**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 82.10 Mb 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 4.58 Mb 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

**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 checked using Picodrop PET01 (Picodrop Ltd., Cambridge U.K). The DNA was enzymatically fragmented to construct a library of 260 bp, which was further used for template preparation. Sequencing was carried out using Ion-314 chip in Ion Torrent Personal Genome Machine (PGM™) 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]. Contigs 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.
Whole Genome Sequencing of Drought Stress Tolerance Endophytic Bacterium Enterobacter sp. Mr1

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 59,767 bp and GC content of 52.8% (Table 1).

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    | 4,633,861 bp| 4,633,861 bp  |
| Largest Contig      | 59,767 bp   |               |
| N50                 | 10,618 bp   | 10,618 bp     |
| N90                 | 3,528 bp    | 3,528 bp      |
| N95                 | 2,420 bp    | 2,420 bp      |

The assembled contigs sequences were submitted to RAST (Rapid Annotations using Subsystems Technology) system, Rfammer 1.2 [17], and ARAGORN software [18] for further analysis. Based on Rfammer 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 to be 4.58 Mb 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 confirmed 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 Rfammer-predicted 16S rRNA sequence. All the contigs were submitted to the Gen bank and NCBI has published sequence data in April-2013.

Nucleotide Sequence Accession Numbers

This Whole Genome Shotgun project has been deposited at DDBJ/EMBL/GenBank under the accession ARPV000000000. The version described in this paper is the first version, NZ_ARPV000000000.1 GI: 499134312. Bioproject registered under Accession: PRJNA2030941D: 203094.

Acknowledgment

This Research work was funded by Junagadh Agricultural University, Junagadh, Gujarat. The sequencing was performed at Department of Biotechnology, JAU, Junagadh.

References

1. Parakhia MV, Tomar RS, Malaviya BJ, Dhingani RM, Rathod VM, et al. (2014) Draft genome sequence of the endophytic Bacterium Enterobacter sp. MR1, isolated from drought tolerant plant (Buteamonosperma). Indian J Microbiology 54(1): 118-119.
2. Bell CR, Dickie GA, Harvey WLG, Chan JWYP (1995) Endophytic bacteria in grapevine. Can J Microbial 41(1): 46-53.
3. Arshad M, Frankenberger WT (1991) Microbial production of plant hormones. Plant Soil 133: 1-8.
4. Cho SM, Kang BR, Han SH, Anderson AJ, Park JY, et al. (2008) 2R, 3R- butanediol, a bacterial volatile produced by Pseudomonas chlororaphis O6, is involved in induction of systemic tolerance to drought in Arabidopsis thaliana. Mol Plant Microbe Interact 21(8): 1067-1075.
5. Brimecombe MJ, De Leij, Lynch JM (2007) The Rhizosphere, Biochemistry and Organic Substances at the Soil-Plant Interface. In: Pinton ZV R & Nannipiere P (Eds.), Boca Raton.
6. Sprent JI, Defaria SM (1998) Mechanisms of infection of plants by nitrogen-fixing organisms. Plant Soil 110(2): 157-165.
7. Benhamou N, Belanger RR, Paulitz TC (1996) Pre-inoculation of Ri T-DNA transformed pea roots with Pseudomonas fluorescens inhibits colonization by Pythium ultimum trow: An ultrastructural and cytochemical study. Planta 199(1): 105-117.
8. Pan MJ, Rademan S, Kunert K, Hastings JW (1997) Ultrastructural studies on the colonization of banana tissue and Fusarium oxysporum f. sp. cubense race 4 by the endophytic bacterium Burkholderia cepacia. J Phytopathology 145(11-12): 479-486.
9. Wiebe W, Hechtbouchholz C, Hoflich G (1994) Electron-microscopic investigations on root colonization of Lepinus albus and Pusum sativum with 2 associative plant-growth promoting rhizobacteria, Pseudomas fluorescens and Rhizobium leguminosarum bvTrifolii. Symbiosis 17: 15-31.

Citation: Parakhia MV, Tomar R, Vala AG, Rathod VM, Kheni JV, et al. (2016) Whole Genome Sequencing of Drought Stress Tolerance Endophytic Bacterium Enterobacter sp. Mr1. J Bacteriol Mycol Open Access 3(2): 00055. DOI: 10.15406/jbmoa.2016.03.00055
Whole Genome Sequencing of Drought Stress Tolerance Endophytic Bacterium Enterobacter sp. Mr1

10. Taghavi S, Garafola C, Monchy S, Newman L, Hoffman A, et al. (2009) Genome survey and characterization of endophytic bacteria exhibiting a beneficial effect on growth and development of poplar trees. Appl Environ Microbiol 75(3): 748-757.

11. Germaine K, Keogh E, García Cabellos G, Borremans B, van der Lelie D, et al. (2004) Colonisation of poplar trees by gfp expressing bacterial endophytes. FEMS Microbiol Ecol 48(1): 109-118.

12. Kluepfel DA (1993) The behavior and tracking of bacteria in the rhizosphere. Annual Review of Phytopathology 31: 441-472.

13. Ashbolt NJ, Inkerman PA (1990) Acetic-acid bacterial biota of the pink sugar-cane mealybug, Saccharococcus sacchari, and its environs. Appl Environ Microbiol 56(3): 707-712.

14. Franke IH, Fegan M, Hayward C, Leonard G, Sly LI (2000) Molecular detection of Gluconacetobacter sacchari associated with the pink sugarcane mealybugSaccharicoccus sacchari (Cockerell) and the sugarcane leaf sheath microenvironment by FISH and PCR. FEMS Microbiol Ecol 31(1): 61-71.

15. Chevreux B (2005) MIRA: an automated genome and EST assembler. German Cancer Research Center, Germany.

16. Aziz RK, Bartels D, Best AA, DeJongh M, Disz T, et al. (2008) The RAST Server: Rapid annotations using subsystems technology. BMC Genomics 9: 75.

17. Lagesen K, Hallin P, Rødland EA, Staerfeldt HH, Rognes T, et al. (2007) RNAmmer: Consistent and rapid annotation of ribosomal RNA genes. Nucleic Acids Res. 35(9): 3100-3108.

18. Laslett D, Canback B (2004) ARAGORN, a program to detect tRNA genes and tmRNA genes in nucleotide sequences. Nucleic Acids Res 32(1): 11-16.