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Lab resource

Metagenome analysis of the root endophytic microbial community of Indian rice (*O. sativa* L.)

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ABSTRACT

This study reports the root endophytic microbial community profile in rice (*Oryza sativa* L.), the largest food crop of Asia, using 16S rRNA gene amplicon sequencing. Metagenome of OS01 and OS04 consisted of 11,17,900 sequences with 300 Mbp size and average 55.6% G + C content. Data of this study are available at NCBI Bioproject (PRJNA360379). The taxonomic analysis of 843 OTU's showed that the sequences belonged to four major phyla revealing dominance of Proteobacteria, Firmicutes, Cyanobacteria and Actinobacteria. Results reveal the dominance of *Bacillus* as major endophytic genera in rice roots, probably playing a key role in Nitrogen fixation.

Resource table

Name of resource	Metagenome of rice root endophytic community
Institution	[a]Bidhannagar College [b]AIIST, PALTA - 743122 [c]The Biome, Kolkata – 700064
Person who created resource	Subhadipa Sengupta[a], Sayak Ganguli[b] and Pankaj K Singh[c]
Contact person and email	bansubha@gmail.com
Date archived/stock date	31/08/2016
Type of resource	Raw sequence reads of rice root endophytic metagenome
Link to directly related literature that employed/validated this resource	https://www.ncbi.nlm.nih.gov/sra/SRX2524586 https://www.ncbi.nlm.nih.gov/sra/SRX2527833
Information in public databases	https://www.ncbi.nlm.nih.gov/sra?linkname=bioproject_sra_all&from_uid=360379

1. Resource details

As rice yield is enormously affected by large number of pathogenic organisms, nematodes, fungi, insects and virus hence, understanding and exploitation of the root endophytic community for this high demand Asian crop can result in the promotion of crop health. This could be an alternative approach towards eco-friendly potential natural source for biological control in disease management [1]. Advances in high-throughput environmental genomic DNA sequencing or metagenomic sequencing as well as various analytical tools and data resources has enabled us to understand the vast diversity of microorganisms, specially rare and uncultured microorganism and their phylogeny in community analysis. It also gives us insight into the enormous amount of functional gene diversity of a microenvironment [2]. The low cost of this technology and easy generation of draft genomes from complex dataset has made metagenomics study a much popular technique bypassing the need for isolation and lab cultivation of individual microorganism.

In this study, we thoroughly investigated the root endophytic microbial community present in the local cultivar of rice (*Oryza sativa* L.) at different field condition of West Bengal. The rice plants were selected at 60 days stage and were dug out from some selected wet land local rice fields which produces the bulk of the requirement of the population of Kolkata. Average temp of the area was 86 °F and soil pH ranges from pH 7.2 to pH 8.1.

Among 10 collected samples, two samples OS01 and OS04 were randomly selected for metagenomic sequencing. The total number of reads obtained for sample OS01 was 5,66,012 and that for sample OS04 was 5,51,888 (Fig. 1). In both the samples, the community study revealed an abundance of over 50% for the members of Firmicutes. In sample OS01 the percentage was found to be 58.4% while in sample OS04 it was 97%. At the genus level *Bacillus* was the most dominant microbial

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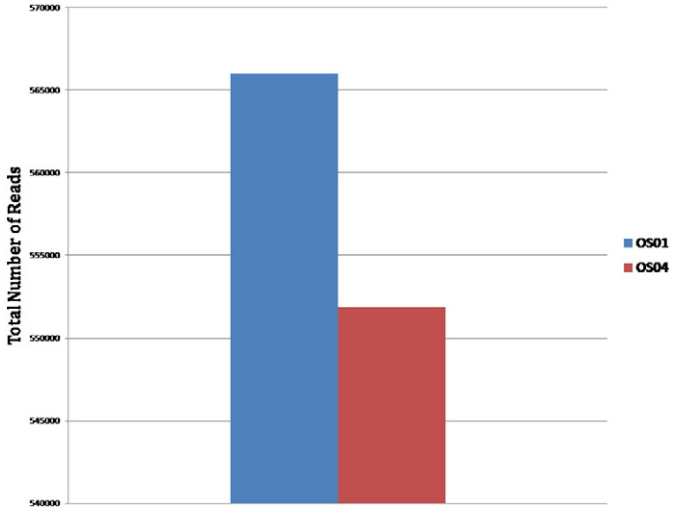

Fig. 1. Histogram representing Total number of reads for both samples (OS01 and OS04).

member with abundance of over 50% in both the samples. In sample OS01 gammaproteobacteria had a high abundance of 34.9% but in sample OS04 the percentage was as low as 0.2% as evidenced in the heat map prepared with OTU abundance of 200 or more (Fig. 2).

2. Materials and methods

Samples were collected directly from field in and around Kolkata in triplicates and were stored in sterile plastic bags. Root samples were thoroughly washed in tap water, several times. Surface sterilization of the root tips were then performed with 70% ethanol for 1 min followed by 1.2% (w/v) NaOCl solution for 15 min. Samples were then washed three times with sterile distilled water with shaking (10 min). Root samples were finally dried and stored at −20 °C. The DNA of each sample was isolated according to the protocol reported by Bonet et al. 2012 [3].The DNA was quantified using QubitdsDNA HS Assay kit (Life Tech). 1 μl of each sample was used for determining concentration using Qubit®2.0 Fluorometer. The amplicon libraries were prepared using Nextera XT Index Kit (Illuminainc.) as per the 16S Metagenomic Sequencing Library preparation protocol (Part # 15044223 Rev. B). Primers for the amplification of the V3-V4 hyper-variable region [V3 Forward Oligo: CCTACGGGNBGCASCAG and V4 Reverse Oligo: GACTACNVGGGTATCTAATCC] of 16S rDNA gene of bacteria and Archaea were used. The amplicons with the Illumina adaptors were amplified by using i5 and i7 primers that add multiplexing index sequences as well as common adapters required for cluster generation (P5 and P7) as per the standard Illumina protocol. The amplicon libraries were purified by 1× AMpureXP beads and checked on Agilent DNA 1000 chip on Bioanalyzer 2100 and quantified on fluorometer by QubitdsDNA HS Assay kit (Life Technologies). The library size of Sample OS01 and Sample OS04 were 634 bp and 622 bp respectively. The libraries were sequenced using the Illumina sequencing chemistry to generate ~150 Mb of data per sample. After obtaining the Qubit concentration for the library and the mean peak size from Bioanalyzer profile, library was loaded onto Illumina Platform at appropriate concentration (10–20 pM) for cluster generation and sequencing. The kit reagents were used in binding of samples to complementary adapter oligos on paired-end flow cell. The adapters were designed to allow selective cleavage of the forward strands after re-synthesis of the reverse strand during sequencing. The copied reverse strand was then used to perform sequencing from the opposite end of the fragments.

2.1. Verification and authentication

Our study revealed that in both the samples, the dominant member of rice root microbiome is *Bacillus* and this data is well supported by other literatures [4,5].QIIME analysis indicated that Shannon α-Diversity = 3.10 and no. of observed species = 420 and the Shannon α-Diversity = 2.40 and no. of observed species = 297 for Sample OS01and for sample OS04 respectively. At phylum level, both the samples are enriched with *Firmicutes* followed by Proteobacteria, *Bacilli*, whereas Gammaproteobacteria were the most abundant at class level in both the samples. At genus level, *Bacillus* and *Acinetobacter* were found to be the most abundant genus enriched in both the root samples. Moreover, our findings were also consistent with the reports of Ji et al. 2014 [6] where three major diazotrophic endophytic communities were identified as Actinobacteria, Gammaproteobacteria and *Bacillus*. Although, the functional annotations of the endophytic bacterial community of our samples are still pending, however, the dominant groups suggests their probable role of atmospheric N_2 fixation, a primary requisite for plant growth particularly in rice.

Consensus Lineage	OS01	OS04	taxID
Bacteria;Firmicutes;Bacilli;Bacillales;Bacillaceae;Bacillus;Bacillus horikoshii	328	468	denovo95
Bacteria;Firmicutes;Bacilli;Bacillales;Bacillaceae;Bacillus;uncultured soil bacterium	10	570	denovo117
Bacteria;Firmicutes;Bacilli;Bacillales;Planococcaceae;Solibacillus	3152	47	denovo144
Bacteria;Firmicutes;Bacilli;Bacillales;Bacillaceae;Bacillus	338	431	denovo164
Bacteria;Firmicutes;Bacilli;Bacillales;Bacillaceae;Bacillus	168	163	denovo188
Bacteria;Firmicutes;Bacilli;Bacillales;Bacillaceae;Bacillus	1259	492	denovo193
Bacteria;Firmicutes;Bacilli;Bacillales;Bacillaceae;Bacillus	377	358	denovo204
Bacteria;Firmicutes;Bacilli;Bacillales;Bacillaceae;Bacillus	37	3441	denovo245
Bacteria;Firmicutes;Bacilli;Bacillales;Planococcaceae;Lysinibacillus	2784	62	denovo292
Bacteria;Proteobacteria;Alphaproteobacteria;Rickettsiales;Mitochondria	6493	2871	denovo325
Bacteria;Firmicutes;Bacilli;Bacillales;Bacillaceae;Bacillus	784	1479	denovo337
Bacteria;Proteobacteria;Gammaproteobacteria;Pseudomonadales;Moraxellaceae;Acinetobacter	68340	392	denovo371
Bacteria;Firmicutes;Bacilli;Bacillales;Bacillaceae;Bacillus;Bacillus megaterium	70	1110	denovo394
Bacteria;Firmicutes;Bacilli;Bacillales;Bacillaceae;Bacillus;Bacillus megaterium	295	2	denovo401
Bacteria;Firmicutes;Bacilli;Bacillales;Bacillaceae;Bacillus;Bacillus flexus	243	113	denovo405
Bacteria;Firmicutes;Bacilli;Bacillales;Bacillaceae;Bacillus;uncultured bacterium	325	38	denovo484
Bacteria;Proteobacteria;Gammaproteobacteria;Pseudomonadales;Moraxellaceae;Acinetobacter;uncultured bacterium	248	6	denovo498
Bacteria;Firmicutes;Bacilli;Bacillales;Bacillaceae;Bacillus	11471	17835	denovo508
Bacteria;Firmicutes;Bacilli;Bacillales;Bacillaceae;Bacillus	585	489	denovo511
Bacteria;Firmicutes;Bacilli;Bacillales;Bacillaceae;Bacillus;Bacillus infantis	82	3074	denovo547
Bacteria;Firmicutes;Bacilli;Bacillales;Bacillaceae;Bacillus;Bacillus cereus	176	1347	denovo564
Bacteria;Firmicutes;Bacilli;Bacillales;Bacillaceae;Bacillus	446	406	denovo592
Bacteria;Firmicutes;Bacilli;Bacillales;Bacillaceae;Bacillus	10	425	denovo638
Bacteria;Firmicutes;Bacilli;Bacillales;Bacillaceae;Bacillus;Bacillus niabensis	118	59579	denovo640
Bacteria;Firmicutes;Bacilli;Bacillales;Bacillaceae;Bacillus;Bacillus megaterium	109	168	denovo643
Bacteria;Firmicutes;Bacilli;Bacillales;Bacillaceae;Bacillus	237	211	denovo654
Bacteria;Proteobacteria;Betaproteobacteria;Burkholderiales;Oxalobacteraceae;Herbaspirillum;Herbaspirillum huttiense subsp. huttiense	1094	18	denovo658
Bacteria;Firmicutes;Bacilli;Bacillales;Paenibacillaceae;Paenibacillus	736		denovo691
Bacteria;Firmicutes;Bacilli;Bacillales;Bacillaceae;Bacillus	52589	107372	denovo709
Bacteria;Firmicutes;Bacilli;Bacillales;Bacillaceae;Bacillus	98	1988	denovo723
Bacteria;Firmicutes;Bacilli;Bacillales;Bacillaceae;Bacillus	87	187	denovo728
Bacteria;Firmicutes;Bacilli;Bacillales;Bacillaceae;Bacillus	20088	261	denovo745
Bacteria;Firmicutes;Bacilli;Bacillales;Bacillaceae;Bacillus	305		denovo751
Bacteria;Firmicutes;Bacilli;Bacillales;Bacillaceae;Bacillus	13186	956	denovo782
Bacteria;Firmicutes;Bacilli;Bacillales;Bacillaceae;Bacillus	9	192	denovo786
Bacteria;Firmicutes;Bacilli;Bacillales;Bacillaceae;Bacillus	354		denovo794
Bacteria;Cyanobacteria;Chloroplast;Oryza sativa Indica Group	4601	1967	denovo818
Bacteria;Firmicutes;Bacilli;Bacillales;Bacillaceae;Bacillus	7	865	denovo822
Bacteria;Firmicutes;Bacilli;Bacillales;Bacillaceae;Bacillus	798	942	denovo826

Fig. 2. Heatmap representing OTUs of 200 or more hits.

2.2. Nucleotide sequence accession numbers

Metagenome sequence data from this study are available at the NCBI Sequence Read Archive (SRA) and Biosamples under accession numbers: SAMN06209694 and SAMN06209718.

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