



# Bioprospecting of phosphorus solubilizing bacteria from Renuka Lake Ecosystems, Lesser Himalayas

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## ABSTRACT

In the present study, diversity and plant growth promoting traits of bacteria isolated from Renuka Lake, Himachal Pradesh was investigated. A total of three samples, including sediment (pH-7.3), sub-surface water (pH-8.1), and surface water (pH-8.2), were collected. A total of 65 bacterial isolates were obtained on different growth media. Among 65 isolates 18 isolates were found to be solubilizers of phosphorus. The maximum amount of phosphorus was solubilized by EU-RL 54 ( $7,976 \pm 0.01 \mu\text{g/l}$ ) followed by EU-RL 53 ( $6,322 \pm 0.01 \mu\text{g/l}$ ). Phosphorus solubilizers also possessed other plant growth promoting traits, such as the production of ammonia, hydrogen cyanide, zinc solubilization, production of hydrolytic enzymes. The isolates were identified belonging to different genera *Acinetobacter*, *Bacillus*, *Enterobacter*, *Klebsiella*, *Proteus*, *Pseudomonas*, and *Staphylococcus*. This is the first report for these plant growth promoting bacteria to solubilize a considerable amount of phosphorus isolated from Ecosystems, Lesser Himalayas.

## 1. INTRODUCTION

The earth's surface is mostly covered with water and about 96% consist of marine ecosystems. Both marine and freshwater environment are rich in biodiversity and important for proper functioning as well as maintaining health of the environment [1]. Furthermore, microbial communities in both water as well as sediment have been shown to make a great contribution to health of the lakes [2]. The ever-present microorganisms are the chief players in cycling of all the elements between sediments, waters, and the atmosphere which is actually known to be key driver of the microbial processes as well as range of natural ecosystems [1]. Many studies have been carried out on lake ecosystems which show that growth and distribution of sediment microbial communities are greatly affected by the availability of carbon sources [1]. Microbial communities, especially in sediment, are chiefly important because of their greater cell density as well

as taxonomic diversity and they can respond quickly to their surrounding environmental [3–5].

Renuka Lake is the largest lake in Himachal Pradesh, with a circumference of about 3,214 m. The lake is oval shaped and has water spread of 670 ha [6]. The annual rainfall in this region is estimated to be between 150 and 199.9 cm [7]. The length and breadth of Renuka Lake is 10.50 and 204 m, respectively, with a maximum depth of ~13 m and the catchment area ~254.3 ha. The silt and debris are deposited in the lake all around the catchment of the lake which is leading to ecological deterioration, eutrophication, and habitat degradation [8]. Due to biological richness of Lake, it has been designated as the wetland of national importance by the Wetland Management Committee [8]. The reports on study of Renuka Lake is very scanty and the literature on Renuka Lake available is either on its geochemistry or this lake as newfound residence for migratory birds, or fluctuations in zooplanktons, and ion chemistry [6, 8–10] and studies on its microbial diversity has not been yet done. Thus, besides investigating flora and fauna of Lake Ecosystem, microbial diversity is also of utmost importance. Therefore, this area was chosen for the present study to investigate the microbial communities present in this particular ecosystem and the roles they may contribute in promoting growth of the plants.

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## 2. MATERIALS AND METHODS

### 2.1. Sampling, Enumeration, and Isolation of the Bacteria

The study area was Renuka Lake located in Sirmour district of Himachal Pradesh, 672 m above the sea level bound by 30°36'30"N latitude and 77°27'6" E longitude. A total of three samples, including sediment, sub-surface water, and surface water, were collected in sterile polyethene bags and bottles, respectively, and stored at 4°C until analysis. The pH of the samples was also recorded. The culturable bacteria in sediment and water samples were isolated through enrichment using the standard serial dilution plating technique. Different growth media were employed for isolation, such as: Nutrient agar medium, King's B agar, Tryptic soy agar, R<sub>2</sub>A medium, Ammonium salt agar medium, and Jenson agar [11]. After appearance of the colonies, they were purified by repeated re-streaking on nutrient agar to obtain isolated colonies. Once the pure cultures were obtained, they were further maintained in slants and 25% glycerol stock at 4°C and -80°C, respectively.

### 2.2. Screening and Identification of P-solubilizing Microbes

All the isolates obtained were screened qualitatively for solubilization of the phosphorus on Pikovskaya agar using three different insoluble substrates of phosphorus, including rock phosphate, apatite, and tricalcium phosphate [12]. The phosphorus solubilizers were quantitatively assayed for phosphate solubilization by method described by Murphy and Riley [13]. P-solubilizing bacteria were screened for other plant growth promoting attributes using the standard methods. All P-solubilizers were screened for solubilization of potassium, zinc, production of indole-3-acetic acid, ammonia, hydrogen cyanide (HCN), amylases, cellulases, pectinases, and proteases using standard

method as described earlier [14–16]. The isolates were identified on biochemical basis using standard methods. The various tests performed were indole test, methyl red test, Voges–Proskauer test, citrate utilization, fermentation of glucose, sucrose, maltose, and production of catalase and oxidase.

## 3. RESULTS

### 3.1. Enumeration and Isolation

The population of heterotrophic bacteria was enumerated in three samples that were isolated from Renuka Lake in Sirmour district of Himachal Pradesh. A considerable variation was observed in number of colonies isolated on each media used. The maximum isolates were obtained from surface water followed by sediment and sub-surface water. A total number of 98 bacterial isolates were obtained from three samples on different media.

### 3.2. Characterization and Identification of P-solubilizing Bacteria

All strains screened for phosphorus solubilization showed variable results on different insoluble sources of phosphorus used. Out of 98 strains, only 18 strains solubilized phosphorus (Table 1). Phosphorus solubilization was found in the range of 3,440 ± 0.01 µg/l to 7,976 ± 0.01 µg/l (Fig. 1). The maximum amount of phosphorus was solubilized by *Staphylococcus* sp. EU-RL 54 followed by *Enterobacter* sp. (EU-RL 53) solubilizing 6,322 ± 0.01 µg/l (Table 1). The downfall in pH were recorded highest EU-RL 53 (Fig. 2). Screening of P-solubilizers for diverse direct and indirect plant growth promoting attributes; it was found that, 18, and 5 showed the P-solubilizing and zinc solubilizing activity, respectively. The production of

**Table 1.** Phosphorus solubilizing bacteria from Renuka Lake ecosystems with multifarious plant growth promoting attributes.

Strains	P- solubilizers	Source	P-solubilization		Amy	Pec	Pro	Zinc	HCN	NH <sub>3</sub>	IAA
			Amt (µg/l)	pH							
EU-RL 2	<i>Klebsiella</i> sp.	SD	3,764 ± 0.00	5.9	-	-	-	-	-	-	+
EU-RL 8	<i>Enterobacter</i> sp.	SD	4,170 ± 0.01	6.0	-	-	+	+	-	-	-
EU-RL 9	<i>Pseudomonas</i> sp.	SD	3,677 ± 0.01	6.6	-	-	+	+	-	+	+
EU-RL 10	<i>Proteus</i> sp.	SD	4,970 ± 0.01	6.3	-	-	-	-	-	-	-
EU-RL 11	<i>Proteus</i> sp.	SD	3,511 ± 0.00	6.5	-	-	-	-	-	-	+
EU-RL 25	<i>Klebsiella</i> sp.	SS	5,188 ± 0.00	5.8	-	-	-	-	+	-	-
EU-RL 35	<i>Proteus</i> sp.	S	4,590 ± 0.01	6.4	-	-	-	+	+	+	-
EU-RL 39	<i>Enterobacter</i> sp.	S	5,023 ± 0.01	6.8	-	-	+	+	-	+	+
EU-RL 49	<i>Enterobacter</i> sp.	S	4,000 ± 0.01	6.8	-	-	+	-	-	-	-
EU-RL 51	<i>Enterobacter</i> sp.	S	4,275 ± 0.01	6.4	-	-	-	-	-	-	+
EU-RL 52	<i>Enterobacter</i> sp.	S	3,566 ± 0.01	6.6	-	-	-	-	-	-	-
EU-RL 53	<i>Enterobacter</i> sp.	S	6,322 ± 0.01	6.1	-	+	-	-	-	-	-
EU-RL 54	<i>Staphylococcus</i> sp.	S	7,976 ± 0.01	6.6	+	-	-	-	-	-	-
EU-RL 56	<i>Bacillus</i> sp.	S	3,440 ± 0.01	6.8	-	-	+	+	-	+	-
EU-RL 57	<i>Acinetobacter</i> sp.	S	5,511 ± 0.00	6.7	-	-	-	-	-	+	-
EU-RL 62	<i>Enterobacter</i> sp.	S	5,346 ± 0.02	6.1	-	-	+	-	-	+	+
EU-RL 63	<i>Enterobacter</i> sp.	S	3,913 ± 0.03	6.5	-	-	-	-	-	-	-
EU-RL 64	<i>Enterobacter</i> sp.	S	3,511 ± 0.01	6.8	-	-	-	-	-	-	-

Surface-S; Sub-surface-SS, Sediment-SD.

indole-3-acetic acid (IAA), ammonia, and hydrogen cyanide (HCN) was found in 6, 6, 2 isolates, respectively. Amylase activity, pectinase, and protease activity were shown by 1, 1 and 6 isolates, respectively. None of the P-solubilizer showed cellulase activity. Among 18 P-solubilizers, only six isolates possessed multiple plant growth promoting traits (Table 1). Based on biochemical characterization, the isolates belonged to genera *Acinetobacter*, *Bacillus*, *Enterobacter*, *Klebsiella*, *Proteus*, *Pseudomonas*, and *Staphylococcus*. *Enterobacter* sp. was the most abundant species isolated followed by *Klebsiella* sp. and *Proteus* sp. (Fig. 3).

#### 4. DISCUSSION

Freshwater lakes are extremely biodiverse natural water reservoirs, with many important recreational and landscape qualities [17] though freshwater environments have been studied to a lesser extent than marine ones [18]. In aquatic ecosystems, bacteria play an important role in degradation of the organic matter, assimilation of carbon, nitrogen, and phosphorus which are catalyzed by enzymes produced by microorganisms [19–22]. Furthermore, bioprospecting microbial diversity of freshwater lakes is important as they are an important source of microbes which produce diverse enzymes of agricultural and industrial importance, various bioactive compounds, etc.

Renuka Lake in the Lesser Himalaya, Himachal Pradesh is in a valley which is completely surrounded by mountains. It further comprises of highly crumpled, shattered, crushed, folded, and dislocated rocks consisting of carbonaceous shales–slates often pyrite-ferrous, limestone, quartzites, and boulder beds [10]. There

are many high altitude sub-glacial lakes in Indian Himalayas that represent hot spots of microbial biodiversity. Huge diversity of microbes has already been reported from Himalayas [23–25]. Yadav et al. [26] reported diverse culturable psychrophilic and psychrotolerant Bacilli producing various cold active hydrolytic enzymes from Chandratat, Dashair, and Pangong Lake of Indian Himalayas. In the present study, we obtained 18 P-solubilizing bacterial strains from sediment, sub-surface water, and surface water. In our study, *Enterobacter* sp. was the most dominant P-solubilizer. The maximum diversity of P-solubilizers was obtained from surface water followed by sediments. The major source of dissolved materials in the Renuka Lake has been known to be primarily from the weathering of rocks [10], whereas organic source is the major contributor of relative enrichment of  $P_2O_5$  in lake sediments [6], which may have contributed to such diversity from surface water and sediments, respectively. In our study, P-solubilizers included *Acinetobacter* sp., *Bacillus* sp., *Enterobacter* sp., *Klebsiella* sp., *Proteus* sp., *Pseudomonas* sp., and *Staphylococcus* sp., which could solubilize phosphorus in the range  $3,440 \pm 0.01$  to  $7,976 \pm 0.01$   $\mu\text{g/l}$ . The study of Gen-Fu and Xue-Ping [27] characterized phosphorus-releasing bacteria which also included *Acinetobacter* sp., *Bacillus* sp., *Enterobacter* sp., and *Staphylococcus* sp. from a small eutrophic shallow lake, Eastern China, and amount of phosphorus released was 50  $\mu\text{g/ml}$  by inorganic phosphorus solubilizing bacteria whereas organic phosphorus-mineralizing bacteria released less than 2  $\mu\text{g/ml}$  water-soluble inorganic phosphorus every day. Qian, et al. [28] characterized phosphorus solubilizing bacteria, including *Acinetobacter* sp., *Burkholderia* sp., *Citrobacter* sp., *Cupriavidus*

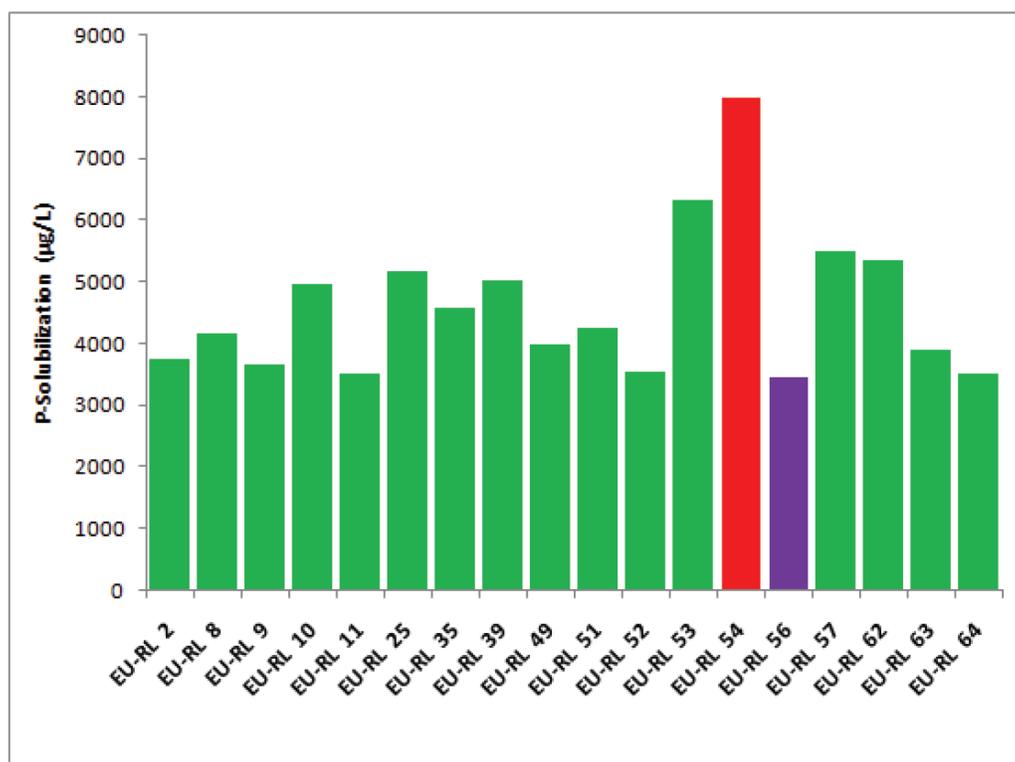


Figure 1: Relative distribution of P-solubilizing capability exhibited by bacteria isolated from Renuka Lake ecosystems, Lesser Himalayas.

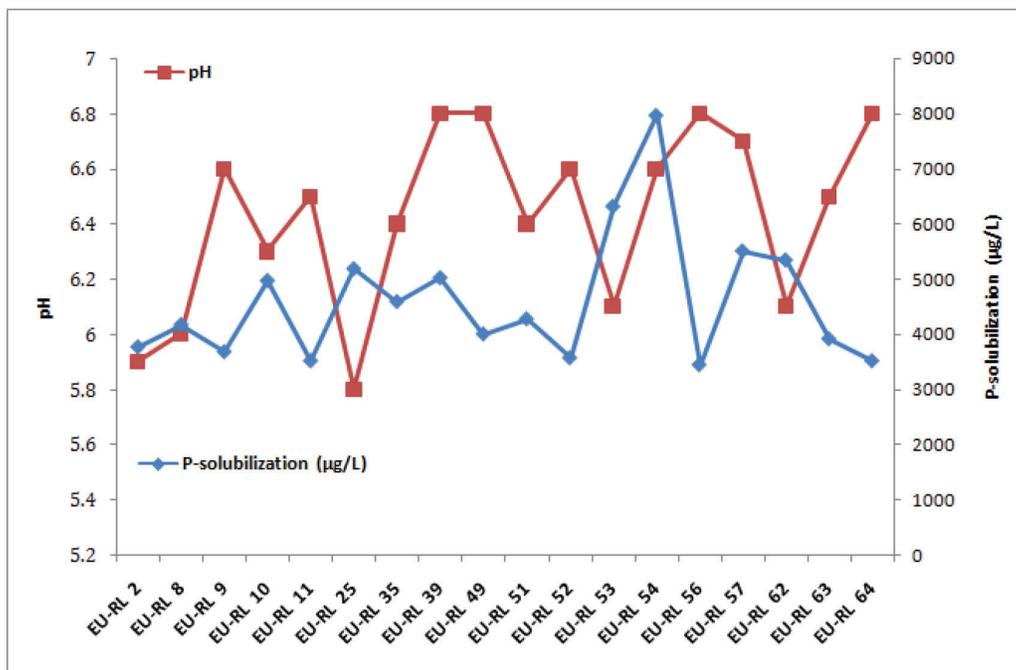


Figure 2: P-solubilization and downfall in pH by bacteria isolated from Renuka Lake, Lesser Himalayas.

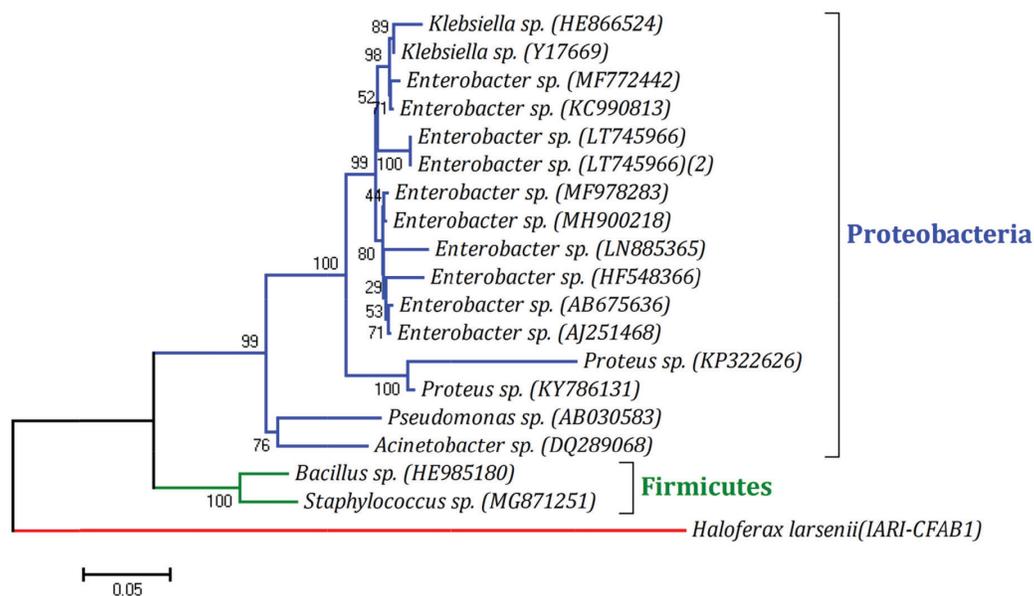


Figure 3: Phylogenetic tree showed the relationship among 18 P-solubilizing bacteria isolated from Renuka Lake, Lesser Himalayas. The sequence alignment was performed using the CLUSTAL W program and trees were constructed using Neighbor Joining (NJ) with algorithm using MEGA4 software.

sp., *Enterobacter* sp., *Pantoea* sp., and *Pseudomonas* sp. from sediments from a Shallow Eutrophic Lake and a wetland. The most efficient inorganic phosphorus solubilizing bacteria strain could accumulate over  $170 \text{ mg/l}^{-1}$  orthophosphate, while the equivalent organic phosphorus-mineralizing bacteria liberated less than  $4 \text{ mg/l}^{-1}$  orthophosphate in liquid culture. Maitra et al. [29] isolated *Bacillus*, *Brevibacillus*, *Enterobacter*, *Agrobacterium*, *Pseudomonas*, *Acinetobacter*, *Microbacterium*, *Curtobacterium*, *Stenotrophomonas*, and *Novosphingobium* as phosphorus

solubilizers releasing 6.3–68.8 mg P/L from Oxbow Lakes of West Bengal, India. Li et al. [30] reported a novel species of phosphate-solubilizing bacterium from Sanchahu Reservoir which is the second biggest freshwater lake in China.

The 18 P-solubilizers were further screened for diverse direct and indirect plant growth promoting attributes. The six P-solubilizers possessed multifarious plant growth promoting attributes. Among these, six P-solubilizers, *Enterobacter* sp. was the most dominant

one showing the production of proteases, ammonia, IAA, and solubilization of zinc followed by *Pseudomonas* sp. (EU-RL 9) which also possessed same attributes. *Proteus* sp. (EU-RL 35) and *Bacillus* sp. (EU-RL 56) also solubilized zinc and produced ammonia, whereas HCN production was shown by strain EU-RL 35 and protease production by EU-RL 56. Among 6 P-solubilizers with multiple plant growth promoting attributes, five solubilized zinc which may be attributed to fact that the lake has high content of zinc [6]. Another common trait found among these bacterial strains was production of ammonia and HCN which is important trait against phytopathogens. Thus, these bacterial strains with plant growth promoting attributes may have potential applications in agriculture to be used as biofertilizers mainly for the acquisition of unavailable nutrients and as biocontrol agents. To the best of our knowledge, this is the first report of plant growth promoting bacteria from Renuka Lake.

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#### CONFLICT OF INTEREST

The authors declared that they have no conflicts of interest.

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