5. SUMMARY

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- The present study deals with "Isolation and characterization of Phosphate Solubilising Microbes from Darjeeling Soils for their use as potential inoculants in upland farming systems".
- After a short introduction to the work, a brief review of literature related to the significance of phosphate solubilising microorganisms has been presented. This section deals with observations of the previous workers in concord with the present line of investigation. The review is presented in selective manner rather than comprehensive. For convenience, the review has been grouped separately under subheadings like different forms of phosphorus in soil system and its availability to plants; PGPR and its importance; diversity and occurrence of phosphate solubilising microorganisms; mechanisms and genetics of phosphate solubilisation; effect of PSM on crop production; interaction of PSM with other beneficial microorganisms; assessment of phosphate solubilisation by microorganisms.
- The objectives of the study were:
    1. Isolation of phosphate solubilising microorganisms from the rhizosphere of dominant crop plants of Darjeeling Hills
    2. Screening of phosphate solubilising microorganism isolates for efficient phosphate solubilisation, production of other plant growth promoting substances *in vitro*
    3. Study of survivality of superior phosphate solubilising microorganisms *in vitro* under different growth conditions
    4. On farm activities of superior phosphate solubilising microorganism isolates

- Different experimental procedures and techniques used during the present study are described in materials and methods section.
- Rhizospheric soil samples were collected from principal crops like tea, potato, large cardamom, orchid, orange, maize and forest trees were collected from different altitudinal locations of Darjeeling hills.

- All the soil samples showed acidic pH in the range of 5.11 to 6.10 which is considered common in hill location. Moisture content and

water holding capacity of soil were moderate to high depending upon the cropping practices. The soil were found moderately fertile with respect to organic carbon; nitrogen, phosphorus and potassium content.

- Soil samples were screened for the occurrence of phosphate solubilising microorganisms in Pikovskaya's agar. Twenty four isolates were selected on the basis of their ability to dissolve insoluble tricalcium phosphate in the media and subjected to further study. The isolates were designated as GCM1, GCM2, GCM3, GCM4, GCM5, GCS1, GCS2, GCS3, GCS4, GCH1, GCH2, GCH3, GCH4, GCO1, GCO2, GCO3, GCO4, GCO5, GCO6, GCF1, GCF2, GCF3, GCF4 and GCF5; and four of them GCM1, GCS2, GCM2 and GCS1 were identified as *Bacillus* sp., *Bacillus cereus*, *Pseudomonas* sp. and *Kurthia* sp. respectively in Indian Institute of Microbial technology, Chandigarh , India. All the isolates were bacteria, no fungi could be isolated as phosphate solubilisers during the present study.
- Pure cultures of phosphate solubilising bacteria (PSB) isolates were reinoculated on Pikovskaya's agar and observed for the clearing zones around their colonies. The clearing zones were measured and used for calculation of solubilising index and solubilising efficiency of each of the isolates. *Pseudomonas* sp. (GCM2) was found efficient showing highest solubilisation index as well as solubilisation efficiency.
- All the 24 PSB isolates were studied for their ability to solubilise tricalcium phosphate in Pikovskaya's broth upto 28 days. Increase in soluble phosphate content of the broth was associated to decrease in pH with increasing days of incubation was shown by 2/3rd of the isolates. Among the isolates *Pseudomonas* sp. (GCM2) was able to solubilise maximum phosphate (213.80 ppm) at the end of incubation period (28 days). *Bacillus cereus* (GCS2) was also able to yield good amount of phosphate (193.29 ppm) during the study.
- The isolates were further tested for their ability to produce growth hormone (IAA) in tryptophan supplemented medium. All the isolates were able to produce considerable quantity of IAA where *Bacillus* sp. (GCM1) was most potent producer with 40 ppm, followed by *Pseudomonas* sp. (GCM2) with 35 ppm. This property of the isolates could be an added advantage for plant growth

- Twelve superior PSB isolates selected on the basis of their performance in PKV broth were further studied for their ability to solubilise phosphate using different concentrations of tricalcium phosphate (TCP) in the broth. All the tested isolates were efficient in phosphate solubilisation even at lowest tested concentration of TCP (1g/l). There was detectable increase in the amount of solubilised phosphate with increased concentrations of TCP in PKV broth. Majority of PSB isolates have shown direct correlation between drop in pH and increase in solubilised phosphate in the media. Maximum phosphate solubilisation occurred with 5g/l TCP which is prescribed concentration in PKV broth.
- Six isolates were further allowed to grow in PKV broth with varying concentrations of glucose and observed for phosphate solubilisation. All the strains were capable of yield soluble phosphate in increasing order with increasing concentrations of glucose.
- The isolates were studied for their response to 28 biochemical parameters and the diverse pattern of response was observed among the isolates. Isolates belonging to same species, *Bacillus* sp. (GCM1) and *Bacillus cereus* (GCS2) showed common response to majority of parameters. Less homology even among Gram positive as well as Gram negative members reflected the diversity of phosphate solubilising bacteria in the soils of Darjeeling hills.
- Morphologically 22 isolates were rod shaped and rests 2 were cocci and the isolates were found comprised of equal number of Gram positive and Gram negative members. This reflects the contribution of diverse groups of microorganisms in solubilising fixed phosphate in soil.
- Antibiotic sensitivity test of the isolates to 20 different antibiotics also showed scattered pattern of response. Gram positive members responded well to β-lactam antibiotics and majority of members were sensitive to amino glycosides, macrolides and DNA-synthesis inhibitors. As a whole, antibiotic sensitivity pattern of PSB isolates reflected the intrinsic genotypic property.
- Similarity percentage values of protein bands of less than 70% in native polyacrylamide gel electrophoresis among six selected isolates

showed the homology of isolates determining the important characteristics including phosphate solubilisation.

- Growth response of PSB isolates to acidic to alkaline conditions was tested. All the isolates showed wide pH tolerance and majority of them performed better in acidic pH. These isolates are expected to survive will in acidic soils of hills as phosphate solubilisers. Additionally the isolates were also tolerant to winter soil temperature.
- Increased germination rate of PSB treated fenugreek seeds over the control were found in the range of 6% to 20%. *Pseudomonas* sp. (GCM2) found superior to all the isolates.
- PSB inoculated planting material of principal cash crops like pea and potato showed better yield over the untreated ones. The range of increase of pea yield was 9.23% to 17.60% and potato was 3.52 to 10.58%. *Pseudomonas* sp. (GCM2) performed better among the isolates.
- There was a stimulatory effect of all the isolates on seedling growth of orange. Here PSB isolates promoted the growth of seedlings significantly, as evidenced by increased plant height, root length, number of leaves and branches. *Bacillus* sp. (GCS1) was found most efficient among the isolates.
- The findings of present study have been discussed in detail and compared with the results of other prominent works.
- In conclusion it may be said that indigenous population of phosphate solubilising bacteria in the soil of Darjeeling hills are efficient in phosphate solubilisation and IAA production. The present study retains its importance due to the habitat from which the microbial strains were isolated and collected. Since these bacteria have been isolated from higher altitudes, they would appear to be better adapted for establishing in the rhizosphere, if reintroduced in the form of inoculants.