



Genome Sequences of 15 *Klebsiella* sp. Isolates from Sugarcane Fields in Colombia's Cauca Valley

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ABSTRACT Members of the *Klebsiella* genus promote plant growth. We report here draft whole-genome sequences for 15 *Klebsiella* sp. isolates from sugarcane fields in the Cauca Valley of Colombia. The genomes of these isolates were characterized as part of a broader effort to evaluate their utility as endemic plant growth-promoting biofertilizers.

The genus *Klebsiella* belongs to the family *Enterobacteriaceae* and includes nonmotile rod-shaped Gram-negative bacteria with polysaccharide capsules. Members of the *Klebsiella* genus are exceptionally widespread in nature; *Klebsiella* spp. inhabit both water and soil environments, and they are associated with numerous plant and animal species (1). *Klebsiella* spp. are known to promote plant growth by colonizing plant tissues (roots) and providing essential nutrients to their plant hosts (2). For example, *Klebsiella* spp. encode the biochemical capacity to fix nitrogen, i.e., to convert molecular nitrogen to organic nitrogen in the form of ammonium (3). Plant growth promotion can also be facilitated via a number of other mechanisms, including phosphate solubilization, the production of phytohormones, an increase in nutritional uptake, and control of environmental stress (4, 5). The aim of this project was to use the analysis of *Klebsiella* sp. isolate genome sequences to evaluate their potential as biofertilizers. Given the fact that some *Klebsiella* spp. are known (opportunistic) pathogens, genome sequence analysis can also be used to mitigate the potential risk they pose to human populations if included as part of a bioinoculum.

The 15 *Klebsiella* sp. isolates characterized here were isolated from INCAUCA sugarcane fields, either from plants' root zones or directly from plant tissue. All isolates were grown overnight on LB medium (Difco) at 37°C. Genomic DNA was isolated using the E.Z.N.A. bacterial DNA kit (Omega Bio-tek), and paired-end fragment libraries were constructed using the Nextera XT DNA library preparation kit (Illumina), with a fragment length of 1,000 bp. Libraries were sequenced on an Illumina MiSeq platform using V3 chemistry, yielding approximately 400,000 paired-end 300-bp sequence reads per sample. Sequence read quality control was performed using the program FastQC version 0.11.5 (6). Adapter/primer sequences and low-quality bases and reads (Q < 20) were removed using Trimmomatic version 0.35 (7).

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TABLE 1 Strains obtained from *Saccharum* hybrid cultivars in 2014 from Colombia and sequenced in this study

Strain	GenBank accession no.
A-Nf5	PIOG00000000
B-Nf7	PIOH00000000
C-Nf10	PIOJ00000000
C1-16S-Nf17	PIOI00000000
D-Nf1	PIOK00000000
E-Nf3	PIOL00000000
F-Nf9	PIOM00000000
G-Nf4	PION00000000
G2-16S-Nf13	PIOO00000000
H-Nf2	PIBL00000000
I-Nf8	PIBM00000000
J-Nf11	PJDI00000000
K-Nf6	PIBN00000000
T11	PJDJ00000000
X1-16S-Nf21	PJDK00000000

The 15 *Klebsiella* sp. isolate genomes were assembled using the *de novo* assembler SPAdes version 3.6 (8). The summary statistics for the resulting assemblies indicate the completeness of the work. The genome coverages range from 50× to 88×, with an average of 64× coverage, which is more than sufficient to produce reliable assemblies. Accordingly, the genome assembly metrics are robust; N_{50} values range from 65,329 bp to 614,324 bp, with an average N_{50} of 290,406 bp, and L_{50} values range from 3 to 29, with an average value of 8.9. Finally, the genome size and GC content values inferred from the assemblies are consistent with what is expected for *Klebsiella* species. Assembled genome sizes range from 5.46 Mb to 6.09 Mb, with an average size of 5.64 Mb, and the GC content values range from 56.7% to 57.5%, with an average GC content of 57.1%.

Isolate genome sequences were annotated using the Rapid Annotations using Subsystems Technology (RAST) Web server (9–11). Functional predictions will be used to prioritize strains that are simultaneously enriched for nitrogen fixing and other plant growth-promoting genes while containing minimal antibiotic resistance genes and virulence factors.

Accession number(s). This whole-genome shotgun project has been deposited at DDBJ/ENA/GenBank under the accession numbers shown in Table 1.

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REFERENCES

1. Bagley ST. 1985. Habitat association of *Klebsiella* species. *Infect Control* 6:52–58. <https://doi.org/10.1017/S0195941700062603>.
2. Zahir ZA, Arshad M, Frankenberger WT. 2003. Plant growth promoting rhizobacteria: applications and perspectives in agriculture. *Adv Agron* 81:97–168. [https://doi.org/10.1016/S0065-2113\(03\)81003-9](https://doi.org/10.1016/S0065-2113(03)81003-9).
3. Postgate JR. 1982. Biological nitrogen fixation: fundamentals. *Philos Trans R Soc Lond B Biol Sci* 296:375–385. <https://doi.org/10.1098/rstb.1982.0013>.
4. Gamez RM, Rodriguez F, Bernal JF, Agarwala R, Landsman D, Marino-Ramirez L. 2015. Genome sequence of the banana plant growth-promoting rhizobacterium *Bacillus amyloliquefaciens* B5006. *Genome Announc* 3:e01391-15. <https://doi.org/10.1128/genomeA.01391-15>.
5. Gamez RM, Rodriguez F, Ramirez S, Gomez Y, Agarwala R, Landsman D, Marino-Ramirez L. 2016. Genome sequence of the banana plant growth-promoting rhizobacterium *Pseudomonas fluorescens* PS006. *Genome Announc* 4:e00329-16. <https://doi.org/10.1128/genomeA.00329-16>.
6. Andrews S. 2010. FastQC: a quality control tool for high throughput sequence data <https://www.bioinformatics.babraham.ac.uk/projects/fastqc/>.
7. Bolger AM, Lohse M, Usadel B. 2014. Trimmomatic: a flexible trimmer for Illumina sequence data. *Bioinformatics* 30:2114–2120. <https://doi.org/10.1093/bioinformatics/btu170>.
8. Bankevich A, Nurk S, Antipov D, Gurevich AA, Dvorkin M, Kulikov AS, Lesin VM, Nikolenko SI, Pham S, Pribelski AD, Pyshkin AV, Sirotkin AV, Vyahhi N, Tesler G, Alekseyev MA, Pevzner PA. 2012. SPAdes: a new

- genome assembly algorithm and its applications to single-cell sequencing. *J Comput Biol* 19:455–477. <https://doi.org/10.1089/cmb.2012.0021>.
9. Aziz RK, Bartels D, Best AA, DeJongh M, Disz T, Edwards RA, Formsma K, Gerdes S, Glass EM, Kubal M, Meyer F, Olsen GJ, Olson R, Osterman AL, Overbeek RA, McNeil LK, Paarmann D, Paczian T, Parrello B, Pusch GD, Reich C, Stevens R, Vassieva O, Vonstein V, Wilke A, Zagnitko O. 2008. The RAST server: Rapid Annotations using Subsystems Technology. *BMC Genomics* 9:75. <https://doi.org/10.1186/1471-2164-9-75>.
 10. Overbeek R, Olson R, Pusch GD, Olsen GJ, Davis JJ, Disz T, Edwards RA, Gerdes S, Parrello B, Shukla M. 2013. The SEED and the Rapid Annotation of microbial genomes using Subsystems Technology (RAST). *Nucleic Acids Res* 42:D206–D214. <https://doi.org/10.1093/nar/gkt1226>.
 11. Wattam AR, Abraham D, Dalay O, Disz TL, Driscoll T, Gabbard JL, Gillespie JJ, Gough R, Hix D, Kenyon R. 2013. PATRIC, the bacterial bioinformatics database and analysis resource. *Nucleic Acids Res* 42:D581–D591. <https://doi.org/10.1093/nar/gkt1099>.