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Whole genome sequencing of drought stress tolerance endophytic bacterium *enterobacter sp.* mr1

Abstract

Bacteria which live inside the plant and not affected to plant are called Endophytic bacteria. Endophytes help plant to the growth, health and development of their host plant. The present studies on isolation of endophytic bacteria from drought tolerance plant *Butea monosperma.* Genome sequencing was performed in Ion-torrent (Personal Genome Machine). A total of 82.10Mb data with 505,210 reads was obtained. GC content of the genome is 52.8%. A total of 80 RNA sequence were identified, of which 8 genes were responsible for 5S rRNA synthesis, 1 gene for 16S rRNA synthesis and 1 gene for 23S rRNA synthesis.[1] The genome size of *Enterobacter sp.* MR1 was found to be 4.58Mb and its closest neighbors were *Enterobacter sp.* 638 (Genome ID: 399742.10) and *Enterobacter cloacae subsp.* cloacae ATCC 13047 (Genome ID: 716441.4).

Keywords: bacteria, endophytic bacteria, *butea monosperma*, *enterobacter sp.*, cytokinins

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Introduction

Endophytic bacteria live inside living tissues of living plant without harming it. They promote the plant growth, health and development of their host plant by providing protection to the host against biotic (diseases) and abiotic (drought and salinity) stresses.[2] Beneficial effects of plant growth promoting endophytic bacteria on plant drought tolerance is caused by changes in hormonal content, mainly that of abscisic acid, ethylene and cytokinins.[3,4]

In plant the roots are the main site of endophytic colonization. Root colonization by bacteria was described to involve several stages of plant growth.[5] In the initial step bacteria move towards the plant roots either passively via soil water fluxes. Specific or complex interactions between the bacterium and the host plant, such as the secretion of root exudates compounds, may arise resulting in the induction of bacterial gene expression. Finally, endophytic bacteria can enter the plant at sites of tissue damage, which naturally arise as the result of plant growth, through root hairs and at epidermal conjunctions.[6] In addition, plant exudates compounds given off through these wounds provide a nutrient source for the colonizing bacteria and thus create favorable conditions for endophytes. This model of endophytic root colonization was confirmed by several microscopic studies for a number of plants.[7–9] including poplar trees by genome sequencing.[10,11] Alternatively, endophytic bacteria can use vector organisms to gain entrance to the apoplastic spaces to colonize the host plant.[12–14]

Materials and methods

Isolation endophytes

Root samples were collected from plant *Butea monosperma* at the farm of Junagadh agricultural university, Junagadh, Gujarat. In the end of summer season because highly drought at that time.

Genome sequencing

For genome sequencing, DNA of *Enterobacter sp.* MR1 was isolated using Phenol-Chloroform method.[10] The DNA concentration and purity was cheked using Picodrop PET01 (Picodrop Ltd., Cambridge U.K). The DNA was enzymatically fragmented to construct a library of 260bp, which was further used for template preparation. Sequencing was carried out using Ion-314 chip in Ion Torrent Personal Genome Machine (PGMTM) from Life Technologies, at Department of Biotechnology, Junagadh Agricultural University, Junagadh, India as per the manufacture's guidelines.

Genome annotation

Raw reads of the sequence were processed for the quality control through default plug-in in Ion Torrent Software Server (FastQC). The quality reads were assembled in MIRA v 3.4.1 by using Smith-Waterman algorithm.[15] Contings were ordered through the tool Mummer and were aligned with reference genome *E. cloacae* ATCC 13047 and *Enterobacter* sp. 638 using Mauva software. Putative coding sequences (CDS) were initially identified by RAST automated annotation software.[16]

Results and discussion

Twelve morphologically different endophytic bacteria isolate form root samples *Butea monosperma* by using nutrient agar medium to check plant growth promoting (PGP) activity of all isolates and based on the function characteristics one bacterium selected which show high PGP activity compare to other. This bacterium identified by using the 16s rRNA sequencing.

Library load in Ion-314 chip, we obtained 71% loading Figure 1 and the library mean read length is 162bp and longest read is 344bp (Figure 2). The MIRA assembler v3.4.1.0 was used for assembling the data which resulted in 640 contigs, with the largest contig size of 59,767bp and GC content of 52.8% (Table1).

The assembled contings sequences were submitted to RAST (Rapid Annotations using Subsystems Technology) system, RNAmmer 1.2,[17] and ARAGORN software[18] for further analysis. Based on RNAmmer analysis obtained total of 80 RNA sequence were identified, of which eight genes were responsible for 5S rRNA synthesis, one gene for 16S rRNA synthesis and one gene for 23S rRNA synthesis. RAST analysis shows the genome size of *Enterobacter sp.* MR1 was found

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to be 4.58Mb and its closest neighbors were *Enterobacter* sp. 638 (Genome ID: 399742.10) and *Enterobacter cloacae subsp. cloacae* ATCC 13047 (Genome ID:716441.4). We have also conformed the 16S rRNA gene sequence by Sanger's sequencing and found 98% identity with *Enterobacter aerogenes* KCTC 2190 strain and 16S rRNA sequence 100 % identity was found between the sequence obtained from whole genome sequence and RNAmmer-predicted 16S rRNA sequence. All the contigs were submitted to the Gene bank and NCBI has published sequence data in April-2013.

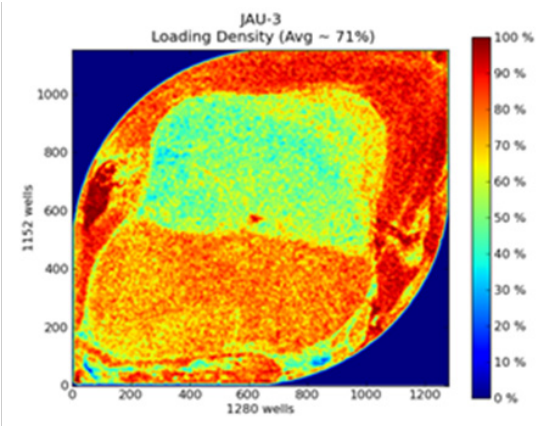

Figure 1 Loading density.

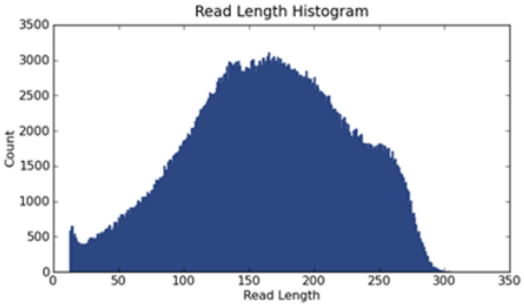

Figure 2 Library read length histogram.

Table 1 Assembly result

Assembly statistics		
	All contigs	**Large contigs**
Assembled Reads	469,225	
Coverage	15.69 X	
Number of Contigs	643	643
Consensus Length	4,633,861bp	4,633,861bp
Largest Contig	59,767bp	
N50	10,618bp	10,618bp
N90	3,528bp	3,528bp
N95	2,420bp	2,420bp

Nucleotide sequence accession numbers

This Whole Genome Shotgun project has been deposited at DDBJ/EMBL/GenBank under the accession ARPV00000000. The version described in this paper is the first version, NZ_ARPV00000000.1 GI: 499134312. Bioproject registered under Accession: PRJNA203094 ID: 203094.

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Conflict of interest

The author declares no conflict of interest.

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