 to 454.6µg/mL and 46.3 to 126µg/mL in PVK broth on the 8th day of incubation, respectively (p≤0.05). These findings are in agreement with Hui et al. [17] who exhibited that 15 PSB strains isolated from the poplar rhizosphere in China could solubilize inorganic P ranging between 376.62 and 669.56 mg L^{-1} on the third day after inoculation.

The process of releasing soluble P from TCP and HAP in the medium was started from day 4 after inoculation for FRPF6. FRPF12 as well as FRPF6 start to convert insoluble HAP to soluble form on the 4th day. However, FRPF4 and FRPF12 started to solubilize TCP from the second day of the incubation. HAP dissolution was also recorded from the second day for FRPF4 (Fig. 2). Besides, Samavat et al. [18] found that not only phosphate solubilizing ability varied among different rhizobacterial isolates, but also some of them showed this ability more slowly than the others. Similar to these findings, we indicated that both the quantity and start time of solubilizing inorganic P depended not only on bacterial isolate type but also on the source of P in the culture media.

It was found that all the three selected isolates resulted in decreasing the pH value of PVK broth from the initial value of 7 to a pH value between 4.2 and 6 on the 8th day of incubation. All the isolates changed the acidity of the medium from the second day except FRPF6. A reduction in pH value by FRPF12 continued until the 6th day and then a steady trend was observed till the 8th day (Fig. 3). Muleta et al. [19] observed that TCP solubilization by a *Pseudomonas chlororaphis* strain and two *Erwinia* species resulted in a reduction in broth medium acidity. Our results were also revealed that TCP dissolution in PVK broth by the selected FP isolates has coincided with a decrease in medium pH. Moreover, most selected isolates except for FRPF6 and FRPF12 showed a decreasing trend for associated pH value from the second day to the end of the incubation period. In

other words, FRPF12 caused a steady trend from the 6th day and FRPF6 started to decrease the medium acidity from the fourth day.

Acidification of the culture medium, whether through the release of H^+ or the production of a variety of organic acids, is one of the main mechanisms of these beneficial rhizobacteria in increasing the solubilization of insoluble mineral phosphate complexes [14].

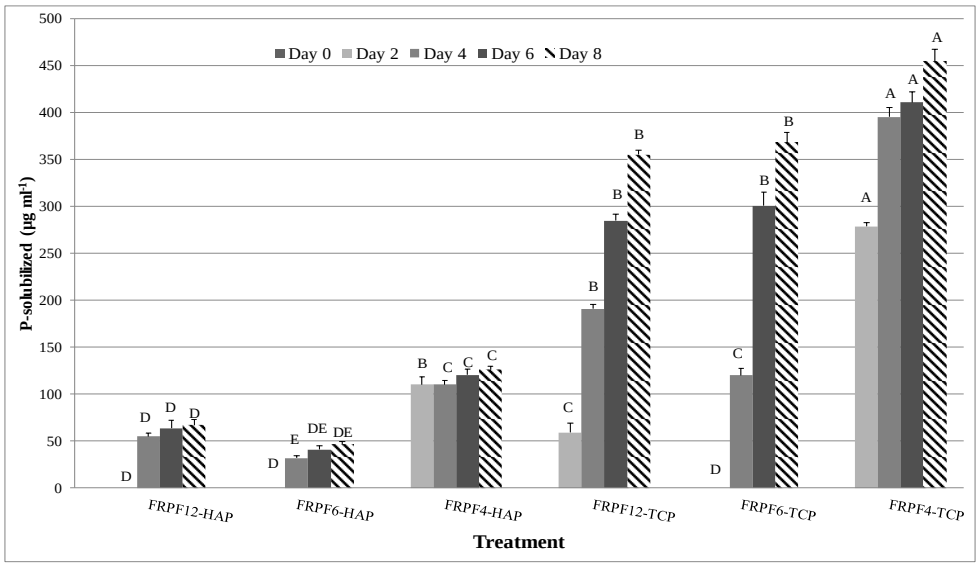

Fig. 2. *Solubility (µg/mL) of TCP and HAP in PVK broth medium by the selected FP isolates on 2nd, 4th, 6th and 8th day after inoculation. Values marked with the same letters are not statistically different according to DMRT (n=3) (p≤0.05). Bars represent standard deviation.*

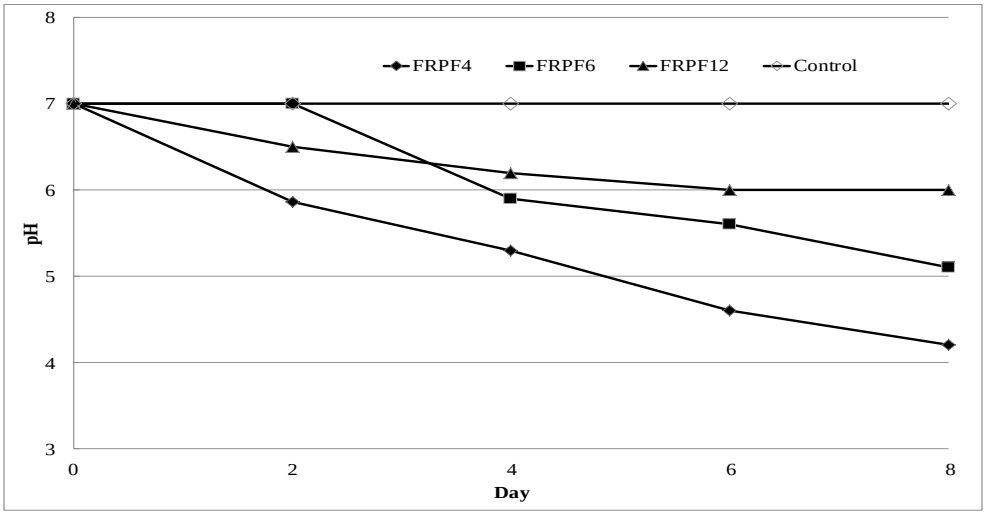

Fig. 3. *Effect of bacterial isolates FRPF4, FRPF6, and FRPF12 on pH value of PVK broth containing TCP during zero, 2, 4, 6, and 8 days after inoculation. Each value is the mean of three replicates.*

Analysis of organic acids

According to the HPLC results, all the three FP isolates were capable of producing several organic acids in PVK broth on the 8th day of incubation. Out of eight tested organic acids, fumaric acid, citric acid, and maleic acid were detected in all the filtrates. Moreover, nicotinic acid was only found in the filtrates of FRPF4 and FRPF6 (Table 2). Furthermore, some unknown organic acids in smaller quantities and aspartic acid were also detected in the filtrates. HPLC chromatograms of the detected organic acids were shown in Fig. 4.

The growth of all the selected FP isolates in the presence of TCP resulted in the gradual accumulation of maleic acid and aspartic acid in comparison with the controls. The amount of fumaric acid, citric acid, and nicotinic acid was decreased for FRPF4 and FRPF6 during the 8 days incubation compared to their controls. Additionally, FRPF12 showed an increase in the amount of citric acid and fumaric acid compared to the control (Table 2). According to the retention times, aspartic acid, citric acid, fumaric acid, nicotinic acid, and maleic acid were faster to exit the HPLC column, respectively (Table 3).

Table 2. *Quantity of the five organic acids detected in the culture filtrates of FRPF4, FRPF6, and FRPF12 isolates and their related controls (FRPF4-C, FRPF6-C, and FRPF12-C).*

Treatment	Aspartic acid (µg/mL)	Citric acid (µg/mL)	Fumaric acid (µg/mL)	Nicotinic acid (µg/mL)	Maleic acid (µg/mL)
FRPF4-C	2260±109.20E	16.9±2.34A	0.61±0.03B	0.45±0.02C	3.6±0.18B
FRPF4	6563±294.12C	11.77±1.39AB	0.53±0.09B	1.83±0.14A	7.70±1.07A
FRPF6-C	1447±89.3E	7.1±0.78BC	0.6±0.03B	0.81±0.17B	1.35±0.08C
FRPF6	9120.67±584.91B	5.9±1.11C	0.16±0.14C	0.17±0.01D	3.68±0.11B
FRPF12-C	5464±355.66D	6.2±1.14C	0.05±0.02C	0.01±0.01D	1.9±0.21C
FRPF12	17756.67±550.72A	7.43±1.65BC	2.41±0.04A	0.01±0.01D	2.59±0.16BC

In each column, values marked with the same letters are not statistically different according to DMRT (n=3) (p≤0.05). Data is shown as mean±standard deviation.

Table 3. *Retention times of the five organic acids produced by the three isolates cultured in PVK with TCP and without TCP (as their related controls: FRPF4-C, FRPF6-C, and FRPF12-C) (n=3).*

Treatment	Aspartic acid	Citric acid	Fumaric acid	Nicotinic acid	Maleic acid
FRPF4-C	2.65±0.02	3.73±0.03	3.85±0.01	4.20±0.02	4.73±0.04
FRPF4	2.60±0.05	3.67±0.06	3.80±0.05	4.23±0.00	4.77±0.03
FRPF6-C	2.65±0.01	3.58±0.08	3.87±0.06	4.21±0.02	4.61±0.07
FRPF6	2.60±0.04	3.51±0.05	3.85±0.05	4.25±0.06	4.77±0.08
FRPF12-C	2.58±0.02	3.75±0.05	3.90±0.08	-	4.73±0.03
FRPF12	2.57±0.01	3.72±0.02	3.83±0.04	-	4.73±0.02

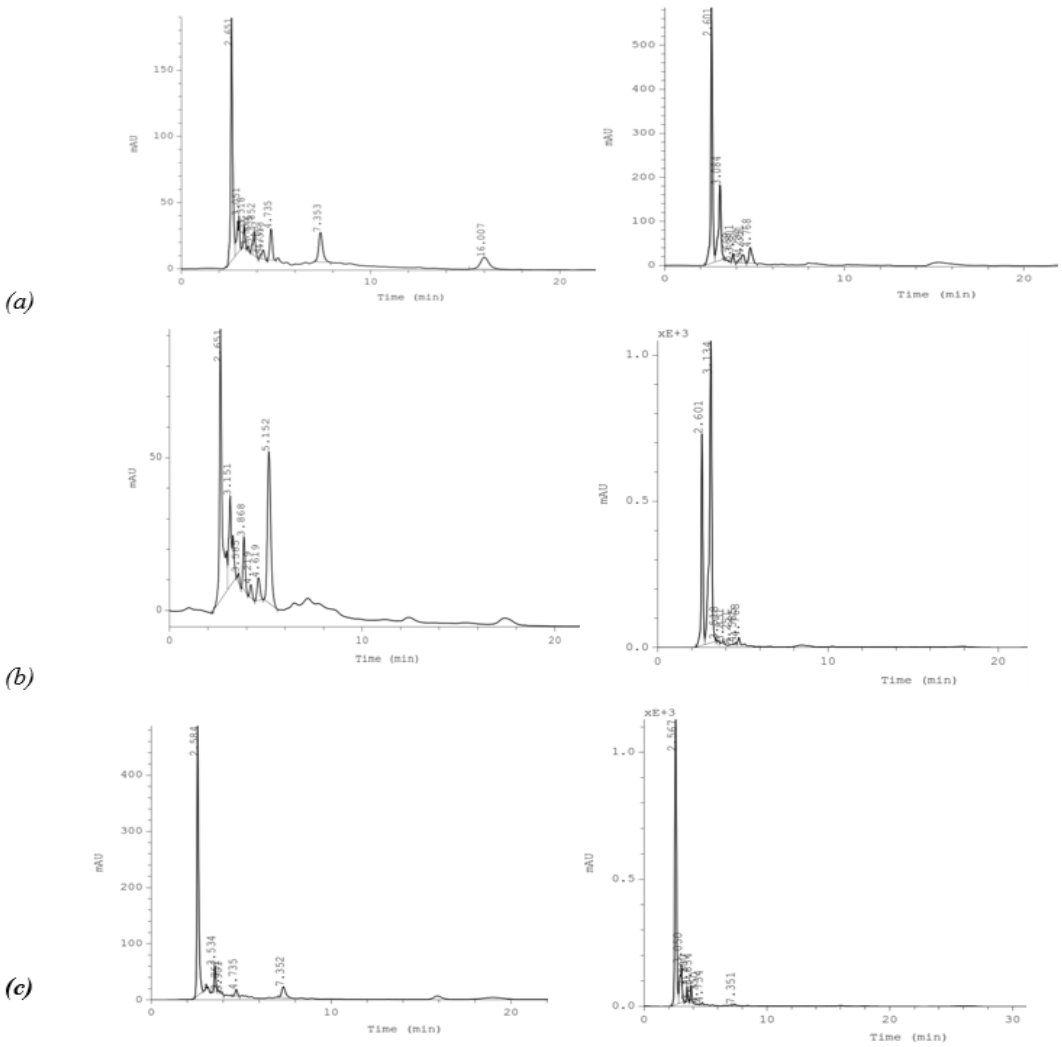

Fig. 4. *HPLC chromatograms of FRPF4(a), FRPF6(b), and FRPF12(c) filtrates in PVK without TCP (control) (left) and with TCP (right).*

It has been proven by several studies that many organic acids such as gluconic acid, butyric acid, acetic acid, citric acid, lactic acid, succinic acid, maleic acid, 2-ketogluconic acid, fumaric acid, isovaleric acid, glycolic acid, propionic acid, formic acid, and many others have been produced by the PS rhizobacteria [14, 20]. In this regard, our HPLC results confirmed that some organic acids like acetic acid, benzoic acid, propionic acid, and succinic acid were not found in the isolates filtrates. However, citric acid, fumaric acid, and maleic acid were detected in all the tested filtrates. Moreover, nicotinic acid could be only detectable in some PSB filtrates. This result similar to Pantigoso et al. [21] who reported that nicotinic acid, malic acid, and 3-hydroxypropionic acid were active in solubilizing TCP from both liquid and solid media.

Aspartic acid also represented the largest amount in all the tested filtrates. This important amino acid plays a significant role in amino metabolism. It has been known

that aspartic acid is chemically synthesized by the reaction of ammonia with fumaric acid or with maleic acid. On the other hand, fumaric acid could be a precursor for the production of other acids like aspartic [22]. So, these acids can be potentially transformed into each other during the incubation .

Compared to their controls, bacterial isolates treated differently when exposed to TCP for changing the produced organic acid quantity. Furthermore, it seems that different organic acids and their interactions with others are responsible for the mineral P solubilizing activity of each tested bacterial isolate. In this regard, Chen et al. [23] reported that a combination of multiple organic acids showed better performance in solubilizing inorganic P and decreasing pH better than a single organic acid .

Consequently, indigenous bacterial isolates with a dual activity of solubilizing mineral P and producing siderophores could be potentially applied as an efficient biofertilizer for black poplar cultivation. However, as previously described by Chung et al. [24], plant P accumulation has not always direct correlation with in-vitro P solubilization or even soil available P. So, further studies under in vivo conditions should be conducted to test the feasible use of such beneficial bacteria as biofertilizer.

CONCLUSION

It was found that some FP isolates screened from the black poplar rhizosphere could efficiently dissolve mineral P from both sources of TCP and HAP. The main P solubilization mechanisms of these superior isolates were to decreasing the acidity of the media as well as producing various organic acids. Accordingly, these superior isolates can be introduced as possible bioinoculant candidates for preparing formulations of biofertilizers, for black poplar cultivation.

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REFERENCES

[1] Isebrands, J. G., Richardson, J. (2014): Poplars and Willows: Trees for Society and the Environment. Boston, MA: Rome: CABI; FAO, 650 p.

[2] Khan, A., Jilani, V., Akhtar, M. S., Naqvi, S. M. S., Rasheed, M. 2009. Phosphorus solubilizing bacteria: occurrence, mechanisms, and their role in crop production. Journal of Agriculture and Biological Sciences 1: 48-58.

[3] Halford, I. C. R. (1997): Soil phosphorus, its measurements and its uptake by plants. Australian Journal of Soil Research 35: 227-239.

[4] Lambers, H., Plaxton, W. C. (2018): P: back to the roots. Annual Plant Review 48: 3-22.

[5] Schnug, E., Haneklaus, S. H. (2016): "The Enigma of Fertilizer P Utilization," in P in Agriculture: 100% Zero, eds Schnug E., De Kok L. (Dordrecht: Springer), 7–26.

[6] Richardson, A. E. (2001): Prospects for using soil microorganisms to improve the acquisition of phosphorus by plants. Australian Journal of Plant Physiology 28:897-906.

[7] Rodríguez, H., Fraga, R. (1999): Phosphate solubilizing bacteria and their role in plant growth promotion. Biotechnology Advances 17: 319-339.

[8] Kumar, A., Shukla, U. K., Singh, A., Poonam, A. K., Prasad, S., Singh, S. K., Kumar, D. (2014): Evaluation of Pseudomonas isolates from wheat for some important plant growth promoting traits. African Journal of Microbiology Research 8: 2604-2608.

[9] Illmer, P. A., Barbato, A., Schinner, F. (1995): Solubilization of hardly soluble $AlPO_4$ with P-solubilizing microorganisms. Soil Biology and Biochemistry 27:260-270.

[10] Kalayu, G. (2019): Phosphate solubilizing microorganisms: promising approach as biofertilizers. International Journal of Agronomy 1-7.

[11] Sarkar, D., Haldar, A. (2005): Physical and Chemical Methods in Soil Analysis: Fundamental Concepts of Analytical Chemistry and Instrumental Techniques. Ed. Indian: New Age international 176.

[12] Sperber, J.I. (1958): The incidence of apatite-solubilizing organisms in the rhizosphere and soil. Australian Journal of Agricultural Research 9: 778-781.

[13] Simon, A., Ridge, E. H. (1974): The use of ampicillin in a simplified selective medium for the isolation of fluorescent pseudomonads. Journal of Applied Bacteriology 37: 459-460.

[14] Pande, A., Pandey, P., Mehra, S., Singh, M., Kaushik, S. (2017): Phenotypic and genotypic characterization of phosphate solubilizing bacteria and their efficiency on the growth of maize. Journal of Genetic Engineering and Biotechnology 15 (2): 379-391.

[15] Jha, B. K., Pragash, M. G., Cletus, J., Raman, G., Sakthivel, N. (2009): Simultaneous phosphate solubilization potential and antifungal activity of new fluorescent pseudomonad strains, *Pseudomonas aeruginosa*, *P. plecoglossicida*, and *P. mosselii*. World Journal of Microbiology and Biotechnology 25:573-581.

[16] Safari, M., Motamedi, E., Kari Dolatabad, H., Modarres Sanavy, S. A. M. (2020): Nano-carriers effects on the viability and efficiency of *Pseudomonas* strains as phosphate solubilizing bacteria. Heliyon 6 (10).

[17] Hui, L., Xiao-Qin, W. U., Jia-Hong, R. E. N., Jian-Ren, Y. E. (2011): Isolation and identification of phosphobacteria in poplar rhizosphere from different regions of China. Pedosphere 21(1): 90-97.

[18] Samavat, S., Samavat, S., Mafakheri, S., Shakouri, M. J. (2012): Promoting common bean growth and nitrogen fixation by the co-inoculation of *Rhizobium* and *Pseudomonas fluorescens* isolates. Bulgarian Journal of Agricultural Science 18 (3): 387-395.

[19] Muleta, D., Assefa, F., Borjesson, E., Granhall, U. (2013): Phosphate-solubilizing rhizobacteria associated with Coffea arabica L. in natural coffee forests of southwestern Ethiopia. Journal of the Saudi Society of Agricultural Sciences 12(1): 73-84.

[20] Sharma, S. B., Sayyed, R. Z., Trivedi, M. H., Gobi, T. A. (2013): Phosphate solubilizing microbes: sustainable approach for managing phosphorus deficiency in agricultural soils. SpringerPlus 2 (587): 1-14.

[21] Pantigoso, H. A., Yuan, J., He, Y., Guo, Q., Vollmer, C., Vivanco, J. M. (2020): Role of root exudates on assimilation of phosphorus in young and old *Arabidopsis thaliana* plants. PLOS One 15(6): e0234216.

[22] Martin-Dominguez, V., Estevez, J., Ojembarrena, F. D. B., Santos, V. E., Ladero, M. (2018): Fumaric acid production: a biorefinery perspective. Fermentation 4 (33): 1-22.

[23] Chen, Y. P, Rekha, P. D., Arun, A. B., Shen, F. T., Lai, W. A., Young, C.C. (2006): Phosphate solubilizing bacteria from subtropical soil and their tricalcium phosphate solubilizing abilities. Applied Soil Ecology 34 (1): 33-41.

[24] Chung, H., Park, M., Madhaiyan, M., Seshadri, S., Song, J., Cho, H., Sa, T. (2005): Isolation and characterization of phosphate solubilizing bacteria from the rhizosphere of crop plants of Korea. Soil Biology and Biochemistry 37: 1970-1974.