

Draft Genome Sequence of the Endophytic Bacterium *Pantoea ananatis* MR5 Isolated from *Cenchrus biflorus*- A Drought Tolerant Plant

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An endophytic plant growth promoting bacterium *Pantoea ananatis* MR5 was isolated from roots of *Cenchrus biflorus*, a drought tolerant plant. Genome sequencing of *Pantoea ananatis* MR5 was carried out in Ion Torrent (PGM), Next generation Sequencer. The data obtained revealed 242 contigs with genome size of 4.1 Mb and G+C content of up to 55%. The size of cell was measured with average of 1.56 μm by using Scanning electron microscope. This bacterium had contain genes responsible for drought tolerance in plant, including phosphate solubilisation, IAA, siderophore production and ACC deaminase activities for plant.

Key words: Drought tolerance endophyte, Plant growth promoting bacterium, Scanning electron microscope, Whole genome sequencing.

Plants normally associated with diverse microorganism. 'Endophytic bacteria' are the population of bacteria that reside within the living organism without doing substantive harm or gaining benefit other than securing residency. Drought is one of the prevalent environmental conditions that cause adverse effects on the growth of plants. Water deficit more than other stresses limit the growth and productivity of crops. Plant adaptation to environmental stresses is controlled by cascades of molecular networks resulting in a combination of metabolic, physiological and morphological changes¹. Xerophytes plant species have mechanisms to overcome drought stress. In some xerophytes, a mechanism could be considered the endophytic association between plant and rhizobacteria able to improve the plant growth in abiotic stress conditions. Sustainable systems

require the understanding of interactions between plants and microorganisms, especially those having a direct influence on plant growth and stress tolerance². The use of Plant growth promoting drought tolerant bacteria may prove useful in developing strategies to facilitate crop growth in drought area.

In the present study, a total 14 bacteria isolated from *Cenchrus biflorus* from banni region of kutch. They were differ in their morphology and plant growth promoting activities. The *Pantoea ananatis* MR5 was selected for whole genome sequencing, based on its highly efficient plant growth promoting activities.

Genome DNA was extracted from selected bacterium culture using the DNA extraction kit (Invitrogen)³. Whole-genome sequencing of was done using Ion Torrent (PGM) Next Generation Sequencer (NGS) (Life technology) at Department of Biotechnology, JAU, Junagadh according to the manufacturer's recommended protocol. Using 400bp chemistry library was generated and library was enriched by using ion-one touch enrichment system. Enriched library was loaded on 314 chip, a total of 81.1 Mb data with a 415,383 reads was

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obtained and coverage of 15.48X. The assembly of reads was done by using MIRA v.3.0.5 (Bastien Chevreux, Rheinfelden)⁴. The result of MIRA assembly found the 242 contigs while largest contig was of 96,232bp with N50 and N90 value of 32,253bp and 9,960bp and GC content of up to 55% respectively.

The data was annotated using Rapid Annotations using Subsystems Technology (RAST) (The computation institute, Chicago)⁵. The majority of *Pantoea ananatis* MR5 genes (657 counts) are responsible for carbohydrate metabolism, followed by amino acids and derivatives; stress response with 476 and 182 counts, respectively. The cofactors, vitamins, prosthetic groups and pigment production counts 275 genes. Generally, these genes are responsible for the basic life-sustaining needs of the bacterial cell. Apart from the presence of the basic necessary genes in *Pantoea ananatis* MR5, there are 96 genes responsible for virulence, disease and defence. Among these 135 genes, 212 were found to play a role in respiration and cell wall & capsule formation. The 283 genes for protein metabolism followed by 77 for sulphur, 68 for phosphorus, 27 for nitrogen and 25 gene for potassium metabolism.

The RNAmmer prediction server, a version 1.2 (Centre for Biological Sequence Analysis, Lyngby)⁶ and ARAGORN software (Murdoch University, Perth)⁷ established the ribosomal RNA present in *Pantoea ananatis* MR5. A total 79 RNA sequence were identified, in that five genes were responsible for 5S rRNA synthesis, two genes for 16S and 23S rRNA synthesis. The genome size of *Pantoea ananatis* MR5 was found to be 4.1 Mb and its closest neighbours were *Pantoea ananatis* LMG 20103 (genome ID: 706191.3) followed by *Pantoea* sp. At-9b (genome ID: 592316.4) and *Pantoea* sp. aB (genome ID: 517433.4). All the contigs were submitted to the Gene bank.

Nucleotide sequence accession numbers

This whole genome shotgun project has been deposited at DDBJ/EMBL/Genbank under accession LBFU00000000. Bioprojet registered under accession was PRJNA280811 and the biosample number was SAMN03470247.

Scanning Electron Microscopy

The cell morphology of *Pantoea*

ananatis MR5 strain was observed in SEM with 21 k magnification (X). It was observed in rod shape with round and pointed end. The size of cell was measured in range of 1.35 to 2.1 μm with average of 1.56 μm .

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