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Shift in Mineral Phosphate Solubilizing Bacterial Communities and Function across Fertility Gradient of Soils Cultivated with Maize in Lower Gangetic Plains of India

Suparna Sen[1] and Niharendu Saha[2]

[1]Ministry of water Resources, River Development & GanGa Rejuvenation, Govt. of India
[2]AICRP on Soil Test Crop Response Correlation Directorate of Research Bidhan Chandra Krishi Viswavidyalaya Kalyani-741235, Nadia, West Bengal, India
E-mail: 1suparna.datta.agri@gmail.com, 2nihar_bckv@rediffmail.com

Abstract—*The present communication deals with the assessment of phosphate solubilizing bacterial community structure and function under the influence of fertilizer intensification. To assess bacterial community structure under different level of fertilizer intensification, an artificial fertility gradient was created in the experimental site in regards to N, P and K status of soil. Twenty randomly isolated phosphate solubilizing bacteria from each of fertility gradient were purified and characterized for carbon-source utilization pattern. Twenty-eight carbon sources having diverse chemical natures were utilized to differentiate the culturable phosphate solubilizing bacteria into different communities. Data, thus, obtained were subject to compute community structure of phosphate solubilizing bacteria through principal component analysis (PCA). Size of isolates of fallow showing greater utilization efficiency towards diverse carbon sources reduced along the gradient of fertility. The result was substantiated by higher substrate diversity of culturable isolates of low fertility gradient soils. On the whole, excepting in high fertility gradient soil, there was practically no change in community composition of phosphate solubilizing bacteria. However, community function in terms of mineral phosphate solubilization and acid as well as alkaline phosphatases activity depressed across the fertility gradients.*

1. INTRODUCTION

The rapid pace of intensification of agriculture during the recent years has resulted in an overall deterioration in vital natural resources, particularly, soil-based ecosystem/ecology. Such change in agriculture goes with changing soil life. It is unknown in which way the soil biocoenosis will change and what the effects on the nutrient circle will be. It is globally hypothesized that in agro-ecosystem biodiversity so as to ecosystem function declines with the intensification of agriculture [1]. The more the degree of intensification the more will be the effect on biodiversity. Phosphate solubilizing bacterial communities, a clad of total soil bacterial, are likely to be affected by heavy phosphatic fertilizer application in currently adopted intensive agriculture. Due attention, thus, has to be paid to diversity of PSB and their function under intensive agriculture.

Soil is diverse in respect of nature of utilizable C sources. The structure and functionalities of phosphate solubilizing microbial communities differ based on soil ecological condition. Therefore, physiological profiling of PSB is essential for biological management of this community *in situ* for formulating P-nutrition management strategies. Understanding the relationships among bacteria through physiological profiling advances our knowledge of bacterial ecology and community structure. Community-level physiological profiling (CLPP) using Eco-plates is often described as a method of determining the functional diversity or functional potential of microbial communities [2,3]. CLPP is effective to establish spatial and temporal changes in microbial communities [4] as well as providing insight into functional ability of microbial community members [3]. Carbon utilization is central to survival, growth and competitiveness of bacteria in any community [5]. By virtue of diverse array of compounds available for metabolism and requirements of specific organisms, C utilization can be used as a discriminating means to characterize and identify bacterial community if those requirements are specific for that community [6].

The objective of the present work was to evaluate the effect of intensification in agriculture specially fertilizer intensification on PSB communities and function in terms of sole C-source utilization pattern in different fertility gradient soils.

2. MATERIALS AND METHODS

3.1 Experimental sites and establishment for fertility gradient

The experiment was laid down in the Central Research Institute for Jute and Allied Fiber (CRIJAF), Barrackpore, 24 pgs. (N) to establish fertility gradient of low, medium and high in respect of available N, P and K in a close boundaries of same soil types following the technique suggested by Ramamurthy *et al*. [7]. An area of low to medium nutrient status and responsive to nutrient application was selected for this experiment. The area was divided along its width into three equal strips. Three fertilizer schedules; $N_{50}P_{25}K_{25}$ (low), $N_{100}P_{50}K_{50}$ (medium) and $N_{200}P_{100}K_{100}$ (high) for growing an exhaustive crop like fodder maize (var. A-De-Cuba) were applied to the three strips. After the harvest of the gradient crop, soils were collected from number of points in a zigzag fashion. Soils were then mixed and representative soil samples were prepared by following soil testing protocol. The variability in soil fertility in terms of available nitrogen, phosphorus and potassium developed in the experimental site was estimated and recorded. A portion of live soil was preserved in a freezer (4°C) for microbiological studies. The gradient, artificially established has been maintaining for the last 13 years by renewing fertility gradient experiments under the supervision of the All India Coordinated Research Project on Soil Test Crop Response Correlation, CRIJAF, Indian Council of Research (ICAR) Barrackpore. To evaluate and compare the effect of fertilizer intensification, soil was also collected from an undisturbed fallow soil from the same experimental sites.

3.2 Preparation of pure cultures of phosphate solubilizing bacteria

The soil suspension of 10^{-5} dilution prepared from soils of four fertility gradients (fallow, low, medium and high) was inoculated on solid Pikovskaia's medium and incubated for 4 days at 30°C. Then, some colonies were selected randomly and allowed to grow on solid Pikovskaia's medium again. After 4 days of incubation at 30°C, the colonies forming a clear zone surrounding it were selected and transferred into a test tube containing solid nutrient agar (NA) medium. Single cells were obtained by repeated streaking in a plate containing NA medium. The colonies not forming clear zones were also selected, assuming they were also phosphate solubilizing bacteria (PSB), as able to grow in Pikovskaia's medium repeatedly [8]. The pure cultures of PSB were preserved in a culture tube containing Pikovskaia's medium.

The PSB cultures from CRIJAF fallow, low, medium and high fertility gradient soils are abbreviated as CF, CL, CM, CH, respectively. Subscripts 1, 2, 3… 20 denote isolate number.

3.3 Sole Carbon sources

Potential substrates including sugars, amino acids, organic acids; especially those may differentiate various taxa in question were selected for this experimental purpose. This selection was broad for the examination and compare of general functional groups within closely related genera. Ecologically meaningful substrates (i.e. those that are likely to be found in soil habitats in nature) were included for more appropriate use of this method for soil microbial community's assay [9,10]. As the present work has been carried out to investigate the phosphate solubilizing bacterial community in terms of sole C-source utilization pattern, it would be worthy to customize the substrates in such a way that it would reflect ecological significance [9]. To carry out this experiment, 28 common and readily available C-sources from different groups were used (see Table-1). One percent solution of each of carbon source after being filter sterilized was mixed in to sterilized characterization medium [11]. Requisite amount of (20 ml) CM medium was poured aseptically into sterile petri plate and allowed for solidification. After 20 minutes, the plate turned inversely and was kept for a day. The reverse surface of petri plate was then divided into ten small squares with marker. Isolates from each gradient were then inoculated into those small squares singly and allow growing for 3-4 days at 30^{o}C.

Table 1: Different carbon source used in the experiment

Sugar	Carboxylic acid	Amino acid	Sugar alcohol	Polymer	Amide	Surfactant
Glucose	Aceetic acid	Asparagine	Glycerol	Starch	Acetamide	Tween-80
Fructose	Citric acid	Phenyl alanine	Mannitol	Cellulose		
Lactose	Creatine monohydrate	Histidine	Inositol			
Mannose	Glutamic acid	DL-alanine	Sorbotol			
Xylose	Succinic acid					
Arabinose	Propionic acid					
Maltose	Malonic acid					
Dextrose						
Sucrose						

3.4 Data analyses

Data were expressed in two different ways. First, a code was used to represent either a positive (growth) or negative (no growth) response of isolates to different carbon sources in terms of growth. Alternatively, a quantitative code was used to

indicate the magnitude or extent of growth in different phosphate sources. A 0 to 9 scale (0 - no growth; 1 - 0.25 mm diameter of colony; 3 - 0.5 mm diameter of colony; 5 - 1 mm diameter of colony; 7 - 1.5 mm diameter of colony and; 9 - 2.0 mm diameter of colony) was used to indicate the relative colony size in the plate. The intensity with which the C-sources were being utilized (percent C-source utilization intensity-PCSUI) by different phosphate solubilizing bacterial isolates were calculated by following the formulae used to calculate percent C-source utilization intensity :

$$PCSUI = \frac{\left(\sum_{i=1}^{N_C}\{X_i|0, 1, 3, 5, \dots, S_{max}\}\right)100}{N_C S_{max}}$$

Where, N_C = Total number of C-source and S_{max} = Highest scale used.

Community composition analyses

Data generated from the investigation were filed to analyze the community composition of soils under different fertility gradient through principal component analysis (PCA). PCA analyses were performed on the basis of correlation matrix involving previously mentioned variables [12]. The score of each of the isolate on the principal component was plotted in a bi-variate scatter gram to allow visual assessment of the position of these isolates in the direction of these components.

As a measure of the number of substrates utilized (substrate richness) and diversity of the extent of utilization of particular substrates (substrate evenness), the Shannon index is used [13]:

$H= -\sum p_i (\ln p_i)$ if $p_i > 0$ or 0, if $p_i = 0$ (Yan *et al*, 2000)

$i = 1$ to N

Where, H denotes substrate diversity, *pi* is the ratio of the activity on a particular substrate to the sum of activities on all the substrates, and *N* is the number of substrates on a plate.

The substrate evenness measures the equitability of activities across all utilized substrates and was assessed according to Pielou (1975):

$E = H/H_{max}$

Where, E denotes substrate evenness, H the substrate diversity, and H_{max} the maximum value of diversity for the number of substrate present.

3.Result and discussion

Creation of fertility gradients in experimental site

Bibliographic antecedent shows very little information about the assessment of phosphate solubilizing bacterial communities through carbon source utilization study except very recently Valverde *et al.* [14] utilized a range of carbon sources namely arabinose, mannose, mannitol, N-acetyl-D-glucosamine, glyconate, caprate and malate to differentiate phosphate solubilizing bacterial diversity in soil. Most of the studies so far conducted on phosphate solubilizing bacteria in agro-ecosystems based mostly on the enumeration of colony forming units on an agar plate using serial dilution and pour plate technique where the community composition rarely analyzed. Recently, few phylogenetic studies of phosphate solubilizing bacterial communities publish [15,16]. Many authors have pointed out the limitation of molecular approaches for diversity study, in general, more expensive; in particular [1]. Moreover, such species diversity of phosphate solubilizing bacteria has little importance in agro ecosystems for sustainability in the solubilization of insoluble phosphatic minerals because there exists a redundancy among phosphate solubilizers. Thus, functional diversity is of great importance from the point of sustainability [1,17]. The more powerful approaches to unraveling the relationships between fertilizer intensification, phosphate solubilizing bacterial communities and their function are: (1) to study the soil phosphate solubilizing bacterial communities and their mineral phosphate solubilization along fertility gradients where trends can be explored or (2) to study soil phosphate solubilizing bacterial communities and functional relationships in field experiments which compare agricultural practices of differing fertilizer intensity. Giller et al. [1] and Suzuki, et al. [18], suggested such novel approaches. Nevertheless, practically, there is often difficulty in finding representative fields in same site with same soil type having different fertility gradients. Therefore, creation of fertility gradient artificially in same ecological boundary is the alternative approach. Long-term exercise with exhaustive crop cultivation is required to create fertility gradient. Ramamurthy *et al.,* [7] introduced this approach during late seventies to assess crop response to applied fertilizers for developing targeted yield equation for different crops.

The present communication deals with the assessment of phosphate solubilizing bacterial community structure based on their sole carbon source utilization pattern along gradients of fertility where trends of phosphate solubilizing bacterial community can be explored [1,18]. Fourteen years old artificially created fertility gradient experimental site of low, medium and high along with a fallow in a close boundaries of same soil types in Central Research Institute of Jute and Allied Fiber (CRIJAF), Indian Council of Agricultural Research (ICAR), Barrackpore, West Bengal, India were selected for the current experiment. The gradient artificially established has been maintained by renewing fertility gradient experiment

under the supervision of All India Coordinated Project on Soil Test Crop Response Correlation, CRIJAF Centre. A distinct gradient in terms of available phosphate established in the experimental sites (see Fig. 10). Thus, a broad range of different soil ecological situations was in the experimental site as characterized by soil properties (see Table 2). The fertility gradient with respect to P, developed by maize crop, was more stiff and uniform while such gradients developed for soil N and K was rather non-uniform. Therefore, it is logical to expect to harbour recognizable diverse phosphate solubilizing bacterial communities under a long term, stable and distinct soil ecological conditions.

Table 2: Characteristics of experimental soil at Central Research Institute of Jute and Allied Fibre (CRIJAF)

Parameter	Fertility gradient of soil			
	Fallow	Low	Medium	High
pH	6.5	7.12	6.15	6.0
EC	0.081	0.123	0.133	0.132
Organic carbon (%)	0.82	0.81	0.8	0.72
Available Nitrogen (Kg/ha)	143.42	175.1	198.2	212.5
Available Phosphorus (P2O5 Kg/ha)	46.5	81.3	92.12	129.91
Available Potassium (K2O Kg/ha)	112.20	115.5	125.6	139.50
Textural class	Sandy loam	Sandy loam	Sandy loam	Sandy loam
Previous crop rotation	Rice-Jute-Lentil			

Carbon source utilization pattern of phosphate solubilizing bacteria across the fertility gradients

The preference of isolates towards C-source utilization was estimated. It revealed that 100% of the isolates from fallow soil utilized glucose, cellulose, lactose, asparagine, sucrose, inositol, glutamic acid, succinic acid as their sole C-source. Three isolates of i.e.15% of total isolates collected from fallow utilized all the twenty eight C-sources used in the experiment while it increased to four isolates i.e. 20% isolates in low and medium fertility soil but the number reduced to one i.e. 5% in high fertility soil. It revealed that while 85% of isolates from fallow soil utilized lactose as their C-source, it reduced sharply along the gradient of fertility to 60% at the highest fertility gradient. Similar trend was also obtained in case of glycerol, citric acid and acetic acid. However, preferential utilization by the isolates towards other C-sources like mannitol, starch, dextrose, asparagines, cellulose and fructose declined from fallow to high fertility gradient but not sharply as in case of rest of four C-sources. Substrate utilization pattern by the isolates decreased in high fertility gradient soil (Fig.1).

For example, cellulose, a β→(4) glycosidically linked relative recalcitrant polymer, was utilized differentially by different phosphate solubilizing bacterial isolates across the fertility gradients. 100% isolates of fallow while preferred it, only 55% isolates of high fertility gradient soils were supported by cellulose. Such discriminating utilization of cellulose by different isolates from different fertility gradients strongly differentiated the nature of community constituted by diverse group of bacteria. Similar pattern of carbon source utilization was exhibited by the isolates for most of the substrates used in the experiment. Out of 28 substrates used, isolates from medium fertility gradient soils utilized the highest number of C-sources with the greatest extent. Thus, diverse community with different metabolic activities reasonably expected to harbor in medium fertility gradient soils. Less stress arising from necessarily balance nutrition and good crop stand possibly supported diverse rhizospheric bacterial flora. Decreasing amount of organic carbon in high fertility soil probably increasingly limit substrate availability to microorganisms which in turn affected functional diversity of phosphate solubilizing bacteria in soils.

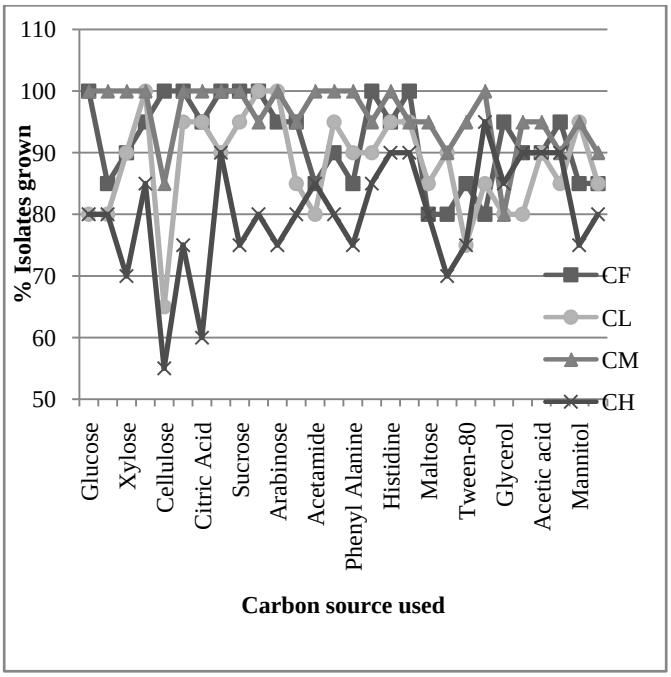

Fig. 1 : Preferential utilization of different carbon sources by culturable phosphate solubilizing bacterial isolates across fertility gradients

It was indicated by the physiological analyses of the isolates that the phosphate solubilizing bacterial communities composed of diverse lineages of bacteria living accros fertility gardients should be able to play an extensive role in P-cycle, in general, P-solubilization, in particular, in agroecosystem. It

was strongly suggested that various species of phosphate solubilizing bacteria coexisted in soil by competing for common substrates as well as taking priority in favourable or specific substrates for each species. It, thus, may be concluded that overall pattern of utilization of organic substrates differed depending on the affiliation of the strain. Furthermore, bacterial communities from fallow and medium fertility comprised more divergent species and, thus, utilized more diverse substrates than low and high fertility soils with more simplified substrate utilization finger prints. On the whole, substrate utilization potential was heterogenously distributed among fertility levels of soils.

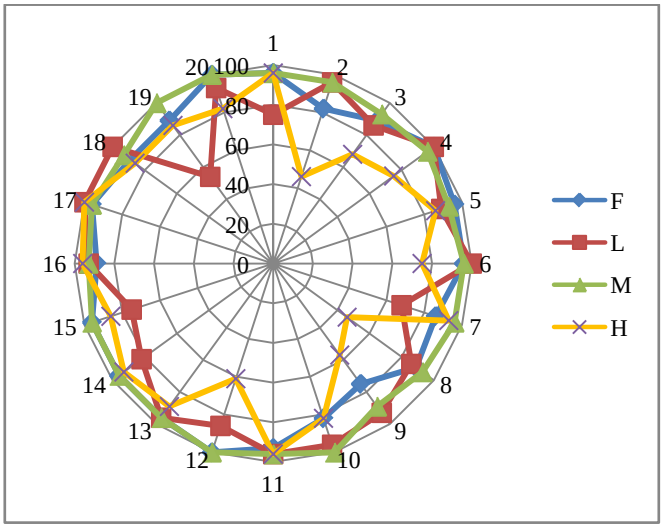

Fig. 2: Percent carbon sources utilized by phosphate solubilizing bacterial Isolates from different fertility gradient soils

Relative distribution of percent carbon sources being utilized by diverse group of PSB from different fertility gradients were shown in radar or spider chart (Fig-2). The whole radial field was subdivided into 20 different axis representing different isolates. The value of percent carbon sources utilized of each isolates was plotted along the separate axis designated for each isolate. The position of respective values of percent carbon sources being utilized (0 – 100%) by isolates across the axis up to the farthest outer ring can be used as an indication of extent of the substrates being utilized by the isolates of different fertility gradients. In this case, substrates those were least utilized were located at axis nearest to the center while those located at the outer most ring were the substrates utilized extensively by the isolates. For example, phosphate solubilizing microbial communities in samples from medium fertility gradient soil had significantly more potential for utilizing diverse carbon sources to the greatest extent as the values were mostly in the outermost periphery across the axis of the radar. On contrary, the corresponding values for high fertility gradient soils were lying toward the center of the radar indicating lower rate of carbon source utilization.

However, Simple appearance of growth of isolates in 28 substrates on the plate was not sufficient in differentiating the four sites, since different substrates were utilized differently by tested phosphate solubilizing bacterial isolates. In this regard, extent of substrate utilized by the isolates expressed here in terms of percent carbon source utilization intensity (PCSUI) is more reasonable option to distinguish the community composition of the tested isolates. Because, this trait of isolates is intimately related to functional/metabolic capacity of the respective bacterial species.

To understand this trait of the tested isolates, PCSUI was computed. It was observed that the highest PCSUI was scored by isolate CF_{20} (52%) followed by those of CF_{14}, CF_{15}, CF_{1}, CF_{16}, CF_{19}, CF_{11}, CF_{13}, CF_{13}, CF_{3}, CF_{18}, CF_{7}, CF_{9}, CF_{2}, CF_{10}, CF_{6}, CF_{4}, respectively. Whereas in low, medium and high fertility gradient soil the highest PCSUI was exhibited by CL_{2} (38%), CM_{10} (51%) and CH_{19} (39%) ,respectively. It was observed that in most of the isolates, PCSUI was higher in medium fertility gradient soil and the minimum values observed in high fertility gradient soil (see Fig. 3). Results, thus, substantiated that even if several strains used the same substrates, the growth rates were often different depending on the strains and its metabolic efficiency. Results showed a linear decrease in substrate versatility and a decline in substrate utilization rates as fertility increased.

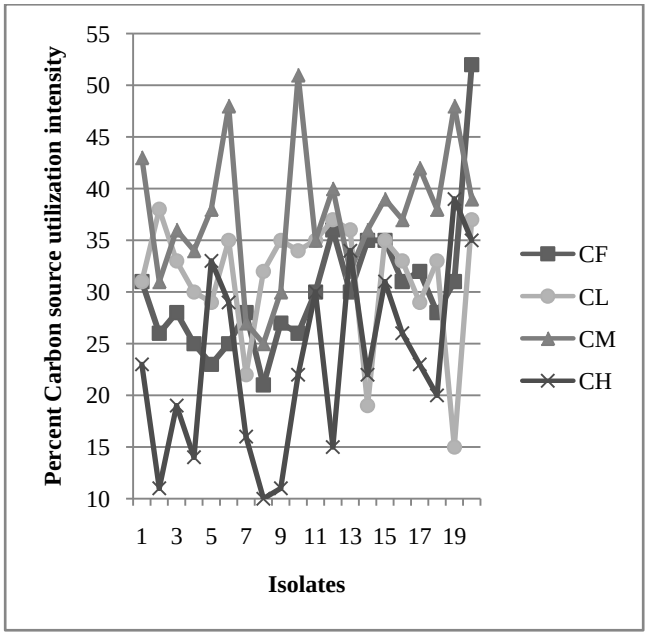

Fig. 3: Percent Carbon source utilization intensity by the isolates from different fertility gradient soil

Utilization of C-sources by the bacterial isolates and their subsequent across the fertility gradient are due to reduced catabolic evenness of phosphate solubilizing bacterial isolates.

Higher number of phosphate solubilizing bacterial population utilized different C-sources in fallow soil. This supports by the findings of Dhillon *et al.* [19] that substrate diversity was higher in long-term fallow. This is due to bacterial communities in fallow soil have high catabolic evenness than that of cultivated soil [20] with different degree of fertilization. The results, thus, supported by the findings of Meyer *et al.* [21] that catabolic diversity of culturable bacteria could be less in soils subjected to stress and disturbance. Moreover, it suggests that a diverse plant community in fallow soils favours effective microbial communities by decreasing their energy demand [22].

Community structure along fertility gradient

To adjudge the responses of each of twenty isolates from fallow, low, medium and high fertility gradient soils towards sole carbon source utilization intensity, physiological profiles of those bacteria were analyzed using Principal Component Analysis (PCA). PCA is a multivariate statistical analysis technique used to project the maximum variance of the bacterial isolates optimally in multiple dimensions (PC_1 and PC_2) in an unconstrained ordination [23,10]. PCA calculated orthogonal axes (principal components) through the data matrix in the direction of highest variance. The number and the categories of carbon source utilized as well as the extent of activity on such a source constitutes a data set which helps to assess the functional diversity of phosphate solubilizing bacterial community. The score of each of the isolate on the principal component plotted in bi-variate scatter gram to allow visual assessment of the position of these isolates in the direction of these components. Thus, all isolates were categorized into different PCA groups based on substrates utilization intensity. Using multivariate statistical analysis of the primary data, the sample sites were differentiated on the basis of pattern of substrates being utilized by the isolates of different fertility gradients. The data matrix consisted of 20 isolates from each gradient and a fallow representing the samples and 28 carbon sources exemplifying the substrates. The mean score of components 1 and 2 were plotted in Fig. 4-7. Two axes accounted for most of the variation in carbon-source utilization among the isolates. In a PCA ordination diagram, isolates with similar response towards sole carbon source utilization intensity were located close to one another, and those dissimilar were located far apart. This represented the divergence of the isolates resulting different community composition marked by circle.

Principal Component Analyses (PCA), using all 28-carbon sources, can indicate more intuitively the pattern of microbial community functional diversity from soils and gave good discrimination among soils. PC_1 and PC_2 explained 29.509% and 14.642% of the variance, respectively, in fallow soil.

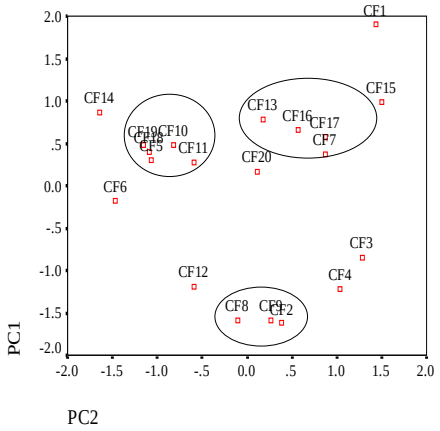

Fig. 4: Scatter diagram based on PC1 and PC2 showing the existing Predominant Communities of CRIJAF fallow soil

In low fertility gradient, PC1 accounted for 27.664% and PC2 accounted for 18.321% of the variation on the correlation matrix.

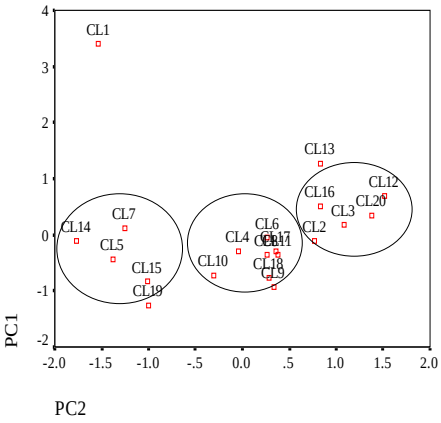

Fig. 5: Scatter diagram based on PC1 and PC2 showing the existing Predominant Communities of CRIJAF low fertility gradient soil

In medium fertility gradient PC_1 accounted for 17.555% and PC2 accounted for 15.058% of the variation on the correlation matrix.

Fig. 6 Scatter diagram based on PC1 and PC2 showing the existing Predominant Communities of CRIJAF medium fertility gradient soil

In CRIJAF high fertility gradient, PC_1 and PC_2 accounted for 27.547% and 14.952% of the variation on the correlation matrix respectively. This indicates that there were significantly different patterns of potential carbon utilization.

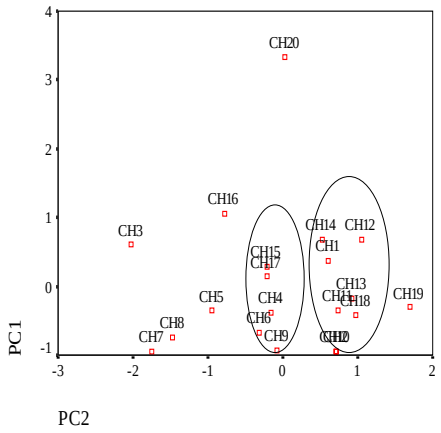

Fig. 7 Scatter diagram based on PC1 and PC2 showing the existing Predominant Communities of CRIJAF high fertility gradient soil

From the diagram (Fig. 4), it is clear that isolates CF_5, CF_{10}, CF_{11}, CF_{18} and CF_{19} from fallow soil revealed a very tight grouping resulting in a distinct community. The ordination result showed more similarities among the isolates. Similarly, isolates CF_7, CF_{13}, CF_{16}, and CF_{17} together constructed another community. Likewise, isolates CF_2, CF_8 and CF_9 formed additional two small communities in fallow soil. PC analyses based on the sole carbon source utilization intensity revealed consistent relationships among the isolates of identified groups. Overall, fallow soils revealed three distinct phosphate solubilizing bacterial communities (Fig. 4). These three clusters in Fig. 4 show the site loadings (scores) mapped into the subspace defined by the principal components PC_1 and PC_2. Like this, in low, medium and high fertility gradient soil, the number of clusters were 3, 2 and 2, respectively. Thus, the number of phosphate solubilizing bacterial communities in CRIJAF soils followed the order like CF=CL>CM=CH. This indicates the reduction of phosphate solubilizing bacterial communities in high fertility gradient soil as compared to fallow soil. Statistically, it was also computed that 18 components having corresponding Eigen vales >1 were required to explain 100% variance in sole carbon source utilization intensity by the isolates of high fertility gradient soils. Whereas 19 components were needed to explain the same level of variance in case of fallow, low and medium fertility gradient soils. These results necessarily explain the lower diversity of PSB in high fertility gradient soils.

This, finding, thus, corroborates the result of having lower substrate diversity (indicated by Shannon index) with maximum fluctuation in high fertility gradient soils. Decreasing Shannon index in high fertility soil probably indicates increasingly limiting substrate availability to more diverse microorganisms which in turn affected functional diversity of phosphate solubilizing bacteria in soils. Moreover, low index in high fertility gradient soils may be due to low carbon content and this low C might be critical factor controlling the growth of microorganisms.

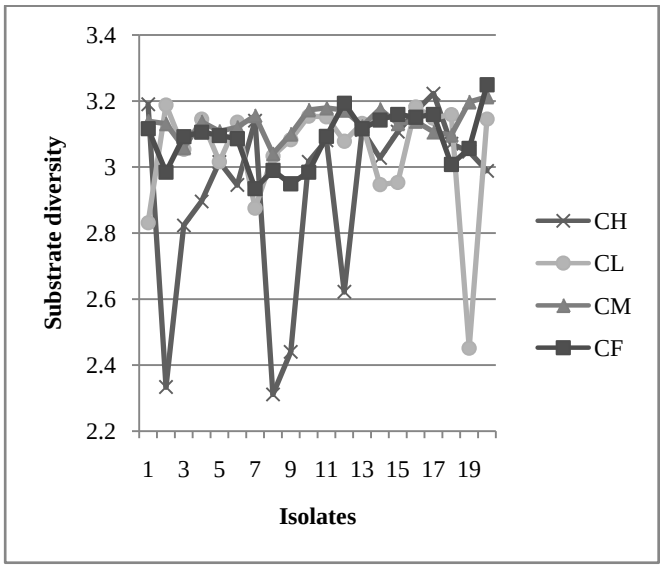

Fig. 8: Substrate diversity of Phosphate Solubilizing Bacteria across fertility gradients

Table 3: Principal Component to explain 100% variables for carbon source utilization intensity of the Phosphate solubilizing bacterial cultures in the experimental site

Fertility gradient CRIJAF	Components having corresponding Eigen >1	Variance explained
CF	19	100%
CL	19	100%
CM	19	100%
CH	18	100%

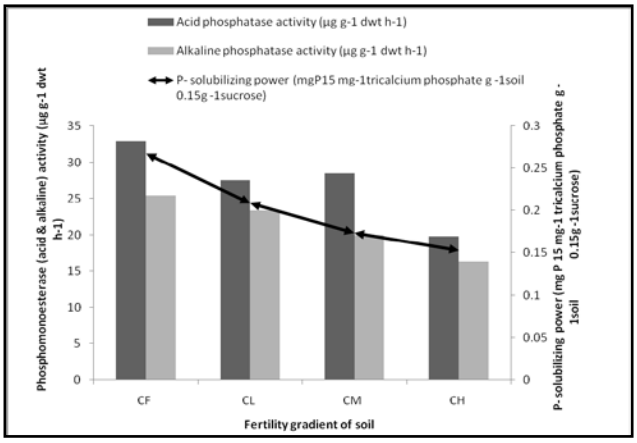

Fig. 9 Function of phosphate solubilizing bacterial isolates across fertility gradient of CRIJAF soil

In addition, low functional diversity in high fertility gradient soil may be due to stress arising from excess soluble phosphate ions in high fertility gradient soils. Similar results were also observed by Zhong *et al*. [24] that long term mineral fertilization had great effect on microbial diversity and activities.

Function of phosphate solubilizing bacterial isolates across fertility gradient of CRIJAF soil

To understand the activities of phosphate solubilizing bacterial communities across fertility gradients, insoluble inorganic phosphate solubilizing power as well as acid and alkaline phosphatases activities of soil were assessed (Fig. 9). The highest P-solubilizing power was obtained from fallow soils. The magnitude of P solubilization gradually decreased along the fertility gradient of soils. Such decrease in P-solubilization was associated with the decrease in pH drop of the liquid medium. Highest P-solubilization with least pH drop was observed with fallow soil.

Thus, it appears that production of organic acids and subsequent pH drop is not the sole mechanism for insoluble inorganic phosphate solubilization. The protons associated with extracellular polysaccharides secreted by the microbes are also responsible for dissolution of TCP in the Pikovskaya's broth [25]. A large number of saprophytic bacteria and fungi acting on sparingly soluble soil phosphates, mainly by chelation-mediated mechanisms [26], carries out phosphorus solubilization.

Such change in mineral phosphate solubilization across fertility gradients may be explained by the fact that p-solubilization activity of microorganisms was affected PSB community, their function and the presence of soluble phosphate ions [27] in different gradients created in the present investigation. Lower PSB diversity as indicated by Shannon index under high fertility gradient soils led to lower phosphate solubilization which was further influenced by the presence of excessive soluble phosphate ions present in high fertility gradient soils [28]. In this context, Goldstein and Liu [29]showed that mineral phosphate solubilizing activity is genetically coded in a gene cluster on plasmids of the microorganisms possessing this activity. It was also reported that gene expression and mineral phosphate solubilization of bacteria is affected by the presence of soluble phosphate due to feedback regulation. While assessing the enzyme activities it was found that, acid phosphatase activity of fallow soil was the highest, which was drastically reduced when the soils were brought under cultivation without fertilizer. This trend was maintained along the gradient of fertility with the modest change in their activity.

In the present investigation establishment of fertility gradient with regard to available phosphorus was highly uniform. Such high level of phosphate in soil solution might cause repress the phosphatase synthesis by the soil microorganisms [30]. Moreover, orthophosphate is a competitive inhibitor of acid and alkaline phosphatase activity [31]. Thus, the phosphatase activity along the path of gradient decreased. Higher phosphate solubilization and phosphatase activities in fallow soils with lesser amount of soluble P suggest higher microbial activities under lower P status. This supports the earlier report by Gyaneshwar *et al.*, [32].

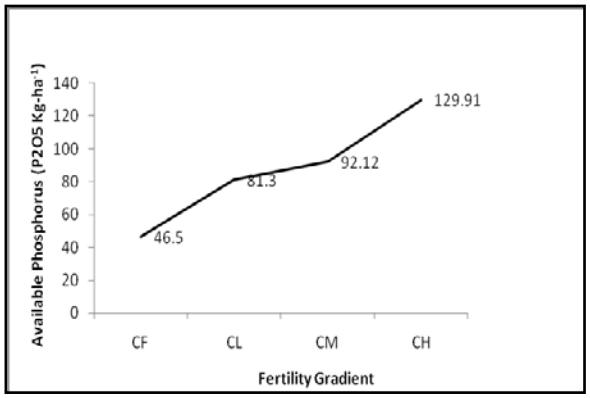

Fig. 10: Establishment of fertility gradient in respect of Phosphorus in CRIJAF soil

3. CONCLUSION

Different pattern of C-source utilization and their rate by culturalble phosphate soulibilizing bacterial isolates were achieved under the influence of artificially created 13 years old fertility gradient experimental sites. But the primary dataset while were subject to principal component analysis for distinguishing PSB communities could not differentiate the experimental sites sufficiently in regard to PSB communities. Such lacunae may be arisen from insufficient number of c-sources used in the experiment. Moreover, single approach based on sole-C-source utilization is unable to differentiate the existing PSB into different communities in finer scale. It would be more meaningful if polyphasic approach like C-source utilization pattern along with nucleic acid and fatty acid profiling followed.

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