

Genome Sequence of the Plant Growth-Promoting Bacterium Enterobacter cloacae GS1

Manoharan Shankar, Paramasivan Ponraj, Devaraj Ilakiam, Jeyaprakash Rajendhran, and Paramasamy Gunasekaran Department of Genetics, Center for Excellence in Genomic Sciences, School of Biological Sciences, Madurai Kamaraj University, Madurai, Tamilnadu, India

Here, we present the genome sequence of *Enterobacter cloacae* GS1. This strain proficiently colonizes rice roots and promotes plant growth by improving plant nutrition. Analyses of the *E. cloacae* GS1 genome will throw light on the genetic factors involved in root colonization, growth promotion, and ecological success of this rhizobacterium.

Eenriched *in planta* from a rhizospheric microbial suspension. This strain has been deposited in the Microbial Type Collection Centre, Chandigarh, India, with the accession number MTCC 5698. Earlier, we have shown that *E. cloacae* GS1 outcompetes other innate microbial flora, colonizes rice roots, and promotes plant growth (6). We sequenced the genome of *E. cloacae* GS1 to gain a better understanding of the genes involved in rhizosphere colonization.

Total genomic DNA from a stationary-phase culture of *E. cloacae* GS1 was isolated using the Qiagen DNeasy protocol according to the manufacturer's instructions. A total of 298,439 reads with an average read length of 526 bp were generated by Roche 454 pyrosequencing at the Research and Testing Laboratory, Lubbock, TX. The reads added up to 157,085,169 sequenced bases, indicating an ~34-fold coverage of the ~4.5-Mb genome. The obtained reads were *de novo* assembled using MIRA (Mimicking Intelligent Read Assembly) version 3.4 (2), which yielded 60 contigs (N_{50} length = 150 kb). The assembly was visualized using the Staden package version 2.0 (7), screened for misassemblies, and manually curated. Contigs with significant overlaps were joined based on consensus quality and coverage at the ends. Finally, 48 contigs were obtained, the longest and shortest of them being 1,527,101 bp and 545 bp, respectively.

The draft genome of E. cloacae GS1 is 4,500,707 bp long with a G+C content of 55.5%. The genome was annotated using the Rapid Annotations using Subsystems Technology (RAST) (1) server employing the GLIMMER gene caller. A total of 4,683 protein-encoding genes (PEGs) distributed in 548 metabolic subsystems were predicted along with 119 RNA coding regions. E. cloacae GS1 contains genes vital for motility, chemotaxis, adhesion, polysaccharide biosynthesis, and biofilm formation, which are required in various stages of root colonization (4). In the rhizosphere, survival of a bioinoculant depends on its ability to elicit density-dependent behavior and compete with rhizospheric microflora for the niche and nutrients. E. cloacae GS1 has the luxS gene encoding S-ribosylhomocysteine lyase, which produces the autoinducer-2 quorum sensing (QS) signal that regulates population density-dependent gene expression (5). Similar to other enteric bacteria, E. cloacae GS1 lacks a luxI homolog required for biosynthesis of N-acyl homoserine lactone (NAHL) QS signals. However, seven LuxR family transcriptional regulators were identified. The best studied of them is *sdiA*, which is involved in the detection of and response to environmental NAHLs (3). E. cloacae GS1 contains seven predicted β-lactamases, fusaric acid resistance

determinants, and enterobactin production and uptake mechanisms, which could help in competition against soil microbiota. The existence of pathways for the synthesis of root elongation factors like indole acetic acid, 2,3-butanediol, and phosphate-solubilizing organic acids correlates with the rice growth-promoting ability of this strain. Thus, the *E. cloacae* GS1 genome encodes the traits essential for a successful bioinoculant.

Nucleotide sequence accession numbers. The *Enterobacter cloacae* subsp. *cloacae* GS1 whole-genome shotgun project has been deposited at DDBJ/EMBL/GenBank under the accession no. AJXP000000000. The version described in this paper is the first version, AJXP010000000.

ACKNOWLEDGMENTS

This work was jointly supported by the Indian Council for Agricultural Research [NBAIM/AMAAS/2007-2012/MG (5)/PG/BG/3] and the University Grants Commission-Center for Excellence in Genomic Sciences.

We thank Bastien Chevreux, Developer, MIRA, for technically supporting the assembly process. The central facilities, CAS, NRCBS, and DBT-IPLS, at the School of Biological Sciences, Madurai Kamaraj University, Tamilnadu, India, are acknowledged. M.S. gratefully acknowledges constructive discussions with Manoharan Ramasamy, Chitralekha Manoharan, and Madhankumar Anandhakrishnan.

REFERENCES

- 1. Aziz RK, et al. 2008. The RAST server: rapid annotations using subsystems technology. BMC Genomics 9:75.
- 2. Chevreux B. 2005. MIRA: an automated genome and EST assembler. PhD thesis. German Cancer Research Center Heidelberg, Heidelberg, Germany.
- Dyszel JL, et al. 2010. *E. coli* K-12 and EHEC genes regulated by SdiA. PLoS One 5:e8946. doi:10.1371/journal.pone.0008946.
- Espinosa-Urgel M, Kolter R, Ramos JL. 2002. Root colonization by *Pseudomonas putida*: love at first sight. Microbiology 148:341–343.
- Schauder S, Shokat K, Surette MG, Bassler BL. 2001. The LuxS family of bacterial autoinducers: biosynthesis of a novel quorum-sensing signal molecule. Mol. Microbiol. 41:463–476.
- Shankar M, Ponraj P, Ilakiam D, Gunasekaran P. 2011. Root colonization of a rice growth promoting strain of *Enterobacter cloacae*. J. Basic Microbiol. 51:523–530.
- Staden R, Beal KF, Bonfield JK. 2000. The Staden package, 1998. Methods Mol. Biol. 132:115–130.

Received 31 May 2012 Accepted 5 June 2012 Address correspondence to Paramasamy Gunasekaran, gunagenomics@gmail.com. Copyright © 2012, American Society for Microbiology. All Rights Reserved. doi:10.1128/JB.00964-12