International Journal of Experimental Research and Review (IJERR)

ISSN: 2455-4855 (Online) *Original Article*
Received: 29th June, 2017; **Accepted:** 15th August, 2017; **Published:** 30th August, 2017

DOI: https://doi.org/10.52756/ijerr.2017.v12.007

Exploring microbial community structure and their interrelationship in tomato Rhizosphere

Sangram Sinha
Department of Botany, Vivekananda Mahavidyalaya, Haripal, Hooghly, West Bengal, Pin code-712405, India

Author's E-mail: sinhasangramvm@gmail.com

Abstract

The Rhizosphere is the small zone surrounding plants' root surface is now considered as hot spot for microbial diversity and pivotal for plant-microbe interaction. The plant-microbe interaction is very vital for plant growth, productivity and stress tolerance. The present study attempted to explore the culturable microbial diversity in the tomato Rhizosphere from agricultural fields of Haripal block of West Bengal. The study found that the Rhizosphere is rich in gram-positive rods, and further biochemical characterisation predicted *Bacillus cereus* as the signature genus consisting of 26% of the total bacteria characterised in this study. Pearson’s correlation coefficient of different important adaptive characters of the bacterial population revealed strong correlations between salt tolerance, exo-polysaccharide (EPS) production, acid tolerance and phosphate solubilizing activity. These interactions may be crucial for Rhizosphere colonisation and overcoming hostile environment like salinity, drought, soil acidity and ultimately promote plant growth under diverse environmental stress.

Key words: *Bacillus cereus,* microbial diversity, Rhizosphere, Signature genus, tomato.

Introduction

Applying chemical fertilizers and pesticides in the agricultural fields to enhance productivity and control diseases has been a serious problem in recent years. It has increased the risk of environmental pollution and loss of soil fertility (Chen, 2006; Sarkar, 2017). Soil health has been increasingly affected by the use of agrochemicals with unfavourable alteration in soil pH, salinity and other beneficial parameters. In addition, chemical fertilizers and pesticides may lead to the extinction of beneficial soil microorganisms, which could hamper bio-geochemical cycling of essential nutrients (Aktar et al., 2009). Amidst this urgency of high productivity to meet the demand for food and restriction imposed on chemical fertilizers, the development and application of bio-fertilizers have been of tremendous importance. Many beneficial microorganisms reside in the Rhizosphere and improve plant growth in many different ways are termed plant growth-promoting Rhizosphere (PGPR) (Vacheron et al., 2013). Scientists worldwide are now pursuing excessive research on the development of effective biofertilizers for sustainable agricultural practice. Plant growth-promoting rhizobacteria (PGPR) has been proved as efficient biofertilizers (Vessey, 2003). Therefore, attempts must be made to formulate eco-friendly biofertilizer using PGPR to restore soil health.

Some microorganisms could produce plant growth promoting substances such as phytohormones like auxin, gibberreline, cytokonin, ethylene, and siderophores (Sinha and Mukherjee, 2008; Glick 2012). Microbes often convert insoluble mineral salts into soluble forms and, therefore, their easy absorption by plants. For example, phosphate solubilising bacteria promote plant growth by making phosphate available to the plants and hence could act as an alternative to phosphate fertilizers (Ahemad and Khan, 2012). These microbes exhibit their plant growth-promoting property either associated with plants' Rhizosphere or as free-living microbes. These beneficial microbes of the former type have been collectively termed plant growth-promoting rhizobacteria bacteria or simply PGPR (Kloepper et al., 1989). Biofertilizers are preparations containing living cells or latent cells of efficient strains of microorganisms (PGPR) that help crop plants uptake nutrients by their interactions in the Rhizosphere when applied through seed or soil (Smith et al., 2015). Tomato is a very important economic crop in tropical countries, and the application of PGPR in tomatoes has been reported to improve plant growth and yield in many studies (Narendra et al., 2015).

Therefore, microbiologists have used the tomato plant as a model plant to study plant-microbe interaction and growth promotion activities.

Nevertheless, the diversity of Rhizosphere microorganisms and their distinctive characters in plant-microbe interaction remained largely unexplored. However, few reports are published to underpin the microbiological community structure and their interaction in the tomato Rhizosphere (Lee et al., 2016). Microbial interaction is pivotal for proper colonisation and survival of the introduced biofertilizer strain. Therefore, the present work has been carried out to decipher the microbial community structure in the Rhizosphere of tomatoes and their mutual interaction before the formulation and application of microbial biofertilizer.

Materials and methods

Field study and analysis of soil

Soil samples from the plough layer (0-15cm) have been selected randomly in a zig-zag manner. The sample collected from the Rhizosphere of Tomato plants grown in agricultural fields of Haripal of Hooghly district of West Bengal, India mixed thoroughly in a container, ground and sieved. A representative sample, about 500 gm, has been taken out and air-dried under shade from this lot.

The air-dried sample was transferred into a clean cloth bag bearing a slip with a mention of complete address, field number, cropping sequence being followed, source of irrigation (tube well/canal), soil type (coarse-textured, fine-textured, alkali or waterlogged), fertilizer/ manure schedule followed in the preceding crops and disease incidence. The soil samples were then analyzed for different parameters such as salinity, pH, NPK content from Vivekananda Institute of Biotechnology, Nympith, West Bengal.

Isolation of bacteria from tomato Rhizosphere

Tomato plants grown in agricultural fields of Haripal of Hooghly district of West Bengal; India were uprooted carefully. One gram of the root adhering soil was taken to prepare soil suspension in sterilized distilled water for enumerating and characterizing the Rhizosphere bacterial population.

Enumeration of the total bacterial population

The total bacterial population was estimated using standard dilution plating technique in nutrient agar (NA) medium. Then, the colonies having distinctive features were counted and selected for further analysis.

Enumeration of salt-tolerant bacteria

Enumeration of salt-tolerant Rhizosphere bacteria was carried out following the conventional method. Briefly, one ml of the previously prepared soil suspension was plated on nutrient agar media supplemented with 0.5M NaCl by serial dilution and colonies that developed were observed after 72 h of incubation at 30^0C.

Enumeration of pH tolerant bacteria

Enumeration of pH tolerant Rhizosphere bacteria was carried out similarly as before in NA plates with a pH of 5. The number of colonies that appeared was recorded after 72 h of incubation at 30^0C.

Enumeration of exo-polysaccharide producing bacteria

One ml of the soil suspension was plated on yeast extract mannitol agar (Sayyed et al., 2015) through dilution plating, and the number of mucoid colonies that formed was detected as exo-polysaccharide producing bacteria (EPS) and counted.

Enumeration of temperature resistant bacteria

One ml of the soil suspension was plated on a nutrient agar medium through dilution plating, and the plates were incubated at 42^0C for three days. The appearance of the number of colonies was recorded.

Enumeration of phosphate solubilizing bacteria

One ml of the soil suspension was plated on Pikovskaya agar medium (Hi-Media, India). Colonies forming halo zone were detected as a positive test for phosphate solubilizing bacteria (PSB) and counted.

Morphological and Biochemical characterization of bacterial isolates

The morphological identification of the bacterial isolates was made based on colony characteristics such as shape, size, colour, margin and elevation that appeared on NA plates. Biochemical identification of the bacterial strains isolated from the Rhizosphere were carried out by Gram staining and standard biochemical tests according to Bergey' Manual of Determinative Bacteriology, 9th edition (Holt et al., 1994).

Statistical analysis

Pearson's correlation matrix of the physio-chemical characters of the Rhizosphere bacterial population was done and signified at a 1% probability level at (p= 0.01).

Result and Discussion

The soil survey of three mouzas, Hara, Raghubati and Narayanpur of Haripal block of North Hooghly District revealed that the soil

Table 1. Soil analysis of various Mouza.

BLOCK	MOUZA	pH	E.C. (dSm)	O.C. (%)	N (k/h)	P_2O_5 (k/h)	K_2O (k/h)
Haripal	Hara	5.69	0.21	0.22	141.9	162.91	242.74
Haripal	Raghubati	5.42	0.24	0.25	223.3	149.65	100.72
Haripal	Narayanpur	5.61	0.31	0.36	187.2	201.43	121.22

Table 2. Microbial response to environmental factors and EPS and PSB activities.

Block	Mouza	Total count (tc)	pH resistant	Salt resist.	EPS	PSB	Tem. Resistant
Haripal	Hara	$5x10^8$	$1.62x10^2$	$1.69x10^2$	$1.65x10^2$	$1.94x10^2$	13
Haripal	Raghubati	$21x10^8$	$9.77x10^2$	$9.54x10^2$	$6.45x10^2$	$8.91x10^2$	22
Haripal	Narayanpur	$11x10^8$	$8.31x10^2$	$16x10^2$	$12.58x10^2$	$1.28x10^2$	16

Table 3. Colony morphology of the isolated strains.

Shape	Colour	Texture	Elevation	Margin	Gram charac.	Spore form-ing	Moti-lity	Bacte-rial Iden-tity
Circular	Off white	Slimy	Flat	Irregular	+ve, Rod	+	+	*B.P.*
Round medium sized colony	Grey-white	Drying	Flat	Entire	+ve, Rod	+	_	*B.S.*
Large round colony	Grey-yellow	Creamy	flat	Lobed	+ve, Rod	+	+	*B.C.*
Circular	Off white	Slimy	Raised	Entire	+ve, Rod	+	+	*B.M.*
Circular	Colour-less	waxy	Flat	Entire	+ve, Rod	-	+	*A.C.*
Circular	transparent	creamy	convex	entire	-ve, Rod	-	+	*P.A.*
Circular	Light greenish yellow in sun light	creamy	convex	entire	-ve, Rod	-	+	*P.F.*
Circular	yellow	creamy	convex	entire	+ve, cocci in pairs	-	-	*M.*
Circular	Pale yellow	creamy	convex	entire	-ve, Coccobacilli	-	-	*A.*
Circular	White	creamy	Flat	entire	-ve, Rod	-	+	*E.*

B.P.: Bacillus polymyxa; B.S: Bacillus subtilis; B.C.: Bacillus cereus; B.M.: Bacillus megaterium; A.C.: Azotobacter chroococcum; P.A.: Pseudomonas aeruginosa; P.F.: Pseudomonas fluorescens; M.: Micrococcus; A.: Acinetobacter; E.: Enterobacter.

Table 4. Biochemical characterization of the isolates.

Bacte-Rial Iden-tity	Cata-lase	Oxid-ase	Amyl-ase	Prote-ase	Gelati-nase	Citrate	Gas production	Indole	MR/VP	Ure-ase
E.	+	-	+	+	-	+	+	-	-/+	-
B.P.	+	-	+	+	+	+	+	-	+/+	+
B.S.	+	+	+	+	+	+	-	-	-/+	-
B.C.	+	-	+	+	-	+	-	-	-/+	
B.M.	+	-	+	+	+	+	+	-	+/-	+
A.C.	+	-	+	-	-	+	+	-	+/-	-
P.A.	+	-	-	+	+	+	+	-	-/-	-
P.F.	+	-	-	-	+	+	-	-	-/-	-
M.	+	+	-	-	-	-	-	-	+/-	+
A.	+	-	+	-	-	+	-	-	-/-	-
+: xxx; ++: xxx; -: xx; +/-: xxx										
E.: Enterobacter; B.P.: Bacillus polymyxa; B.S: Bacillus subtilis; B.C.: Bacillus cereus; B.M.: Bacillus megaterium; A.C.: Azotobacter chroococcum; P.A.: Pseudomonas aeruginosa; P.F.: Pseudomonas fluorescens; M.: Micrococcus; A.: Acinetobacter.										

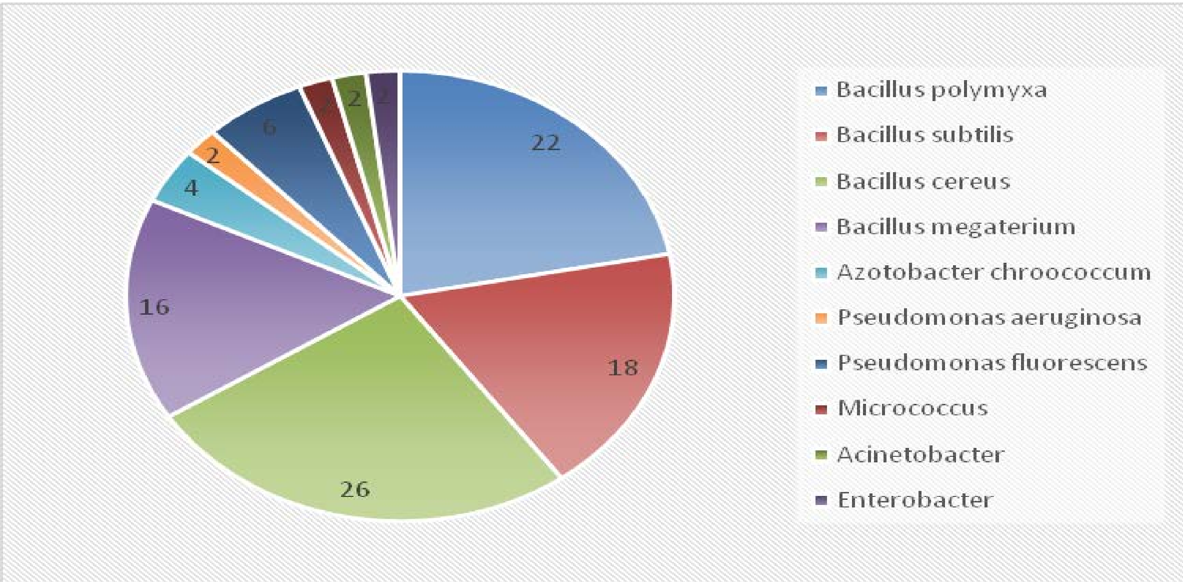

Fig 1. Diversity of tomato Rhizosphere bacteria in percentage.

Table 5. Pearson's correlation matrix of tomato Rhizosphere bacterial community.

	TC	*pH*	*Salt RST*	*EPS*	*PSB*	*TEM RST*
TC	1.000					
Ph	-0.143	1.00				
Salt RST	-0.117	0.977**	1.000			
EPS	-0.112	0.979**	0.974**	1.000		
PSB	-0.093	0.929**	0.956**	0.915**	1.000	
TEM RST	-0.082	0.321	0.380	0.255	0.229	1.000
The statistically significant correlations at p=0.01 are super scribed with double Asterix marks						

is slightly acidic and nitrogen, phosphorous and potassium (NPK) ratio are inconsistent with the nation's recommended NPK ratio of 4:2:1 (Table 1). The Rhizosphere soil collected from the tomato Rhizosphere were evaluated for total bacterial count (TC) by dilution plating technique in nutrient agar (NA) medium. The bacterial colonies that appeared on NA plates were calculated in colony-forming units. It was found that bacterial numbers varied slightly and ranging between $5x10^8$ to $21x10^8$, and the result is commensurable with the observation of other researchers (Torsvik et al., 2002). Bacterial isolates resistant to heat, salt stress, and pH were found to be much less than the total bacterial community. Microbial isolates were also found to produce exo-polysaccharides (EPS) and be able to solubilize inorganic phosphate. Microbial activities and response to environmental factors have been tabulated (Table 2) and found to possess some good plant growth-promoting characteristics phosphate solubilizing activities as reported earlier (Hazra and Pratiwi, 2013) and exo-polysaccharide synthesizing ability (Mu'minah et al., 2015). Pearson's correlation analysis (Table 5) showed that there exists a strong correlation between salt tolerance, EPS production, acid tolerance and phosphate solubilizing activity. Therefore, these interactions are probably the driving factors for Rhizosphere colonization in tomato plants. It has been reported in many studies that EPS production protects the microbes from the hostile environment like salinity, drought, soil acidity, as revealed from earlier works (Marvasi et al., 2010; Sandhya and Ali, 2015). Therefore, the present study corroborates with the earlier findings.

The bacterial isolates were characterised using bacterial colony characters (Table 3) and biochemical tests (Table 4). It was discovered that the majority of them (around 82%) (Fig. 1) belong to the *Bacillus* genus (Garbeva et al., 2003), with *Bacillus cereus* accounting for 26% of the total bacterial population identified in this study, followed by *Bacillus polymixa* (22%), *Bacillus subtilis* (18%), and *Bacillus megaterium* (16%)).The rest 18% comprised of the genera *Pseudomonas*, *Azotobacter*, *Acinetobacter*, *Micrococcus* and *Enterobacter*.

Conclusion

Rhizosphere has recently been identified as a hotspot for microbial diversity and a plethora of biotechnologically important microbes. Mining this microbial diversity may lead to the discovery of many agriculturally important microbes for sustainable agriculture. The present work aimed to study the culturable microbial diversity in the tomato Rhizosphere and found that *Bacillus* is the most predominant genera with phosphate solubilizing property and exo-polysaccharide synthesizing ability to protect the plant from bacterial pathogens and environmental stress. The Pearson's correlation coefficient of the physio-chemical properties of the bacterial population revealed that the community probably evolved strategies to combat hostile environments for perpetuating under stress.

Acknowledgement

The author is thankful to Department of Botany, Vivekananda Mahavidyalaya, Haripal, for providing laboratory facilities for this work.

Conflict of interest

The author declares that there is no conflict of interest.

References

Ahemad, M. and Khan, M. S. (2012) Evaluation of plant-growth-promoting activities of rhizobacter-ium, *Pseudomonas putida* under herbicide stress. *Annals of Microbiology*. 62: 1531–1540.

Aktar, M. W., Sengupta, D. and Chowdhury, A. (2009). Impact of pesticides use in agriculture: their benefits and hazards. *Interdiscipl. Toxicology*. 2(1): 1–12.

Chen, J. H. (2006). The combined use of chemical and organic fertilizers and/or biofertilizer for crop growth and soil fertility. Proceedings of International Workshop on Sustained Management of the Soil-Rhizosphere System for Efficient Crop Production and Fertilizer Use. Bangkok. Pp. 1-11.

Garbeva, P., van Veen, J. A. and van Elsas, J. D. 2003. Predominant *Bacillus*spp. in agricultural soil under different management regimes detected via PCR-DGGE. *Microbial Ecology*. 45 (3): 302-316.

Glick, B. R. (2012). Plant Growth-Promoting Bacteria: Mechanisms and Applications. *Scientifica*. PMID: 24278762.

Hazra, F. and Pratiwi, E. (2013). Isolation, Characterization, and Molecular Identification of Phosphate Solubilizing Bacteria from Several Tropical Soils. *Journal of Tropical Soils*. 18(1): 67.

Holt, J. G., Krieg, N. R., Sneath, P. H. A. and Williams, S. T. (1994). Bergey's Manual od Determinative Bacteriology, 9th ed. Baltimore, Md: Williams & Wilkins.

Kloepper, J. W., Lifshitz, R. and Zablotowicz, R. M. (1989). Free-living bacteria linocula for enhancing crop productivity. *Trends.Biotech*. 7: 39–43.

Lee, S. A., Park, J., Chu, B., Kim, J. M., Joa, J. H., Sang, M. K., Weon, H. Y. (2016). Comparative analysis of bacterial diversity in the rhizosphere of tomato by culture-dependent and -independent approaches. *Journal of Microbiology*. 54(12): 823–831.

Marvasi, M., Visscher, P. T. and Casillas, M. L. (2010). Exopolymeric substances (EPS) from *Bacillus subtilis*: polymers and genes encoding their synthesis. *FEMS Microbiology Letters*. 313(1): 1–9.

Mu'minah, B., Subair, H. and Fahruddin. (2015). Isolation and Screening Bacterial Exopolysaccharide (EPS) from Potato Rhizosphere in Highland and the Potential as a Producer Indole Acetic Acid (IAA). *Procedia Food Science*. 3: 74–81.

Narendra, B. A., Jogaiah, S., Ito, S., Kestur, N. A. and Tran, L. S. (2015). Improvement of growth, fruit weight and early blight disease protection of tomato plants by rhizosphere bacteria is correlated with their beneficial traits and induced biosynthesis of antioxidant peroxidase and polyphenol oxidase. *Plant Science: an International Journal of Experimental Plant Biology*. 231: 62–73.

Sandhya, V. and Ali, S. Z. (2015). The production of exo-polysaccharide by *Pseudomonas putida* GAP-P45 under various abiotic stress conditions and its role in soil aggregation. *Microbiol.*. 84: 512–519.

Sarkar, B. (2017). Traditional use of medicinal plants and its biodiversity in India. *International Journal of Experimental Research and Review*. 10: 23-26.

Sayyed, R. Z., Patel, P. R. and Shaikh, S. S. (2015). Plant growth promotion and root colonization by EPS producing

Enterobacter sp. RZS5 under heavy metal contaminated soil. *Indian Journal of Experimental Biology*. 53(2): 116-23.

Sinha, S. and Mukherjee, S. K. (2008). Cadmium-induced siderophо repro-duction by a high Cd-resistant bacterial strain relieved Cd toxicity inplants through root colonization. *Current Microbiology* 56(1): 55-60.

Smith, D. L., Praslickova, D. and Ilangumaran, G. (2015). Inter-organismal signalling and management of the phytomicrobiome. *Frontier in Plant Science*. 6: 722.

Torsvik, V., Ovreas, L. and Thingstad, T. F. (2002). Prokaryotic diversity: Magnitude, dynamics, and controlling factors. *Science*. 296: 1064–1066.

Vacheron, J., Desbrosses, G., Bouffaud, M. L., Touraine, B., Moënne-Loccoz, Y., Muller, D., Legendre, L., Wisniewski-Dyé, F. and Prigent-Combaret, C. (2013). Plant growth-promoting rhizobacteria and root system functioning. *Frontiers in Plant Science*. 4 (356): 1-19.

Vessey, J. K. (2003). Plant growth promoting rhizobacteria as biofertilizers. *Plant Soil*. 255: 571–586.