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Kurthia Sp, a Novel Member of Phosphate Solubilising Bacteria from Rhizospheric Tea Soil of Darjeeling Hills, India

[1]Binod Chandra Sharma, [2]Rashi Subba, [3]Aniruddha Saha
[1, 2]P.G. Department of Botany, Darjeeling Government College, Darjeeling -734101, India
[3]Department of Botany, University of North Bengal, Raja Rammohanpur, Dt. Darjeeling, India

Abstract: *Rhizospheric soil from tea [Camellia sinensis (L.) Kuntze] bushes of Darjeeling hills was screened for the presence of phosphate solubilising bacterial populations on Pikovskayas agar. One of the potent strains was identified as Kurthia sp. In vitro tricalcium phosphate solubilising ability of this strain was determined as 40.62±1.1 mg/l with a drop in pH from 7.0 to 5.60 indicating the importance of acid production in the solubilisation process. This strain also produced growth regulating substance (IAA) under in-vitro conditions in the presence of precursor tryptophan. The amount of IAA released was 17.5 mg/l. This genus is considered as new novel member as phosphate solubilisers which may be developed as phosphatic biofertilizers after further characterisation in field conditions.*

Key-Words: *Darjeeling tea, rhizosphere, Kurthia sp., phosphate solubilisation*

I. Introduction

After nitrogen phosphorus (P) is the major plant growth-limiting nutrient despite being abundant in soils in both inorganic and organic forms. However, many soils throughout the world are P-deficient because the free phosphorus concentration (the form available to plants) even in fertile soil is generally not higher than 10 µM even at pH 6.5 where it is most soluble [1].

Phosphate solubilizing microorganisms (PSMs) is an ecologically special functioning group of soil microorganisms which play an important role in the turnover of organic P and insoluble inorganic phosphate, and in the cycling of P in soil [2, 3]. The composition and dynamics of this functional group was influenced greatly by vegetation type, soil texture, soil chemical elements, and pH in soil solution [4, 5, 6, 7].

PSMs convert these insoluble phosphates into soluble forms through the process of acidification, chelation, exchange reactions and production of gluconic acid [8].

Phosphate solubilising bacteria (PSB) produce amino acids, vitamins and growth promoting substances [9, 10] which promote plant growth. Although the mechanisms by which plant growth promoting rhizobacteria (PGPR) promote plant growth are not yet fully understood, many different traits of these bacteria are responsible for growth promotion activities [11]. It includes the ability to produce or change the concentration of the plant hormones indole acetic acid (IAA), gibberellic acid, cytokinins, and ethylene; fix dinitrogen; suppress the growth of deleterious microorganisms by production of siderophore, β-1, 3-glucanase, chitinases, antibiotics, and cyanide; and dissoIve phosphates and other nutrients.

IAA produced by bacteria improves plant growth by increasing the number of root hairs and lateral roots [12]. Microbial biosynthesis of IAA in soil is enhanced by tryptophan from root exudates or decaying cells [13, 14]. Tea is regarded as an important plantation crop of very high economic and commercial value in North-Eastern India. The studies on physico-chemical and microbiological soil properties under tea plantation crop are scanty [15]. Therefore the objectives of our research were to isolate the phosphate solubilizing bacteria from the rhizosphere of tea plants, further screen them for their performance under *in vitro* conditions.

II. Materials and Methods

2.1 Isolation of phosphate solubilizing bacteria

Rhizospheric soil from healthy tea (*Camellia sinensis* (L.) Kuntze) bushes of Singla Tea Estate, Darjeeling was collected and studied in the laboratory. Ten gram (10 g) of soil sample was suspended in 90 ml of sterile distilled water serial dilutions were prepared until the 10^{-7} dilution was obtained. The Pikovskaya agar (10 g Glucose, 5 g tricalcium phosphate, 0.5 g ammonium sulphate, 0.2 g potassium sulphate, 0.1 g magnesium sulphate, 0.5 g yeast extract, trace amount of manganese sulphate and ferrous sulphate, 20 g agar, 1000 ml distilled water, pH : 6.8) medium was used for isolation and maintenance of PSB. Bacterial colonies causing clear phosphate solubilizing zones by a turbid white background were selected and purified for further study. The colony diameter of PSB colony (halo zones) was measured by using metric scale. PSB isolates were preliminarily identified based on the morphological tests such as motility, cell shape and size and biochemical tests such as glucose fermentation, urea hydrolysis, nitrate reduction, citrate utilization, indole production,

Voges-proskaeur and methyl-red reaction and a potent isolate was sent to type culture collection for identification [16].

2.2 Quantification of P solubilization

The phosphorus solubilizing potential of selected PSB strain was tested *in vitro* by estimating available phosphorus in the Pikovskaya's broth amended with known amount of tricalcium phosphate as a substrate. A control without any inoculation was also maintained. The organisms were allowed to grow for 7 days at 30°C and centrifuged at 10,000 rpm for 10 min in a cooling centrifuge (REMI-C30BL, Remi, India). Phosphorus was determined in supernatant following the procedure of Fiske and Subbarow[17] using UV-VIS Spectrophotometer (SHIMADJU UV-1700 Pharmaspec, Shimadju, Japan).

2.3 Measurement of pH

A change in pH of the medium due to the growth of PSB was measured with a digital pH meter (Elico, India) after 7 days of incubation.

2.4 IAA Production

The production of IAA was determined following the standard protocol [18]. The tested bacterial strains was grown in LC medium in the presence of tryptophan (100 µg/l) and incubated at 30^0C for 3 days. A two ml culture was removed from each tube and centrifuged at 10,000 rpm for 15 minutes. One ml of supernatant fluid was transferred to fresh tube to which 100 µl of 10 mM orthophosphoric acid and 2 ml of reagent consisting of 1 ml of 0.5 $FeCl_3$ in 50 ml of 35% $HClO_4$ were added. The absorbance of the developed pink colour was read at 530 nm after 25 min in UV-Vis Spectrophotometer (SHIMADJU UV-1700 Pharmaspec, Shimadju, Japan). the IAA concentration in the culture was determined by using a calibration curve of pure IAA as a standard, following linear regression analysis [18].

III. Results

One of the colonies which produced halo around it on the Pikovskaya agar was selected for characterisation and designated as GCS1.

Table 1 summarizes the values of P (mg/l) solubilised in liquid culture and the change in pH of the corresponding medium after seven days of incubation as well as the IAA production *in vitro* after 3 days of incubation in presence of tryptophan.

Table 1: *In vitro* phosphate solubilisation and IAA production by PSB strain GCS1

PSB Strain (GCS1)	pH	Available phosphorus (mg/l) after 7 days of incubation	IAA production (mg/l) after 3 days of incubation
Kurthia sp.	5.60	40.62 ± 1.1	17.5

Phosphate solubilisation was accompanied by a decrease in the pH of the medium by the selected isolate DTS02. Amount of available phosphate was determined as 40.62 ± 1.1 mg/l from 0.5% tricalcium phosphate with decrease in pH from an initial value of 6.8 to 5.60 after 7 days of incubation.

Table 2: Biochemical characteristics of PSB strain GCS1

Biochemical characteristics	Results
Gram's reaction	+
Methyl-red test	+
Voges-Proskaeur	+
Citrate Utilization	+
Nitrate reduction	+
Catalase test	+
Arginine dihydrolase	+
Xylose	+
Dextrose	+
Galactose	+
Mannose	+
Indole test	-
H_2S production	-
Urea test	-
Gas production from glucose.	-

(+) = positive response, (-) = negative response

The amount of IAA produced was found to be 17.5 mg/l by this isolate after 3 days of incubation. Eight PSB strains isolated also from tea rhizospheric soil produced IAA in the range of 10-30 mg/l as reported earlier[19].The biochemical characterization of the PSB isolate (Table 2) showed that it was gram positive, rod shaped and positive for methyl red, voges-proskaeur, citrate utilization, nitrate, catalase, arginine dihydrolase, xylose, dextrose, galactose, mannose and was negative for indole production, H_2S production, starch hydrolysis, urea hydrolysis and gas production from glucose. The isolate GCS1 was sent to IMTECH, Chandigarh, India and identified as *Kurthia* sp.

IV. Discussion

The results obtained in this study throw light on the existence of phosphate solubilizing bacteria in rhizosphere soils of tea plants. Baby *et. al.,* [20] carried out an investigation on microbial dynamics in the rhizosphere of tea plants and reported that there was a significant difference on the population level of PSB in different clones/seedlings of tea. Further, they reported that the population of nitrogen fixing *Azospirillum* and PSB were higher in young tea fields than older fields.

In general, Ca-phosphate solubilisation seems to be link with pH decrease of the medium but this pH decrease was not strictly proportional to the amount of the phosphate solubilised. During present study, it was found that there is slight correlation between the decrease of pH and the phosphate solubilised. These findings were supported by the reports of [21] who reported that sometimes the culture filtrate pH was relatively high and yet in the medium high P solubilization occurred, this may occur due to the chelation of organic acids with Ca^{++} ion in tricalcium phosphate.

Similarly, it has been reported [22, 23, 24] that pH had no affect on P solubilization [25] also found no correlation between the pH and solubilization of P over an incubation period of 7 days. Similar observation was reported with *P. aurantiogriseum* [21], with a *Penicillium* isolate [26] and with *P. radicum* [24].
The pH drop in PSM liquid cultures have been reported in several researches which supports the pH change in present study [27, 28, 29, 30].

Bacteria isolated from rhizosphere soils are known to produce growth-promoting substances [31] and some of them are capable of dissolving phosphate [32]. Phosphate solubilising bacteria are capable of producing physiologically active auxins that may have pronounced effects on plant growth. The cultures release greater quantities of IAA in the presence of a physiological precursor, tryptophan, in a culture medium. Production of IAA varies greatly among different species and is also influenced by culture conditions, growth stage and availability of substrate(s) [31, 33].

The amount of IAA produced by this isolate was higher than one reported earlier [34] which range from 2.31 to 9.43 µmol/ ml and was lower than that have been reported by [35] which range from 34.02 to 45.31 mg/l. The amount of IAA biosynthesis by the novel strain of *Kurthia* sp. is found to be considerably higher than reported *Bacillus* isolates which ranged from 2.04 to 2.78 mg/l [36].

V. Conclusion

In conclusion, results of this study have shown that *Kurthia sp.* which was isolated from tea garden of Darjeeling hills are capable of producing plant growth promoting substance IAA, capable of solubilizing inorganic phosphates thereby decreasing the pH of the medium. Further studies are required to evaluate the phosphate solubilising efficiency of this strain *in vivo.* This strain may further be characterized to use as a potential novel biofertilizer in organic farming.

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