Scholars Academic Journal of Biosciences (SAJB) **ISSN 2321-6883 (Online)**
Sch. Acad. J. Biosci., 2013; 1(7):346-351 **ISSN 2347-9515 (Print)**

(An International Publisher for Academic and Scientific Resources)
www.saspublishers.com **DOI:** 10.36347/sajb.2013.v01i07.001

Research Article

Isolation of *Pseudomonas fluorescens* from rhizospheric soil of faba bean and assessment of their Phosphate solubility: in vitro study, Ethiopia

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Abstract: Soil fertility improvement is an important area which needs attention since most of the hazardous inputs added into the agricultural system are in the form of chemicals. Since crop production and soil normal flora are affected by input of chemical fertilizer. Biofertilizer microorganisms make the nutrients available to plants. The present study was concerning to isolate of some *Pseudomonas fluorescens* species that possess a promising properties which make it a better bio-fertilizer bacteria. Twelve *Pseudomonas fluorescens* species was isolated from rhizospheric soil of faba bean and tested for phosphate solubilization. As result, all tested isolates *Pseudomonas fluorescens* species have a potential of phosphate solubilization on Pikovskaya media by formed a clear halo zone. So it could be summarized that all *Pseudomonas fluorescens* isolate species can be used as bio-fertilizers for soil fertiliy improvement, as a result increase crop growth and yield.
Keywords: Bio-fertilizer, Clear halo zone, Crop, Phosphate solubilization, Pikovskaya media, *Pseudomonas fluorescens*

INTRODUCTION

The diversity and beneficial activity of the plant-bacterial association and its understanding is important to sustain agro-ecosystems for sustainable crop production [6]. Plant growth promoting rhizobacteria are known to rapidly colonize the rhizosphere and suppress soil borne pathogens at the root surface [24] and also be beneficial to the plant by stimulating growth [3,18].

The plant growth promoting ability of these bacteria is generated mainly by the phosphate solubilize [25] in various species of *Pseudomonas* [10, 16].

Phosphorus contributes to the biomass construction of micronutrients, the metabolic process of energy transfer, signal transduction, macromolecular biosynthesis, photosynthesis, and respiration chain reactions [28]. Phosphorus is generally deficient in most natural soils, because it is fixed as water-insoluble iron and aluminum phosphates in acidic soils or calcium phosphate in alkaline soils [30].

Involvement of microorganisms in the solubilization of insoluble phosphate was first shown by [31]. A large number of heterotrophic and autotrophic microorganisms including bacteria (*Pseudomonas, Bacillus, Enterobacter, Rhizobium*) [25], fungi (*Aspergillus* and *Penicillium*) [11, 27, 35] and cyanobacteria (*Phormidium*) [17] have been mechanism of phosphate solubilization studied the for their ability to solubilize hydroxyapatite, tricalcium phosphate, and rock phosphate due to the production of organic acids such as citric, glutamic, lactic, oxalic, malic, fumaric, tartaric, propionic, glycolic and succinic acid [33, 1], which through their hydroxyl and carboxyl groups chelate the cations bound to phosphate [13] by acidification, exchange reactions, and polymeric substances formation to soluble forms HPO^{2-}_4 and $H_2PO^-_4$ [5].

Phosphate solubilize bacteria are important components of soil and directly or indirectly influence the soil's health through their useful activities [22]. Phosphorus is known to improve root growth and nodulation of legumes thereby improve the N content of plants through nitrogen fixation [20]. Rhizospheric bacteria promote the plant growth by different mechanisms. One of the important mechanisms is the solubilization of mineral phosphates in the rhizosphere and provides soluble P to plants [36]. Phosphate solubilizing bacteria are potential to increase available P for plant, especially in soils with large amounts of precipitated phosphate [8]. The amount of soluble P in soil is generally very low, normally at levels of mg kg^{-1} or less [9]. Phosphate solubilizing bacteria play a crucial role in making available solubilized fraction of various phosphate minerals in soils to growing plants.

Application of the phosphate-solubilizing microbes *Agrobacterium, Bacilus, Enterobacter, Pseudomonas, Aspergillus, Trichoderma,* and *Glomus* around the roots of plants, in soils and in fertilizers has been shown to release soluble phosphorus, promote plant growth, and protect plants from pathogen infection [2, 19, 25, 26, 37, 38]. Biofertilizers such as microbial inoculants promote plant growth; productivity and increase the nutrient status of the host plant have internationally been accepted as an alternative source of chemical fertilizers [34]. This study was introduce, the

alternative as well as save use of *Pseudomonas fluorescens* species as biofertilizer through inoculum preparation. So it was create more awareness to use these bacteria and invite the scholars to search for further investigation. The present study was designed to isolate certain rhizospheric bacteria of *Pseudomonas fluorescens* from rhizospheric soil of health faba bean and tested their Phosphate solubilization potential.

MATERIALS AND METHODS

Soil sample collection

The rhizospheric soil samples were collected in an envelope from fields growing faba bean (*Vicia faba* L.) from five Kebales of Salele zone: Mechale wartsu at altitude of 2560 meters above sea level, Wachale at altitude of 2540 meters above sea level, Gore kateme at altitude of 2590 meters above sea level, Eveno at altitude of 2510 meters above sea level and Gago at altitude of 2520 meters above sea level of North Showa of Oromiya Region of Salele zone, Ethiopia as shown in fig 1. The soils were brought to Mycology Laboratory, Department of Microbial, Cellular and Molecular Biology, College Natural Sciences, Addis Ababa University.

Figure 1. Soil samples

Isolation of Pseudomonas fluorescens

Isolation of *Pseudomonas fluorescens* isolates studies were carried out on King's B medium (KBM) [12]. 1g of rhizosphere soil sample was suspended in 99 ml of sterile distilled water. Samples were serially diluted and 0.1 ml of sample was spreaded on King's B medium plates. After incubation at 28^{o}C for 48 h the plates were exposed to UV light at 365 nm for few seconds and the colonies exhibiting the fluorescence were picked up and streaked on to the slants for maintenance, purified on King's B medium plates and also desigented as P f1-12 which stands for *Pseudomonas fluorescens* isolates used for further studies.

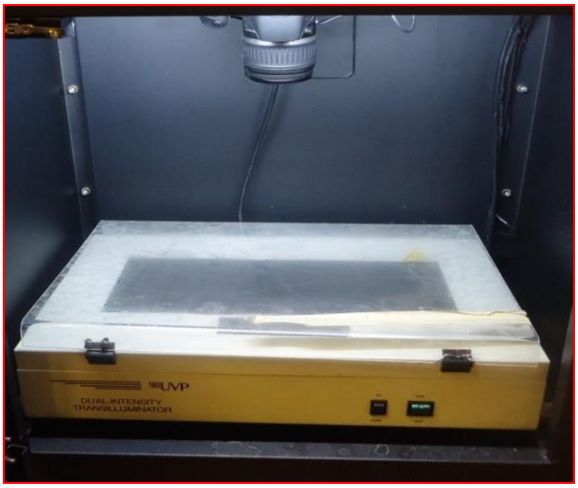

Figure 2. UV lamp apparatus set up

Assay for Phosphate-Solubilization

Phosphate-solubilization test was conducted qualitatively by plating the *Pseudomonas fluorescens* isolates in agar containing precipitated tricalcium phosphate. The medium was a modification of Pikovskaya medium [32], consisted of 10 g glucose, 5 g tribasic phosphate ($Ca_5HO_{13}P_3$), 0.5 g $(NH_4)_2SO_4$, 0.1 g $MgSO_4.7H_2O$, 0.2 g KCl, trace of $MnSO_4$ and $FeSO_4$, 0.5 g yeast extract, and 15 g agar, in 1,000 ml distilled water. *Pseudomonas fluorescens* isolates culture were streaked on the surface of agar plates and incubated at 28°C for 3 days. After 3 days, the colonies showing the clear zones around them were considered as positive for positive P-solubilization.

RESULTS AND DISCUSSION

Isolation of Pseudomonas fluorescens

During this research study, 12 *Pseudomonas fluorescens* were isolated from rhizospheric soil of healthy faba bean from five Kebales of Salale zone of Oromiya Region on King's B medium and observed under UV light at 365 nm for few seconds and pigment producer was screened as indicated fig 3. Then it was purified again on same medium and observed under UV light as shown in fig 4. All the rhizospheric isolates were named as *Pseudomonas fluorescens* isolate 1= P f1, *Pseudomonas fluorescens* isolate 2= P f2, *Pseudomonas fluorescens* isolate 3=P f3, *Pseudomonas fluorescens* isolate 4= P f4, *Pseudomonas fluorescens* isolate 5=P f5, *Pseudomonas fluorescens* isolate 6= P f6, *Pseudomonas fluorescens* isolate 7=P f7, *Pseudomonas fluorescens* isolate 8= P f8, *Pseudomonas fluorescens* isolate 9=P f9, *Pseudomonas fluorescens* isolate 10= P f10, *Pseudomonas fluorescens* isolate 11= P f11, *Pseudomonas fluorescens* isolate 12=P f12, and maintained on Nutrient Agar slants for further testing.

Figure 3. *Pseudomonas fluorescens* was isolated based on their pigment production under UV light at 365 nm

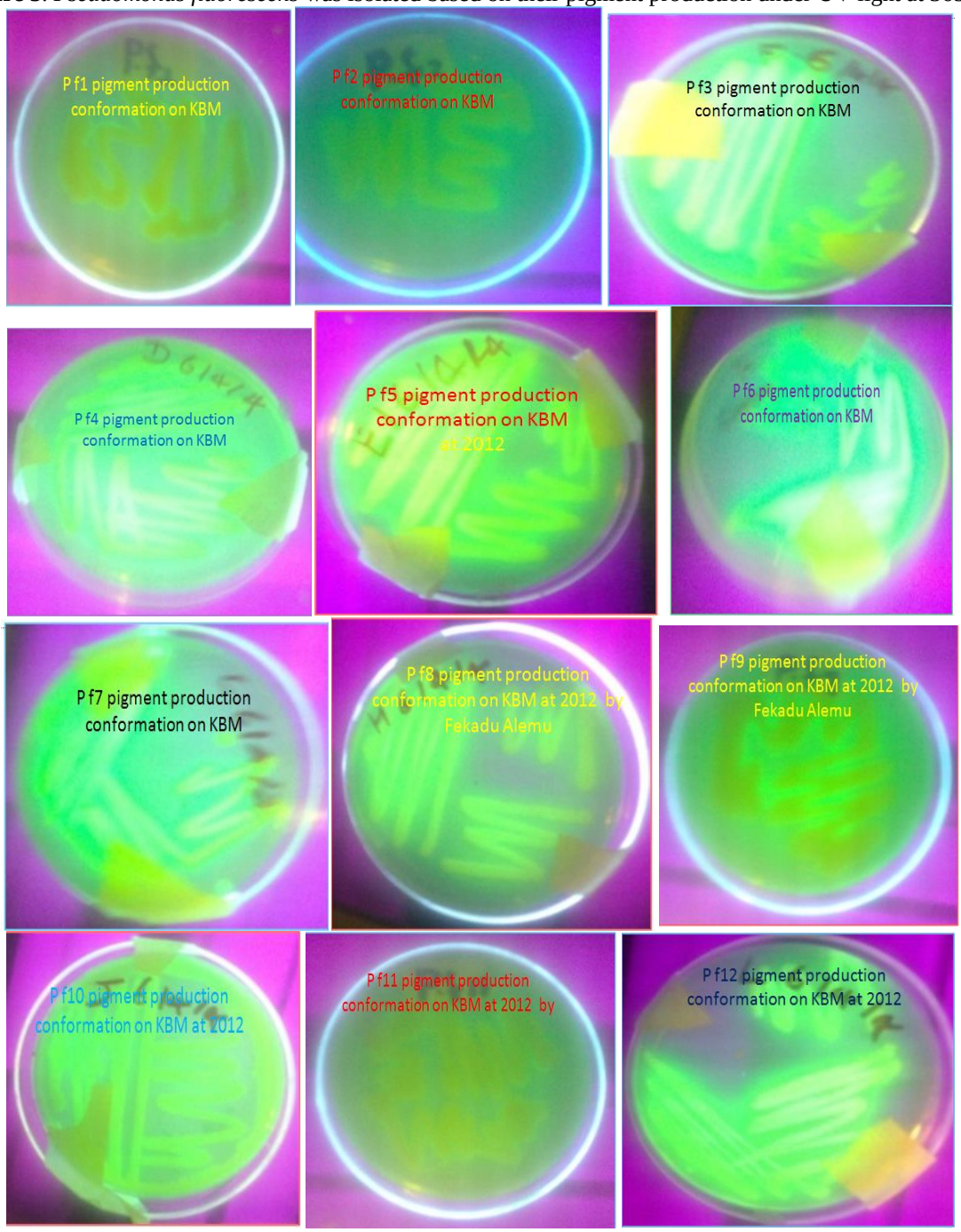

Figure 4. *Pseudomonas fluorescens* isolates was confirmed again under UV light at 365 nm

Assay for Phosphate-Solubilization

Twelve *Pseudomonas fluorescens* were isolated from the rhizospheric soil of healthy faba bean plants and were tested for their solubilization of Phosphate. Those isolate of *Pseudomonas fluorescens* were tested Phosphate solubilizing activity on Pikovskaya media plates, and some them was formed a clear halo zone indicating P-solubilizing activity as indicated in fig 5.

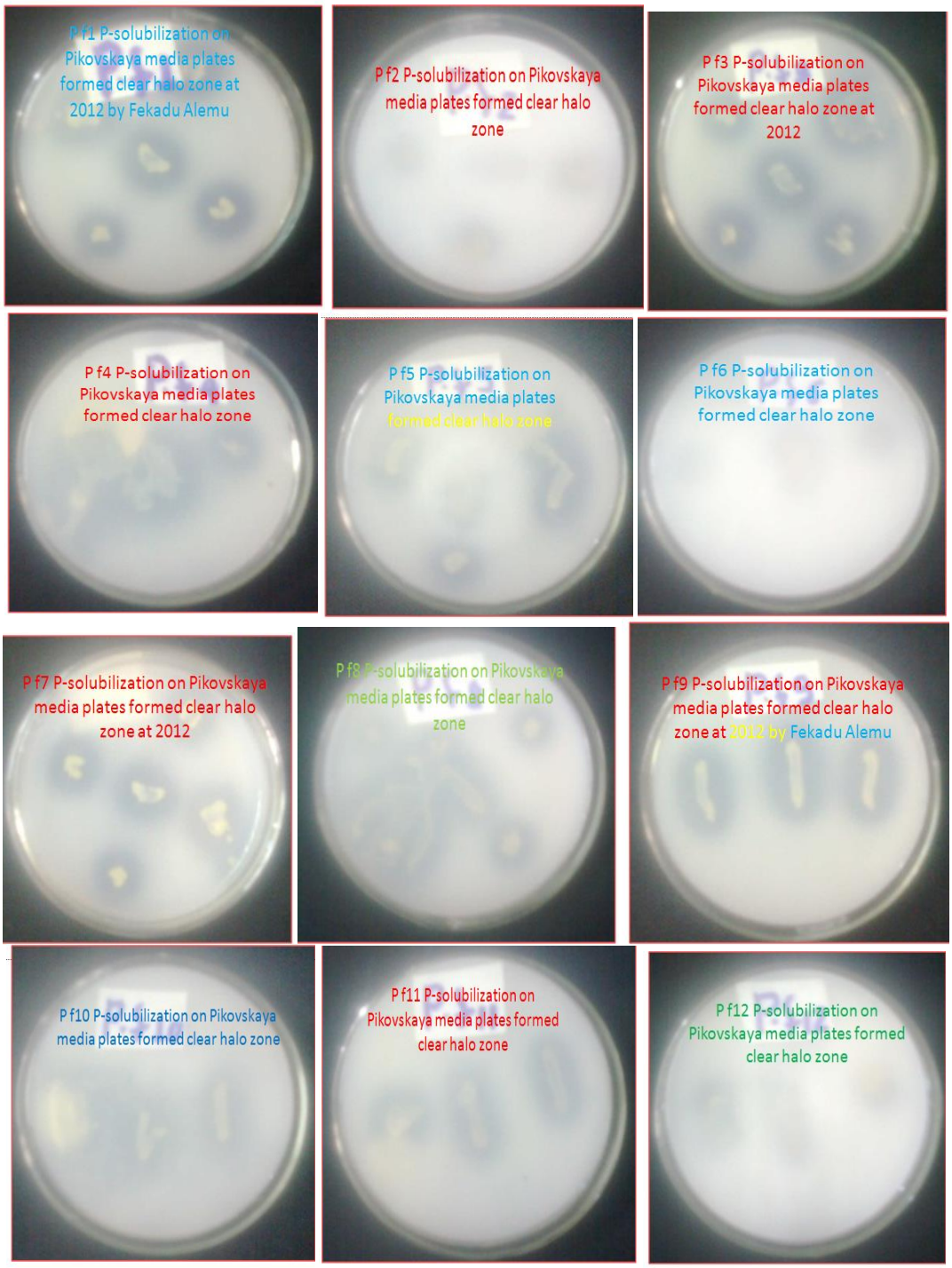

Figure 5. Phosphate solubilizer of *Pseudomonas fluorescens* isolates forming clear zone on Pikovskaya media plates after 3 day

All isolates of *Pseudomonas fluorescens* bacteria showed clear halo zone of phosphate solubilization. Isolate 1, 3 7, and 9 of *Pseudomonas fluorescens* showed highest the clearer zone in PVK medium. Similarly, [21] reported *Pseudomonas corrugata* as phosphate solubilizer. Highest phosphate solubilization zone was also recorded by *Pseudomonas* spp. as reported by [14] on PVK medium. The microorganisms capable to form a halo zone due to organic acids production in the media plates [29] and

are selected as potential phosphate solubilizers [4]. Production of phosphatase enzyme by Phosphate Solubilize bacteria and microbial phytases activity was reported by [23]. This solubility of P might be the activity of certain microbes in preferable phosphate sources or due to the activity of phosphatase enzyme. Some plant growth promoting rhizobacteria of colonize roots of plant and promote plant growth and development through a variety of mechanisms. The exact mechanism by which plant growth promoting rhizobacteria stimulate plant growth is not clearly known, although the mechanisms such as activation of phosphate solubilization and promotion of the mineral nutrient uptake are usually believed to be involved in plant growth promotion [7, 15].

CONCLUSION

It has been observed that applications of chemical fertilizer for increase crop yield are largely affecting human health, normal flora of soil and environment. For this reason, seed inoculation with *Pseudomonas fluorescens* isolates as a bio-fertilizer that is an acceptable alternative to chemical fertilizer application. Based on present studies, *Pseudomonas fluorescens* isolates under investigation possess a variety of promising properties which make them better bio-fertilizer agents that are capable of producing plant growth promoting substances and subsequent enhancement of yield of the crop. Such type of study is necessary as it advocates that the environmental friendly bio-fertilizer use for increase soil fertility and production of faba bean crop. Therefore, this study can be further exploited for the commercial production of an inoculum to use as biofertilizers that are an efficient approach to replace chemical fertilizers and its incorporation in the production system of crop (*Vicia faba)*.

ACKNOWLEDGMENTS

I am grateful thanks to Addis Ababa University, Department of Microbial, Cellular, and Molecular Biology, College of Natural Sciences which are giving facilities to conduct this study.

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