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Original research

Isolation of Phosphate Solubilizing Bacteria from the rhizosphere of the balloon vine (Cardiospermum Halicacabum) grown in Saluga and Ghazal Protected Area, Aswan, Egypt

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Abstract:
Phosphate Solubilizing Bacteria (PSB) may aid in the process of solubilizing phosphorous elements from their binding so that they are readily accessed by plants, increasing their effectiveness and the soil's availability of phosphorus. In Saluga and Ghazal Protected Area, Aswan, Egypt, the *Cardiospermum Halicacabum* (balloon vine) plants are being studied with the aim of isolating and defining the morphological properties of the P solubilizing bacteria colony there. Thirteen bacterial isolates from *Cardiospermum Halicacabum* plant were found exhibited P-solubilization potential. The morphological characteristics of the isolates, inclusive the form, margin, shape, elevation, and color of the colony, were gained. The bacteria found were gram + and gram -, which are ٦٩.٢٣% and ٣٠.٧٧% . Furthermore, Using thirteen different bacterial isolates, a clean zone measuring 11–26 mm was produced. Phosphate's dissolving index ranged from 2.11 to 2.38, while its solubility ranged from 111.11 to 137.50.

Keywords: Rhizospheric Soils, Plant Growth-promoting Rhizobacteria, Phosphate Solubilizing Bacteria, Saluga and Ghazal Protected Area

1- Introduction

The rhizosphere, which is tucked away in the soil form, is a small still dynamic area where plant roots and soil-borne microbes interact significantly on a biotic and abiotic levels **(Mueller *et al*., 2019).** In comparison to the bulk soil, The rhizosphere provides a more hospitable environment for the growth of microorganisms and plants. **(Valentine, 2007)**. As evidenced by the effective biogeochemical cycling of various soil nutrients, these intricate and diverse connections led to greater soil health and optimal plant growth within the root environment. **(Lecomte *et al*., 2018)**. The rhizosphere is made up of three separate layers: (1) The zone with the highest concentration of rhizosphere activity is the endo-rhizosphere (the outer layer of plant root surface). (2) the ecto-rhizosphere, which is the highest layer of the rhizosphere, up to bulk soil, and (3) the rhizoplane, which is the inner layer of the intermediate zone in contact with the root epidermis and mucilage. **(Ryan *et al*., 2009)**.

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For the best plant establishment, growth, and development, phosphorus (P), like nitrogen (N), is one of the most limited nutrients and one of the necessary elements. **(Dickerson *et al*., 2000)**. The second most important macroelement after nitrogen, phosphorus, is necessary for proper plant nutrition (**Sharma *et al*., 2013**). It is essential for several plant metabolic activities, including photosynthesis, energy transmission, and others (**Umar *et al*., 2021**). P is typically present in abundance but not in a form that is useful to plants. Only 0.1% of the phosphorus in the soil may be used by plants (**Adnan *et al*., 2019**). Through intricate interactions with several cations, such as Ca^{2+} and Mg^{2+} in alkali soils and Fe^{3+} and Al^{+} in acidic soils, phosphorus becomes immobilised in soil **(Alaylar *et al*., 2020)**. After significant levels of nitrogen, phosphorus is a crucial nutrient for plants since it is involved in many aspects of plant metabolismsuch include cell division, glucose metabolism, and the activation and deactivation of enzymes. (**Razaq *et al*., 2017**). Phosphorus is present in large amounts in soil in both organic and inorganic forms. (**Sapkota *et al*., 2017**). It is mostly in an indissoluble form (nearly 95-99%), so that it can no longer be imbibe by plants. Phosphorus ions in the form of $H_2PO_4^{-1}$ and HPO_4^{-2} can be absorbed by plants. Phosphorus requires the cooperation of soil bacteria, one of which is PSB, to breakdown into an accessible form. Phosphatase enzymes are formed as a result of the interaction among PSB and plant root (in the event that soil phosphate supply is inadequate), although microorganism-derived phosphatase is more prevalent. Phosphates bound to organic substances (citric acid, glutamate, succinate, lactate, oxalate, glycolate, fumarate, tartaric, and alpha-ketobutiric acid) and can be absorbed by plant roots when they are released by the phosphatase enzyme into these accessible forms. Other nutrients are also released by (PSB) in addition to dissolving and releasing P from insoluble molecules. (**Oteino *et al*., 2015**). One of the microorganisms that can be utilised to replace soil P availability is the (PSB), making it potentially highly useful for the development of biological fertilizer by direct soil inoculation (**Ghorchiani *et al*., 2018**).

Additionally, chemical fertilizers can be replaced by via means of (PSB) in combination with a number of different fertilizers. (**Mukherjee and Sen, 2015**). There are several different kinds of PSB, including *Enterobacter* sp. (**Jiang *et al*., 2019**) *Enterococcus* sp., *Bacillus* sp., *Serratia* sp., *Staphylococcus haemolyticus* (**Biswas *et al*., 2018**), *Aneurinibacillus aneurinilyticus* (**Chauhan *et al*., 2017**), and others. The potential of PSB (both fungi and bacteria) as biological fertilizers or biological agents is the most effective and efficient approach to improve crop production because it can replace the use of chemical fertilizers and is ecologically benign. Additionally, it promotes agriculture and the sustainable management of biological resources. (**Su *et al*., 2019**). The purpose of this study was to identify and characterise phosphate solubilizing bacteria (PSB), which are effective and environmentally friendly and can remove insoluble phosphate from the endophyllosphere, endorhizosphere, and ectorhizosphere of balloon vine plants grown in Saluga and Ghazal protected areas.

2- Materials and Methods

2.1. Site of samples

The Saluga and Ghazal protected area are situated in middle of the Nile, three km north of the Aswan Dam, 24°04 N, 32°52 E) (**Abdel-Wahab *et al*., 2019**). It has remnants of ancient Nile flora, particularly *Acacia* trees, of which there are 20 species worldwide. Twenty-seven samples from nine of the balloon vine plants, three on Ghazal Island and six on Saluga Island were taken for Ectorhizosphere, endorhizosphere and endophyllosphere.

2.2. Preparing of samples.

Dilutions after gently removing excess dirt, sufficient parts of root systems were transferred into sampling glasses containing saline solution to create samples of the ectorhizosphere. A 60-minute shake of the glass was followed by more serial dilutions. The fresh root system underwent a thorough cleaning with tap water, followed by treatments with 95% ethanol for 30 second and 3% sodium hypochlorite for 1.5 h. Surface-sterilized roots were carefully washed in sterile water. For the purpose of counting bacterial groups in the endorhizosphere, further dilutions were made. For the manufacture of 1: 10 (w/v), A leaf, top, and sheath, as well as stems, were aseptically divided into tiny pieces that ranged in size from 0.5 to 10 cm. (**Othman *et al.*, 2003**)

2.3. Isolation of phosphate solubilizing bacteria

0.1 ml of an aliquot from previously diluted decimal solutions from Ectorhizosphere, endorhizosphere and endophyllosphere of *Acacia* trees was inoculated on Picovskaya' s agar media (**Rao and Sinha, 1963**), comprised by 2.5mg $MnSO_4.2H_2O$; 5g $Ca_3(PO4)_2$; 2.5 mg $MgSO_4.7H_2O$; ten g glucose; 2.5 mg $FeSO_4.7H_2O$; 0.2 g NaCl; 20 g agar; 5 g yeast extract, 1 L of distilled water, when diluted, pH 7. Colonies suggesting halo zones with a large relative diameter were selected and purified on Picovskaya's agar medium after incubation for 7 days at 30 C in order to evaluate their characterizations.

2.4. Bacteria's Capability to Solubilize Phosphate

Spot inoculation was used to develop bacterial isolates, which were inoculated with 4 mm of the isolate and incubated for 7 days at 30 ^{0}C. Picovskaya's media can be used to observe the creation of clear zones around the colony, which can be used to gauge the bacteria's capacity to dissolve phosphate. By taking a closer look at the expanding colonies that can create a distinct zone surrounding them, the qualitative testing was put to rest. This proved the isolates' ability to break down phosphate. The following formulas were used to calculate the phosphate solubilization index (PSI) and phosphate solubilization efficiency (PSE): (**Sudewi *et al.*, 2020**)

$$PSI = \frac{\text{The colony diameter + The diameter of clear zone}}{\text{The colony diameter}}$$

$$PSE = \frac{\text{The diameter of clear zone}}{\text{The colony diameter}}\ x\ 100$$

2.5. Characterization bacterial isolates

The bacterial isolates which are presumably capable of phosphates solublization are preliminarily identified by the cell morphological, Gram reaction, cell motility, colony characteristics and spores' formation (**Tripathi and Sapra 2020**), The hanging drop method was used to investigate the movement of microorganisms. as described by (**Bertrand *et al.* 2001**).

3. Results and Discussion

3.1. PSB Isolate Isolation and Morphology Characterization

Thirteen bacterial isolates from *Cardiospermum Halicacabum* plant were found exhibited P-solubilization potential. Table 1 show sphere tested of isolates and spore or non-spore -forming bacteria. Figure 1 reveals the conclusions of the (PSB) isolate's qualitative analysis. The bacterial isolates show variable levels of phosphate solubility when grown on Pikovskaya agar media. The presence or absence of bacterial isolates dissolving phosphate on the media is shown by the clear zone that appears around the colony. When compared to other colonies, The greatest area of the

halo zone diameter can be produced by the best phosphate solvent bacteria.. Isolates that don't produce distinct zones, however, are unable to dissolve phosphate.

Results of the tested colony morphological, cell shape, gram reaction and cell motility of the bacterial isolates are shown in Table 2. These isolates have nine circular shapes, two punctiform and one spindle. Including eight with the convex elevation, ٣ raised, and ٢ umbonate. While the margin resulted are ١١ entire, ١ curried, and one undulate. The coloring of the colonies seen in white isolating isolates. It differs from gram-positive and gram-negative bacteria, according to the results of the gram reaction test., which are ٦٩.٢٣% and 30.77%. These isolates have five long rod, four short rod, three rod and one coccus. Motile reactions account for 53.85% of the test's results, while non-motile reactions make for 46.15 % (Table 2).

Fig 1. Colonies of Bacterial isolate (PSB 5) grown for 7 days on Picovskaya's agar media showing the clear zone of phosphate dissolution.

Table 1. Sphere tested of isolates and Spore or non-spore -forming bacteria.

Isolate Code	Sphere tested	**Spores formation**
PSB 1	Endorhizosphere 1	-
PSB 2	Endorhizosphere 2	-
PSB 3	Endorhizosphere 3	-
PSB 4	Endorhizosphere 1	+
PSB 5	Ectorhizosphere 1	-
PSB 6	Ectorhizosphere 2	-
PSB 7	Ectorhizosphere 3	-
PSB 8	Ectorhizosphere 4	-
PSB 9	Ectorhizosphere 5	-
PSB 10	Phyllosphere 1	+
PSB 11	Ectorhizosphere 6	-
PSB 12	Ectorhizosphere 7	-
PSB 13	Ectorhizosphere 8	+

Table 2. Phosphate solubilizing isolates that have undergone some morphological characterization analysis

isolates	morphology of colonies				Gram reaction	Cell Shape	Motility
	Shape	Elevation	Margin	Color			
PSB 1	circular	convex	entire	white	G +	Rod	Non motile
PSB 2	circular	convex	entire	white	G -	long Rod	motile
PSB 3	punctiform	convex	entire	white	G +	long Rod	motile
PSB 4	circular	convex	entire	white	G +	long Rod	Non motile
PSB 5	punctiform	convex	entire	white	G +	short Rod	motile
PSB 6	spindle	umbonate	curied	white	G +	coci	Non motile
PSB 7	circular	convex	entire	white	G -	short Rod	Non motile
PSB 8	circular	convex	entire	white	G -	short Rod	Non motile
PSB 9	Irregular	umbonate	undulate	white	G +	short Rod	Non motile
PSB 10	circular	Raised	entire	white	G +	Rod	motile
PSB 11	circular	Raised	entire	white	G -	Rod	motile
PSB 12	circular	Raised	entire	white	G +	long Rod	motile
PSB 13	circular	convex	entire	white	G +	long Rod	motile

3.2. The outcome of a quantitative examination of bacterial isolates' capacity to dissolve phosphate.

Table 3 displays quantitative capability of PSB. Thirteen bacterial strains were found to be capable of dissolving phosphate, according to Phosphate Solubilization Index PSI test data., which ranges from 2.11 to 2.38. Better PSI are result by PSB 11, which is 2.38 with PSE 137.50. At the same time, the least PSI result by PSB 9, which is 2.11, with PSE of 111.11. (Table 3) and **Fig. 2&3.**

Table 3. The quantitative evaluation of bacterial isolates' capacity to dissolve phosphate.

Isolate code	Diameter of Halo Zone (mm)	Diameter of Colony (mm)	Phosphate Solubilization Efficiency (PSE)	Phosphate Solubilization Index (PSI)
PSB 1	**23**	**20**	**115**	**2.15**
PSB 2	**23**	**19**	**121.05**	**2.21**
PSB 3	**26**	**21**	**123.81**	**2.24**
PSB 4	**19**	**15**	**126.67**	**2.27**
PSB 5	**14**	**11**	**127.27**	**2.27**
PSB 6	**24**	**20**	**120**	**2.2**
PSB 7	**17**	**14**	**121.43**	**2.21**
PSB 8	**18**	**15**	**120**	**2.2**
PSB 9	**20**	**18**	**111.11**	**2.11**
PSB 10	**22**	**18**	**122.22**	**2.22**
PSB 11	**11**	**8**	**137.5**	**2.38**
PSB 12	**15**	**13**	**115.38**	**2.15**
PSB 13	**21**	**17**	**123.53**	**2.24**

Each bacterial isolate's PSI demonstrates its capacity to dissolve phosphate. The enzyme phosphatase is more active in releasing P from organic molecules when the value of PSI is higher, and a broader clear zone indicates an insoluble phosphate that has been converted by PSB into a soluble form.

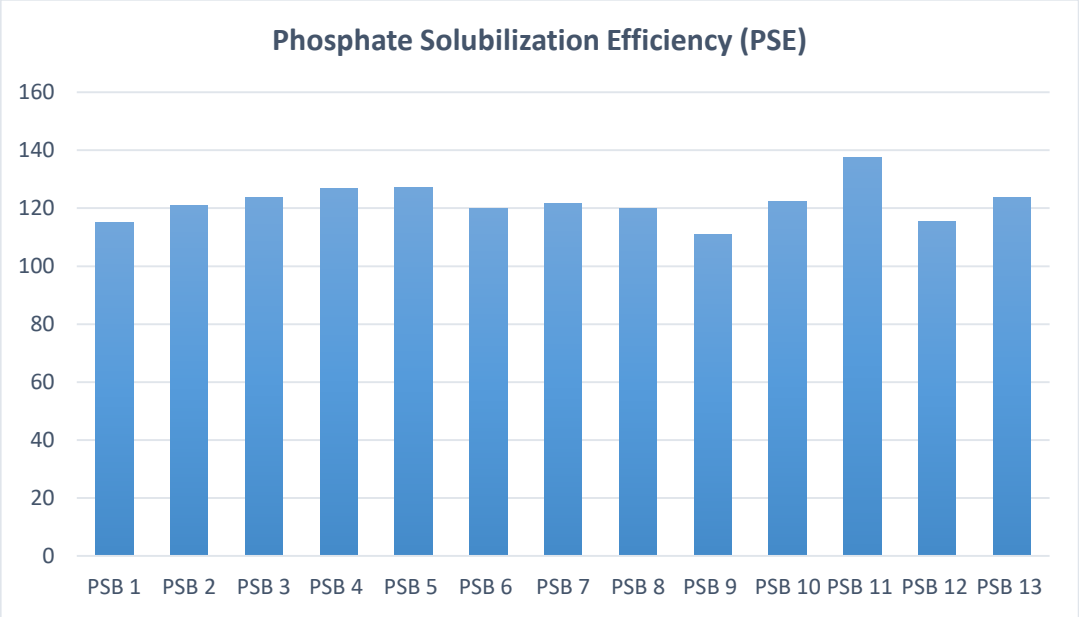

Fig 2. Phosphate Solubilization Efficiency (PSE)

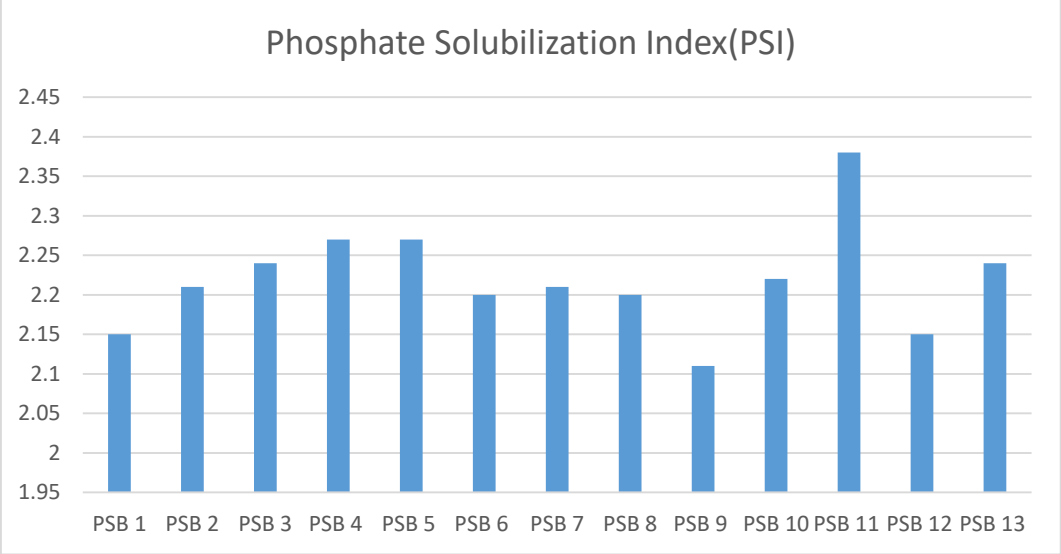

Fig 3. Phosphate Solubilization Index (PSI)

The complex process of phosphorus solubilization is regulated by a number of variables, including the nutritional quality and the bacterium's physiological and growth state **(Reyes *et al.*, 1999)**. The two most significant of the many hypotheses that have been put up to explain the mechanism of phosphate solubilization are the theories of acid generation and enzymes. The process of phosphate solubilization by PSB is attributed to the generation of low molecular weight organic acids, which was accompanied by the medium's acidification, according to the acid production **(Puente *et al.*, 2004)**, and those organic acids can chelate the cation with their carboxyl and hydroxyl groups **(Kpomblekou *et al.*, 1994).** The analysis of PSB culture filtrates revealed the presence of a number of organic acids, including gluconic, succinic, tartaric, malic, oxalic, alpha keto butyric, and 2-ketogluconic acid. **(Kpomblekou *et al.*, 2002; Cuningham and Kuiak 1992)**.

Phytases release phytic acid, phosphonatases and C-P lyases, enzymes that carry out C-P cleavage of phosphonates, release the organic phosphorus from the organic material, and phosphohydrolase, which dephosphorylates the phospho-ester bonds. **(Behera *et al*., 2014).** The results obtained from this research were consistent with **(Sudewi *et al*., 2020**; **Annizah *et al*., 2021).** Because they create a clean zone around their colony when cultivated on Pikovskaya agar media, these bacteria have the ability to dissolve phosphate. $Ca_3(PO_4)^2$ is an insoluble phosphate present in the Pikovskaya agar media. Bacteria bind the molecule, releasing the H_2PO^{4-}ion. Ions released by the colony cause a clearance zone to form. **(Sudewi *et al.*, 2020)**. The phosphatase enzyme's activity led to the formation of the clear zone. Also, it was discovered that the clear zone happens as a result of the phosphatase enzyme's activity causing the pH of a medium to decrease. Moreover, these microorganisms produce organic acids that have the ability to lower pH and interact with P-binding substances like Al^{3+}, Fe^{3+}, Ca^{2+}, and Mg^{2+} to form organic chelates that allow the release of free phosphate ions (PO^{4-}) **(Annizah *et al*., 2021).**

4. Conclusions

By extracting phosphate solubilizing bacteria from the rhizosphere of *Cardiospermum Halicacabum* at Saluga and Ghazal Protected Area, we have shown that the isolates studied can produce a variety of phosphate solubility activities. In terms of clear zone diameter and PSI, PSB is superior to other bacterial isolates since it has the greatest clear zone area (11 mm) and the highest PSI value (2.38 mm).

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