The Pharma Innovation Journal 2022; 11(11): 1135-1141

www.ThePharmaJournal.com

The Pharma Innovation

ISSN (E): 2277-7695
ISSN (P): 2349-8242
NAAS Rating: 5.23
TPI 2022; 11(11): 1135-1141

www.thepharmajournal.com
Received: 23-08-2022
Accepted: 01-10-2022

E Venu
Department of Plant Pathology, Bidhan Chandra Krishi Viswavidyalaya, Mohanpur, Nadia, West Bengal, India

H Nazneen
Department of Plant Pathology, Bidhan Chandra Krishi Viswavidyalaya, Mohanpur, Nadia, West Bengal, India

K Ray
Department of Plant Pathology, Bidhan Chandra Krishi Viswavidyalaya, Mohanpur, Nadia, West Bengal, India

SK Ray
Department of Plant Pathology, Bidhan Chandra Krishi Viswavidyalaya, Mohanpur, Nadia, West Bengal, India

Corresponding Author:
E Venu
Department of Plant Pathology, Bidhan Chandra Krishi Viswavidyalaya, Mohanpur, Nadia, West Bengal, India

Isolation and characterization of native rhizobacteria and their tolerance to different levels of pH

E Venu, H Nazneen, K Ray and SK Ray

Abstract
Soil acidity is one of the major constraints for crop production in the lateritic regions of West Bengal, such as Birbhum, Bankura, parts of Barddhaman, West Medinipur and entire Purulia districts of West Bengal. In order to alleviate the soil acidity by utilizing native potential rhizospheric PGPR (Plant Growth Promoting Rhizobacteria) in the laterite regions of West Bengal, the survey was conducted for collection of rhizospheric soil samples from various cultivated and wild plants from different locations of Birbhum district. In the present investigation, attempts were made for the enumeration, isolation and characterization of the most efficient native proteobacteria inoculants for accruing benefit to agricultural crop production in the problematic acid soils. A total number of forty six bacteria were isolated from lateritic regions of West Bengal. Highest number of total bacterial population was observed from rhizosphere of Paddy followed by rhizosphere of pointed gourd. The root exudates of cucurbit rhizosphere harbor the highest number of bacterial population under lateritic region as compared to the rhizosphere of other crops. Finally 46 isolates were purified and maintained for the studies. Dendogram was generated based on hierarchical clustering and grouped these 46 isolates into two major groups on the basis of their growth at pH 4.0. Among 46 bacterial isolates, 41% isolates were found to be highly acid tolerant at pH level 4.0 indicating isolates are fit to sustain under low pH conditions.

Keywords: Plant growth promoting rhizobacteria, lateritic soils, soil acidity, characterization, plant health promotion activity

Introduction
Lateritic soils are the significant and prevalent soils in tropical and subtropical climates. These are highly weathered soils in the classification system. The significant features of the lateritic soils are their unique colour, poor fertility, high clay content, lower cation exchange capacity and soil acidity. Excess amount of iron and aluminum oxides are present in this soil. Oxides of iron prevailing primarily in the amorphous and crystalline inorganic forms are one of the crucial components in many soil orders. Lateritic soil profiles which are developed due to continuous leaching characteristically suffer from limitations of acidity, low base saturation, deficiency of organic matters and water erosion. Management of acidic soils include both indirect and direct ways to ensure that production potential of a particular soil is reclaimed. Reclamation of acidic soil includes addition of lime and efficient handling of agricultural practices for optimal crop yield (Yirga *et al*., 2019) [15]. On the other hand, the use of PGPR is gaining importance to address soil acidity stress. Extensive efforts have been made concerning the use of PGPR as an approach for dealing with many environmental stresses of plants. Present understanding indicates that about 2–5% of rhizospheric bacteria which can be cultured are plant growth promoters, either directly and or indirectly (Dutta and Bora, 2019) [6] are the pre requisite to utilize this resource in agriculture is increasing.
The acid-soil tolerant rhizobacteria can improve the survival and productivity of crop plants (Zhang *et al*., 2020) [16]. Rhizospheric proteobacteria isolated from acidic soils have been found more ability to colonize and improve plant growth under acidic conditions. Their abundance responded variably to soil pH and their diversity increased with increasing pH (Rousk *et al*., 2010) [14]. Changes in the relative abundance of subgroups within the *Acidobacteria*, and increase in *Bacteroidetes*, *Nitrospira*, *Alphaproteobacteria*, *Betaproteobacteria*, *Gammaproteobacteria*, and *Deltaproteobacteria* across the pH gradient were also observed by Jones *et al*., (2009) [9]. It is evident that the plant rhizospheres are more promising microhabitats for microorganisms compared to surrounding bulk soils, and these microbes directly or indirectly influence the growth and development of the plant.

The occurrence of several beneficial rhizobacteria like *Rhizobium*, *Bradyrhizobium*, *Azotobacter*, *Azospirillum*, *Pseudomonas*, *Bacillus* etc. have been reported from stressed environments like desert ecosystems, acid soils, saline and alkaline areas and also from highly eroded hill slopes, and these are assumed to be involved in natural reclamation process of the soil. PGPR's can also confer some degree of tolerance to plants, towards abiotic stress.

The utilization of rhizospheric proteobacteria is becoming a vital component for plant growth-promotion and biological control. They are capable of reducing disease severity in many crops through induced resistance mechanism. Induced systemic resistance (ISR) in crop plants is characterized by the induction of host-defense responses including, defense related enzymes synthesis and accumulation of phenolics. Therefore, efforts are necessary to figure out alternative, innovative, environmental friendly options to grow the crop more efficiently in laterite soil and as well to reduce the use of costly and non-environmental friendly chemical fertilizers. In this context, efforts are made to enumerate, isolate and characterize the native plant growth promoting proteobacteria from rhizospheres of different crops from red and lateritic region of West Bengal.

Material and Methods

Survey and collection of soil samples

Soils of this study were collected from different fields from the laterite region (Birbhum district) of West Bengal, India during summer, monsoon and winter seasons of 2017-18 (Table-1). The soils were collected from different sites having variable p^H and from the rhizospheres of different crops. Soil samples with three replications from each site were collected randomly (0–15 cm depth) and plant roots, evident faunas, stones, debris etc. were removed. Approximately 1 kg of soil was carried from each site into the laboratory in labeled sterile containers. These samples were retained at 4^0C before going to microbial analyses of the moist soils and further samples were used for soil pH analysis. During survey, soil samples were collected from different hosts in lateritic zone of West Bengal for isolation of the rhizospheric microorganism(s) in laboratory for future use. *In vitro* laboratory works have been done at Bacteriological laboratory, Department of Plant Pathology, Bidhan Chandra Krishi Viswavidyalaya, Mohanpur, Nadia, West Bengal, India.

Determination of pH f Soil

Determination of pH of collected soil samples were done in 1:2.5 soil water suspension using glass electrodes. Electrical conductivity of the soil saturation extract (EC) is done by measuring the electrical conductance of soil water saturation extract using conductivity meter (USDA, 1954).

Isolation, purification and enumeration of culturable bacterial count of soils

The collected rhizospheric soil samples were air-dried under shade, properly ground, sieved and stored at 4°C up to 48 hr before used for isolation of proteobacteria using nutrient agar by serial dilution and pour plate technique. Colony morphology for proteobacteria were selected by observing their colonies from the plates containing dilution of 10^{-5} and 10^{-6} following standard bacteriological method. Colony count was taken after 72 hours of incubation at 28–30 °C. Different colony types appeared on nutrient agar medium were marked and individual colony was picked up followed by streaking on separate nutrient agar plates to obtain single isolated colony. The cultures were maintained in nutrient agar slants for detailed characterization. The pure cultures thus obtained were labeled with designation.

Morphological Characterization and Gram Staining of the Isolates

Colony characteristics *viz*. colony shape, margin, colour, elevation and opacity of the isolates were done using nutrient agar (NA) on Petri plates and cellular morphology using standard Gram staining method.

Determination of acid tolerant ability of rhizobacteria

The intrinsic resistance of the isolated rhizobacteria against different P^H was evaluated by observing the growth on NA medium amended with 0.1M concentration of *hydrochloric acid* (HCl) @ 2, 4, 6, 8 and 10% (w/v). Control plates was also maintained without HCl. Following streaking of individual isolate under aseptic condition plates were incubated for 48 h at 28± 2 ºC and the growth of bacteria on amended plates were observed comparing with control plates.

Results and Discussion

Survey and collection of soil samples

Result presented in Table-1 revealed that a total twenty two rhizosphere soil samples were collected from six different blocks namely Suri-1, Bolpur, Illambazar, Dubrajpur, Suri-2 and Rajnagar comprising of 14 mouzas of Birbhum district of West Bengal. Laboratory assay of the collected soil samples exhibited varied range of soil pH from 4.8 to 6.9. Among the collected samples lowest pH (4.8) was found in sample LHU-19 collected from paddy root rhizosphere at Chandrapur Mouza of Rajnagar block while highest pH (6.9) was recorded from paddy root rhizosphere at Mallickpur Mouza of Suri-1 block. Soil texture studies among 22 soil samples showed that maximum (15 nos.) belong to sandy loam category followed by clay loam (5 nos.) and one (1no.) each of sandy and loam category. Among the samples, 15 nos. were collected from cultivated crops followed by forest plant root rhizosphere (3 nos.), grass land (2 nos.) and one each from pasture land and fallow land respectively. Result indicated that samples were collected, from six (6 nos.) cultivated crops like Paddy (*Oryza sativa*), Cucumber (*Cucumis sativus*), Pumpkin (*Cucurbita moschata)*, Sugarcane (*Saccharum officianarum*), Pointed gourd (*Trichosanthes dioica*) and Colocasia (*Colocasia esculenta*). The soil ID numbers, origins and GPS locations of the twenty two soil samples were presented in Table-1.

Rhizosphere Bacterial Population Dynamics

Five ((LHU-2, LHU-3, LHU-10, LHU-17 and LHU-21) out of twenty two collected soil samples did not showed any bacterial colony in laboratory assay thus Log value of bacterial population, total number of rhizobacteria, colony shape and selected isolate(s) /sample were estimated from representative 17 nos. of samples and result is shown in Table-2. Results obtained from Table-2 revealed that soil sample LHU-19 collected from paddy crop exhibited maximum log value (5.49) of bacterial population and it was minimum (4.69) from samples of LHU-4, LHU-13 and LHU-14 collected from Paddy, fallow land and forest respectively.

Perusal of the result showed that a total 206 rhizobacteria was observed on media among which 81(39.32%) colonies were found round while 125 (60.68%) colonies were observed as irregular in shape. Maximum number of rhizobacteria was obtained from soil sample LHU-19 (31 nos.) followed by LHU-22 (24 nos.), LHU-18 (23 nos.) and LHU-11 & LHU-12 (20 nos.) whil minimum was recorded in sample LHU-13 (4 nos.). Round shaped rhizobacterial colony was found maximum from sample LHU-19 (14 nos.). Followed by LHU-12 (13 nos.) and LHU-18 (12 nos.) and it was minimum in sample LHU-4 (2 nos.). No round shaped bacterial colony was observed in soil sample from LHU-5, LHU-6, LHU-9, LHU-14 & LHU-22.

Similarly out of 125 irregular colony forming rhizobacteria, maximum was recorded from sample of LHU-22 (24 nos.) followed by LHU-19 (17 nos.) and LHU-11 (12 nos.) while it was minimum from soil samples LHU-4 & LHU-7 (3 nos.). No irregular shaped colony was found from isolates in sample of LHU-20 & LHU-13. Total 46 isolates were isolated from the selected colonies and used for further studies (Table-2). Among them, maximum isolates were obtained from sample LHU-18 & LHU-18 (6 nos.) followed by LHU-8, LHU-11 & LHU-12 (4 nos.) and minimum from the samples of LHU-5, LHU-9, LHU-13 & LHU-14 (1 no.). Dendrogram was formed by hiararchial cluster analysis to group the type of colonies from all soil samples and depicted in (Fig.1, Fig.2 &Fig. 3). A total number of forty six total bacteria were isolated from lateritic regions of West Bengal. Highest number of total bacterial population was observed from rhizosphere of Paddy followed by rhizosphere of forest soils. The root exudates of cucurbit rhizosphere harbor the highest no. bacterial population under lateritic region as compared to the rhizosphere of other crops.

Morphological characteristics of native rhizobacteria

Morphological observation of 46 isolates were made on colony color, shape, margin, elevation and opacity which was recorded after 72 hrs of incubation. Morphological parameters recorded are presented in Table-3. All isolates was also grouped into three cluster based on their colony shape using K-mean cluster (Table 4 and 5) and total three clusters were formed. Result from Table -3 revealed that among 46 bacterial isolates, 60.87% possessed irregular shape while 39.13% were found round shape. Maximum isolates (42.22%) showed lobate colony margin followed by entire margin (31.11%), curled (20.00%), undulate (4.44%) and filiform margin (2,22%). Colony colour of the isolates was varied in the following categories like off-white (28.88%),white (55.55%), yellowish white (8.88%), yellow (4.44%) and orange (2.22%). Elevation of colony among the isolates exhibited as raised (28.88%), flat (57.77%), convex (8.88%) and umbonate (4.44%).Among the collected isolates, 53.33% was found transparent while 46.66% was opaque.

Table 1: Locations of soil samples collected from different plant rhizosphere at Birbhum district of West Bengal

Block	Mauza	Longitude	Latitude	Soil Texture	Crop	Soil ID	pH
SURI-1	Thilpada village	87°31'54"	23°55'26"	Sandy loam	Cucumber	LHU1	5.7
SURI-1	Thilpada village	87°31'54"	23°55'26"	Sandy loam	Pumpkin	LHU2	6.2
SURI-1	Shaktipur	87˚52'27"	23˚93'25"	Sandy loam	Pointed gourd	LHU3	5.3
SURI-1	Shaktipur	87˚52'27"	23˚93'25"	Sandy loam	Paddy	LHU4	6.1
SURI-1	Abdarpur village	87˚33'04"	23˚53'53"	Sandy loam	Paddy	LHU5	6.5
BOLPUR	Gopalnagar	87˚37'48"	23˚38'32"	Clay loam	Pasture land	LHU6	6.8
ILLAMBAZAR	Borouilla (Forest)	87˚36'16"	23˚38'23"	Sandy loam	Forest	LHU7	5.2
ILLAMBAZAR	PayerIlamnagar (Sripur)	87˚31'10"	23˚38'43"	Sandy	Paddy	LHU8	5.6
DUBRAJPUR	Dakshin Chandipur (Birori)	87˚25'40"	23˚43'44"	Clay loam	Sugarcane	LHU9	4.9
DUBRAJPUR	Narayanpur Chirpai)	87˚28'7"	23˚48'59"	Loam soil	Colocasia	LHU10	6.3
DUBRAJPUR	Narayanpur Chirpai)	87˚28'7"	23˚48'59"	Sandy soil	Paddy	LHU11	6.4
SURI-2	Sekhampur	87˚59'19"	23˚82'28"	Sandy loam	Paddy	LHU12	5.7
SURI-2	Purandarpur	87˚57'58"	23˚86'01"	Clay loam	Fallow land	LHU13	5.4
SURI-1	Mallickpur	87˚51'04"	23˚87'68"	Sandy loam	Forest	LHU14	5.6
SURI-1	Mallickpur	87˚48'65"	23˚88'99"	Sandy loam	Paddy	LHU15	6.9
SURI-1	Karidhya G.P	87˚48'72"	23˚91'47"	Sandy loam	Grass	LHU16	6.4
SURI-1	Karidhya G.P	87˚48'72"	23˚91'46"	Clay loam	Pointed gourd	LHU17	5.7
SURI-1	Nagari	87˚46'05"	23˚91'44"	Clay loam	Paddy	LHU18	5.2
RAJNAGAR	Chandrapur	87˚40'28"	23˚91'78"	Sandy loam	Paddy	LHU19	4.8
RAJNAGAR	Chandrapur	87˚38'69"	23˚92'55"	Sandy loam	Paddy	LHU20	5.2
RAJNAGAR	Chandrapur	87˚38'63"	23˚92'59	Sandy loam	Grass	LHU21	6.6
RAJNAGAR	Chandrapur	87˚38'64"	23˚92'61"	Sandy loam	Forest (Sorajhar)	LHU22	5.5

Table 2: Number of rhizospheric bacterial colonies, round and irregular colonies per soil sample at the dilution of 10^{-5} and number of bacteria isolated from soil

Soil	Log value of bacterial population	Total No. of rhizobacteria	Round colonies	Irregular colonies	No. of isolates	Name of the isolates
LHU1	4.90	8	4	4	2	VE1, VE25
LHU4	4.69	5	2	3	2	VE22,VE24
LHU5	4.84	7	0	7	1	VE26
LHU6	4.77	6	0	6	2	VE8,VE11
LHU7	4.84	7	4	3	3	VE19,VE7,VE18
LHU8	5.00	10	4	6	4	VE14,VE20,VE21,VE10
LHU9	5.00	10	0	10	1	VE2
LHU11	5.30	20	8	12	4	VE15,VE31,VE12,VE33
LHU12	5.30	20	13	7	4	VE3,VE27,VE5,VE29
LHU13	4.69	4	4	0	1	VE6
LHU14	4.69	5	0	5	1	VE23
LHU15	5.00	10	4	6	2	VE30,VE16
LHU16	4.90	8	4	4	2	VE9,VE32
LHU18	5.36	23	12	11	6	VE13,VE34,VE28,VE36,VE42,VE43
LHU19	5.49	31	14	17	**6**	VE17,VE35,VE38,VE40,VE45,VE46
LHU20	5.38	8	8	0	3	VE4,VE37,VE41
LHU22	5.38	24	0	24	2	VE39.VE44
					46	

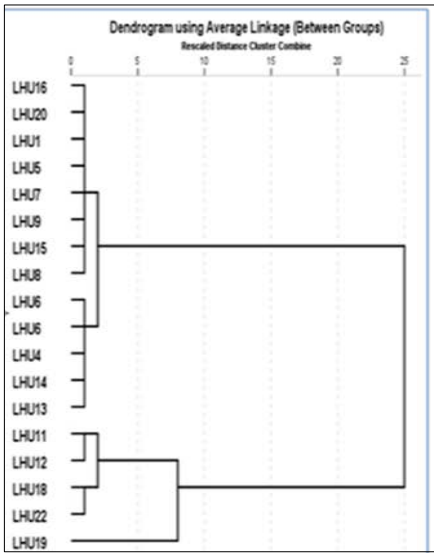

Fig 1: Totalrhizospheric bacteria

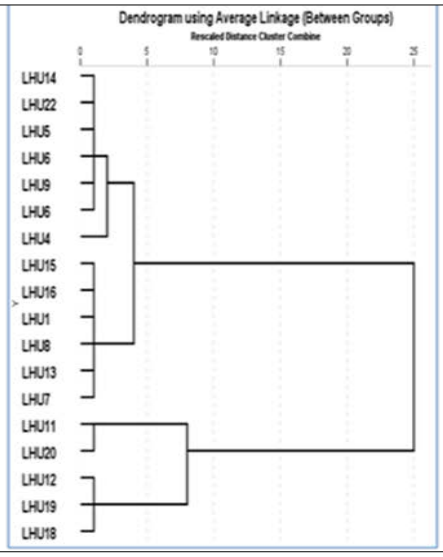

Fig 2: Total round colonies

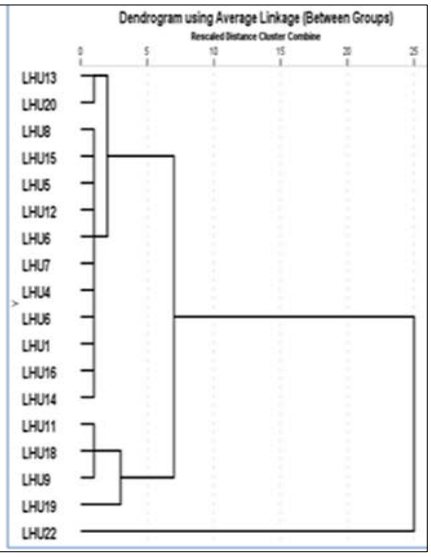

Fig 3: Total Irregular colonies

Table 3: Colony morphology and names of the bacterial isolates isolated from rhizospheric soil samples

Sl. No.	Isolate Name	Shape	Margin	Colour	Elevation	Opacity
1	VE1	irregular	Entire	Off-white	Raised	Opaque
2	VE2	irregular	Entire	white	Flat	Transparent
3	VE3	irregular	Lobate	white	Convex	Transparent
4	VE4	round	Lobate	Off-White	Flat	Opaque
5	VE5	irregular	entire	Off-White	Raised	Transparent
6	VE6	round	curled	white	Flat	Transparent
7	VE7	round	entire	Off -White	Flat	Transparent
8	VE8	irregular	curled	White	Flat	Opaque
9	VE9	irregular	entire	Off-white	Convex	Transparent
10	VE10	irregular	entire	Off-White	Flat	Opaque
11	VE11	irregular	lobate	Off-white	Flat	Transparent
12	VE12	round	Lobate	White	Raised	Opaque
13	VE13	round	lobate	Off-white	Umbonate	Opaque
14	VE14	round	curled	Orange	Flat	Opaque
15	VE15	irregular	lobate	Yellowish-white	Flat	Opaque
16	VE16	round	entire	White	Raised	Transparent
17	VE17	irregular	lobate	Off-white	Raised	Transparent
18	VE18	irregular	curled	Off-white	Flat	Opaque
19	VE19	irregular	lobate	white	Umbonate	Opaque

20	VE20	irrregular	entire	White	Raised	Transparent
21	VE21	round	lobate	white	Raised	Opaque
22	VE22	irregular	curled	White	Raised	Transparent
23	VE23	irregular	lobate	White	Flat	Transparent
24	VE24	irregular	undulate	Yellowish-white	Flat	Opaque
25	VE25	irregular	lobate	White	Flat	Transparent
26	VE26	irregular	entire	Yellow	Flat	Opaque
27	VE27	round	lobate	Yellow	Raised	Opaque
28	VE28	round	curled	White	Flat	Transparent
29	VE29	round	entire	White	Flat	Transparent
30	VE30	irregular	lobate	White	Flat	Transparent
31	VE31	round	filiform	Yellowish white	Flat	Opaque
32	VE32	round	curled	White	Flat	Transparent
33	VE33	irregular	lobate	White	Flat	Transparent
34	VE34	round	entire	White	Flat	Transparent
35	VE35	round	lobate	Yellowish white	Flat	Opaque
36	VE36	irregular	lobate	Off-White	Raised	Opaque
37	VE37	irregular	undulate	Off-White	Flat	Opaque
38	VE38	irregular	lobate	White	Convex	Transparent
39	VE39	irregular	curled	White	Flat	Transparent
40	VE40	irregular	lobate	white	Raised	Opaque
41	VE41	round	entire	Off-White	Raised	Opaque
42	VE42	irregular	lobate	White	Raised	Transparent
43	VE43	irregular	curled	White	Flat	Transparent
44	VE44	irregular	entire	white	Flat	Opaque
45	VE45	round	entire	White	Convex	Transparent
46	VE46	round	-	-	-	-

Table 4: K-mean clustering of soil samples based on the number of total rhizobacteria, round and irregular colonies

Soil	Clusters no.		
	Total rhizobacteria	Round	Irregular
LHU1	3	1	2
LHU4	3	3	2
LHU5	3	3	2
LHU6	3	3	2
LHU7	3	1	2
LHU8	3	1	2
LHU9	3	3	1
LHU11	2	1	1
LHU12	2	2	2
LHU13	3	1	2
LHU14	3	3	2
LHU15	3	1	2
LHU16	3	1	2
LHU18	2	2	1
LHU19	1	2	1
LHU20	3	1	2
LHU22	2	3	3

Table 5: Distribution pattern of isolates in each cluster

Cluster No.	Parameters	Total Bacteria	Round colonies	Irregular colonies
1	Range (SD±)	31	8-4 (1.85)	17-10 (3.11)
	Cluster center	31	5	12.5
	No. of soil	1	4	13
2	Range(SD±)	24-20 (2.06)	14-12 (1.00)	7-0 (2.29)
	Cluster center	21.75	13	3.92
	No. of soil	8	3	7
3	Range(SD±)	10-3 (2.55)	2-0 (0.76)	24
	Cluster center	6.77	0.29	24
	No. of soil	4	13	1

p^H tolerance activity

In the current experiment, pH tolerance activity of rhizospheric isolates was tested by culturing them in nutrient agar plates with various pH levels and subsequently measuring the growth of the bacteria. The growth area of the isolates was measured in different pH levels and analyzed.

Acidic pH tolerance (pH 4 – 6) of the rhizobacterial isolates were screened in nutrient agar media adjusted the pH with drops of 0.1 N HCl. The NA medium adjusted to pH level 4 to 6 was inoculated with different rhizobacteria for screening their acid tolerance potentiality. After incubation of 48hours at 28±1 °C, growth of the colonies was visualized and based on the growth, 1-3 rating was given to the isolates, 1 being the no growth, 2 being the moderate growth and 3 being good growth.

Dendogram generated based on hierarchical clustering could group 46 isolates of rhizospheric bacteria into two major groups on the basis of growth at pH 4 (Fig.4, Plate-1). Group A contained nineteen isolates with high level of pH tolerance at pH 4.0. Group B contained moderate and no acid tolerant

isolates. Sub-group B1contained eleven isolates with moderate level of acid tolerance at pH 4 and sub-group B2 contained sixteen isolates having no growth at pH 4.0. The result indicated that, although samples for the isolates were collected from different acid affected regions, their acid tolerance ability varies significantly. Increase in acidity outside the cell membrane increases the chance of destroying bacterial cell wall and membrane integrity which may be one of the major reasons for reduced growth of PGPR. The determinants of soil microorganisms are based on properties such as C and Na availability, organic matter content, water availability and pH (Bossio *et al*., 1998; Drenovsky *et al*., 2004; Garcia-Pausas and Paterson, 2011) [3, 5, 7] as well as bio-geographic patterns including soil type and seasonality (Kristin and Miranda, 2013) [10].

Discussion

In our present experiment, acid tolerance test was done under *in vitro* condition at pH level 4 to 6 with the 46 rhizospheric bacterial isolates from the lateritic region of six different blocks of Birbhum district of West Bengal. Among 46 bacterial isolates 41% isolates were found to be highly acid tolerant at pH level 4 that mean this isolates sustain under low pH conditions.

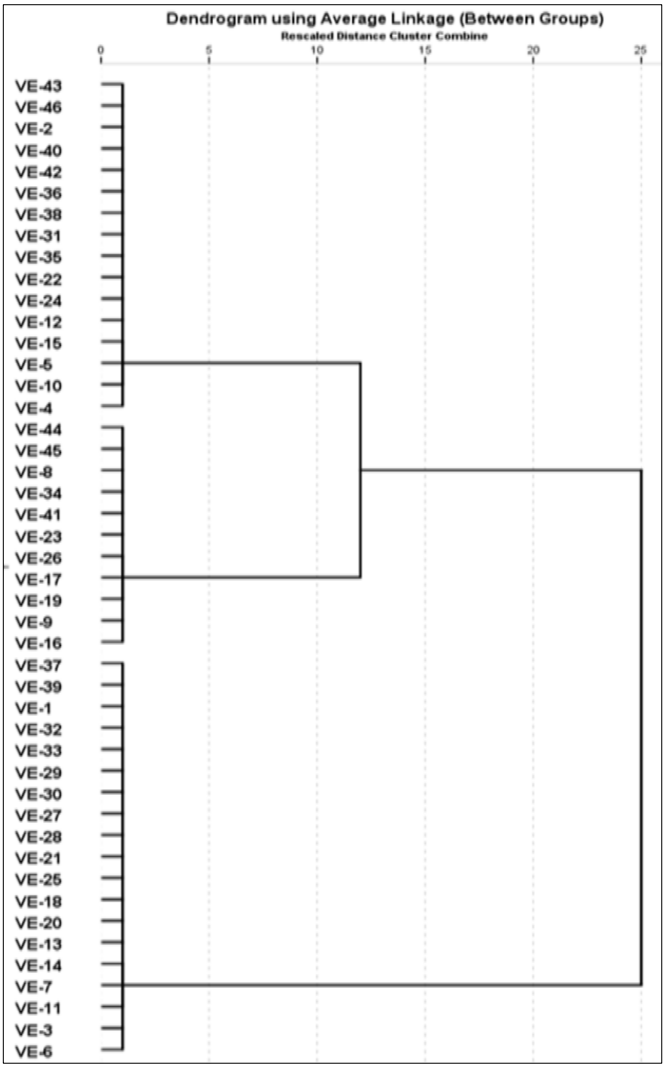

Fig 4: Distribution of isolated rhizospheric proteobacteria bacteria based on growth at pH=4

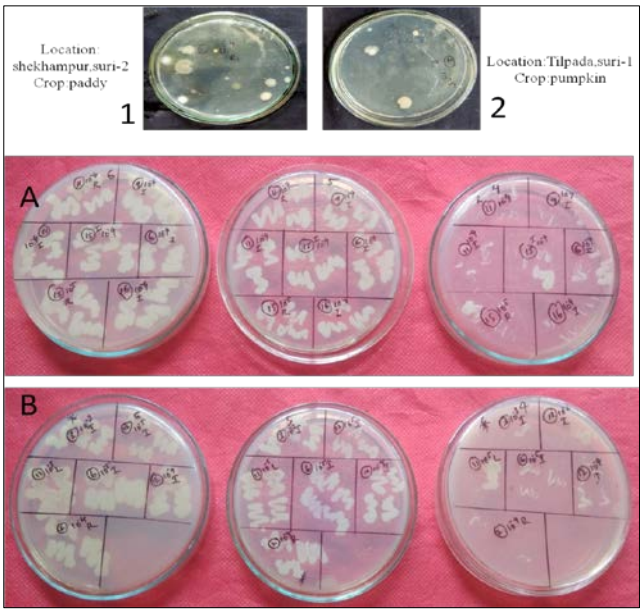

Plate 1: Isolation of rhizospheric proteobacteria from different soils (1 and 2). Activity of rhizospheric proteobacteria lisolates at different pH conditions (A-B) (pH=6, pH=5 and pH=4)

Conclusion

Research works on PGPR has already been reported that rhizosphere bacteria /or soil bacteria were found to play an important role towards plant growth promotion and enhanced crop production. But in problematic soil like red and lateritic belt of Birbhum district of West Bengal might be reclaimed through introduction of locally acclimatized, native P^H tolerant PGPR bacteria. Further research of antagonistic efficacy against different soil borne plant pathogen with the native rhizospheric proteobacteria and production of secondary metabolites responsible for antagonistic efficacy are in progress.

References

1. Backer R, Rokem JS, Ilangumaran G, Lamont J, Praslickova D, Ricci E, *et al*. Plant growth-promoting rhizobacteria: context, mechanisms of action, and roadmap to commercialization of biostimulants for sustainable agriculture. Frontiers in plant science. 2018;9:1473.
2. Bais HP, Weir TL, Perry LG, Gilroy S, Vivanco JM. The role of root exudates in rhizosphere interactions with plants and other organisms. Annu. Rev. Plant Biol. 2018;57:233-266.
3. Bossio DA, Scow KM, Gunapala N, Graham KJ. Determinants of soil microbial communities: effects of agricultural management, season, and soil type on phospholipid fatty acid profiles. Microbial ecology. 1998;36(1):1-12.
4. Di Benedetto NA, Campaniello D, Bevilacqua A, Cataldi MP, Sinigaglia M, Flagella Z, *et al*. Isolation, screening, and characterization of plant-growth-promoting bacteria from durum wheat rhizosphere to improve n and p nutrient use efficiency. Microorganisms. 2019;7(11):541.
5. Drenovsky RE, Vo D, Graham KJ, Scow KM. Soil water content and organic carbon availability are major determinants of soil microbial community composition. Microbial ecology. 2004;48(3):424-430.

6. Dutta J, Bora U. Role of PGPR for Alleviating Aluminum Toxicity in Acidic Soil. Plant Growth Promoting Rhizobacteria for Sustainable Stress Management. Berlin: Springer; c2019.
7. Garcia-Pausas J, Paterson E. Microbial community abundance and structure are determinants of soil organic matter mineralisation in the presence of labile carbon. Soil Biology and Biochemistry. 2011;43(8):1705-1713.
8. Holtz JD. Bergey's manual of determinative bacteriology, 9th edn. Williams and Wilkins, Baltimore; c1993.
9. Jones RT, Robeson MS, Lauber CL, Hamady M, Knight R, Fierer N. A comprehensive survey of soil acidobacterial diversity using pyrosequencing and clone library analyses. The ISME journal. 2009;3(4):442-453.
10. Kristin A, Miranda H. The root microbiota—a fingerprint in the soil. Plant and Soil. 2013;370(1):671-686.
11. Majeed A, Abbasi MK, Hameed S, Imran A, Rahim N. Isolation and characterization of plant growth-promoting rhizobacteria from wheat rhizosphere and their effect on plant growth promotion. Frontiers in microbiology. 2015;6:198.
12. Msimbira LA, Smith DL. The roles of plant growth promoting microbes in enhancing plant tolerance to acidity and alkalinity stresses. Frontiers in Sustainable Food Systems. 2020;4:106.
13. Nandi S, Adhikari A, Dutta S, Chattopadhaya A, Nath R. Potential effects of plant growth promoting rhizobacteria (*Pseudomonas fluorescens*) on cowpea seedling health and damping off disease control. African Journal of Biotechnology. 2013;12(15):1853-1861.
14. Rousk J, Baath E, Brookes PC, Lauber CL, Lozupone C, Caporaso JG, *et al*. Soil bacterial and fungal communities across a pH gradient in an arable soil. The ISME Journal. 2010;4(10):1340-1351.
15. Yirga C, Erkossa T, Agegnehu G. Soil Acidity Management. Addis Ababa: Ethiopian Institute of Agricultural Research; c2019.
16. Zhang J, Singh D, Guo C, Shang Y, Peng S. Rhizobia at Extremes of Acidity, Alkalinity, Salinity, and Temperature. Microbial Versatility in Varied Environments. Berlin: Springer; c2020.