





## Genome Sequence of a Multidrug-Resistant Strain of *Bacillus pumilus*, CB01, Isolated from the Feces of an American Crow, *Corvus brachyrhynchos*

R. Lee Nelson, Michael A. Castro, Madhusudan Katti, Jonathan A. Eisen, Tricia A. Van Laara

Department of Biology, College of Science and Mathematics, California State University, Fresno, Fresno, California, USA<sup>a</sup>; Department of Evolution and Ecology, Department of Medical Microbiology and Immunology, University of California, Davis, Davis, California, USA<sup>b</sup>

Avian species have the potential to serve as important reservoirs for the spread of pathogenic microorganisms. Here, we report the genome sequence of a drug-resistant strain of *Bacillus pumilus*, CB01, isolated from the feces of an American crow, *Corvus brachyrhynchos*.

Received 22 June 2016 Accepted 24 June 2016 Published 18 August 2016

Citation Nelson RL, Castro MA, Katti M, Eisen JA, Van Laar TA. 2016. Genome sequence of a multidrug-resistant strain of *Bacillus pumilus*, CB01, isolated from the feces of an American crow, *Corvus brachyrhynchos*. Genome Announc 4(4):e00807-16. doi:10.1128/genomeA.00807-16.

Copyright © 2016 Nelson et al. This is an open-access article distributed under the terms of the Creative Commons Attribution 4.0 International license.

Address correspondence to Tricia A. Van Laar, tvanlaar@csufresno.edu

Work in avian species, including gulls (1), crows (2), and other birds (3), has shown the potential for the transmission of pathogenic organisms to humans and human-associated settings, including poultry and dairy operations. More importantly, birds (4, 5) have been identified as being reservoirs for antimicrobial-resistant microorganisms. Birds, such as gulls and crows, are of particular concern as reservoirs for antimicrobial resistance due to their association with human habitation and tendency to feed on raw sewage and garbage (2, 3), which are likely contaminated with enteric pathogens and other pathogenic microorganisms. Also, birds are highly migratory, with approximately 300 species migrating to and from North America every year (3), leading to the potential spread of antimicrobial-resistant organisms to/from multiple countries.

Bacillus pumilus is a Gram-positive, aerobic, spore-forming bacterium found commonly in soil. The spores allow this organism to be resistant to many adverse environmental conditions, including heat, desiccation, and disinfection (6). *B. pumilus* has been shown to produce and secrete antifungal agents, promote plant growth, degrade pesticides, and control insect larvae (7–10). More recently, *B. pumilus* has also been shown to be associated with infection, including sepsis in neonates and cutaneous and catheter infections (11–14).

B. pumilus strain CB01 was isolated from the fecal matter from an American crow, Corvus brachyrhynchos, collected approximately 200 m from Fresno Community Regional Medical Center in Fresno, CA. Feces were resuspended in phosphate-buffered saline (PBS) and streaked for isolation on various differential media. This colony was selected from orange serum agar and grown in LB broth overnight at 37°C. Total genomic DNA was purified using the Wizard genomic DNA purification kit from Promega (Madison, WI, USA). This strain was identified as B. pumilus through 16S rRNA sequencing (15). As a major concern with bacterial infections is their potential for antimicrobial resistance, we determined the minimum inhibitory concentrations (MICs) of various antibiotics to generate a resistance profile for B. pumilus CB01.

Our analysis found resistance to numerous antibiotics, including cefotaxime, hygromycin, methicillin, and spectinomycin.

De novo genome sequencing of B. pumilus CB01 was provided by MR DNA (Shallowater, TX) using an Illumina MiSeq, generating 3,217,896 300-bp paired-end reads. The sequence was processed and assembled using the A5-miseq assembly pipeline (16). Assembly yielded a genome with 3,830,520 bp and 41.5% G+C content, with a coverage of 169.89×. There were 37 contigs, with an  $N_{50}$  contig size of 442,844 bp. The genome was annotated using the RAST annotation server (17) and was predicted to contain 3,988 protein-coding sequences and 100 noncoding RNAs.

Continued study of microorganisms associated with avian species, particularly the American crow, will provide insight into the potential for these birds to contribute to pathogenesis and antimicrobial resistance in human populations.

**Accession number(s).** This whole-genome shotgun project has been deposited at DDBJ/ENA/GenBank under the accession no. LYXP000000000. The version described in this paper is version LYXP01000000.

## **FUNDING INFORMATION**

This work, including the efforts of R. Lee Nelson, was funded by California State University Council on Ocean Affairs, Science & Technology (COAST). This work, including the efforts of Tricia A. Van Laar, was funded by California State University, Fresno start-up.

## **REFERENCES**

- 1. Benton C, Khan F, Monaghan P, Richards WN, Shedden CB. 1983. The contamination of a major water-supply by gulls (*Larus* sp.)—a study of the problem and remedial action taken. Water Res 17:789–798. http://dx.doi.org/10.1016/0043-1354(83)90073-8.
- Weis AM, Miller WA, Byrne BA, Chouicha N, Boyce WM, Townsend AK. 2014. Prevalence and pathogenic potential of *Campylobacter* isolates from free-living, human-commensal American crows. Appl Environ Microbiol 80:1639–1644. http://dx.doi.org/10.1128/AEM.03393-13.
- 3. Reed KD, Meece JK, Henkel JS, Shukla SK. 2003. Birds, migration and emerging zoonoses: west Nile virus, Lyme disease, influenza A and enteropathogens. Clin Med Res 1:5–12. http://dx.doi.org/10.3121/cmr.1.1.5.

- Pinto L, Radhouani H, Coelho C, Martins da Costa P, Simões R, Brandão RM, Torres C, Igrejas G, Poeta P. 2010. Genetic detection of extended-spectrum beta-lactamase-containing *Escherichia coli* isolates from birds of prey from Serra da Estrela natural reserve in Portugal. Appl Environ Microbiol 76:4118–4120. http://dx.doi.org/10.1128/ AFM.02761-09.
- Silva N, Igrejas G, Rodrigues P, Rodrigues T, Gonçalves A, Felgar AC, Pacheco R, Gonçalves D, Cunha R, Poeta P. 2011. Molecular characterization of vancomycin-resistant enterococci and extended-spectrum betalactamase-containing *Escherichia coli* isolates in wild birds from the Azores archipelago. Avian Pathol 40:473–479. http://dx.doi.org/10.1080/ 03079457.2011.599061.
- Brown KL. 2000. Control of bacterial spores. Br Med Bull 56:158–171. http://dx.doi.org/10.1258/0007142001902860.
- 7. Leifert C, Li H, Chidburee S, Hampson S, Workman S, Sigee D, Epton HA, Harbour A. 1995. Antibiotic production and biocontrol activity by *Bacillus subtilis* C27 and *Bacillus pumilus* CL45. J Appl Bacteriol 78: 97–108. http://dx.doi.org/10.1111/j.1365-2672.1995.tb02829.x.
- 8. Anwar S, Liaquat F, Khan QM, Khalid ZM, Iqbal S. 2009. Biodegradation of chlorpyrifos and its hydrolysis product 3,5,6-trichloro-2-pyridinol by *Bacillus pumilus* strain C2A1. J Hazard Mater 168:400–405. http://dx.doi.org/10.1016/j.jhazmat.2009.02.059.
- Garcia-Ramon DC, Molina CA, Osuna A, Vílchez S. 2016. An in-depth characterization of the entomopathogenic strain *Bacillus pumilus* 15.1 reveals that it produces inclusion bodies similar to the parasporal crystals of *Bacillus thuringiensis*. Appl Microbiol Biotechnol 100:3637–3654. http:// dx.doi.org/10.1007/s00253-015-7259-9.
- 10. Gutiérrez-Mañero FJ, Ramos-Solano B, Probanza A, Mehouachi J, Tadeo FR, Talon M. 2001. The plant-growth-promoting rhizobacteria bacillus *pumilus* and *Bacillus licheniformis* produce high amounts of phys-

- iologically active gibberellins. Physiol Plant 111:206 211. http://dx.doi.org/10.1034/j.1399-3054.2001.1110211.x.
- Bentur HN, Dalzell AM, Riordan FA. 2007. Central venous catheter infection with *Bacillus pumilus* in an immunocompetent child: a case report. Ann Clin Microbiol Antimicrob 6:12. http://dx.doi.org/10.1186/ 1476-0711-6-12.
- 12. Grass G, Bierbaum G, Molitor E, Götte N, Antwerpen M. 2016. Genome sequence of *Bacillus pumilus* strain Bonn, isolated from an anthrax-like necrotic skin infection site of a child. Genome Announc 4(1):e01741-15. http://dx.doi.org/10.1128/genomeA.01741-15.
- 13. Kimouli M, Vrioni G, Papadopoulou M, Koumaki V, Petropoulou D, Gounaris A, Friedrich AW, Tsakris A. 2012. Two cases of severe sepsis caused by *Bacillus pumilus* in neonatal infants. J Med Microbiol 61: 596–599. http://dx.doi.org/10.1099/jmm.0.033175-0.
- 14. Tena D, Martinez-Torres JA, Perez-Pomata MT, Sáez-Nieto JA, Rubio V, Bisquert J. 2007. Cutaneous infection due to *Bacillus pumilus*: report of 3 cases. Clin Infect Dis 44:e40–e42. http://dx.doi.org/10.1086/511077.
- Dunitz MI, Lang JM, Jospin G, Darling AE, Eisen JA, Coil DA. 2015.
  Swabs to genomes: a comprehensive workflow. PeerJ 3:e960. http://dx.doi.org/10.7717/peerj.960.
- Coil D, Jospin G, Darling AE. 2015. A5-miseq: an updated pipeline to assemble microbial genomes from Illumina MiSeq data. Bioinformatics 31:587–589. http://dx.doi.org/10.1093/bioinformatics/btu661.
- 17. 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. http://dx.doi.org/10.1186/1471-2164-9-75.