Lab resource

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

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A B S T R A C T

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.

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Keywords:
Endophytic microbial community
Food crop
Metagenome
OTU
Rice

Resource table

| Name of resource | Metagenome of rice root endophytic community |
|------------------|---------------------------------------------|
| Institution      | aBidhannagar College
bAIIST, PALTA - 743122
"The Biome, Kolkata - 700064" |
| Person who created resource | Subhadipa Sengupta a, Sayak Ganguli b and Pankaj K Singh b |
| 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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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 QubitsDNA 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 (Illumina) as per the 16S Metagenomic Sequencing Library preparation protocol (Part # 15044223 Rev. B).

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 OS01 and 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 N2 fixation, a primary requisite for plant growth particularly in rice.
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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