



Complete Genome Sequence of a *Klebsiella pneumoniae* Strain Isolated from a Known Cotton Insect Boll Vector

Enrique G. Medrano, Marissa M. Forray, Alois A. Bell

Insect Control and Cotton Disease Research Unit, U.S. Department of Agriculture, Agricultural Research Service, College Station, Texas, USA

Klebsiella pneumoniae (associated with bacterial pneumonia) was previously isolated from *Nezara viridula*, a significant vector of cotton boll-rot pathogens. We provide the first annotated genome sequence of the cotton opportunistic strain *K. pneumoniae* 5-1. This data provides guidance to study the bases of cotton pathogenesis by bacteria associated with vectors.

Received 29 July 2014 Accepted 30 July 2014 Published 21 August 2014

Citation Medrano EG, Forray MM, Bell AA. 2014. Complete genome sequence of a Klebsiella pneumoniae strain isolated from a known cotton insect boll vector. Genome Announc. 2(4):e00850-14. doi:10.1128/genomeA.00850-14.

Copyright © 2014 Medrano et al. This is an open-access article distributed under the terms of the Creative Commons Attribution 3.0 Unported license.

Address correspondence to Enrique G. Medrano, gino.medrano@ars.usda.gov.

Klebsiella pneumoniae causes bacterial pneumonia, and is a recognized source of nosocomial and community-acquired infections (1). An emerging problem in treatment of the bacteria is strain resistance to multiple antibiotics including those of the beta-lactamase family (1). Furthermore, although virulence factors have been identified, there is limited knowledge concerning the pathological pattern of infection. Notably, *Klebsiella* strains have been recovered from the southern green stink bug, *Nezara viridula* (2, 3), a known vector of cotton disease-causing agents (4). Potential correlations have been documented between increasing populations of *N. viridula* and seed necrosis. The strain sequenced here is capable of causing appreciable boll damage.

The sequenced K. pneumoniae subsp. pneumoniae strain Kp 5-1 (K3/K8 serotype) was isolated from N. viridula collected from a cotton field (3). A Roche 454 GS-Junior DNA analyzer was used to generate the draft genome sequence (16-fold coverage) of strain Kp 5-1. A Roche GS Titanium shotgun library was prepared, and two runs were pyrosequenced, yielding 42 Mb (208,251 reads with an average length of 317 bases) and 37 Mb (232,738 reads with an average length of 457 bases). Using the same genomic DNA stock, 3- and 8-kb paired-end Titanium libraries were constructed and pyrosequenced, producing 7 Mb (183,274 reads with 99,378 paired reads) and 42 Mb (20,765 reads with 16,641 paired reads), respectively. The genome was constructed using GS De Novo Assembler 454 version 2.7 and the CLC Genomics Workbench Linux platform, resulting in two scaffolds totaling 5.5 Mb. Both the largest scaffold (5.4 Mb) and an identified plasmid (186 kbp) contained contig gaps that were closed by cloning of PCR products and Sanger sequencing by employing an ABI PRISM 3100. Putative coding sequences were predicted by manual annotation using the NCBI BLAST program and the Prokaryotic Genome Annotation Pipeline program at the NCBI; both sets of results were manually curated.

In total, the genome contained 4,918 predicted coding sequences (CDSs), 6 rRNA operons, and 77 tRNAs. The annotation data revealed type IV and VI secretion systems, which are reportedly involved in plant disease in other bacteria (5) and potentially play a role in cotton infirmity. Sequencing the genome of strain Kp 5-1 will allow for the discovery of purported genes implicated in the pathogenesis of cotton bolls for the purpose of bypassing current traditional isolation and infectivity methods. This will allow for direct detection of the genes and their respective products involved in disease production.

Nucleotide sequence accession numbers. This complete genome sequence has been deposited at DDBJ/EMBL/GenBank under accession numbers CP008700 and CP008701.

ACKNOWLEDGMENTS

Thanks are due to Cyndy Menn for both her technical and computational support. Whitney Mantooth, Stephanie Thornton, and Anne Purgason assisted with the manual annotation of the genome.

This work was supported by USDA-ARS project 6202-22000-031-00D.

REFERENCES

- Calbo E, Freixas N, Xercavins M, Riera M, Nicolás C, Monistrol O, del mar Sole M, Sala MR, Vila J, Garau J. 2011. Foodborne nosocomial outbreak of SHV1 and CTX-M-15-producing *Klebsiella pneumoniae*: epidemiology and control. Clin. Infect. Dis. 52:743–749. http://dx.doi.org/ 10.1093/cid/ciq238.
- Hirose E, Panizzi AR, De Souza JT, Cattelan AJ, Aldrich JR. 2006. Bacteria in the gut of southern green stink bug (Heteroptera: Pentatomidae). Ann. Entomol. Soc. Am. 99:91–95. http://dx.doi.org/10.1603/0013 -8746(2006)099[0091:BITGOS]2.0.CO;2.
- Medrano EG, Esquivel J, Bell A, Greene J, Roberts P, Bacheler J, Marois J, Wright D, Nichols R, Lopez J. 2009. Potential for *Nezara viridula* (Hemiptera: Pentatomidae) to transmit bacterial and fungal pathogens into cotton bolls. Curr. Microbiol. 59:405–412. http://dx.doi.org/10.1007/ s00284-009-9452-5.
- 4. Medrano EG, Esquivel JF, Bell AA. 2007. Transmission of cotton seed and boll rotting bacteria by the southern green stink bug (*Nezara viridula* L.). J. Appl. Microbiol. 103:436–444. http://dx.doi.org/10.1111/j.1365 -2672.2006.03262.x.
- Medrano EG, Bell AA. 2012. Genome sequence of *Pantoea* sp. strain Sc 1, an opportunistic cotton pathogen. J. Bacteriol. 194:. http://dx.doi.org/ 10.1128/JB.00450-12. 3019.