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Endophytic bacterial community of rice (*Oryza sativa* L.) from coastal saline zone of West Bengal: 16S rRNA gene based metagenomics approach

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ABSTRACT

Rice is one of the most important cereal crops and a staple food for more than half of the world's population and West Bengal is its leading producer in India. But several abiotic conditions, like draught, soil salinity affect rice productivity. A novel approach using endophytic bacteria to ameliorate various stress conditions is gaining popularity for the betterment of agriculture. However, work on endophytic bacteria isolated from rice has not been properly evaluated in India. In this study we wanted to explore the diversity of bacterial endophytes inhabiting roots of rice plants growing in the Coastal saline zone of Sundarbans, West Bengal. This has been achieved through amplicon metagenomics of bacterial 16S rRNA gene in the IlluminaMiSeq platform. In our investigation endophytic bacterial community was exclusively dominated by the order *Rickettsiales* followed by *Enterobacteriales*. Our result identified the genera, some of whom have potential plant growth promotion (PGP) abilities as well as role in defense mechanisms of plants (viz. *Pantoea, Enterobacter, Paenibacillus*, etc.). Few genera (viz. *Aerinimonas, Arcobacter, Chitinophaga, Hydrogenispora, Sulfospirillum,* etc.) identified in our study have not been reported previously as endophytes and most probably are unique endophytes of this region. This study has enumerated the diversity of endophytic bacteria from rice grown in the saline zone of West Bengal that would help us to design a better strategy for cultivation under abiotic stress condition.

1. Introduction

In the last decade many researchers have established that plants serve as a host to a wide range of microorganisms namely bacteria, fungi, archaea and unicellular eukaryotes like algae and amoebae that inhabit the interiors of plant and are termed as endophytes (Compant et al., 2010; Hardoim et al., 2015). The presence of bacteria as endophytes were first recognized in the 19th century (Hardoim et al., 2015). Endophytic bacteria colonize plants mainly through roots but their occurrence has also been established in leaf, stem, reproductive organs as well as in fruits and seeds (Pirhadi et al., 2016; Dombrowski et al., 2017). Symbiotic endophytic bacteria could provide immense benefits to their host plant by promoting their growth via nutrient mobilization, synthesis of plant growth hormones and also helping them to combat other pathogens and pests (Compant et al., 2010; Pirhadi et al., 2016; Dombrowski et al., 2017). The relationship between an endophyte and its hosts is intricate and an elaborate study is necessary to interpret the association and mode of interaction. Endophyte selection is influenced by soil condition, composition and amount of root exudates by plants (Dimkpa et al., 2009). For better understanding of the mechanism of interaction between plants and endophytes, metagenomics approach along with other omics approach has paved a promising role in decoding the microbial composition (Meena et al., 2017). Understanding the community composition of bacterial endophytes is essential to generate a complete picture of endophytes intrinsic to their particular host. Hence, studying diversity becomes the first necessary step for identifying potential endophytes inhabiting the host to understand their functions and mode of interaction with host. Recently, next-generation sequencing approaches have paved a better way to decipher the bacterial communities than traditional fingerprinting and Sanger sequencing methods (Reinhold-Hurek & Hurek, 2011; Tian et al., 2015). While, numerous studies have been conducted across the world, studies from India on understanding of endophytic bacterial communities are rare (Hardoim et al., 2015; Reinhold-Hurek & Hurek, 2011; Bulgarelli et al., 2013). So far, investigations from India focus primarily on culture dependent isolation of endophytes and conducting bioassays to investigate their role in biocontrol against important pests and diseases (Pirhadi et al., 2016; Nagendran et al., 2014) except few notable exceptions who have studied diversity (Sengupta et al., 2017; Chaudhry et al., 2016).

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In spite of the huge economic and agricultural importance of rice in India, its endophytic bacterial community has not been explored extensively. Bacterial endophytes of rice plants benefit their host by fixing nitrogen, regulating phytohormones production, solubilizing phosphate, producing siderophores, increasing water utilization efficiency, reducing sulphate, oxidizing ammonia and inducing systemic resistance in plants thereby stimulating plant growth as well as contributing to sustainable rice production (Hardoim et al., 2011a; del Castillo et al., 2015). Till date there are no such reports on endophytic bacterial diversity of rice from saline regions of India. Rice is grown in all the six agro ecological zones of West Bengal, including the Coastal saline zone. West Bengal possess the highest area of coastal saline lands in India and one of the most important problems in coastal regions is the high salt salinity (Bandyopadhyay et al., 2003). Among other abiotic stresses, soil salinity is a major impediment for rice production (Yaish et al., 2016). Salinity not only has profound effects on vegetative growth by affecting photosynthesis, inhibiting plant growth but also hampers the reproductive development of plants. Use of potential microorganisms to ameliorate salt stress and improve plant growth is a low cost beneficial strategy in recent times (Shrivastava & Kumar, 2015). It is reported that endophytic bacteria play a pivotal role and assist the host in coping up with environmental stresses like salinity (Pirhadi et al., 2016; Yaish et al., 2016; El-Awady et al., 2015). To enhance our knowledge on how endophytes mitigate stress it is essential to decipher the bacterial communities inhabiting these hosts. Since symbiotic interaction of endophytes with their hosts also depends on specific habitat. Thereby understanding the microbiome composition will help to decode the mechanism by which microorganism influence plant salt resistance (Yuan et al., 2016). Therefore, this paper aims to investigate the diversity of rice root inhabiting bacteria in saline coastal zone of Sunderbans, South 24 Parganas, West Bengal using high throughput sequencing of their 16S rRNA gene on IlluminaMiseq platform.

2. Material and methods

2.1. Site description and sample collection

Rice plant samples (cultivar WGL20471, locally known as LalMiniket) at their vegetative stage were collected in Spring season (March) from four rice fields labelled as SPI1, SPI2, SPII4 and SPII5 at two different sites, Kasarichak Mirpara (site1: N 21.96922°, E 088.33285°) and Bahirchak (site2: N 21.96933, E 088.34173°) in PatharPratima, Coastal saline zone of Sunderbans, South 24 Parganas, West Bengal (Table 1). From each field 3 plants were sampled. The plants were dug out carefully to prevent any damage to the roots. Immediately after collection the samples were kept in autoclaved plastic bags (Himedia) placed on ice and brought back to the laboratory for further processing within 24 h. The plants from each field were pooled together and a representative sample was selected for each of the four fields. The salinity status of the soil which is indicated as a measure of the electrical conductivity (EC) of the saturation extract (EC_e) in the root zone is also mentioned in Table 1. For a soil to qualify as a saline soil a value greater than 4 dS m^{-1} at 25 °C is generally taken into account (Shrivastava & Kumar, 2015).

Table 1
Details of sampling information and salinity status of the soil.

Site ID	Sample ID	Location		
		Name of the site	GPS data	EC_e (dS m^{-1})
Site 1	SPI1	Kasarichak Mir Para Field - 1	21.96922°, 088.33285°	9.63
	SPI2	Kasarichak Mir Para Field - 2	21.96922°, 088.33285°	10.44
Site 2	SPII4	Bahirchak Field - 1	21.96933°, 088.34173°	13.02
	SPII5	Bahirchak Field - 2	21.96933°, 088.34173°	14.99

2.2. Surface sterilization and metagenome extraction

The roots were surface sterilized following the protocol by Sessitch et al., (2012) with few modifications (Sessitsch et al., 2012). Briefly, the adhering soil particles of the roots were removed by washing thoroughly under tap water then with sterile distilled water followed by 0.1% tween 20 solutions. Surface sterilization was done by shaking the roots with 75% ethanol for 2 min followed by sodium hypochlorite (4% available chlorine) for 2 min and repeatedly washed with sterile distilled water to remove traces of hypochlorite. For sterility check, 100 µl of the last washed water was plated on nutrient agar plates and incubated at 30 °C for 4 days to observe growth of any microorganism. The surface sterilized roots were frozen with liquid nitrogen and grounded to a fine powder using sterile mortar and pestle. Metagenomic DNA was extracted in duplicates using Power Plant Pro DNA isolation kit (Mo Bio) following manufacturer's instructions.

2.3. Amplicon metagenomic sequencing

The replicated metagenomic DNA were pooled and the hypervariable V3-V4 regions of the bacterial 16S rRNA gene were amplified using universal primers 341F (GCCTACGGGNGGCWGCAG) and 806R (ACTACHVGGGTATCTAATCC) to generate amplicon library using Nextera XT Index kit (Illuminainc.) and Nextera XT DNA Library Prep Kit (Part # 15044223 Rev. B). The sequencing was performed on Illumina Miseq platform in a 2 × 300 bp paired-end run. The raw paired-end primer trimmed sequences were provided by Eurofins, Germany. Although the library was prepared with bacterial specific universal primers we know that still there is a chance of contamination with mitochondrial /and chloroplast DNA in this amplified samples. Therefore, other strategies were adapted further to exclude these contaminations.

2.4. Sequence processing

For all the samples the raw FastQ dataset (R1- forward read & R2-reverse read) were processed following Hassenruck et al. (Hassenrück et al., 2016) protocol. Sequences were trimmed based on minimum quality score 15 and window size of 4 bases by using trimmomatic v0.32. Then trimmed sequences were merged using PEAR v0.9.5 and OTU (operational taxonomic unit) clustering was performed using swarm v2.0. The singleton removed OTUs were taxonomically assigned using SINA (SILVA Incremental Aligner) v1.2.11 of the SILVA rRNA project reference database (release 128) with a minimum similarity alignment of 0.9 and a last common ancestor consensus of 0.7. The data obtained were further curated to exclude the OTUs that showed affiliation towards mitochondria/ chloroplast/ eukaryotic DNA using well standardised *R* scripts.

2.5. Statistical analysis

In order to estimate species richness and evenness of the Bacterial community composition in each of the samples, α-Diversity indices were calculated. The α-Diversity indices represented by OTU number, Chao 1 estimator, Shannon diversity index and inverse Simpson diversity index were measured using repeated random subsampling of the amplicon sequence datasets. All statistical analysis were performed in R using the core distribution and an additional package “vegan” (Oksanen et al., 2016).

2.6. Nucleotide accession number

The raw sequence data reported in this paper were submitted to