

Draft Genome Sequence of the Endophytic Bacterium *Providencia rettgeri* MR4 Isolated from *Abuliton indicum*- A Salt Tolerant Plant

R.A. Bhadania, B.A. Golakiya, D.L. Akbari, M.V. Parakhia and H.N. Bhalani

Department of Biotechnology, Junagadh Agriculture University, Junagadh, India.

(Received: 11 April 2015; accepted: 10 June 2015)

A halotolerant endophytic plant growth promoting bacterium *Providencia rettgeri* MR4 was isolated from roots of *Abuliton indicum*, a plant has better ability to with stand saline conditions from Kutch, India. Draft genome sequencing of *Providencia rettgeri* MR4 was carried out in Ion Torrent (PGM), Next generation Sequencer. This bacterium had contained genes responsible for salt tolerance in plant, including phosphate solubilisation, IAA, siderophore production, nitrate reduction, ACC deaminase activities and antagonism against phytopathogenic fungi.

Key words: Salt tolerant endophytes, *Abuliton indicum*,
Plant growth promoting endophytic bacteria, Whole genome sequencing.

Endophytic bacteria colonize the internal tissue of the plant showing beneficial effects on host plants and no negative effect on their host. Salt tolerant endophytic bacteria could resist the salinity of soil; from root, shoot and leaf of rice plant cultivated in highly saline soil¹. Problems associated with salinity about 20% of cultivated and irrigated lands around the world are severely affected by salinity. In some halophytes, a mechanism could be considered the endophytic association between plant and rhizobacteria able to improve the plant growth in abiotic stress conditions²).

PGPR elicited induced systemic tolerance (IST) aid the growth of plants under abiotic stresses by producing various antioxidants, which result in the degradation of reactive oxygen species (ROS)³. Production of IAA or unknown determinant scan increase root length, root surface

area and the number of root tips, leading to enhanced uptake of nitrate and phosphorous^{4, 5}. PGPRs have also been reported to protect plants from various pathogens by activating defense genes, for example those encoding chitinase, β -1, 3-glucanase (GLU), peroxidase (POX), phenylalanine ammonia lyase (PAL) etc⁶.

Best identified salt tolerant endophytic bacteria having highly PGPR activity will be under such conditions, agriculture will gradually shift towards hitherto uncultivable areas such as coastal areas and waste lands and these microorganisms can contribute to sustainable agriculture under adverse conditions. This work will be useful for the development of salt stress tolerant varieties of our important crops using genetic engineering.

In the present study, a total 17 bacteria isolated from *Abuliton indicum* from kalodungar of Kutch, India. The *Providencia rettgeri* MR4 was selected for whole genome sequencing, based on its highly efficient plant growth promoting activities and tolerant NaCl up to 12.5% concentration in the Nutrient agar⁷. Whole Genome Shotgun (WGS) of haloarchaeon 3A1-DGR is an

* To whom all correspondence should be addressed.

isolate from a salt crystallizer pond of Little Rann of Kutch, India and has been described recently⁸ with accession number ATCR 00000000.

Genome DNA was extracted from selected bacterium culture using the DNA extraction kit (Invitrogen)⁹. 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 450bp 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 80.5 Mb data with a 116,910 reads was obtained and coverage of 6.62X. The assembly of reads was done by using MIRA v.3.0.5 (Bastien Chevreux, Rheinfelden)¹⁰. The result of MIRA assembly found the 642 contigs while largest contig was of 22,063bp, with N50, N90 and N95 value of 4,728bp, 3,078bp and 2,774bp, respectively and GC content of up to 40.47% respectively. The reads were trimmed and assembled mapping using software CLC Genomics Workbench 8.0.1 (CLC bio a QUIAGEN company).

The data was annotated using Rapid Annotations using Subsystems Technology (RAST) (The computation institute, Chicago)¹¹. RAST annotation revealed the association of 131 genes in stress responses in this organism: 26 in osmotic stress (21 in choline and betaine uptake and betaine biosynthesis), 50 in oxidative stress (5 in protection from reactive oxygen species, 21 in oxidative stress, 4 in glutathione: biosynthesis and gamma-glutamyl cycle, 9 in glutathione: non-redox reactions, 3 in rubrerythrin, and 7 in glutaredoxins). The majority of MR4 genes (576 counts) are responsible for amino acids and derivatives, followed by carbohydrate metabolism; RNA metabolism 518 and 265 counts, respectively.

The RNAmmer prediction server, a version 1.2 (Centre for Biological Sequence Analysis, Lyngby)¹² and ARAGORN software (Murdoch University, Perth)¹³ established the ribosomal RNA present in *Providencia rettgeri* MR4. A total of 56 RNA sequence were identified, of which one genes were responsible for 23S rRNA synthesis and one gene for 16S rRNA synthesis. The genome size of *Providencia rettgeri* MR4 was found to be 3.2 Mb and its closest neighbours were closet to *Providencia stuartii*

MRSN 2154 (genome ID: 1157951.4) followed by *Cronobacter sakazakii* ATCC BAA-894 (genome ID: 290339.8), *Klebsiella pneumoniae* MGH 78578 (genome ID: 272620.3) and *Photobacterium luminescens* sub sp. *laumondii* TTO1 (genome ID: 243265.1). 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 the accession LCVM00000000.SUBID: SUB939235. Bioprojet registered under accession was PRJNA283342 and the biosample number was SAMN03646990.

ACKNOWLEDGMENT

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

REFERENCES

1. Tanawy, E. A. Acquainting with salt tolerant endophytic bacteria isolated from rice plant grown in highly saline soil in Egypt. *Intl J Acade Res* 2009; 1(2):72-79.
2. Kloepper, J., Schroth, M. Plant growth promoting rhizobacteria in radish. *Intl Con Pl Patho Bact INRA* 1978; 2:879- 882.
3. Figueredo, M. V. B., Burity, H. A., Martinez, C. R., Chanway, C. P. Alleviation of drought stress in common bean (*Phaseolus vulgaris* L.) by co-inoculation with *Paenibacillus polymyxa* and *Rhizobium tropici*. *Appl Soil Ecol* 2008; 40:182-188.
4. Mantelin, S., Touraine, B. Plant growth-promoting bacteria and nitrate availability impacts on root development and nitrate uptake. *J Exp Bot* 2004; 55: 27-34.
5. Gyaneshwar, P., Kumar, G. N., Parekh, L. J., Poole, P. S. Role of soil microorganisms in improving P nutrition of plants. *Plant Soil* 2002; 245: 83-93.
6. M'Piga, P., Belanger, R. R., Paulitz, T. C., Benhamou, N. Increased resistance to *Fusarium oxysporium* f. sp. *radicis-lycopersici* in tomato plants treated with the endophytic bacterium *Pseudomonas fluorescens* strain 6328. *Physiol Mol Pl Pathol* 1997; 50: 301-320.
7. Chakraborty, U., Roy, S., Chakraborty, A. P.,

- Dey, P., Chakraborty, B. Plant growth promotion and amelioration of salinity stress in crop plants by a salt-tolerant bacterium. *Res Sci Tech* 2011; **3**(11): 61-70.
8. Pal, K. K., Dey, R., Thomas, M., Ghorai, S., Sherathia, D., Vanpariya, S., Rupapara, R., Rawal, P., Mandaliya, M., Sukhadiya, B., Saxena, A. K. Draft genome sequence of an extreme Haloarchaeon 3A1-DGR isolated from a saltern crystallizer of the Little Rann of Kutch, India. *Indian J Microbiol* 2014; **54**(4):471-473.
 9. Parakhia, M. V., Tomar, R. S., Malviya B. J., Dhingani, R. M., Rathod, V. M., Thakkar, J. R., Golkiya B. A. Draft genome sequence of the endophytic bacterium *enterobacter* spp. MR1, isolated from drought tolerant plant (*Butea monosperma*). *Indian J Microbiol* 2014; **54**(1):118-119.
 10. Chevreux, B. MIRA: an automated genome and EST assembler. 2005; Thesis, German Cancer Research Center, Germany.
 11. Aziz, R. K., Bartels, D., Best, A. A., DeJongh, M., Disz, T., Edwards, R. A., Formsma, K., Gerdes, S., Glass, E.M., Kubal, M., Meyer, F., Olsen, G.J., Olson, R., Osterman, A.L., Overbeek, R.A., McNeil, L.K., Paarmann, D., Paczian, T., Parrello, B., Pusch, G.D., Reich, C., Stevens R., Vassieva, O., Vonstein, V., Wilke, A., Zagnitko, O. The RAST server: rapid annotations using subsystems technology. *BMC Genomics* 2008; **9**:75
 12. Lagesen, K., Hallin, P., Rodland, E.A., Staerfeldt, H.H., Rognes, T., Ussery, D.W. RNAmmer: consistent and rapid annotation of ribosomal RNA genes. *Nucleic Acids Res* 2007; **35**:3100-3108.
 13. Laslett, D., Canback, B. ARAGORN, a program to detect tRNA genes and tmRNA genes in nucleotide sequences. *Nucleic Acids Res* 2004; **32**:11-16