DOI: 10.5281/zenodo.10682302

CHAPTER 10

Studies on Phosphate Solubilizing Microorganisms Isolated From Soil

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Received: 23 January, 2024; Accepted: 30 January, 2024

Abstract

In the present study phosphates solubilizing microbial (PSM) cultures were isolated from the collected soil samples. Rhizospheric microflora such as *Trichoderma*, *Aspergillus*, *Penicillium*, *Rhizobium*, *Pseudomonas*, *Azotobacter* sp. were isolated as a phosphate solubilizing strains using selective agar media. The isolated bacterial cultures were recognized by culture dependent method and the fungal species were identified by morphological characters. The bacterial isolates were incubated at 35°C for 24-48 hrs in incubator while fungal cultures were incubated at 35°C for 48-72 hrs in incubator. The serial dilution as 10^{-4}, 10^{-5}, 10^{-6} and 10^{-7} were preferred for the isolation of PSM. On Pikovaskays agar plate, the zone of clearance verified phosphate solubilization. The identified strains may be useful in enhancing crop yield, preventing plant diseases, and promoting growth.

Keywords: PSM, Zone of clearance, Pikovaskays agar medium

Introduction

Biofertilizers have the most potential microflora which organizes different elements in the soil. Such bio-inoculants actively involved in mineralization of innumerable micro elements such as phosphorus, zinc, potash, iron etc. and fixed the environmental nitrogen by symbiotic or non-symbiotically hence improve the soil productiveness. The Rhizospheric microorganisms produce the plant growth producing hormones, acts as a defense mechanism of plant against pathogens. The second most important nutrient for plants is phosphate. To supplement phosphates in the soil, chemical phosphatic fertilisers are commonly utilised. (D.V. Pathal & Mukesh Kumar, 2016). Study was conducted to describe walnut phosphate-solubilizing bacteria (PSB). The application of W24 or W12 increased the walnut seedlings' root dry weight, height, and uptake of P and nitrogen. (Xuan Yu, *et al.*, 2011).

ISBN: 978-93-95369-85-5

Methods and materials

Soil Sampling

The three samples soil were collected and transported to autoclaved screw cap tube and stored at room temperature (Aneja KR, 2005, Dubey RC and Mahesgwari DK 2019).

Soil Microorganisms Medium (Soil extract agar/broth)

This medium was used for the enrichment of the microorganisms present in the collected soil. The soil extract medium contains (g/lit) K_2HPO_4: 0.5, Glucose: 1, Agar: 20.0, Soil extract (Stock): 100 ml, D/W: 900 ml adjust the pH: 7.2. (Aneja KR, 2005, Dubey RC and Mahesgwari DK 2019).

Serial Dilution method

The collected soil samples from Bhokar region were used to dilute it serially as 1 gm soil sample mixed in ringer's solution (9 ml) and serially make dilutions up to 10^{-7}. After inoculating the plates with 0.1 ml of the diluted suspension on selective media, the plates were incubated at 35°C for 1-2 days for bacteria and 2-3 days for fungi. ((Aneja KR, 2005).

Culture dependent characterization of isolate

The isolated colonies were purified by pure culture methods and characterize it all the cultures by culture dependent methods. (Aneja KR, 2005, Dubey RC and Mahesgwari DK 2019).

Screening of Phosphate solubilizing Microorganisms

Prepare soil suspensions and spread on the Pikovaskays agar medium for isolation of efficient PSM for the preparation of the bioinoculant. The Pikovaskays agar medium contains (g/lit) Dextrose: 10.0, Yeast extract: 0.5, ammonium sulphate: 0.5, Calcium phosphate: 5.0, Potassium chloride: 0.2, Magnesium sulphate: 0.1, Manganese sulphate: 0.0001g (trace amount), $FeSO_4$: 0.0001 g (Trace amount), Agar-agar: 20 g. (Aneja KR, 2005, Dubey RC and Mahesgwari DK 2019).

Results and Discussion

Phosphate-solubilizing microorganisms were isolated by serial dilution method using Pikovskaya medium. The serial dilution as 10^{-4}, 10^{-5}, 10^{-6} and 10^{-7} were preferred for the isolation of phosphate solubilizing microorganisms. 15 bacterial cultures were isolated and 4 fungal species were purified among them potential phosphate solubilizing microorganisms were studied. After the incubation of plates at 35°C temperature in incubator, the formed zone of clearance reveals presence of PSM in sample. Most of the reference checked the capacity of the isolated effective phosphate-solubilizing organisms to solubilize insoluble phosphate in liquid culture media is evaluated after selection. Ultimately, the effectiveness of the inoculants in pot as well as in farm for crop production is evaluated against a range of crops, using the carefully chosen effective phosphate-solubilizing cultures for their establishment.

ISBN: 978-93-95369-85-5

Fig: Phosphate solubilizing Microorganisms (PSM) on Pikovskaya medium.

The bacterial cultures were identified by culture dependent method and the fungal species were identified by morphological characters. The pure culture of *Pseudomonas, Rhizobium, Azotobacter, Trichoderma, Aspergillus, and Penicillium species.* were enriched using selective media. The present research work may be useful for the farmers and in horticulture fields by preferring the three steps such as firstly isolated the PSM by using above methodology and make a pure cultures. Secondly use the pure cultures of PSM for preparation of mass cultures and thirdly distribute it in concern field. The presence of combined form of phosphate mineralized by the applied PSM effectively and production and nutrient quality of the crop may increase.

As per the investigation by Das, they studied on microorganisms potential of producing a zone of clearance around the colony, utilising either Pikovskaya agar medium (Pikovskaya 1948) or tricalcium phosphate medium, were frequently investigated in the laboratory as possible phosphate solubilizers because of the solubilization of organic acids in the surrounding medium (Das 1989, Nautiyal 1999). According to Richardson (2001), the accessible inorganic P component in acid soils is typically found absorbed to the surface of soil minerals or as precipitates of weakly soluble $FePO_4$ and/or $AlPO_4$. Consequently, the primary sources of P utilised were three distinct synthetic compounds that are characteristic of the phosphate minerals typically found in soil. Solubilization halos around bacterial colonies were only visible on plates containing $Ca_3 (PO_4)_2$, despite the fact that many colonies might grow on the medium. Moreover, the subsequent mobilisation of P from $FePO_4$ or $AlPO_4$ in liquid medium occurred at a significantly reduced rate in comparison to $Ca_3 (PO_4)_2$. Similar results have been discovered in the past by Chung et al. (2005) and Delvasto et al. (2007). This could imply that the PSB cultures isolated from the rhizosphere of walnut trees have a negligible impact on increasing plant development in acidic soils by supplying soluble P to the roots. These worries should lead to the development of more accurate screening techniques for locating mineral-solubilizing bacteria in acid soils that are ecologically significant. On the other hand, objectively assessing the phosphate-solubilizing ability in vitro is necessary to ascertain the cellular P of bacterial cells since PSB could absorb P dissolved from insoluble substances during cell growth. Estimating P levels only in the supernatant has the drawback of not taking into consideration P that the cell uses (Steyaert et al, 2010, Yoav Bashan et al, 2014).

The combination inoculation of PSB and other microbes may have improved the growth and yield parameters of different plants for the following reasons: (a) Phosphate-solubilizing bacteria may alter the composition of root secretion and flexibility, impacting the colonisation and development of other bacteria. (b) Mixed inoculants, which included higher concentrations of N and P as well as growth-promoting compounds generated by these organisms, provided plants with more balanced nutrition and (c) The utilisation of weakly soluble P sources may be made possible by PSB's interactions with other microorganisms, which could have a synergistic effect. More attention should be paid to the research and use of novel PSB and other microbe combinations in order to achieve better outcomes. Importantly, more research into the processes underlying synergistic interactions is needed to understand the molecular foundation of these interactions. (Xuan Yu *et al,* 2011, Maureen O'Callaghan 2016, Kale GJ et al. 2018).

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The ability of microorganisms to produce and release organic acids, which chelate cations (mostly Ca^{2+}) attached to phosphate and transform them into soluble forms via their carboxylic groups, is known as P-solubilizing activity (Kpomblekou and Tabatabai 1994).

Conclusion

Present investigation illustrates the metabolically diverse and healthy community of microorganisms in the rhizosphere must for agricultural fields. The isolated phosphate solubilizing microorganisms (PSM) could be highly potential to involve in biogeochemical cycle. This research is continued for the isolation of most efficient PSM necessary for improvement of plant including growth and nutrient quality.

Acknowledgement

I thankful to Swami Ramanand Teerth Marathwada University, Nanded (MS) for providing the fund and Department of Microbiology, Shri Shivaji College Parbhani for availing the microbiology lab for research work.

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ISBN: 978-93-95369-85-5