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Diversity and distribution of rhizospheric bacteria associated with Devil's cotton (*Abroma augusta* L.) along with alterations induced by the abiotic environment

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ABSTRACT

Recent research on the pharmacological aspects of Devil's cotton (*Abroma augusta* L.) plant has increased its impetus, notwithstanding its usage by the traditional practitioners, in face of its over-exploitation. We attempted a study of the association ecology of pedospheric bacteria associated with its rhizosphere. A total of 9 (nine) bacteria were isolated from rhizospheric soil collected from 8 natural locations of the Brahmaputra valley, distributed in 4 districts of Assam, India. The association between rhizospheric bacteria and fungal biota (including endo-mycorrhizae and non-mycorrhizal fungi) with abiotic soil properties can be exclusively advocated for a broader utilization as an ecological indicator for the conservation of target plant species as discussed in the present paper.

Keywords: Abiotic factors; Culture-dependent approach; Microbiome; Plant health; Rhizosphere.

1. INTRODUCTION

The rhizosphere, or the narrow zone of soil affected by the presence of plant roots [1], with respect to species richness and community size is considered one of the most diverse microbial habitats [2]. The bacterial community dominating rhizosphere by playing important roles in soil formation, biogeochemical cycling of carbon, nitrogen, phosphorus, and other elements [3], nutrient acquisition, protection against adverse biotic-abiotic factors, and in plant growth promotion through, for instance, the production of plant hormones [4, 5] affect plant health; apart from removal or degradation of toxic and/or recalcitrant organic contaminants [6, 7]. Although, researchers have displayed widespread interest in understanding the diversity and function of the rhizopsheric microbial communities, most studies regarding rhizosphere microbiome have centered on crop/ model plants. Consequently, the rhizosphere of wildly occurring plant species for the most part remains unknown [3].

Abroma augusta L. also known as the *Devils' cotton plant* (English) is a medicinally important plant included in the angiospermic family Malvaceae (previously under family Sterculiaceae), mainly used for the treatment of various types of disorder in the traditional systems of medicine [8-10]. The medicament utility coupled with its wild, limited distribution has led to overexploitation of this plant; which showcases the urgent need to conserve this plant species, both in *in situ* as well as *ex situ* conditions [9, 10]. In terms of vegetative morpho-taxonomy; it is evergreen, quick-, pubescent shrub or a small tree with spreading, velvety branches. It occurs naturally in tropical Asia, South and eastern Africa and Australia [11]; in both wild and cultivated areas, throughout the warm and moister parts of the Indian sub-continent ranging from Punjab and Uttar Pradesh eastwards to Arunachal Pradesh, Assam, Meghalaya and Tripura, ascending to 1,200 m, and southwards in peninsular India [12, 13]. Nonetheless, aside from studies of the arbuscular mycorrhizal [9] and non-mycorrhizal fungal communities [10] associated with this plant, little is known of the bacterial diversity of the other microbial communities present in its rhizosphere.

A World Health Organization (WHO) survey, depicts that 70-80% of the world population especially from developing countries rely on natural products of medicinal plants for their health care [14]. With regard to the origin of natural products, these are either produced by plants or their associated microbes, generally phyllospheric, rhizospheric or endophytic bacteria [15]. The aim of the present study was to analyze the bacterial diversity naturally present in the rhizosphere associated with wild *Abroma augusta* L.

2. MATERIALS AND METHODS

2.1. Rhizosphere soil sampling, processing and physico-chemical characteristics

Owing to the scattered distribution of *Abroma augusta* L. [9, 10]; sites from eight natural locations/provenances of Brahmaputra valley, *viz*.- Titabor, Borholla, Namrup, Nagamati, Kokilamukh, Kaziranga, Amsoi and Jagiroad varying in the anthropogenic interferences were used for the study. Rhizospheric soil was collected naturally occurring in these geographic locations and distributed in 4 districts *viz.* Jorhat, Dibrugarh, Golaghat and Nagaon of Assam state in India (latitude 24° 8′ to 24° 2′ N and longitude 89° 42′ to 96° 0′ E). The majority of rain fall (1800 mm to 3000 mm) in these regions occur during monsoon period i.e., March through May, the heaviest precipitation comes with the southwest monsoon, which arrives in June, stays through September, and often causes widespread and destructive flooding [16]. From each individual plant, rhizospheric soil samples (at least three samples) were taken by digging out a small amount of soil (500 g) close to plant roots up to the depth of 15-30cm and these samples were stored in sterilized polythene bags at 10ºC for further processing in the laboratory (maximum time between sampling and processing was 12 h) and physico-chemical analyses of soil.

The pH and soil temperature were measured for all soil samples using electronic digital pH meter (Eutech Instruments, Singapore) and soil thermometer (Jainco, India). Moisture content was determined by oven dry technique [17]. Organic carbon (%) estimation was done by Walkley-Black's method [18].

2.2. Isolation, identification and cultivation of bacteria

To ascertain the diversity of soil bacteria, qualitative analysis involving Warcup's soil plate method [19] and Waksman's soil dilution method [20] was used. Bacterial isolates were characterized using culture-dependent identification (morphology-based), Gram staining as well as biochemical reactions [21]. The isolates were observed using a microscope and were photo-micrographed using a camera.

2.3. Statistical analyses

All the data were analyzed statistically, Analysis of the diversity parameters with respect to Bacterial isolates/ species viz.-quantitative analysis such as density, frequency and abundance of rhizospheric soil myco-flora and diversity indices were computed based on standard methods and protocols [22, 23]. Pearson's coefficient of correlation was calculated to study the relationship

between different variables. For the statistical analyses, MS Excel 2007 was used.

3. RESULTS AND DISCUSSION

The physico-chemical parameters, viz. - *p*H, moisture content, soil temperature, electrical conductivity, humidity and organic carbon of all the locations where *A. augusta* was occured naturally have been discussed in our previous research work [9, 10] where the diversity and distribution of rhizospheric non-mycorrhizal myco-biota as well as arbuscular mycorrhizae associated with the target plant species was assessed.

Our present study revealed the presence of a total of 9 (nine) of 7 (seven) genera of bacteria (see Table 1; Plate 1), *viz*. - *Streptococcus* sp. 1, *Streptococcus* sp. 2, *Streptobacillus* sp., *Pseudomonas* sp., *Bacillus* sp. 1, *Bacillus* sp. 2, *Streptomyces* sp., *Serratia* sp. and *Micrococcus* sp. inhabiting the rhizopshere of *Abroma augusta* L. The morphological characteristics of bacterial isolates reported in the collected soil samples have been enumerated and discussed in Table 1. Habitat was observed to influence the occurrence of bacteria with maximum (8) occurrence of bacterial isolates at roadsides and minimum at foothills, fallows and riverine areas (1, each) (see Table 2). With respect to elevation (expressed in metres above sea level or m asl), maximum inhabitance of diverse bacteria were observed in the low elevation range, i.e. 50-80 m asl (23) with maximum (4) at 57 m asl with *Pseudomonas* sp. exhibiting maximum (8 elevation sites) variation in distribution (See Table 2, Figures 1-4).

Table 1. Morphological and biochemical characteristics of bacterial isolates in the collected soil samples of *Abroma augusta* L.

Sl. No.	Species	Colony morphology	Gram's reaction	Methyl red test	Catalase test	Oxidase test	Glucose fermentation test	Nitrate reduction test
1.	*Streptococcus* sp. 1	Flattened, depressed centre, entire, round, dull white	+	ca	-	ca	+	ca
2.	*Streptococcus* sp. 2	Flattened, depressed centre, wavy, lobate, white	+	ca	-	ca	+	ca
3.	*Streptobacillus* sp.	Pleomorphic, filamentous rod. Fusiform; develop characteristic lateral bulbar swellings	-	-	-	ca	+	ca
4.	*Pseudomonas* sp.	Round, translucent whitish, bright, button shaped colonies	-	-	+	+	-	+
5.	*Bacillus* sp. 1	Punctiform, irregular, opaque, whitish, raised	+	-	+	+	+	+
6.	*Bacillus* sp. 2	Punctiform, irregular, opaque, whitish, flat	+	-	+	-	+	+
7.	*Streptomyces* sp.	Clumpy, depressed, whitish colonies; Rods; form substrate and aerial mycelium	+	+	+	ca	ca	+
8.	*Serratia* sp.	Red coloured, round, irregular, elongated colonies	-	+	+	-	+	+
9.	*Micrococcus* sp.	White coloured, round, small	+	ca	+	+	-	+

N.B.: +: positive result, -: negative result, ca: could not be ascertained.

Table 2. Habitat - and elevation-wise natural occurrence and diversity of bacterial isolates in rhizosphere of *Abroma augusta* L.

Habitat							Elevation (m asl)															
Fallow land	**Foot hills**	**Forest fringe area**	**Paddy field**	**River side**	**Road side**	**Bacterial isolates**	**56**	**57**	**63**	**65**	**66**	**68**	**70**	**72**	**73**	**74**	**75**	**78**	**88**	**117**	**118**	**130**
					+	*Streptococcus* sp. 1		+		+						+						
+			+		+	*Streptococcus* sp. 2	+	+					+	+								
		+			+	*Streptobacillus* sp.					+	+	+		+							
		+	+		+	*Pseudomonas* sp.					+	+	+		+		+	+	+			
	+		+		+	*Bacillus* sp. 1											+		+	+	+	+
					+	*Bacillus* sp. 2		+		+						+						
				+		*Streptomyces* sp.			+													
					+	*Serratia* sp.		+														
					+	*Micrococcus* sp.				+												
1	1	2	3	1	8	Total	1	4	1	3	2	2	3	1	2	2	2	1	2	1	1	1

Table 3. Location wise natural occurrence, frequency and diversity indices of bacterial isolates in rhizosphere of *Abroma augusta* L.

Locations/ Sites	**Titabor**	**Borholla**	**Namrup**	**Nagamati**	**Kokilamukh**	**Kaziranga**	**Amsoi**	**Jagiroad**	**Frequency (%)**
Bacterial isolates									
Streptococcus sp. 1						+			6.25
Streptococcus sp. 2						+		+	18.75
Streptobacillus sp.							+		12.5
Pseudomonas sp.	+	+					+		18.75
Bacillus sp. 1		+	+	+					18.75
Bacillus sp. 2						+			6.25
Streptomyces sp.					+				6.25
Serratia sp.						+		+	6.25
Micrococcus sp.						+	+		6.25
Species richness (Unique)	0	2.5	1	1	0	3.4	3	1	
Diversity Index (Region wise)	0	0.02	0	0	0	0.03	0.07	0.02	

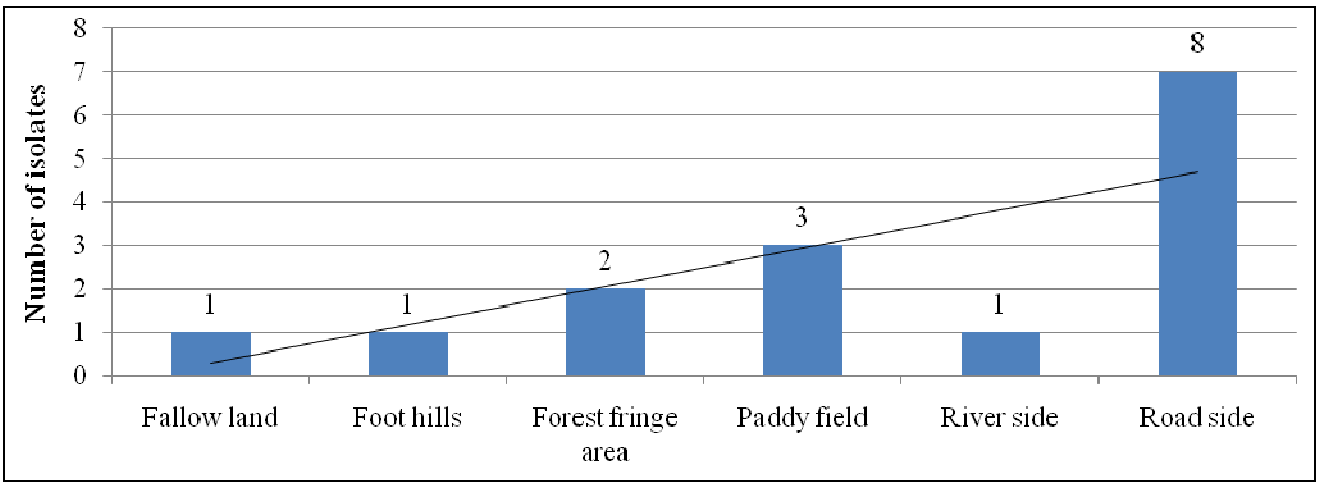

Figure 1. Habitat wise variation in bacterial isolates (on an average data).

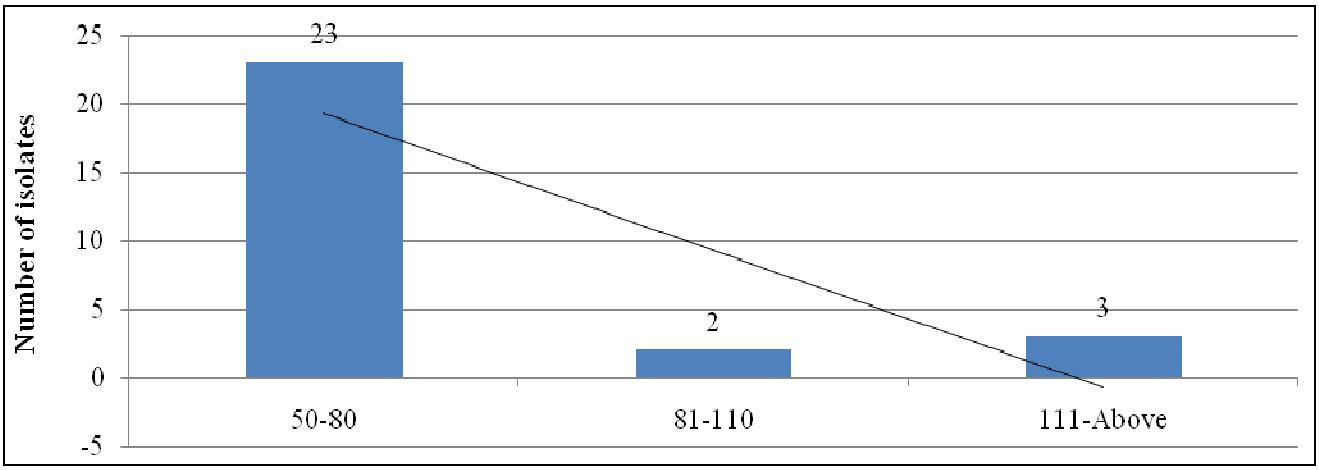

Figure 2. Elevation range wise variation in occurrence of bacterial isolates.

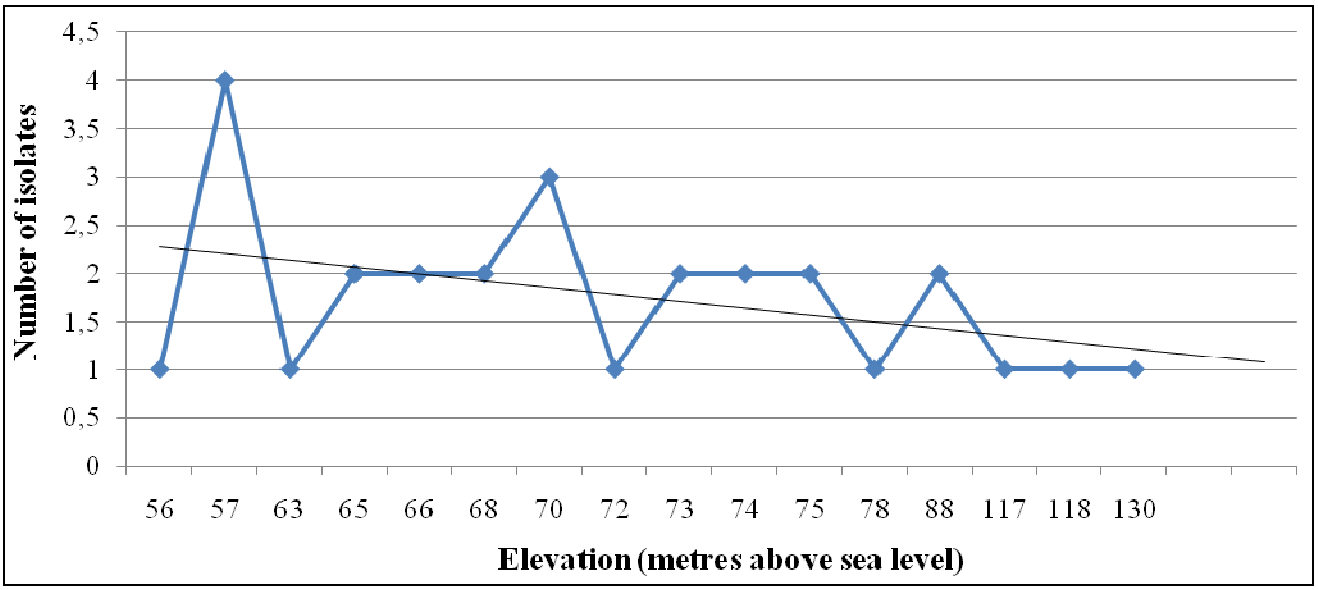

Figure 3. Elevation wise variation in bacterial isolates (on an average data).

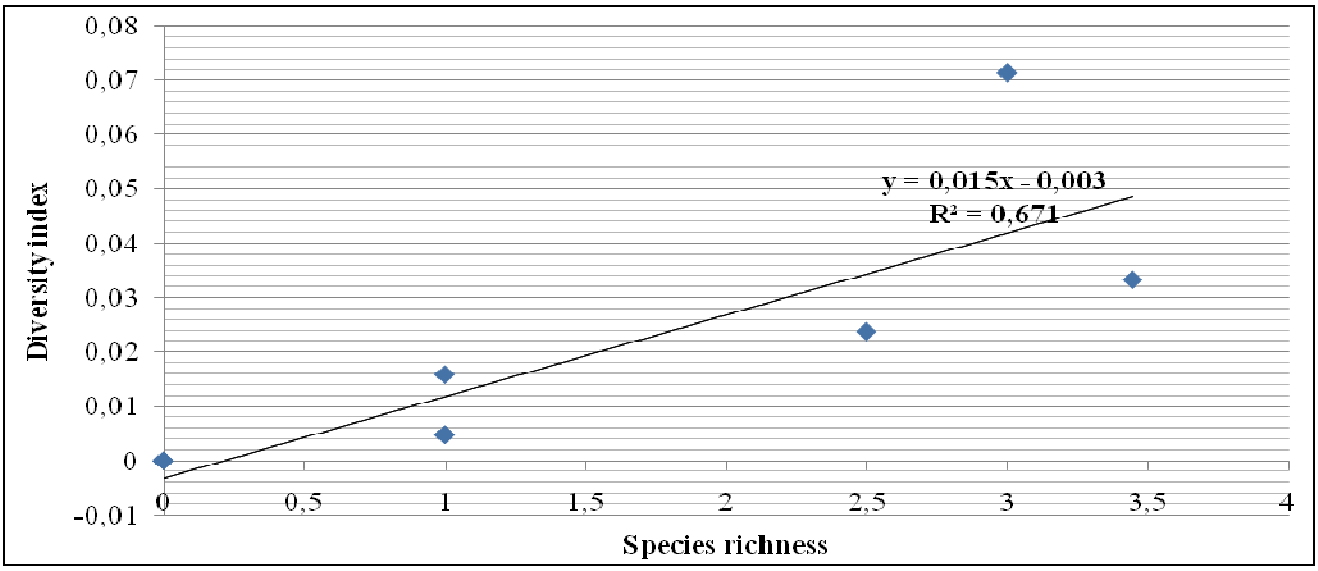

Figure 4. Scatter diagram - Species Richness *v/s* diversity Index.

The bacteria *Streptococcus* sp. 2, *Pseudomonas* sp. and *Bacillus* sp. 1 (3 isolates, each) were observed in larger numbers in contrast to *Streptococcus* sp. 1, *Bacillus* sp. 2, *Serratia* sp. and *Micrococcus* sp. (1 isolate, each). The natural occurrence (%) of bacterial isolates was maximum (18.75) in *Streptococcus* sp. 2, *Pseudomonas* sp. and *Bacillus* sp. 1; while it presented lower values (6.25) in *Streptococcus* sp.1, *Bacillus* sp. 2, *Streptomyces* sp., *Serratia* sp. and *Micrococcus* sp. the location Kaziranga exhibited greater diversity (5 genera) of bacteria, species richness (3.4) and diversity index (0.03); while Titabor and Kokilamukh had lower values (1,0,0 respectively) (see Table 3).

With regard to correlation studies between the abiotic factors (i.e. elevation, pH, electrical conductivity, temperature, organic carbon, humidity and moisture content) and biotic indices (Species richness and Diversity index); it was revealed that elevation and temperature had depressing effect on the bacterial communities, while electrical conductivity had constructive effect (see Table 4).

Table 4. Pearson's correlation matrix amongst various external environmental factors along with ecological indices of rhizospheric bacterial in study sites harboring *Abroma augusta* L.

	Elevation	pH	Electrical conductivity	Temperature	Organic Carbon	Humidity	Moisture content	Species richness	Diversity index
Elevation	1								
pH	-0.320	1							
Electrical conductivity	-0.716	0.091	1						
Temperature	0.071	-0.746	0.381	1					
Organic Carbon	0.039	0.181	-0.094	-0.583	1				
Humidity	-0.363	-0.299	0.720	0.712	-0.342	1			
Moisture content	-0.233	-0.255	0.643	0.562	-0.053	0.938	1		
Species richness	-0.138	-0.199	0.051	-0.058	0.206	0.180	0.296	1	
Diversity index	-0.426	0.129	0.127	-0.220	-0.057	-0.041	-0.059	0.775	1

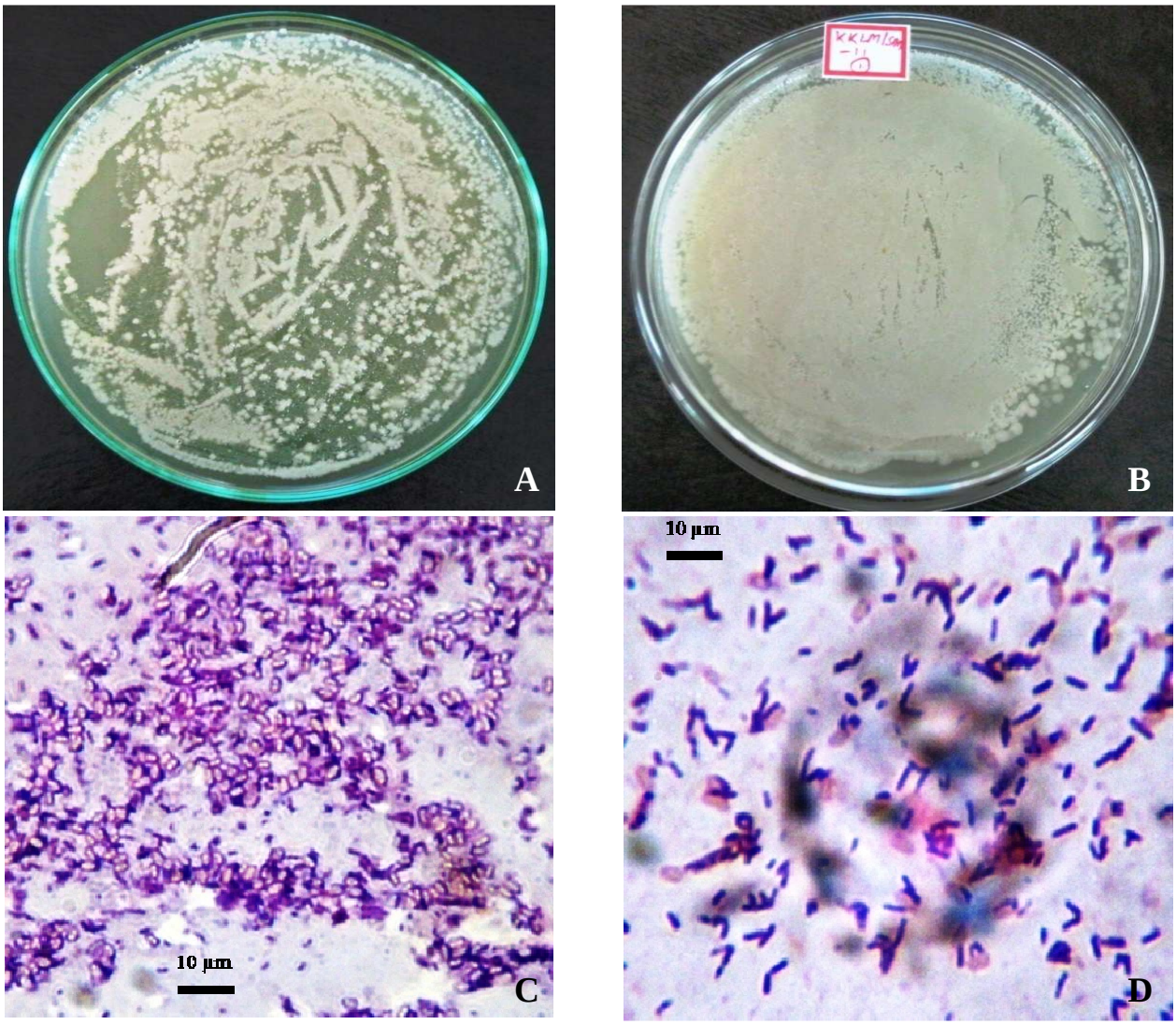

Plate 1. A: Colony of *Bacillus* sp., B: Colony of *Pseudomonas* sp., C: *Bacillus* sp., D: *Bacillus* sp.

In this work, the diversity within the rhizosphere bacterial community associated with the Devils' cotton plant species from the North-Eastern part of Indian sub-continent was explored using culture-dependent approaches. Since, the assembly of microbial communities in the rhizosphere can be affected by human activities such as the input of fertilizers and pesticides [3], sites varying in the anthropogenic interferences were also used for the study. In recent years, bioactive metabolites from medicinal plants have gained global attention. Bioactive metabolites are produced by medicinal plant or associated microbes. These bioactive metabolites are involved in symbiotic association with the host plant [24]. Bacteria produce bioactive metabolites exhibiting activities against phyto-pathogens as well as against bacteria, fungi, viruses, protozoans affecting humans and animals [15].

Out of the isolated, a *Pseudomonas* sp. (MSML/RFRI/Ps-1) was hypothesized as putative and multiplication was carried out through bacterial cultivation technique by using growth curve after specific time intervals of one hour [25]. The inoculum of bacteria was taken in stationery phase (10-11 hrs.) and has been tried for bio-inoculation experiments to assess their effect on accumulation of bioactive phyto-compunds, the data of which will be communicated in future by the authors.

4. CONCLUSIONS

This study confirmed the presence of certain bacteria in the rhizosphere of the target plant species; which can be further utilized for bio-inoculation studies; thereby, enhancing the conser-vation aspect of the plant. The association between

rhizospheric microbial biota including bacteria, endomycorrhizae and non-mycorrhizal fungi vis-à-vis abiotic soil properties can be exclusively advocated for a broader utilization as an ecological indicator for the conservation of target plant species.

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AUTHORS' CONTRIBUTION

VP has conceptualized the study and identified the bacterial isolates; VP and AJS have equally contributed in respect to survey, conduction of laboratory work and manuscript preparation. The final manuscript has been read and approved by both the authors.

TRANSPARENCY DECLARATION

The authors declare no conflicts of interest.

REFERENCES

1. Hrynkiewicz K, Baum C. The potential of rhizosphere microorganisms to promote the plant growth in disturbed soils. Environ Prot Strat Sustain Develop. 2011; 35-64.
2. Mendes R, Garbeva P, Raaijmakers JM. The rhizosphere microbiome: Significance of plant beneficial, plant pathogenic, and human pathogenic microorganisms. FEMS Microbiol Rev. 2013; 37: 634-663.
3. Philippot L, Raaijmakers JM, Lemanceau P, van der Putten WH. Going back to the roots: the microbial ecology of the rhizosphere. Nat Rev Microbiol. 2013; 11: 789-799.
4. Berendsen RL, Pieterse CMJ, Bakker PAHM. The rhizosphere microbiome and plant health. Trends Plant Sci. 2012; 17: 478-486.
5. Bulgarelli D, Schlaeppi K, Spaepen S, Ver Loren van Themaat E, Schulze-Lefert P. Structure and functions of the bacterial microbiota of plants. Annu Rev Plant Biol. 2013; 64: 807-838.
6. Joseph SJ, Hugenholtz P, Sangwan P, Osborne CA, Janssen PH. Laboratory cultivation of widespread and previously uncultured soil bacteria. Appl Environ Microbiol. 2003; 69: 7210-7215.
7. Davis KE, Joseph SJ, Janssen PH. Effects of growth medium, inoculum size, and incubation time on culturability and isolation of soil bacteria. Appl Env Microbiol. 2005; 71: 826-834.
8. Patel DK, Dhanabal SP. Determination of bioanalytical parameters for the standardization of *Abroma augusta*. J Acute Dis. 2013; 2(4): 292-295.
9. Parkash V, Saikia AJ, Bora D. Locational variability of physico-chemical properties of rhizospheric soil and myco-biota associated with *Abroma augusta* L. Adv Biores. 2014; 5(3): 156-165.
10. Parkash V, Saikia AJ. Habitational abiotic environmental factors alter arbuscular mycorrhizal composition, species richness and diversity index in *Abroma augusta* L. (Malvaceae) rhizosphere. Plant Pathol Quarant. 2015; 5(2): 98-120.
11. Das S, Datta R, Nandy S. Phytochemical screening and evaluation of anti-inflammatory activity of methanolic extract of *Abroma augusta* Linn. Asian Pac J Trop Dis. 2012; 2(S1): 114-117.
12. Anonymous. The Wealth of India. NISCAIR Press Publisher, New Delhi, 2006.
13. Gupta B, Nayak S, Solanki S. *Abroma augusta* Linn.: a review. Pharmacia Sinica. 2011; 2(4): 253-26.
14. Akerele O. Nature's medicinal bounty: don't throw it away. 14, World Health Forum, WHO, Geneva, 1993: 390-395.
15. Strobel G, Daisy B, Castillo U, Harper J. Natural products from endophytic microorganisms J Nat Prod. 2004; 67(2): 257-268.
16. Das H. Assam (state, India). Encyclopædia Britannica. http://www.brittannica.com/EB checked/ topic/39101/Assam. 2014 (Accessed on April, 2014).
17. Allen SE. Chemical analysis of ecological materials. Wiley & Sons, New York, 1974.
18. Walkley A, Black IA. An examination of the Degtjareff method for determining organic carbon in soils: effect of variations in digestion conditions and of inorganic soil constituents. Soil Sci. 1934; 63: 251-263.
19. Warcup JH. The soil pate method for isolation of fungi from soil. Nature. 1950; 166: 117-118.

20. Waksman SA. Principles of soil microbiology, Williams and Wilkins Co., Baltimore, Md, 1927: 897.
21. Brenner DJ, Krieg NR, Staley JT. (eds.) Bergey's Manual of Systematic Bacteriology, New York, NY: Springer, 1984: 284-289.
22. Curtis JT, McIntosh RP. The interrelations of certain analytic and synthetic phytosociological characters. Ecology. 1950; 31: 434-455.
23. Parkash V. Utilization of vesicular arbuscular mycorrhizal diversity for the quality stock production of some useful forest plant/s species of Nongkhyllem Reserve forest, Nongpoh, Meghalaya, India. Project Completion Report (RFRI-12/2008-09/SFM). Rain Forest Research Institute, Jorhat, Assam, India, 2012.
24. Strobel GA. Endophytes as source of bioactive products. Microb Infect. 2003; 5(6): 535-544.
25. Herbert D, Elsworth R, Telling RC. The continuous culture of bacteria; a theoretical and experimental study. J Gen Microbiol. 1956; 14: 601-622.