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RHIZOSPHERE OF *HOTTUYNIA CORDATA* AS A NICHE FOR PLANT GROWTH PROMOTING, ANTIBACTERIAL METABOLITES AND HYDROLYTIC ENZYME PRODUCING *PROTEOBACTERIA* AND *FIRMICUTES*

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ABSTRACT

Houttuynia cordata is a perennial aromatic medicinal plant which exhibits a wide range of pharmaceutical activities such as antibacterial, antiviral, anti-inflammatory, immunologic, anticancer, antioxidative and antimutagenic effects. In the present study, we investigated the diversity of culturable rhizospheric bacteria as well as endophytes from root and rhizome. A total of 183 morphologically different isolates were obtained, of which 21, 13 and 149 isolates were isolated from root, rhizome, and rhizosphere respectively. The isolates were characterized for various metabolic, plant growth promoting and other biotechnologically useful activities, based on which they were clustered into four groups by principal component analysis. The restriction fragment length polymorphisms analysis grouped all the isolates into 12 phylotypes and majority of the isolates were found to be associated with Proteobacteria and Firmicutes. Proteobacteria predominantly showed plant growth promoting and antibacterial activities, while Firmicutes constituted a higher proportion of hydrolytic enzyme producers.

***KEYWORDS**: Endophytes, Houttuynia Cordata, Rhizospheric Bacteria*

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INTRODUCTION

Houttuynia cordata Thunb. (*H. cordata*) is a sole member of Saururaceae, a family mainly distributed in Eastern Asia. It has long been used in North East India not only as an edible vegetable but also as traditional medicine. Different studies indicate that *H. cordata* contains abundant nutrients and active components such as volatile oils, flavonoids, and water-soluble polysaccharides (Li et al., 2004; Chen et al., 2004; Cao and Wang, 2005). Also, a wide range of pharmaceutical properties such as antibacterial, antiviral, anti-inflammatory, immunologic, anticancer, anti-oxidative, anti-mutagenic, etc has also been attributed to this herb (Chen et al., 2004).

Rhizosphere constitutes the soil just adhering to roots while endophytes are the organisms living within the plant tissues without causing any symptoms (Bednarek et al., 2010). It is now proved by various researchers that a complex physicochemical and biological interaction exists between plants and these rhizospheric bacteria and endophytes which can enhance plant growth by a wide variety of mechanisms like phosphate solubilization (Hayat et al., 2010), siderophore

production, biological nitrogen fixation, production of 1-aminocyclopropane-1-carboxylate deaminase (ACC), phytohormone production, etc. Furthermore, rhizobacteria can also suppress plant diseases and assist in nutrient cycling and stress tolerance through the production of enzymes and hormones (Hayat et al., 2010).

The main objective of this study was to explore the genetic diversity and metabolic potential of culturable bacterial community inhabiting the rhizospheric soil, roots, and rhizome of *H. cordata* of Assam, North East India.

MATERIALS AND METHODS

Isolation and Enumeration of Bacteria

Houttuynia plants were collected from three different districts of Assam (Figure. 1), *viz*. Kamrup (Guwahati), Dibrugarh and Jorhat district. The details of geographical locations and habitat from where samples were collected are given in Table 1. Rhizospheric soil was collected from the roots of 10 plants after removing loosely attached soil particles and pooled together. Bacteria were isolated by plating serial dilutions (10^{-3} to 10^{-6} in triplicate) of the soil sample prepared in saline water (0.85% w/v NaCl) on agar plates. Two different growth media used in the present study to isolate a broader population of culturable bacteria includes Nutrient Agar (HiMedia, Mumbai, India) and M9 media. M9 minimal medium was prepared by adding 10.2 g Na_2HPO_4, 3g KH_2PO_4, 0.6 g NaCl, 20 g NH_4Cl, 5 g glucose and 15 g agar to 1 liter of deionized water. Plates were incubated at 37˚C for 48 hrs and bacterial counts were expressed as colony forming units (cfu)/g fresh weight of soil. Non-rhizospheric bacterial count from the bulk soil was also determined as above for comparison.

Endophytic bacteria were isolated from roots and rhizome after surface sterilization (Figure. 2). Briefly, the roots and rhizomes were washed under tap water, sterilized with 1% (w/v) sodium hypochlorite followed by three washing in 70% (v/v) ethanol and finally rinsed with sterile saline. These were than homogenized in a mortar pestle and plated on nutrient agar (200µl/plate). Morphologically different colonies were selected, purified by re-streaking on the respective media on which they were isolated and characterized further for various enzyme activities.

Screening of Phylogenetic Diversity

Genomic DNA was isolated from all bacterial isolates according to Sambrook and Russell (2001). Amplification of the 16S rRNA gene was performed using the universal bacterial primers 8F (5'-AGAGTTTGATCCTTGGCTCAG-3') and 1492R (5'-GGTTACCTTGTTACGACTT -3') (Hayes and Lovley, 2002). PCR cocktail (50µl) contained 50 pM of primer, 50 ng of genomic DNA, 1X Taq DNA polymerase buffer, 1 U of Taq DNA polymerase (Promega, Madison, WI, USA), 0.2 mM of each dNTP, and 1.5 mM $MgCl_2$. Amplification was carried out with an initial denaturation at 94°C for 5 min, followed by 30 cycles of 30 s at 94°C, 30 s at 56°C and 1min at 72°C with a final extension at 72°C for 7 min. Amplification was checked by loading 5-µl of the PCR product in a 1.2 % agarose gel. The remaining PCR products were purified using Quick PCR purification column (Promega, Madison, WI, USA) and used for restriction fragment length polymorphisms analysis (RFLP). The purified PCR products were digested with *HinfH*1 and *MspI* (New England Biolabs, England) in separate reactions following the manufacturer's protocol. The digested product was run in a 2% agarose gel to determine the RFLP profile.

All the bacterial isolates were grouped together in phylotypes based on the RFLP analysis. The 16S rDNA amplified and digested fragments were scored manually for their presence (denoted as '1') or absence (denoted as '0') for

both restriction enzymes used. The binary matrix was used to estimate genetic diversity using Jaccard's coefficient [GSj=a/(a+b+c); Jaccard, 1908], where 'GS' is the measure of genetic similarity between individuals 'i' and 'j', 'a' is the number of polymorphic bands that are shared by 'I' and 'j', 'b' is the number of bands present in 'I' and absent in 'j', 'c' is the number of bands present in 'j' and absent in 'I'. The similarity matrix was subjected to UPGMA (Unweighted Pair Group Method of Arithmetic Averages) clustering method. All the above mentioned statistical analysis was performed using NTYSYS-pc software (version 2.02, Rohlf, 1998). The resultant dendrogram was used to establish the degree of similarity or genetic relatedness among all the bacterial isolates. All the isolates clustering together was considered as belonging to a single phylotype and one isolate from each phylotype was randomly selected as a representative of that phylotype and used for 16S rDNA sequencing. 16S rDNA sequencing was done in ABI 3130 automated DNA sequencer (Applied Biosystems, USA) and good quality sequences were selected and blasted using BlastN for searching the closest match sequence. The sequences were aligned pairwise using Clustal X (Thompson et al., 1994) and the phylogenetic tree was constructed under the Kimura two-parameter model and bootstrap analyses with 100 re-samplings were performed with MEGA 4.0. (Tamura et al., 2007). The sequences thus obtained were submitted to GeneBank.

Screening of Metabolic Diversity

The isolated cultures were analyzed for various metabolic activities including hydrolytic enzymes, plant growth promoting and other biotechnologically useful activities. Siderophore production was determined by Chrome Azurol S agar (CAS) assays (Alexander and Zuberer, 1991). Briefly, 5 µl of the overnight grown bacterial isolates (inoculum) was dropped onto the center of a CAS plate. After incubation at 28˚C for 48 hrs, siderophore production was assessed on the basis of change of color of the medium from blue to orange. Phosphate solubilizing ability of the bacterial isolates was determined by streaking on the Pikovskaya Agar medium (HiMedia, India). The plates after inoculation were incubated at 28˚C and the isolates that induced clear zone around the colonies were considered as positive (Katznelson and Bose, 1959). Protease production was determined by skimmed milk agar (Sullivan et al., 1991) where bacterial cells were spot inoculated and after 2 days of incubation at 28˚C, proteolytic activities were identified by clear zone around the cells.

The production of indole acetic acid (IAA) was determined according to Trivedi et al. (2011) with slight modifications. Briefly, nutrient broth containing 5 mM of tryptophan was inoculated with the isolates and after 48 hrs of incubation, IAA production was determined by the development of pink color with the addition of few drops of Salkowski reagent (12 g l^{-1} $FeCl_3$ in 7.9 M H_2SO_4). Urease production was determined by a visible color change from orange to pink after inoculating bacterial cells on Christenson's Urea broth (HiMedia, India). The production of cellulase was determined according to Kasana et al. 2008, by using carboxymethylcellulose (CMC) agar plates. 5 µl of overnight grown culture was spot plated on CMC agar plates and after 48 hrs of incubation at 28˚C, plates were flooded with Gram's iodine solution. Cellulase producing isolates showed distinct zone around the colonies within 3 to 5 minutes.

Amylase production was determined by spot inoculating bacterial isolates on nutrient agar media containing 2% starch and incubating plates at 28˚C for 48 hrs. Bacterial isolates showing clear zone after the addition of iodine solution were recorded as amylase positive. Nitrate reduction ability was determined by inoculating bacterial isolates on nitrate broth and after incubation at 28˚C for 48 hrs, 5 drops of the sulfanilic acid solution followed by 5 drops of the α-naphthylamine solution was added. The appearance of distinct red or pink color within a few minutes indicates nitrate reduction. The production of esterase was done by spot inoculation of overnight grown culture on Tween 20 agar media

and after 48 hrs of incubation, esterase activity was determined by clear zone due to hydrolysis of tween 80 around the colony. Production of lipase was determined by spot inoculation of overnight grown culture on tributyrin agar (HiMedia, India). After 48 hrs of incubation at 28˚C, bacterial isolates showing a clear zone around the colony was recorded positive for lipase production. Production of pectinase was determined by spot inoculation of overnight grown culture on MP-7 medium (HiMedia, India). After 48 hrs of incubation at 28˚C, bacterial isolates showing clear halo around the colony when flooded with 1% polysaccharide precipitant were recorded as positive. Nitrogen-fixing ability of isolates was determined by spot inoculating overnight grown culture on Jensens media (HiMedia, India). Growth on this nitrogen free Jensens media was recorded as positive for nitrogen-fixing ability.

Antimicrobial activity was tested against 4 different test pathogens namely, *Escherichia coli* (MTCC-40) and *Staphylococcus aureus* (MTCC-96), *Micrococcus luteus* (MTCC-1538) and *Pseudomonas aeroginosa* (MTCC-741). The test cultures were obtained from MTCC (Microbial Type Culture Collection), IMTECH, Chandigarh, India. The isolates were grown on nutrient agar (HiMedia, Mumbai, India) for 48 h after which the plates were overlaid with soft agar containing the test organism. The plates were further incubated at 37˚C for 24 h and then observed for inhibition of the test organisms.

Principal component analysis (PCA) was performed in XLStat-Pro 7.5 (Addinsoft, New York, USA) software. Results of the metabolic activities were coded as binary data (1 or 0) for this analysis. PCA was carried out to compare the isolated bacterial genera with respect to their metabolic potential. A Pearson (n) correlation matrix was used to perform PCA analysis.

RESULTS

Isolation of Bacteria

The endophytic and rhizospheric bacterial community of widely used ethnomedicinal plant *viz*., *H. cordata* of Assam was analyzed in this study. The average bacterial densities in the rhizospheric and bulk soils on nutrient agar medium were $3.29 \pm 0.18 \times 10^5$ and $1.21 \pm 0.45 \times 10^5$ cfu/g dry soil, respectively. Thus, *Houttuynia* showed a positive rhizosphere effect.

The rhizosphere soil yielded 141 isolates on nutrient agar (NA population) and 8 isolates on minimal medium (M9 population) (Figure. 3A). Of the total 141 NA population, 62, 43 and 36 isolates were obtained from Guwahati (GH), Dibrugarh (DB) and Jorhat (JR) respectively. Also, of the total 8 M9 population, 4, 3 and 1 isolates respectively were obtained from GH, DB, and JR. Similarly, 21 and 13 morphologically different endophytic isolates (EP) were obtained from roots and rhizome, respectively. Of the total 21 EP isolates from roots, 7, 8 and 6 isolates were obtained from GH, DB, and JR respectively. Similarly, of the total 13 EP isolates from the rhizome, 5, 5 and 3 isolate was obtained from GH, DB and JR respectively. Thus, a total of 183 isolates were obtained and analyzed.

Phylogentic Diversity of the Bacteria

Based on the RFLP analysis of 16S rRNA genes, the isolates were grouped into 12 phylotypes, of which M9, EP, and NA populations were represented by 6, 7 and 11 phylotypes respectively (Table 2). The 16S rDNA sequences obtained from a representative isolate of each phylotype were deposited to GeneBank (Accession numbers KJ021706 to KJ021717). Based on the sequence similarity search, the phylotypes were found to be affiliated to *Proteobacteria*, *Actinobacteria*, and

Firmicutes (Figure. 4). Two phylotypes each were found to be affiliated to *α-Proteobacteria* and *Actinobacteria*. Four phylotypes, each were affiliated to *γ-Proteobacteria* and *Firmicutes* respectively. The α-P*roteobacteria* mainly consisted of isolates belonging two genera i.e., *Rhizobium sp.* and *Mesorhizobium sp*. Fourteen isolates were grouped together in the phylotype consisting of *Rhizobium* species whereas 21 isolates were included in the phylotype *Mesorhizobium*. The *Firmicutes* mainly consisted of *Bacillus sp*ecies and *Gammaproteobacteria* mainly consisted of *Pseudomonas* species. Two *Actinobacterial* genera found in the present study was *Micromonospora* and *Microbacterium.*

Metabolic Diversity of Isolates

All the isolates were analyzed for various metabolic activities such as the production of hydrolytic enzymes, plant growth promoting activities and antimicrobial activity. The response of all isolates to metabolic activities is shown in figure 3b and 3c. Different PGP activities tested in the present study includes IAA production, nitrogen fixation, phosphate solubilizing and siderophore. Out of the total population, the majority of rhizospheric and EP population didn't show IAA production (Figure. 3b). But the majority of the isolates (both rhizospheric and EP) could reduce nitrate (46.4 %) and solubilize inorganic phosphates (30%) (Figure. 3b). Among various phylogenetic groups, *α-Proteobacteria* showed a high frequency of isolates with nitrate reduction (50%), siderophore production (30.6%) and IAA production (24.1%), phosphate solubilizing (56.4%) and nitrogen-fixing (41.9%) activities. *γ-Proteobacteria* showed a high number of nitrate reducing (48.6%), siderophore producing (36.8%) and nitrogen-fixing (42.1%) isolates. Firmicutes showed a more number of urease (92.1%), protease (96%), amylase (76.4%) and cellulase (60.7%) producing isolates.

Although endophytic bacteria constituted small fraction (18.5%) of the total isolates, the frequency of antimicrobial activity producing isolates was highest. A total of 5.3%, 19.4%, 22.1% and 21.4 % of the rhizospheric populations showed antibacterial activity against *P. aeroginosa, E. coli, S. aureus, M. luteus* respectively, whereas, 11.7%, 50%, 47.0% and 41.1% of EP bacterial population showed antibacterial activity against *P. aeroginosa, E. coli, S. aureus, M. luteus* respectively (Figure. 3c).

PCA was performed in order to graphically visualize metabolic diversity of the isolates (Figure. 6). The PCA type that was used during the computations is Pearson's correlation matrix. The plot shows that some metabolic traits are more closely correlated as compared to others (Figure. 6A). It should be noted here that most of the PGP related trait were grouped together whereas traits related to the production of biotechnologically important hydrolytic enzymes were grouped together. Also, the antibacterial activity against *M. luteus, E. coli* and *S. aureus* were more correlated than the antibacterial activity against *P. aeroginosa*. The closely grouped traits suggest that the majority of the isolates showed positive for those traits. Also, the score plots showed no major differences among the metabolic capabilities of the rhizospheric and endophytic populations (Figure. 6b).

DISCUSSIONS

The rhizosphere is the region that is influenced by the plant roots and is usually reported to have high microbial activity. Therefore, the bacterial community composition in the rhizosphere is important for the performance of the plant. Also, endophytic bacteria can promote plant growth by reducing the deleterious effects of plant pathogens through direct or indirect mechanisms. Bacteria can directly antagonize pathogens by competition for nutrients and production of allelochemicals and indirectly through the induction of systemic resistance (ISR) (Lugtenberg and Kamilova, 2009). In the present study, we have characterized the rhizospheric and endophytic bacteria from the root and rhizome of *H. cordata*.

Here, we not only describe the phylogenetic diversity of the bacterial community but also the metabolic diversity among the isolates. Hence, we opted for pure culture technique to isolate bacteria from rhizosphere, roots, and rhizome. The *Houttuynia* plant showed a positive rhizospheric effect with the average bacterial populations in the rhizospheric soil were $3.29 \pm 0.18 \times 10^5$ and bulk soilswere $1.21 \pm 0.45 \times 10^5$ cfu/g soil, respectively. The higher concentration of bacterial population observed in the rhizospheric soil compared to bulk soil may therefore, be due to the release of exudates by the roots of *H. cordata* plants that promotes bacterial growth. Similar types of results were also obtained by Kumar et al. (2012) while studying root-associated bacteria from a medicinal plant *Ajuga bracteosa*.

The RFLP based phylogenetic characterization of bacteria showed that rhizospheric isolates were phylogenetically more diverse than the endophytic isolates (Table 2) which is expected as the later require presence of specific adaptations. The isolates were found to be affiliated to *α-Proteobacteria*, *γ-Proteobacteria*, *Firmicutes*, and *Actinobacteria*. The *γ-Proteobacteria* constituted the most dominant phylum whereas *Actinobacteria* remains the least frequent phylum. This is similar with other reports which show higher frequency of Proteobacteria in the rhizosphere (Trivedi et al., 2011). The *α-Proteobacteria* was represented exclusively by two families: Sphingomonadaceae (1 phylotypes) and Rhizobiaceae (1 phylotypes). The strains of *Rhizobium* and *Sphingomonas* are known to interact positively with plant roots by producing IAA (Tsavkelova et al., 2007). The establishment of Sphingomonadaceae in the rhizosphere has also been shown to be promoted by plant root exudates (Haichar et al., 2008). Interestingly, *γ-proteobacteria* includes four phylotypes consisting of only *Pseudomonas* species. This is consistent with the earlier reports that plant root exudates promote the growth of motile bacteria with higher growth rate, especially Pseudomonas, in the rhizosphere (Gardener and Weller, 2001). *Pseudomonads* are the dominant group of bacteria that prefer living in close vicinity to the root or on the root surface and play a crucial role in soil health and plant development (Kloepper et al., 1980).

This group of bacteria has been reported to influence plant growth directly or indirectly. In the present study, four phylotypes (27.86% of total isolates) were affiliated to *Bacillus sp.* under genera *Firmicutes*. Some other reports have also shown a higher frequency of *γ-Proteobacteria* and *Firmicutes* in the rhizospheric soil in other parts of India (Haldar et al., 2011). It should be noted here that *Pseudomonas* and *Bacillus sp*. are the two most common plant growth promoting rhizobacteria that can enhance biomass, nitrogen and phosphorous uptake, and crop yield (Haldar et al., 2011). In the present study, it was interesting to find that the majority of the EP population was affiliated to *Pseudomonas sp.* and *Bacillus sp*. Endophytic *Bacillus* species have been shown to be predominant in several plant roots such as *Platycodon grandiflorum* (Islam et al., 2010). Endophytic *Pseudomonas* has been reported from the roots of many plants such as *Brassica napus* L. (Misko and Germida, 2002), *Calystegia soldanella* L. (Park et al., 2005), and *Daucus carota* L. (Surette et al., 2003). In this respect, we can safely state that the rhizospheric effect of *H. cordata* led to the selection of some useful groups of bacteria.

Main PGP activities of rhizobacteria include production of IAA which is a phytohormone regulating plant growth, siderophores which solubilize iron and make it available to plant, antibiotics which can inhibit pathogens, nitrogen fixation, and phosphate solubilization leading to increase in availability of the limiting nutrient phosphorus (Babalola, 2010). The isolates obtained in the current study were screened for various metabolic activities to understand their PGP and biotechnological potential. We found that *Proteobacteria* predominantly showed PGP and antibiotic activities, while *Firmicutes* constituted a higher proportion of hydrolytic enzyme producers (Figure. 5). Some isolates, especially the *α-Proteobacteria*, showed multiple PGP activities and might play a very important role in the growth and medicinal value of

Houttuynia cordata. *In vitro* culturing and inoculation of these isolates into the rhizosphere may improve the quality of *Houttuynia* essential oil.

We found 56.6 % of *α-Proteobacteria* were able to solubilize inorganic phosphates. Phosphorous deficiency is a major constraint to crop production. Plants absorb only inorganic form of phosphorous and the level of inorganic phosphorus is very low in the soil because most of the phosphorous are present as insoluble forms. Soil microorganisms transform the insoluble forms of phosphorus into soluble forms and thus influence the subsequent availability of phosphate to plant roots (Illumer and Schinner, 1995). Thus, the phosphates solubilizing isolates obtained in the present study could be used in future as biofertilizers for growing different crop plants in this region of the country. Other isolates showing interesting metabolic profile can be studied further for the selected activities. Since metabolic activities were analyzed qualitatively in this study, secondary screening can further be carried out to get quantitative information. This will help in confirming the potential for industrial application.

To facilitate the selection of potential isolates, a two-dimensional graphical projection via PCA was carried out. Several metabolic traits was found to be correlated with each other (Figure. 6a) Interestingly, we found that plant growth promoting related trait such as IAA production, nitrogen-fixing, siderophore production, phosphate solubilizing, etc., were correlated with each other. On the other hand different hydrolytic enzyme production, related traits such as protease, esterase, amylase, cellulase, etc was correlated to each other. The antimicrobial activities against all the three tested human pathogens also distributed closely. The metabolic traits grouping closely suggest that majority of the isolates were positive to all the traits grouping together. Thus, the PCA based correlation showed that we can group all the isolates into 4 major groups. Group I includes isolates showing positive for PGP traits such as phosphate solubilizing and siderophore production, group II includes isolates having antibacterial activity against selected test microbial pathogens, group III includes isolates producing industrially important enzymes such as protease, cellulase, lipase, etc and group IV includes isolates showing positive for urease activity. Therefore, the present study has also contributed towards the enrichment of microbial repository with many biotechnologically important cultures which are metabolically very versatile having multiple enzyme activities. Some of the isolates in the present study have very promising plant growth promoting activity and has shown to induce root and shoot growth in other crop plants (data unpublished). Thus, exploration of medicinal plant-associated microbes will help us to find novel biotechnologically important microbes.

CONCLUSIONS

The present study reports the presence of genetically and metabolically diverse bacterial population in the rhizosphere, root, and rhizome of the medicinal plant *Houttuynia cordata*. The genetic diversity analysis indicates rhizobacteria mostly belongs to *Proteobacteria*, *Actinobacteria*, and *Firmicutes*. The metabolic diversity indicates that the isolates produced hydrolytic enzymes, showed plant growth promoting and also antimicrobial activity. Proteobacteria predominantly showed plant growth promoting and antibiotic potential while Firmicutes constituted a higher proportion of hydrolytic enzymes producers.

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APPENDICES

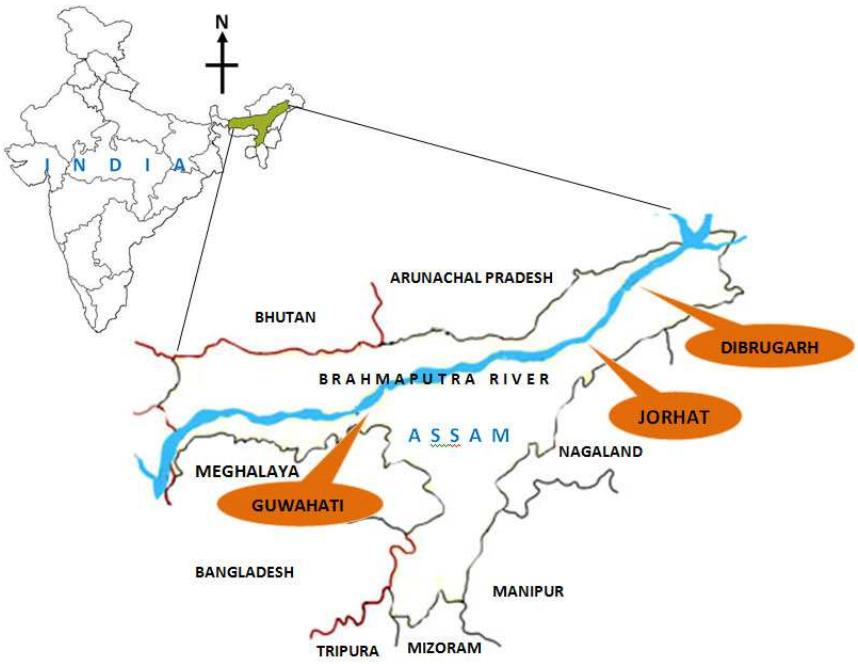


Figure 1

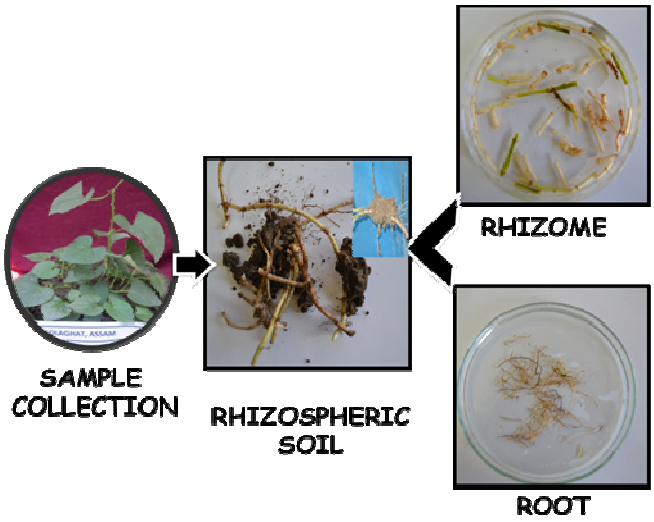


Figure 2

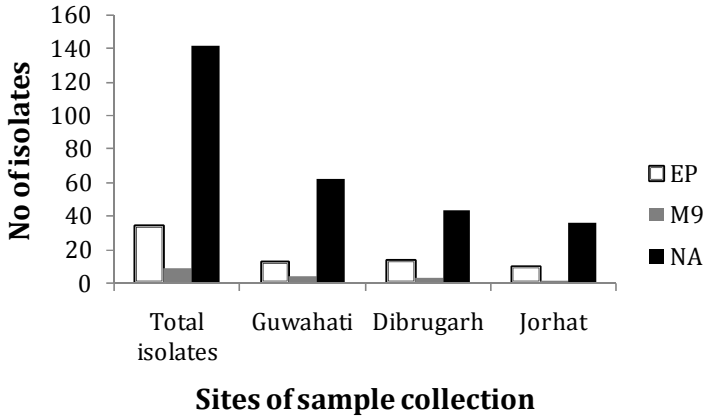


Figure 3

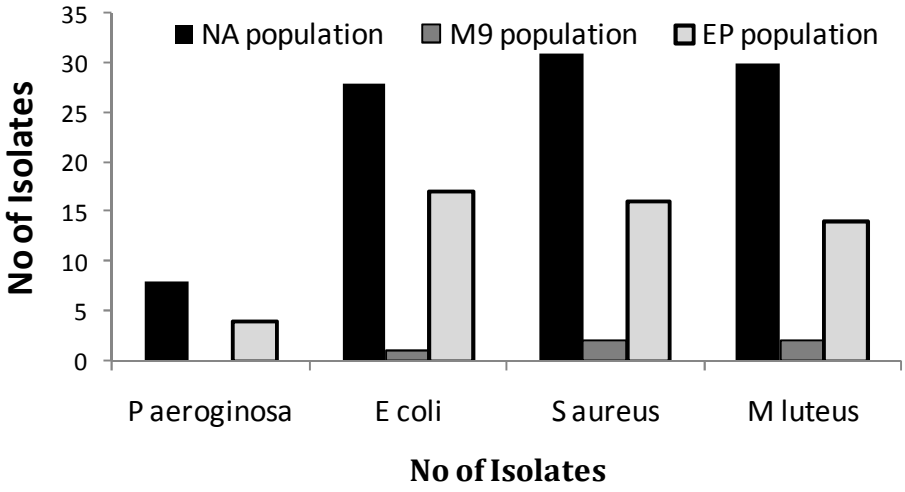
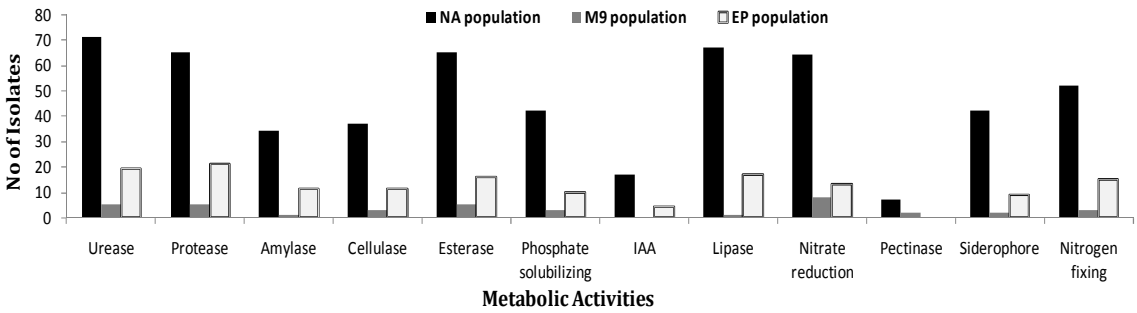


Figure 4



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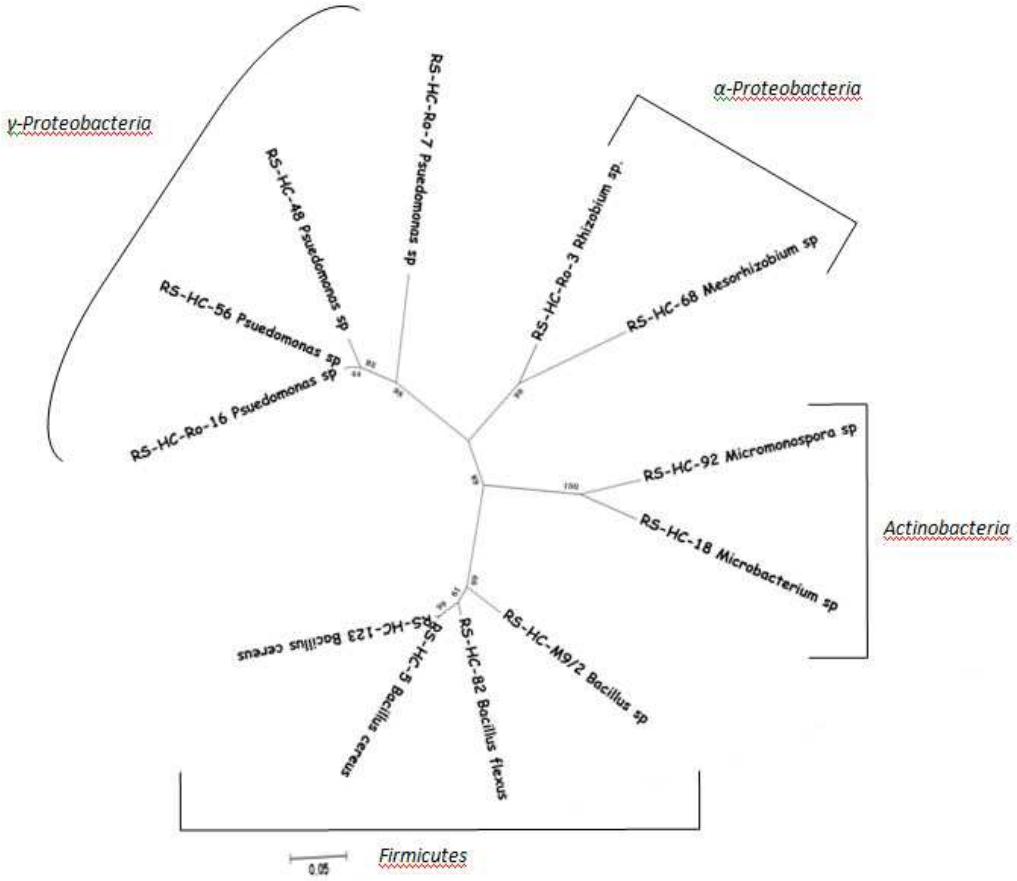

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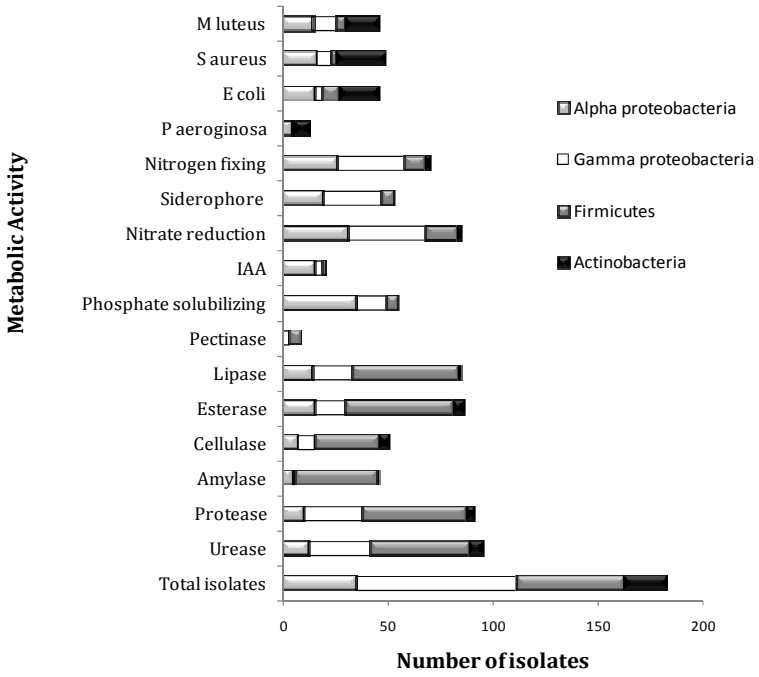

Figure 7

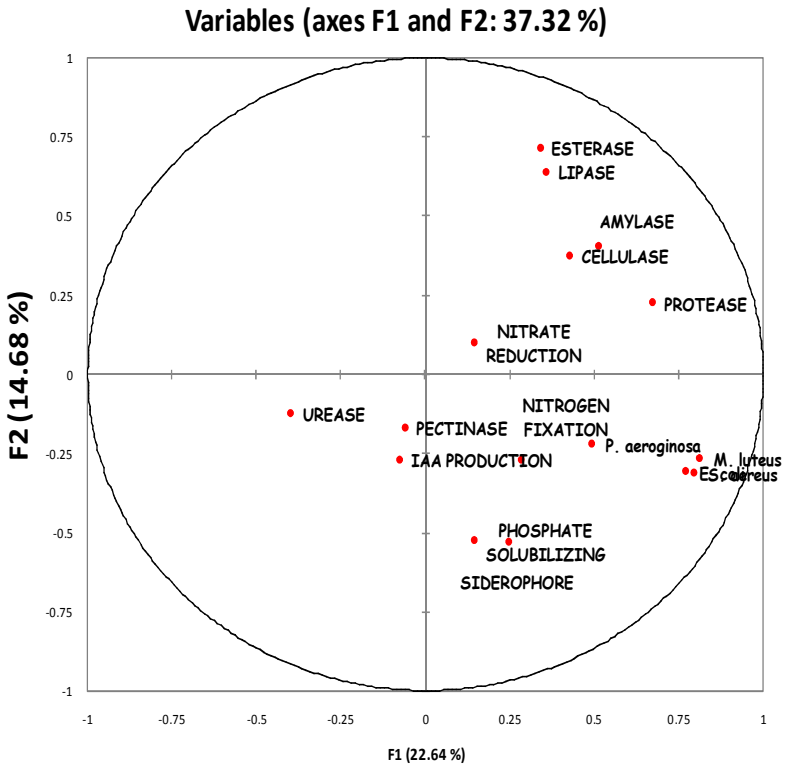

Figure 8

Figure Captions

Figure. 1. The geographical locations selected for collection of *H. cordata* Rhizospheric soil in the Brahmaputra valley of Assam, North East India. The sample was collected from three site ie., Guwahati, Jorhat and Dibrugarh.

Figure. 2. Rhizosphere soil, rhizome and root used for isolation of rhizosphere associated bacteria from *H. cordata*

Figure. 3. Isolation of bacteria from *H. cordata* rhizospheric soil and endophytes from rhizome and roots (a) Number of isolates obtained from the three sites selected in the present study. (b) Metabolic potential of all the isolates (c) Anti-microbial activity of all the isolates.

Figure. 4. Phylogenetic tree constructed from 16S rRNA genes of the representative isolates from *H. cordata* rhizosphere, roots and rhizome using Kimura two-parameter model performed with MEGA 4.0. Scale bar represents 0.05 changes per nucleotide position. Bootstrap values are shown near the nodes that they support.

Figure. 5. Metabolic activities and phylogentic affiliation of the isolates in the total population of 183 morphotypes isolated from *Houttuynia* roots and rhizosphere. The column of total isolates shows phylogenetic affiliation of all the isolates.

Figure. 6. Principal component analysis for metabolic profiles of bacteria isolated from *H. cordata* rhizosphere and endophytes isolated from root and rhizome.

Table 1: Details of Houttuynia Rhizosheric Soil Sample Collection Site

S. No	Collection Site	Geographical Locations	Habitat
1	Guwahati	26° 9'19.76"N 91°39'41.11"E	Botanical garden, growing wild
2	Jorhat	26°43'45.08"N 94°13'42.39"E	Near tea garden, cultivated
3	Dibrugarh	27°28'22.20"N 94°54'43.06"E	Near bamboo woods, cultivated

Table 2: Phylogenetic Affiliation of the Isolates from *H. Cordata* Rhizosphere, Roots And Rhizome Based On RFLP Based Clustering And 16S Rrna Gene Sequencing

Phylotype Representative (Genbank Accession)	No. of Isolates in Phylotype				Blastn Based Similarity Result (Genbank Accession)	Similarity (%)
	M9	EP	NA	Total		
Alphaproteobacteria						
RS-HC-Ro-3 (KJ021706)	1	1	12	14	*Rhizobium sp*. S5-405 (JQ660184)	99 %
RS-HC-68 (KJ021716)	0	0	21	21	*Mesorhizobium sp*. UPJMR1 (FJ965848)	100%
Gammaproteobacteria						
RS-HC-Ro-16 (KJ021712)	3	12	16	31	*Pseudomonas sp.* RN 8 (KF225787)	100 %
RS-HC-56 (KJ021713)	1	0	12	13	*Pseudomonas sp*. A1-R13 (JX994119)	100 %
RS-HC-48 (KJ021714)	0	0	27	27	*Pseudomonas sp*. AU_SW2_M (JN210574)	100 %
RS-HC-Ro-7(KJ021715)	0	3	2	5	*Pseudomonas sp*. A21-65 (KF220437)	100 %
Actinobacteria						
RS-HC-92 (KJ021707)	0	0	12	12	*Micromonospora sp* NEAUCF28(KC200157)	98 %
RS-HC-18 (KJ021708)	0	0	9	9	*Microbacterium sp* S8 (KF134543)	99 %
Firmicutes						
RS-HC-82 (KJ021717)	1	1	15	17	*Bacillus flexus* RN 6 (KF225786)	98 %
RS-HC-M9/2 (KJ021709)	0	2	6	8	*Bacillus sp*. AU_SR14 (JN210577)	99 %
RS-HC-5 (KJ021710)	1	8	9	18	*Bacillus cereus* JN244 (KF687092)	100 %
RS-HC-123 (KJ021711)	1	7	0	8	*Bacillus cereus* AR1 (KF668609)	100 %
Total Count	**8**	**34**	**141**	**183**		