

# Draft Genome Sequences of *Klebsiella variicola* Plant Isolates

Esperanza Martínez-Romero,<sup>a</sup> Jesús Silva-Sánchez,<sup>b</sup> Humberto Barrios,<sup>b</sup> Nadia Rodríguez-Medina,<sup>b</sup> Jesús Martínez-Barnetche,<sup>c</sup> Juan Téllez-Sosa,<sup>c</sup> Rosa Elena Gómez-Barreto,<sup>c</sup> Ulises Garza-Ramos<sup>b</sup>

Centro de Ciencias Genómicas (CCG), Universidad Nacional Autónoma de México (UNAM), Cuernavaca, Morelos, México<sup>a</sup>; Instituto Nacional de Salud Pública (INSP), Centro de Investigación Sobre Enfermedades Infecciosas (CISEI), Departamento de Diagnóstico Epidemiológico, Cuernavaca, Morelos, México<sup>b</sup>; Instituto Nacional de Salud Pública (INSP), Centro de Investigación Sobre Enfermedades Infecciosas (CISEI), Cuernavaca, Morelos, México<sup>c</sup>

**Three endophytic *Klebsiella variicola* isolates—T29A, 3, and 6A2, obtained from sugar cane stem, maize shoots, and banana leaves, respectively—were used for whole-genome sequencing. Here, we report the draft genome sequences of circular chromosomes and plasmids. The genomes contain plant colonization and cellulases genes. This study will help toward understanding the genomic basis of *K. variicola* interaction with plant hosts.**

Received 24 July 2015 Accepted 28 July 2015 Published 10 September 2015

**Citation** Martínez-Romero E, Silva-Sánchez J, Barrios H, Rodríguez-Medina N, Martínez-Barnetche J, Téllez-Sosa J, Gómez-Barreto RE, Garza-Ramos U. 2015. Draft genome sequences of *Klebsiella variicola* plant isolates. *Genome Announc* 3(5):e01015-15. doi:10.1128/genomeA.01015-15.

**Copyright** © 2015 Martínez-Romero et al. This is an open-access article distributed under the terms of the [Creative Commons Attribution 3.0 Unported license](http://creativecommons.org/licenses/by/4.0/).

Address correspondence to Ulises Garza-Ramos, ulises.garza@insp.mx.

**K**lebsiella variicola comprises clinical as well as plant and insect associated bacteria (1–4). The species was described in 2004 mainly on the basis of a distinct phylogenetic position and low DNA-DNA hybridization to related species (4). Now, PCR-multiplex may be used for a quick identification of *K. variicola* (5) and a wide SNP analysis supported the taxonomic status of the species (6). *K. variicola* isolates represent a small proportion of *Klebsiella* recovered from hospitalized patients (6, 7), and thus they seem less virulent than *Klebsiella pneumoniae* (1). However, in other cases, *K. variicola* infections are highly lethal (7). Plant, insect and clinical *K. variicola* strains are capable of fixing nitrogen and promoting plant growth (8); they seemingly have different epidemiological dynamics in comparison to *K. pneumoniae* (9). A genomic analysis would reveal *K. variicola* virulence or antibiotic resistance genes and genes involved in its association with plants.

The endophytic isolates studied in this work were originally obtained from inside tissues from maize shoots (strain 3), banana leaves (strain 6A2), or sugar cane stem (strain T29A) (2, 4). Total genomic DNA from *K. variicola* isolates were extracted and purified using the DNeasy Kit (Qiagen, Germany). Whole-genome sequences were generated using pyrosequencing on the 454 Roche FLX Titanium (T29A and 6A2 strains) and Plus (3 strain) platform. Reads (99.9% above Q40) longer than 500 bp were used for *de novo* assembly with the CLC Genomics Workbench version 4.0 (CLC bio). The total sequence data are as follows. *K. variicola* T29A genome (227,982 reads with 30- to 943-bp length range): a total of 126 contigs, with an  $N_{50}$  of 97,031 bp and estimated genome size of 5,865,668 bp (including the 200-kb and 80-kb plasmids) with 18× coverage; *K. variicola* 3 genome (463,271 reads with 24- to 1,508-bp length range): a total of 24 contigs, with an  $N_{50}$  of 374,829 bp and estimated genome size of 5,512,858 bp with 25× coverage; and *K. variicola* 6A2 genome (205,051 reads with 30- to 943-bp length range): a total of 206 contigs, with an  $N_{50}$  of 49,243 bp and estimated genome size of 5,925,914 bp (including the 200-kb, 150-kb and 140-kb plasmids) with 15× coverage. Gene prediction and annotation were carried out using the bioin-

formatic MicroScope platform (10). In the *K. variicola* T29A, 3, and 6A2 genomes, respectively, a total of 5,751, 5,387, and 5,906 coding DNA sequences; 67, 77, and 60 tRNA genes; and 4, 6, and 2 rRNA were found. The average G+C content was similar in the three genomes (56.9 to 57.3).

A BLAST analysis showed a *nifL-nifQ* cluster (with 20 genes), *pyrG*, acetyl-CoA acetyltransferase (KPK\_0844), *purF*, *argG*, *lysA*, *ivII*, *adA*, *uvrA*, *dinF*, *fumC*, and *acrA* genes. The *K. variicola* T29A and 6A2 genomes contained the *celK* gene that encodes to cellulose 1,4-beta-cellobiosidase protein, and the *bgfH* gene that encodes to Aryl-phospho-beta-D-glucosidase protein is contained in the T29A, 3, and 6A2 genomes. The sequenced genomes are similar to the previously reported *K. pneumoniae* 342 (actually *K. variicola*) genome (1). A total of 4,680 core genes were determined among the *K. variicola* genomes included in this study.

**Nucleotide sequence accession numbers.** The annotated genomes sequences are available at the European Nucleotide Archive under accession numbers CXPA01000001 (*K. variicola* T29A), CXOY01000001 (*K. variicola* 3), and CXPC01000001 (*K. variicola* 6A2).

## ACKNOWLEDGMENTS

This work was funded by the Consejo Nacional de Ciencia y Tecnología (CONACyT), SEP-CONACYT grant number 130224.

## REFERENCES

1. Fouts DE, Tyler HL, DeBoy RT, Daugherty S, Ren Q, Badger JH, Durkin AS, Huot H, Shrivastava S, Kothari S, Dodson RJ, Mohamoud Y, Khouri H, Roesch LF, Krogfelt KA, Struve C, Triplett EW, Methé BA. 2008. Complete genome sequence of the N2-fixing broad host range endophyte *Klebsiella pneumoniae* 342 and virulence predictions verified in mice. *PLoS Genet* 4:e1000141. <http://dx.doi.org/10.1371/journal.pgen.1000141>.
2. Martínez L, Caballero-Mellado J, Orozco J, Martínez-Romero E. 2003. Diazotrophic bacteria associated with banana (*Musa* spp.). *Plant Soil* 257: 35–47. <http://dx.doi.org/10.1023/A:1026283311770>.
3. Pinto-Tomás AA, Anderson MA, Suen G, Stevenson DM, Chu FS, Cleland WW, Weimer PJ, Currie CR. 2009. Symbiotic nitrogen fixation

- in the fungus gardens of leaf-cutter ants. *Science* 326:1120–1123. <http://dx.doi.org/10.1126/science.1173036>.
4. Rosenblueth M, Martínez L, Silva J, Martínez-Romero E. 2004. *Klebsiella variicola*, a novel species with clinical and plant-associated isolates. *Syst Appl Microbiol* 27:27–35. <http://dx.doi.org/10.1078/0723-2020-00261>.
  5. Garza-Ramos U, Silva-Sánchez J, Martínez-Romero E, Tinoco P, Pinagonzales M, Barrios H, Martínez-Barnetche J, Gómez-Barreto RE, Tellez-Sosa J. 2015. Development of a multiplex-PCR probe system for the proper identification of *Klebsiella variicola*. *BMC Microbiol* 15:64. <http://dx.doi.org/10.1186/s12866-015-0396-6>.
  6. Holt KE, Wertheim H, Zadoks RN, Baker S, Whitehouse CA, Dance D, Jenney A, Connor TR, Hsu LY, Severini J, Brisse S, Cao H, Wilksch J, Gorrie C, Schultz MB, Edwards DJ, Nguyen KV, Nguyen TV, Dao TT, Mensink M, Minh VL, Nhu NT, Schultsz C, Kuntaman K, Newton PN, Moore CE, Strugnell RA, Thomson NR. 2015. Genomic analysis of diversity, population structure, virulence, and antimicrobial resistance in *Klebsiella pneumoniae*, an urgent threat to public health. *Proc Natl Acad Sci USA* 112:E3574–E3581. <http://dx.doi.org/10.1073/pnas.1501049112>.
  7. Maatallah M, Vading M, Kabir MH, Bakhrouf A, Kalin M, Naucré P, Brisse S, Giske CG. 2014. *Klebsiella variicola* is a frequent cause of blood-stream infection in the Stockholm area, and associated with higher mortality compared to *K. pneumoniae*. *PLoS One* 9:e113539. <http://dx.doi.org/10.1371/journal.pone.0113539>.
  8. Wei C, Lin L, Luo L, Xing Y, Hu C, Yang L, Li Y, An Q. 2014. Endophytic nitrogen-fixing *Klebsiella variicola* strain dx120e promotes sugarcane growth. *Biol Fertil Soils* 50:657–666. <http://dx.doi.org/10.1007/s00374-013-0878-3>.
  9. Martínez J, Martínez L, Rosenblueth M, Silva J, Martínez-Romero E. 2004. How are gene sequence analyses modifying bacterial taxonomy? The case of *Klebsiella*. *Int Microbiol* 7:261–268.
  10. Vallenet D, Belda E, Calteau A, Cruveiller S, Engelen S, Lajus A, Le Févre F, Longin C, Mornico D, Roche D, Rouy Z, Salvignol G, Scarpelli C, Thil Smith AA, Weiman M, Médigue C. 2013. MicroScope—an integrated microbial resource for the curation and comparative analysis of genomic and metabolic data. *Nucleic Acids Res* 41:D636–D647. <http://dx.doi.org/10.1093/nar/gks1194>.