Journal of Biology and Today's World
ISSN 2322-3308
Journal home page: http://journals.lexispublisher.com/jbtw/

Received: 08 July 2015 • Accepted: 10 August 2015

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doi:10.15412/J.JBTW. 01040503

Isolation and Identification of Phosphate Solubilizing Bacteria from Pistachio Farms of Anar City

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ABSTRACT

Indiscriminate and incorrect use of poisons and fertilizers is very dangerous and has created a lot of problems and examples of these problems include health and environmental damage or economic problems. There are microorganisms in soil that are able to release the phosphorus from the minerals and organic compounds through producing of primary metabolites and their secretion in soil on the minerals and organic compounds. in this research and during two consecutive seasons of the year (spring and winter 2014), sampling was conducted in pistachio farms of Anar city. Identification of phosphate solubilizing bacteria was done using the current biochemical tests. the study results showed that only Bacillus pumilus is able to solve phosphate in pistachio farms. Bio-fertilizers not only have many economic and environmental benefits, but also maintain the stability of available resources in soil, increase the long-term production capacity and decrease the environmental pollutions.

Key words: Phosphate, Bacillus pumilus, Environment, Pistachio farms

1. INTRODUCTION

Phosphorus is one of the important nutrients for plants which are low in soil. Plants absorb their required phosphorus from the soil solution in the form of phosphate anion (HPO_4^{2-} or $H_2PO_4^-$). Of course, there are two form of mineral and organic phosphorus in large quantities and in the range of 400-1200 mg/kg in soil (but the concentration of soluble phosphate in soil is usually very low and in range of 1 mg/kg or lesser. So, there is no problem in most soils in terms of total amount of phosphorus, but its availability and accessibility is a problem (1). Soil microbes have the ability to convert the insoluble forms of phosphorus to its soluble forms. Organic and inorganic compounds extracted from the root result in the increasing of microbial population around the roots. As the phosphate solubilizing microorganisms are naturally available in soil and lead to increase of the available phosphorus and stimulation of plants' growth but they are not enough in soil to compete with other microorganisms in rhizosphere, the inoculation of plants with phosphate solubilizing microorganisms has beneficial effects. Phosphate solubilizing bacteria cause dissolution of insoluble phosphate compounds through three mechanisms of organic acid production, chelating and ligand exchange reactions (2). During the dissolution process, a part of soluble phosphorus is used by the phosphate solubilizing bacteria, but since the amount of soluble phosphorus is more than bacterial needs, this free amount can be available for the plant. Most soils in Iran contain limestone and gypsum and they can stabilize phosphorus. As a result, phosphorus is absorbed by soil colloidal particles and comes out of reach of plants. Therefore, there is no problem in most soils in terms of total amount of phosphorus, but the problem is its accessibility. Phosphorus is absorbed to the elements such as Fe^{3+}, Ca^{2+} and Al^{3+} and causes the formation of insoluble compounds (3). Looking at the nature and mechanisms that we have taken will lead to better management mechanisms such as the adaptations of plants and soil and rhizosphere microflora intervention strategies in the absorption of phosphate. There is a group of phosphate solubilizing microorganisms in nature that, with the gradual release of phosphorus, is absorbed into the plant and reduces the need for phosphate fertilizers. Phosphate solubilizing microorganisms are isolated as a way to reduce the use of chemical fertilizers and therefore reduce environmental pollution (4).

2. MATERIALS AND METHODS

2.1. Sampling from different regions of pistachio agricultural soils

Sampling was conducted from different soils of agricultural lands of pistachio trees and it was done from the surface and the depth of 5 and 10 cm. It should be noted that sampling was done from different soils such as sandy and or salty soils and/or soils where organic fertilizers had been used in them.

2.2. Sampling from the intended soils

Sampling was done from different soils of agricultural lands of pistachio trees and it was done from the surface and the depth of 5 and 10 cm. it should be noted that sampling was done from different soils such as sandy and or salty soils and or soils where organic fertilizers were used in them. Some soil sample was collected from the depth of 5 and 10 cm and the collected samples were transferred to the laboratory in sterile plastic containers (5).

2.3. Diluent solution

Normal saline was used to prepare solutions with different dilutions.

Preparation method:

NaCl .... 8.5 g
Distilled water .... 1000 ml

NaCl was weighted and poured into an Erlenmeyer and then distilled water was added and mixed well to solve. The sample can be stored in room temperature for some months. Then 9 ml normal saline was poured in the test tubes and was sterilized at 121°c for 15 min at 15 PSI pressure.

2.4. Evaluation of phosphate dissolution by the bacteria through measuring the halo diameter and colony diameter

After 72 hour's incubation and due to halo formation around the colony, the efficacy of phosphate dissolution and dissolution index were calculated according to the following formulas (1-2) (5).

$$\text{Solubilizing efficacy (SE)} = \frac{colony\ diameter}{halo\ diameter} \times 100 \quad (1)$$

$$\text{Solubilizing index (SI)} = \frac{colony\ diameter + halo\ diameter}{colony\ diameter} \quad (2)$$

2.5. Identification of phosphate solubilizing microorganisms

Regarding the identification of phosphate solubilizing microorganisms, the evaluation of macroscopic characteristics (appearance) of effective colonies in phosphate dissolution, microscopic characteristics and biochemical tests were used.

3. RESULTS AND DISCUSSION

3.1. Sampling from the soil of pistachio farms in Anar city

Totally, 30 soil samples from different areas from surface and depths of 5 and 10 cm were transferred to the laboratory in sampling procedures.

3.2. The culture results of transferred soil samples to the laboratory

In general, 150 purified and isolated colonies were isolated from the cultivation of transferred soil samples in the laboratory and this isolation was done based on the colonies' characteristics.

3.3. Qualitative evaluation results of phosphate dissolution in PVK solid medium

The evaluation of results of inoculated colonies on the surface of PVK solid medium showed that from a total of 150 inoculated microbial isolates, only some bacteria can do the phosphate bio-dissolution which is detected by the phosphate area around the colony.

3.4. Comparative evaluation of the results

The effective bacterial and fungal isolates in isolated phosphate in screening stage

Comparative evaluation of phosphate dissolution results of isolates by the formula 3.

$$\text{Solubilizing efficiency} = \frac{colony\ diameter}{halo\ diameter} \times 100 \quad (3)$$

Shows that MZ_1 and MZ_2 isolates had the greatest dissolution percentage which were selected and applied for continuation of the work. Results were given in the Table 1.

Table 1. Halo diameter, colony diameter and SI of the superior Ca3P solubilizing isolates in PVK solid medium

Isolated bacterium	Colony diameter(mm)	Halo diameter (mm)	SI	SE
MZ_1	7	14	3	50
MZ_2	6	18	4	33.3

Macroscopic evaluation results of effective isolates in phosphate dissolution showed in Table 2 and Table 3.

Table 2. Characteristics of MZ_1 colony (bacterial isolate)

Color of the colony surface	Cream

Growth speed	Colony establishment in 24 hours
Colony margin	Ridged
Colony surface	With wrinkles

Table 3. Characteristics of MZ_2 colony (fungal isolation)

Color of the colony surface	Black
Growth speed	Medium
Colony margin	Smooth
Colony surface	Fluffy form

The results of microscopic evaluation of MZ_1 isolate by Gram stain showed gram-positive bacilli with spores (spore former) and biochemical tests were performed (Figure 1).

Figure 1. Gram-positive bacilli

Over the past few decades, due to the population growth and increasing demand for food, the use of fertilizers in order to increase production per area unit is severely increased. As phosphorus is one of essential elements for plant growth and, after nitrogen, it is the most important element needed by plants, use of phosphate fertilizers has a long history and dates back to the Green Revolution and introduction of chemical fertilizers (6). Evaluated phosphate dissolution by Pantoea agglomerans R-42 which was isolated from soybean rhizosphere. The effect of concentrations of different salts of KCl, NaCl and $cacl_2$ on phosphate dissolution was studied. He reported that dissolution is decreased by increasing concentration of NaCl. From the above mentioned research, it can be concluded that an increase in percentage of salts may be good for bacteria performance, but further increase reduces the bacterial performance. In general it can be said that low percentage of salinity is better for phosphate dissolution. The indiscriminate use of chemical fertilizers will cause the imbalance of nutrients and elements in the soil, reducing the efficacy of agricultural products and jeopardizing health of human and other living organisms. The need for an adequate replacement for chemical fertilizers feels when we know that limited resources, rising costs and consolidation of a major part of used phosphate fertilizers in the unusable form for plants are the consequences of using these chemical fertilizers in addition to environmental damages caused by using the chemical fertilizers. The elements such as Ca^{2+}, Fe^{3+} and Al^{3+} react with phosphorus and lead to the formation of insoluble compounds and prevent the absorption of phosphorous. This causes that phosphate fertilizers get 10 to 25% (7, 8).

4. CONCLUSION

The need for an adequate replacement to release the accumulated phosphate in soil is felt more when we know that the available phosphate resources in soil have the ability to supply the required phosphate of plants for the optimal production in hundred years. Therefore, it is sufficient to make this huge resource of phosphorus absorbable and usable for plants. For this reason, using bio-fertilizers increases the absorption of nutrients in soil. Phosphate solubilizing bacteria seem efficient for increasing the availability of required phosphorus for plant. We recommended for future researches: Regarding bio-fertilizers, it is more favorable to use synthetic fertilizers that means that there are a number of phosphate solubilizing bacteria. efficient isolates in phosphate solubilizing which obtained from the research must be studied in environmental scale i.e. gardens and farms.

Funding/ Support

Not mentioned any Funding/ Support by authors.

ACKNOWLEDGMENT

Not mentioned any acknowledgment by authors.

AUTHORS CONTRIBUTION

This work was carried out in collaboration among all authors.

CONFLICT OF INTEREST

The authors declared no potential conflicts of interests with respect to the authorship and/or publication of this article.

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