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Study of phosphate Solubilizing activity of Lead tolerant *Pseudomonas aeruginosa* HMT 51 isolated from Zawar mines, Udaipur, India

Sen Monica and Joshi Harshada*
Department of Biotechnology, Vigyan Bhawan, Block B, Mohanlal Sukhadia University, Udaipur, Rajasthan, INDIA

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Abstract

Phosphate is one of the most vital macronutrient required for the growth and development of plants. A large number of microorganisms present in the rhizosphere are known to solubilize the insoluble phosphorus present in the soil, converting it into soluble form and make available to the plants. The present study is aimed to determine the efficiency of phosphate solubilizing activity of lead tolerant Pseudomonas aeruginosa HMT51 isolated from mine spoil of Zawar. The phosphate solubilizing activity was determined using modified Sperber medium containing 10% $CaCl_2$ and 10% K_2HPO_4 by spot assay method. The isolate was found positive as it showed halo zone around its colonies. The efficiency of phosphate solubilization of HMT51 was determined by comparing the diameter of halo zones after 24h, 48h, and 72h. It was found to be 200, 281.81 and 314.28 after 24h, 48h and 72 h respectively. Lead tolerant phosphate solubilizing bacteria will support the plants growing in mine spoil by providing soluble form of phosphate and can thus enhance microbe assisted phytoremediation of metal polluted sites

Keywords: Lead tolerance; *Pseudomonas*; Phosphate; zawar mines.

Introduction

Phosphate solubilizing microorganisms (PSMs) are a group of rhizobacteria that supplements phosphorus to the plants which is otherwise remains unavailable to them[1]. Several heterotrophic and chemoautotrophic bacteria have the capacity to release P from fixed and insoluble mineral and organic phosphate sources, mainly by the production of various organic acids but sometimes by the production of CO_2, H_2S, chelating agents, humic acid, proton extrusion and siderophores [2, 3]. Phosphate solublizing microorganisms (PSM) particularly those belonging to the genera *Pseudomonas* and many others possess the ability to convert insoluble phosphates of soil into soluble forms by secreting organic acids such as formic, acetic, propionic, lactic, glycolic, fumaric and succinic acids [4,5]. Production of organic acids results in acidification of the microbial cell and its surroundings. PSMs can grow on various phosphorus containing medium and play an important role in supplying phosphate to plants in a more environmentally-friendly and sustainable manner [6, 7].

Accumulation of heavy metals in soil is highly toxic to most of the plants growing in such contaminated soil. Heavy metals ions, when present at an elevated level in the environment, are excessively absorbed by roots and translocated to shoot, leading to impaired metabolism and reduced growth[8]. Microbial populations are known to affect heavy metals mobility and availability to the plant through release of chelating agents, acidification, phosphate solubilization, and redox changes[9]. The population, growth, survival activities and phosphate solubilization efficiency of PSM are greatly influenced by soil physical, chemical and biological stresses. The present study is done to isolate such a potential PSM that can tolerant high concentration of lead, get adapted to such stresses along with providing soluble form of phosphate to the plants. Such a PSM may prove to be an ideal microbe that may support microbe assisted phytoremediation.

Materials and Methods

Source of bacteria: The isolate *Pseudomonas aeruginosa* HMT 51 was recovered previously from rhizospheric soil of plants growing in tailing dam of Zawar mines. The isolation was done on nutrient agar supplemented with 0.1mM lead acetate. This isolate was able to tolerate high concentration of lead i.e., upto 2.5mM.

Detection of phosphate solubilizing activity: The isolate *Pseudomonas aeruginosa* HMT 51 was used to study the phosphate solubilizing ability and efficiency. The phosphate solubilizing ability was detected on modified Sperber medium[9] containing 10% $CaCl_2$ and 10% K_2HPO_4 by spot assay method.

Efficiency of phosphate solubilizing activity*:* Efficiency study has been carried out by performing an experiment of halo zone formation around the bacterial growth after inoculated on modified Sperber medium and incubated for 24h, 48h and 72h at 37 ± 2^0C. The zones were then compared to find phosphate solublization efficiency (E)[10]

E = Solubilization diameter (s)/Growth diameter (g) x 100

Optimization of physiological conditions (Temperature and pH): The phosphate solubilization efficiency (PSE) of the isolate was studied on modified Sperber media with constant pH 7.0 and varying incubation temperature 10ºC, 37ºC and 45ºC and modified Sperber media adjusted at different pH values 5, 7and 9,with incubation temperature 37ºC.

Results and Discussion

Bacterial resistance to heavy metals is an important consideration when bacteria are to be introduced into soils for enhancing bioremediation of metal contaminated soils. Phosphate solubilizing ability in addition to metal tolerance may prove to be a suitable combination for promoting plant growth in metal contaminated sites. In the present study, the lead tolerant *Pseudomonas aeruginosa* HMT 51 was studied for its phosphate solubilizing ability on modified Sperber media. It gives halo zone around its colonies on modified Sperber media and hence is considered to be a useful PSB. Venkateswaran and Natarajan[11] while studying the Porto Novo waters found *Pseudomonas* spp. as dominant inorganic phosphorous compounds solubilizing microbe. Heavy metal tolerant *Pseudomonas* with phosphate solubilizing ability had been formerly isolated by few workers[12] but tolerance to lead has been reported rarely.

For the isolation of PSB several types of media could be used for e.g. Pikovskaya agar[13], basal Sperber medium[14], modified Sperber medium[9] etc. In the present study modified Sperber medium was used, on which fairly good phosphate solubilizing activity was found. This medium was used for similar type of work by many other researchers[4, 13].

For determining the phosphate solubilization efficiency; the isolate was spotted on modified sperber medium and incubated for 24h, 48h and 72h at 37$\pm$ 2°C. After 24h, 48h and 72h of incubation the halo zone diameters for *Pseudomonas aeruginosa* HMT 51 were found to be 1.2, 1.9 and 2.6 cm respectively.

Table-1
Table showing zone of phosphate solubilization and efficiency of *Pseudomonas aeruginosa* HMT 51

S. No.	Duration	Colony Diameter(cm)	Zone of phosphate solubilization(cm)	Efficiency
1.	24h	0.8	1.6	200
2.	48h	1.1	3.1	281.81
3.	72h	1.4	4.4	314.28

The efficiency of the isolate *Pseudomonas aeruginosa* HMT51 was determined by using the formula given by Edi-Premono (1996). The results of phosphate solubilization efficiency study showed that for the *Pseudomonas aeruginosa* HMT 51 the efficiencies were 200, 281.81 and 314.28 after 24h, 48h and 72 h respectively. S. Sheshadri *et al.* [15]found *Pseudomonas* as most efficient phosphate solubilizer on Pikovskaya's agar plates with the efficiencies 228.26$\pm$6.12 and 205.86 $\pm$ 4.11 respectively at 7[th] day incubation. The results in the present study are also in concordance to that of S. Shivakumar[16] who reported the efficiency for his isolate *Pseudomonas aeruginosa* FP6to be 226. Generally, halo zone increased with increase in colony diameter. Fluctuations in solubilization index were observed during the seventh day observation period. In most of the cases it gradually increased, while in few cases (*Micrococcus, Alcaligenes, Corynebacterium and Flavobacterium*) increased initially and later decreased.

Solubilization of insoluble phosphates by microorganisms depends upon various factors that are temperature, pH etc.[17]. The metabolic activities of bacteria are linked to the temperature of the environment by getting adapted to their indigenous environment[18, 19]. The phosphate solubilizing bacterial isolate *Pseudomonas aeruginosa* HMT 51 showed optimum growth at 37°C and pH 7.Similar results were also reported in past by few workers.

Conclusion

It is concluded from the present study that lead tolerant *Pseudomonas aeruginosa* HMT 51 was found as an efficient phosphate solubilizer. Hence future use of this isolate as a potential agent for bioremediation will reduce metal pollution in soil and supports plants to grow in contaminated areas by providing soluble form of phosphate.

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