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Full Length Research Paper

Mineral phosphate solubilizing bacterial community in agro-ecosystem

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The present communication deals with the assessment of phosphate solubilizing bacterial community structure across artificially created fertility gradient with regards to N, P and K status of soil in the experimental site. 20 randomly phosphate solubilizing bacteria from each fertility gradient were isolated, purified and characterized through their insoluble mineral phosphate-source utilization patterns. Four insoluble phosphate sources; purulia rock phosphate (PRP), mussourie rock phosphate (MRP), crystalline iron and aluminum phosphate were charged in basic Pikovskaia solid medium. Growth pattern of the isolates on those phosphate sources was recorded. Different communities utilized different P-sources in different magnitudes. Size of isolates of low fertility gradient showing greater utilization efficiency towards diverse phosphate sources gradually reduced along the gradient of fertility. Data thus obtained from the experiment were filed to compute community structure of phosphate solubilizing bacteria through principal component analysis (PCA). On the whole, the community composition of phosphate solubilizing bacteria reduced gradually under the influence of cultivation and fertilization in different fertility gradient soils.

Key words: Fertility gradient, phosphate solubilizing bacteria, insoluble phosphate utilization, principal component analysis, community structure.

INTRODUCTION

Only 10 -25 % of fertilizer phosphorus is acquired by plants; the other main part as a result of chemical changes in soil, transforms into insoluble forms thus making it hard for plants to reach. Thus, "over phosphatization" is a global ecological problem of agriculture, but it is known that a part of soil bacteria called phosphate solubilizing bacteria (PSB) is capable of solubilizing accumulated insoluble phosphatic compound sources in soil by the production of organic acid, phenolic compounds, protons and siderophores (Landeweert et al., 2001).

The structure and functionalities of phosphate solubilizing microbial communities differ on the basis of phosphate sources present in soils. Thus, physiological response of PSB community toward this component is important for formulating management strategies. Understanding relationships among bacteria through physiological profiling advances our knowledge of bacterial ecology and community structure and assists in the investigation of an unknown bacterium.

Phosphate solubilizers belonging to diverse taxonomic groups of microorganisms, especially bacteria are known. The ecological role of these microorganisms in soil is very important, as they take part in biogeochemical cycles of phosphorus in the ecosystems. Thus, it is necessary to study the composition and dynamics of these microbial populations to reach a better understanding of soil microbial diversity, nutrient transformation and uptake by plants. The study of populations of microorganisms, which share the common characteristic of phosphate solubilization has great complexity, because they belong to very diverse groups sometimes not closely related under a phylogenetic point of view. Many PSB species remain unknown and more studies are needed to reveal the high biodiversity of these bacteria. Although the study of rhizosperic bacteria is difficult because of the high number of bacteria present in the soil, the characterization and identification of these bacteria are needed for wide ecological studies of the soil.

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Carbon source utilization technique is widely used for community composition analysis (Garland, 1996a) and functional diversity of soil microbes (Campbell et al., 1997; Yan et al., 2006), but it has its own pitfalls of choosing appropriate C-sources of ecological significance. In this regard, the use of different insoluble mineral phosphatic sources in the present experiment will be ecologically significant and rational to understand the community interacting with that mineral fraction of soil to make it available for plant uptake for sustainable crop production.

MATERIALS AND METHODS

Experimental site and establishment for fertility gradient

The experiment was laid down in the Central Research Farm, Gayeshpur, Bidhan Chandra Krishi Viswavidyalaya 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. (1967). 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. 3 fertilizer schedules; $N_0P_0K_0$ (low), $N_{100}P_{50}K_{50}$ (medium) and $N_{200}P_{100}K_{100}$ (high) for growing an exhaustive crop like fodder maize were applied to the 3 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 (-5°C) for microbiological studies.

The gradient, artificially established has been maintained for several years by renewing fertility gradient experiments under the supervision of the All India Coordinated Research Project on Soil Test Crop Response Correlation, BCKV, Kalyani Centre.

Preparation of pure cultures of phosphate solubilizing bacteria

The soil suspension of 10^{-5} dilution prepared from soils of three fertility gradients (low, medium and high) was inoculated on solid Pikovskaia's medium and incubated for 4 days at 30°C. Then randomly, some colonies were selected 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. The pure cultures of PSB were preserved in a culture tube containing Pikovskaia's medium. Isolates were then subjected to grow in Pikovskaias medium containing different insoluble mineral phosphate sources.

Mineral phosphate sources

As the present work has been carried out to investigate the phosphate solubilizing bacterial community, it would be worthy to customize the substrates in such a way that it would reflect ecological significance (Gorlenko et al., 1994). In this context, insoluble phosphate solubilization pattern of selected isolates will be of ecological significance for differentiating the community concerned for phosphate solubilization. To carry out this experiment, 4 insoluble phosphate sources viz. purulia rock phosphate, mussourie rock phosphate, iron phosphate and aluminum phosophate at the rate of 5% (w/v) were used in Pikovskaia medium. Before using the phosphate sources, soluble phosphate was removed by sequential washing with cold water, hot water and ultimately with 2% citric acid solution. Utilization of insoluble phosphate sources by different isolates of different soils of varying fertility gradients was assessed. 5% (w/v) each of the 4 phosphate sources were charged in basic Pikovskaia solid medium and poured aseptically into sterile Petri plates and allowed to solidify. After 20 min, the plates were turned inversely and kept for a day. The reverse surfaces of the Petri plates were then divided into 16 small squares with glass markers. Isolates from each gradient were then inoculated into the small squares singly and allowed to grow for 3 - 4 days at 30°C.

Data analyses

Data were expressed in 2 different ways. First, a code was used to represent either a positive (growth) or negative (no growth) response of isolates to different mineral phosphate 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- .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 P-sources were being utilized (percent P-source utilization intensity- PPSUI) by different phosphate solubilizing bacterial isolates was calculated by the following formula:

$$PPSUI = (x_1(0) + x_2(1) + x_3(3) + x_4(5) + \ldots\ldots\ldots X\ 100) / (\text{Total No. of P-source X Highest scale used})$$

where x is different P-sources.

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 (William and Goldstein; SPSS 7.5, 1997). The score of each of the isolate on the principal component was plotted in a bi-variate scattergram to allow visual assessment of the position of these isolates in the direction of these components.

RESULTS AND DISCUSSION

Bibliography antecedent reveals that most of the studies so far conducted on phosphate solubilizing bacteria in agroecosystems were based mostly on the enumeration of colony forming units on an agar plate using serial dilution and pour plate technique where the community composition was rarely analyzed. Recently, few phylogenetic studies of phosphate solubilizing bacterial communities were published (Rivas et al., 2006; Valverde et

Table 1. Characteristics of experimental soil.

Parameter	Fertility gradient of soil			
	Fallow	**Low**	**Medium**	**High**
pH	6.88	6.84	6.50	6.25
EC	0.091	0.114	0.117	0.117
Textural class	Sandy clay loam	Sandy clay loam	Sandy clay loam	Sandy clay loam
Organic carbon	0.79	0.78	0.74	0.71
Available Nitrogen (Kg/ha)	137.83	167.86	191.73	200.2
Available Phosphorus (P_2O_5 Kg/ha)	42.15	75.32	87.04	124.34
Available Potassium (K_2O Kg/ha)	125.20	107.20	117.19	130.80

al., 2007; Perez et al., 2007; Peix et al., 2007). Such species diversity of phosphate solubilizing bacteria has little importance in agroecosystems for sustainability in the mineralization of insoluble phosphatic minerals because there exist a redundancy among phosphate solubilizers. Thus, functional diversity is of great importance from the point of sustainability (Giller et al., 1997; Pankhurst et al., 1995). The present communication deals with phosphate solubilizing bacterial community structure on the basis of their insoluble phosphate utilization pattern along gradients of fertility where trends of diversity of bacteria can be explored (Giller et al., 1997; Suzuki et al., 2005). A distinct gradient in terms of available phosphate was established in the experimental sites. Thus, a broad range of different soil ecological situations is presented as characterized by soil properties (Table 1). Therefore, distinct soil ecological conditions would be expected to harbour diverse phosphate solubilizing bacterial communities.

To characterize the phosphate solubilizing bacterial isolates across the fertility gradient, the isolates from different fertility gradients were tested against different insoluble phosphate sources viz. Purulia rock phosphate, Mussourie rock phosphate, crystalline iron and aluminium phosphate. Results showed that, the capacity to utilize all of the phosphate sources by isolates from low, medium and high fertility soils declined gradually. In low fertility gradient soil 45% of the isolates utilized all four P-sources whereas it reduced 30% and 15%, in medium and high fertility gradient soils, respectively (Tables 2, 3 and 4).

It is interesting to note that in medium fertility gradient and onwards two new phosphate solubilizing bacterial community emerged. One of the community utilized only two phosphate sources out of four and the other could not utilize either of the phosphatic source excepting the initial insoluble tricalcium phosphate as P-source (Table 2, 3, 4). Such changes in insoluble inorganic phosphate sources utilization spectrums across fertility gradient is due to the variation of phosphate solubilizing bacterial metabolism towards phosphates solubilization and utilization under the influence of fertility gradient.

Goldstein and Liu (1987) have shown that mineral phosphate solubilizing activity is genetically coded in a gene cluster on the plasmids of 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 feed back regulation. So under the influence of long-term existence of phosphate solubilizing bacteria with soluble phosphate in gradient soils, there might be changes in gene expression among the isolates; thus, new communities might evolve.

Among the phosphate solubilizing bacterial isolates, 75% isolates of low fertility gradient soil favoured Purulia rock phosphate as their phosphate source. But this capacity to utilize Purulia rock phosphate by the isolates of medium and high fertility soils sharply reduced to 65 and 55%, respectively. A similar trend was also noticed in the cases of the other insoluble phosphate sources. Preferential utilization of insoluble phosphate-sources by bacterial isolates and their subsequent reduction across the fertility gradient was due to reduced metabolic activities towards a particular phosphate source. This corroborates earlier reports of Degens et al. (2001) who discussed reduced metabolic diversity in soils under stress conditions.

To estimate the efficacy of phosphate source utilization by the isolates, percent phosphate source utilization intensity (PPSUI) was computed. It was observed that uniformity in the efficacy of mineral phosphate solubilization in low fertility soil gradually declined in medium and high fertility soils (Tables 2, 3 and 4). Relative growth of isolates on different mineral phosphates depends on the degree of solubilization of those mineral phosphates by the respective isolate. This indicates the evenness of phosphatic source utilization, a prominent phenotypic characteristic of phosphate solubilizing bacterial isolates.

Community composition analyses

To discriminate the phosphate solubilizing bacterial community, data from the responses of each 20 isolates from low medium and high fertility gradients towards insoluble phosphate-source were analysed using principal component analysis. PCA is a multivariate statistical analysis technique used to project the maximum variance of bac-

Table 2. Insoluble phosphate source utilization pattern by phosphate solubilizing bacterial isolates of low fertility gradient soil.

Isolate	Purulia rock phosphate	Mussourie rock phosphate	Ferric phosphate	Aluminium phosphate	% phosphate source utilized	PPSUI
L_1	+	+	+	+	100	44.32
L_2	+	+	-	-	50	16.62
L_3	+	+	+	+	100	37.8
L_4	+	+	+	+	100	60.94
L_5	+	+	+	-	75	41.55
L_6	+	+	+	+	100	49.86
L_7	+	+	+	+	100	44.32
L_8	+	+	+	+	100	44.32
L_9	+	+	+	-	75	30.47
L_{10}	+	+	+	+	100	44.32
L_{11}	+	-	-	+	50	22.16
L_{12}	-	+	+	-	50	22.16
L_{13}	+	+	+	+	100	33.24
L_{14}	+	-	+	+	75	24.93
L_{15}	+	-	-	+	50	22.16
L_{16}	-	+	+	-	50	16.62
L_{17}	+	+	+	+	100	44.32
L_{18}	-	+	-	+	50	22.16
L_{19}	-	+	+	+	75	30.47
L_{20}	-	+	+	+	75	38.78
% isolate utilized P-source	75	85	80	75		

Table 3. Insoluble phosphate source utilization pattern by phosphate solubilizing bacterial isolates of medium fertility gradient soil.

Isolate	Purulia rock phosphate	Mussourie rock phosphate	Ferric phosphate	Aluminium phosphate	% phosphate source utilized	PPSUI
M_1	+	+	+	+	100	34.32
M_2	-	+	+	-	50	22.16
M_3	+	+	+	-	75	47.09
M_4	+	+	+	+	100	38.78
M_5	+	+	+	+	100	66.48
M_6	-	+	+	+	75	36.01
M_7	+	+	-	-	50	27.7
M_8	+	+	-	+	75	36.01
M_9	+	+	+	+	100	38.78
M_{10}	+	+	+	+	100	38.78
M_{11}	+	+	+	+	100	38.78
M_{12}	-	+	+	-	50	22.16
M_{13}	+	+	-	+	75	30.47
M_{14}	-	+	+	+	75	30.47
M_{15}	+	-	+	+	75	30.47
M_{16}	-	-	-	-	0	0
M_{17}	+	+	-	+	75	30.47
M_{18}	+	-	-	+	50	22.16
M_{19}	-	-	+	-	25	8.31
M_{20}	-	-	-	+	25	8.31
% isolate utilized P-source	65	70	65	75		

Table 4. Insoluble phosphate source utilization pattern by phosphate solubilizing bacterial isolates of fallow soil.

Isolate	Purulia rock phosphate	Mussourie rock phosphate	Ferric phosphate	Aluminium phosphate	% phosphate source utilized	PPSUI
H_1	+	+	+	+	100	66.48
H_2	-	+	+	+	75	30.47
H_3	+	+	+	+	100	38.78
H_4	-	-	-	-	0	0
H_5	+	+	+	-	75	41.55
H_6	+	+	+	-	75	36.01
H_7	+	+	+	+	100	49.86
H_8	-	+	-	-	25	19.39
H_9	+	+	-	+	75	41.55
H_{10}	-	+	+	+	75	52.60
H_{11}	+	-	-	-	25	13.85
H_{12}	-	+	+	-	50	27.7
H_{13}	+	-	-	+	50	22.16
H_{14}	-	+	-	-	25	13.85
H_{15}	-	-	+	-	25	13.85
H_{16}	+	-	-	+	50	22.16
H_{17}	+	+	-	-	50	22.16
H_{18}	-	-	+	-	25	13.85
H_{19}	+	+	-	-	50	27.7
H_{20}	-	+	-	+	50	22.16
% isolate utilize P-source	55	70	50	55		

Table 5. Eigen value and eigen vectors of principal component analysis of the variables used to differentiate the isolates of low fertility gradient soil.

Variable	Component	
	1	2
PRP	0.59	0.69
MRP	0.76	-0.57
$FEPO_4$	0.86	-0.41
$ALPO_4$	0.50	0.75
Eigen value	1.91	1.52
% of variance	47.82	38.08
Cumulative %	47.82	85.90

terial isolates optimally in multiple dimensions (PC1 and PC2) in an unconstrained ordination. PCA calculated orthogonal axes (principal components) through the data matrix in the direction of the highest variance. The analyses were performed on the basis of correlation matrix involving previously mentioned variables. The score of each of the isolate on the principal component was plotted in a bi-variate scattergram to allow visual assessment of the position of these isolates in the direction of these components.

Table 5 shows the eigen values and vectors for the principal components obtained from the correlation matrix. 85.90% of the divergence among the isolates of low fertility gradient soil was explained by the two components. The first component (PC1) accounted for 47.82% of the variation on the correlation matrix. The variables loading heavily on the PC1 were different insoluble inorganic phosphatic sources like Purulia rock, Mussourie rock, crystalline iron and aluminum phosphates. The second component (PC2) accounted for 38.08% of total divergence and having a positive loading from Purulia rock and aluminium phosphates.

In a PCA ordination diagram, isolates with similar response towards different variables used in the experiment are located close to one another and those dissimilar are located far apart. From the diagram, it is clear that isolates 1, 3, 4, 7, 8, 10, 13 and 17 from the low fertility gradient soil revealed a very tight grouping resulting in a distinct community. Similarly, isolates 2 and 9; 5 and 6; 11, 15 and 18; 12 and 16; 14 and 20 together construct separate five communities. On the whole, low fertility gradient soils revealed 6 distinct phosphate solubilizing bacterial communities (Figure 1).

Table 6 shows the eigen values and vectors for the principal components obtained from the correlation matrix. 76.31% of the divergence among the isolates of medium fertility gradient soil was explained by the 2 components. The first component (PC1) accounted for

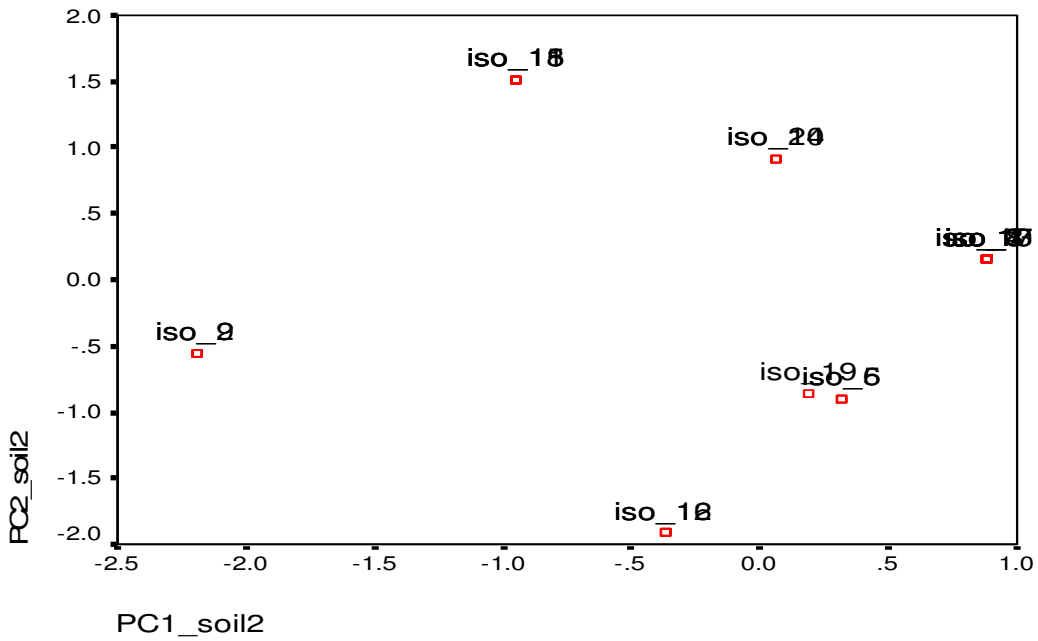

Figure 1. Scatter diagram of regression factor scores due to first two components extracted by principal component analysis of variables to differentiate the isolates of low fertility gradient soil.

Table 6. Eigen values and vectors of principal component analysis of the variables used to differentiate the isolates of medium fertility gradient soil.

Variable	Component	
	1	2
PRP	0.90	-0.27
MRP	0.58	0.54
$FEPO_4$	0.40	0.73
$ALPO_4$	0.81	-0.44
Eigen value	1.96	1.09
% of variance	49.04	27.27
Cumulative %	49.04	76.31

Table 7. Eigen values and vectors of principal component analysis of the variables used to differentiate the isolates of high fertility gradient soil.

Variable	Component	
	1	2
PRP	0.61	0.69
MRP	0.84	-0.20
$FEPO_4$	0.64	-0.55
$ALPO_4$	0.85	0.12
Eigen value	2.22	0.83
% of variance	55.43	20.67
Cumulative %	55.43	76.10

49.04% of the variation on the correlation matrix. The variables loading heavily on the PC1 were different insoluble inorganic phosphatic sources like Purulia rock, Mussourie rock, crystalline iron and aluminum phosphates. The second component (PC_2) accounted for additional 27.27% of the total divergence and having positive loading from Mussourie rock and iron phosphates. The mean score of components 1 and 2 are plotted in Figure 2. In a PCA ordination diagram it is clear that isolates 1, 3, 4, 5, 6, 8, 9, 10 and 11 from medium fertility gradient soil revealed a very tight grouping resulting in a distinct community. Similarly, isolates 2 and 12; 13 and 17; 14 and 15 together construct another 3 communities. On the whole, medium fertility gradient soils revealed 4 distinct phosphate solubilizing bacterial communities.

Table 7 shows the eigen values and vectors for the principal components obtained from the correlation matrix. 76.10% of the divergence among the isolates of high fertility gradient soil was explained by the 2 components. The first component (PC1) accounted for 55.43% of the variation on the correlation matrix. The variables loading heavily on the PC1 different insoluble inorganic phosphatic sources like Purulia rock, Mussourie rock, crystalline iron and aluminum phosphates. The second component (PC2) accounted for additional 20.67% of the total divergence and having positive loading from Purulia rock and aluminum phosphates. The mean score of components 1 and 2 are plotted in Figure 3. In a PCA ordination diagram, it is clear that isolates 1, 2, 3, 6, 7, 8, 9, 10 and 19 from high fertility gradient soil revealed a very tight grouping resulting in a distinct community. Similarly, isolates 11 and 17; 15 and 18 together

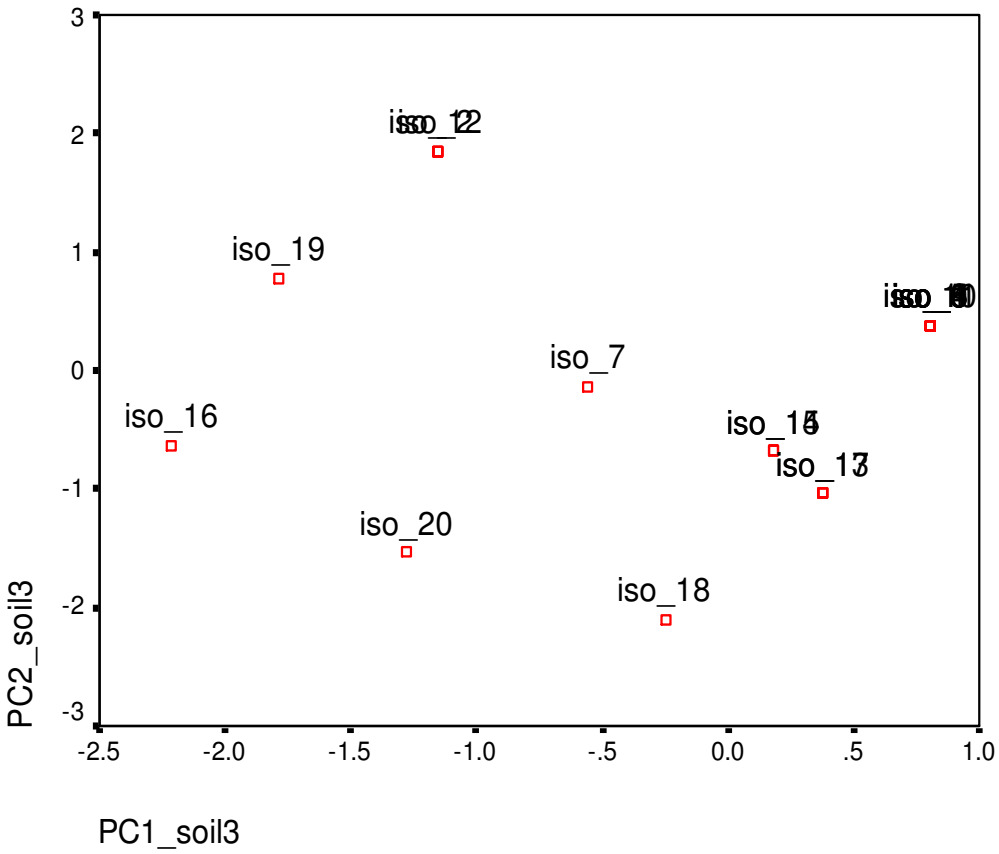

Figure 2. Scatter diagram of regression factor scores due to first two components extracted by principal component analysis of variables to differentiate the isolates of medium fertility gradient soil.

construct 2 communities. On the whole, high fertility gradient soils revealed 3 distinct phosphate solubilizing bacterial communities (Figure 3).

While comparing the phosphate solubilizing bacterial community structure across fertility gradients, it was revealed that application of fertilizer had caused a reduction in phosphate solubilizing bacterial community composition across the fertility gradients. These results were consistent with the hypothesis that microbial communities with reduced catabolic diversity are less resistant to stress and disturbance resulting in less communities.

Tables 2 and 3 showed that sole mineral phosphate utilization (P-metabolism) as well as their intensity (MPUI) reduced through the path of gradients with a drastic reduction in high fertility gradient soil. Stress arising from the higher chemical fertilizers probably reached above the threshold level that enabled only a few species to survive (Grime, 1979; Austin, 1987). This is in line with the observation of O'Donnell et al. (2001) who established fertilizers as the drivers of change in microbial community structures. Fertilizer influences the microbial community either by altering soil reaction (Macrae et al., 1999) or by modifying the structural integrity of soil (Buyanovsky and Wagner, 1987). Other soil factors reported to be greater in low and medium fertility gradient soil as compared to that of high fertility gradient soil (Table 1) such as organic carbon content and pH may also have increased resistance against stress. These factors would offer greater protection to the microbial community and possibly enhance microbial recolonization after stress or disturbance in the low and medium fertility gradient soil. Thus, the hypothesis of the present investigation that phosphate solubilizing bacterial communities will decline with increasing fertility gradient has, thus, been confirmed.

Conclusion

Response of isolates towards the utilization of different insoluble mineral phosphates is not sufficient phenotypic character to differentiate existing phosphate solubilizing bacterial communities in soils. To find out the fine scale of community composition, polyphasic approaches consisting of phenotypic and genotypic characteristics such as sole C sources utilization pattern, intrinsic antibiotic resistance properties, fatty acid profiling and nucleic acid analyses are to be included in community analyses.

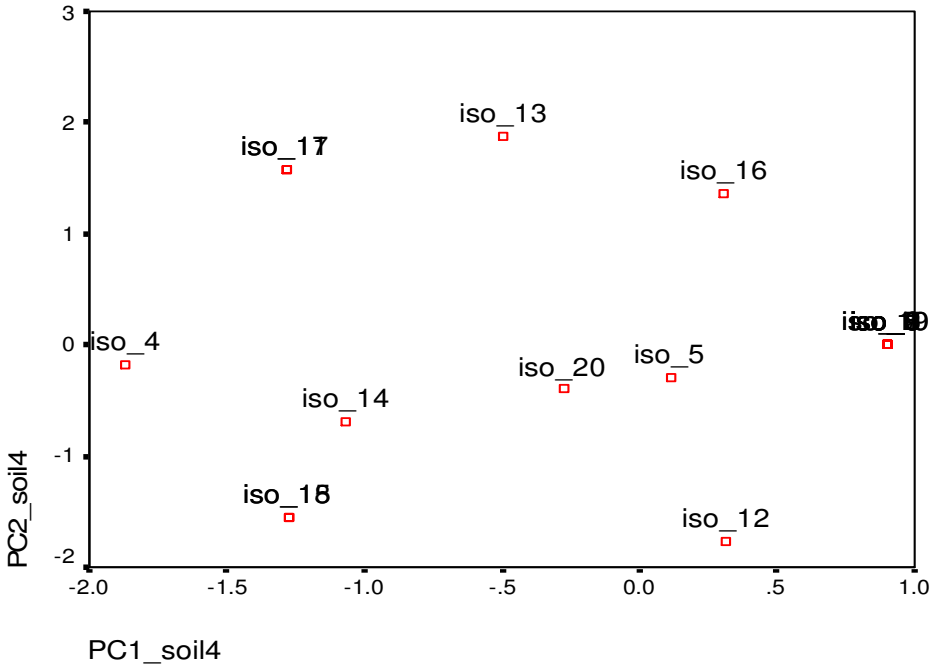

Figure 3. Scatter diagram of regression factor scores due to first two components extracted by principal component analysis of variables to differentiate the isolates of high fertility gradient soil.

REFERENCES

Austin MP (1987). Model for the analysis of species' response to the conventional gradients. Vegetation, 69: 35-45

Buyanovsky GA, Wagner GH (1987). Carbon transfer in a winter wheat (*Triticum aestivum)* ecosystem. Biol. Fertil. Soil, 5: 76-82.

Campbell CD, Grayston SJ, Hirst DJ (1997). Use of rhizosphere carbon sources in sole carbon source tests to discriminate soil microbial communities. J. Microbiol. Meth. 30: 33-41.

Degens BP, Schipper LA, Sparling GP, Duncan LC (2001). Is the microbial community in a soil with reduced catabolic diversity less resistant to stress or disturbance? Soil Biol. Biochem. 33: 1143-1153.

Garland JL (1996a). Analytical approaches to the characterization of samples of microbial communities using patterns of potential C sources utilization. Soil Biol. Biochem. 28: 213-221.

Giller KE, Beare MH, Lavelle R, Izac AMN, Swift JJ (1997). Agricultural intensification, soil biodiversity in agroecosystem. Appl. soil. Ecol. 6: 3-16.

Goldstein AH, Liu ST (1987). Molecular cloning and regulation of a mineral phosphate solubilizing gene from *Erwinia herbicola*. Biotechnol. 5: 72-74.

Gorlenko MV, Majorova TN, Koahevin PA (1994). Disturbances and their influences on substrate utilization patterns in soil microbial communities. Soil science Faculty, Moscow state University, Soil Biology Department, Moscow-119899, Russia.

Grime JP (1979). Plant strategies and Vegetation Processes. Wiely, Chichester. p. 222.

Landeweert R, Hoffland E, Finlay RD, Kuyper TW, Van Breemen N (2001). Linking plants to rocks, ectomycorrhizal fungi mobilize nutrients from minerals. Trends Ecol. Evol. 16: 248-253.

Macrae A, O'Donnell AG, Rimmer DL (1999). Plant induced acidity in `model rhizospheres of *Triticum aestivum* and *Brassica napus*. Leandra. 14: 43-49.

O'Donnell AG, Seasman M, Macrae A, Waite I, Davies JT (2001). Plans and fertilizers as drivers of change in microbial community structure and function in soils. Plant Soil 232: 135-145.

Pankhurst CE, Ophel-Keller K, Doube BM, Gupta VVSR (1995). Biodiversity of soil microbial communities in agricultural system. Biodiv. Cons. 5: 197-209.

Peix A, Velazquez E, Martinez-Molina E (2007). Molecular methods for biodiversity analysis of phosphate solubilizing microrganisms (PSM). First International meeting on Mineral Phosphate solubilization. p. 102. ISBN.978-1-4020-4019-1.

Perez E, Sulbarian M, Ball MM, Yarzabal LA (2007). Isolation and characterization of mineral phosphate solubilizing bacteria naturally colonising a limonitic crust in the south-eastern Venezuela region. Soil Biol. Biochem. 36: 2905-2914.

Ramamurthy B, Narasimham RL, Dinesh RS (1967). Fertilizer application for specific yield target of Sonara 64 wheat . Indian Farming. 17: 43-45.

Rivas R, Velazquez E, Valverde A, Mateos PF, Martinez-Molina E (2006). A two primers random amplified polymorphic DNA procedure to obtain polymerase chain reaction fingerprints of bacterial species. Electrophoresis, 68: 5217-5222.

Suzuki C, Kunito T, Aono T, Liu CT, Oyaizu H (2005). Micribial indices of soil fertility. J. Appl. Microbiol. 98: 1062-1074.

Valverde A, Igual JM, Cervantes E (2007). Polyphasic characterization of phosphate solubilizing bacteria isolated from rhizospheric soil of north-eastern region of Portugal. First International Meeting on Microbial Phosphate Solubilization, 102: 273-276.

William RD, Goldstein M (1997). In: Multivariate Analysis: method and application. Jhon Wiley & Sons. New York.

Yan M, Zhizhou C, Jiantao Z, Xiaomei W, Hongying H, XiaoMei Y, Jianying Z (2006). Studies on microbial community characteristics at the seed germination stage of cucumber. Plant Nutr. Fert. Sci. 12:109-114.