### Washington University School of Medicine Digital Commons@Becker

**Open Access Publications** 

2017

## Draft genome sequences of three $\beta$ -lactamcatabolizing soil Proteobacteria

Terence S. Crofts Washington University School of Medicine in St. Louis

Bin Wang Washington University School of Medicine in St. Louis

Aaron Spivak Washington University School of Medicine in St. Louis

Tara A. Gianoulis *Harvard University* 

Kevin J. Forsberg Washington University School of Medicine in St. Louis

See next page for additional authors

Follow this and additional works at: https://digitalcommons.wustl.edu/open\_access\_pubs

#### **Recommended** Citation

Crofts, Terence S.; Wang, Bin; Spivak, Aaron; Gianoulis, Tara A.; Forsberg, Kevin J.; Gibson, Molly K.; Johnsky, Lauren A.; Broomall, Stacey M.; Rosenzweig, C. Nicole; Skowronski, Evan W.; Gibbons, Henry S.; Sommer, Morten O.A.; and Dantas, Gautam, "Draft genome sequences of three  $\beta$ -lactam-catabolizing soil Proteobacteria." Genome Announcements.5,32. e00653-17. (2017). https://digitalcommons.wustl.edu/open\_access\_pubs/6187

This Open Access Publication is brought to you for free and open access by Digital Commons@Becker. It has been accepted for inclusion in Open Access Publications by an authorized administrator of Digital Commons@Becker. For more information, please contact engeszer@wustl.edu.

#### Authors

Terence S. Crofts, Bin Wang, Aaron Spivak, Tara A. Gianoulis, Kevin J. Forsberg, Molly K. Gibson, Lauren A. Johnsky, Stacey M. Broomall, C. Nicole Rosenzweig, Evan W. Skowronski, Henry S. Gibbons, Morten O.A. Sommer, and Gautam Dantas

#### PROKARYOTES





AMERICAN SOCIETY FOR

# Draft Genome Sequences of Three $\beta$ -Lactam-Catabolizing Soil *Proteobacteria*

#### Terence S. Crofts,<sup>a</sup> Bin Wang,<sup>a,b</sup> Aaron Spivak,<sup>c</sup> Tara A. Gianoulis,<sup>d</sup>† Kevin J. Forsberg,<sup>b\*</sup> Molly K. Gibson,<sup>b\*</sup> Lauren A. Johnsky,<sup>e\*</sup> Stacey M. Broomall,<sup>e</sup> C. Nicole Rosenzweig,<sup>e,f</sup> Evan W. Skowronski,<sup>e\*</sup> Henry S. Gibbons,<sup>e</sup> Morten O. A. Sommer,<sup>g</sup> Gautam Dantas<sup>a,b,h,i</sup>

gen@meAnnouncements™

Department of Pathology and Immunology, Washington University in St. Louis, St. Louis, Missouri, USA<sup>a</sup>; Center for Genome Sciences and Systems Biology, Washington University in St. Louis, St. Louis, Missouri, USA<sup>b</sup>; Department of Genetics, Washington University in St. Louis, St. Louis, Missouri, USA<sup>c</sup>; Wyss Institute for Biologically Inspired Engineering, Harvard, Cambridge, Massachusetts, USA<sup>d</sup>; U.S. Army Edgewood Chemical Biological Center, Aberdeen Proving Ground, Maryland, USA<sup>e</sup>; OptiMetrics, Inc., Abingdon, Maryland, USA<sup>f</sup>; Novo Nordisk Foundation Center for Biosustainability, Technical University of Denmark, Lyngby, Denmark<sup>9</sup>; Department of Molecular Microbiology, Washington University in St. Louis, St. Louis, Missouri, USA<sup>h</sup>; Department of Biomedical Engineering, Washington University in St. Louis, St. Louis, Missouri, USA<sup>h</sup>

**ABSTRACT** Most antibiotics are derived from the soil, but their catabolism there, which is necessary to close the antibiotic carbon cycle, remains uncharacterized. We report the first draft genome sequences of soil *Proteobacteria* identified for subsisting solely on  $\beta$ -lactams as their carbon sources. The genomes encode multiple  $\beta$ -lactamases, although their antibiotic catabolic pathways remain enigmatic.

A ntibiotic synthesis and antibiotic resistance are both ancient and well-studied features of the soil microbiome (1). Missing from our understanding of antibiotic ecology is the ultimate environmental fate of these potential carbon sources. While antibiotic catabolism has been recognized since the discovery of the first compounds (2–4), including in multiple *Proteobacteria* species (5–10), the cellular machinery underlying these phenotypes has eluded discovery. In order to facilitate the study of this phenomenon, we have undertaken the whole-genome sequencing of three soil isolates termed ABC07 (*Pseudomonas* sp. strain PE-S1G-1), ABC08 (*Pandoraea* sp. strain PE-S2R-1), and ABC10 (*Pandoraea* sp. strain PE-S2T-3). These strains were previously described as being capable of utilizing penicillin as their sole source of carbon for growth in minimal medium (9).

Each ABC strain was inoculated into 5 ml of LB from  $-80^{\circ}$ C glycerol stocks (15% in LB) and grown aerobically at room temperature. ABC strain genomic DNA was extracted from cell pellets using the Mo Bio PowerMax soil kit (catalog no. 12988-10) and dissolved in Tris-EDTA (TE) buffer. Genomes were sequenced at the Edgewood Chemical Biological Center Genomics Laboratory using a 454-GS FLX sequencer, and raw .sff files were assembled *de novo* using Newbler version 2.0.01.14 (454 Life Sciences) with the following parameters: SeedStep, 12; SeedLength, 16; MinSeedCount, 1; SeedHit-Limit, 10,000; HitPositionLimit, 200; MinMatchLength, 40; MinMatchIdentity, 90; Match-IdentScore, 2; MatchDiffScore, -3; and MatchUniqThresh, 12. Each sequenced genome resulted in ca. 500,000 reads encompassing ~10<sup>8</sup> bp. ABC07, ABC08, and ABC10 were assembled into 137, 38, and 25 large contigs, respectively, with contig  $N_{50}$  metrics of 98,867, 1,179,846, and 601,724 bp, respectively.

To identify features of the genomes potentially pertinent to antibiotic catabolism, genomes were uploaded to the online KBase server (11) for annotation using RAST (12). RAST predicted 6,594, 5,771, and 5,569 total features, and 10, 8, and 8  $\beta$ -lactamases or

Received 26 May 2017 Accepted 13 June 2017 Published 10 August 2017

**Citation** Crofts TS, Wang B, Spivak A, Gianoulis TA, Forsberg KJ, Gibson MK, Johnsky LA, Broomall SM, Rosenzweig CN, Skowronski EW, Gibbons HS, Sommer MOA, Dantas G. 2017. Draft genome sequences of three β-lactamcatabolizing soil *Proteobacteria*. Genome Announc 5:e00653-17. https://doi.org/10.1128/ genomeA.00653-17.

**Copyright** © 2017 Crofts et al. This is an openaccess article distributed under the terms of the Creative Commons Attribution 4.0 International license.

Address correspondence to Terence S. Crofts, tcrofts@path.wustl.edu.

\* Present address: Kevin J. Forsberg, Division of Basic Sciences, Fred Hutchinson Cancer Research Center, Seattle, Washington, USA; Molly K. Gibson, Flagship Pioneering, Cambridge, Massachusetts, USA; Lauren A. Johnsky, Technology, Plans and Program Office CERDEC Command, Power & Integration (CP&I) Directorate RDER-CPS, Aberdeen Proving Ground, Maryland, USA; Evan W. Skowronski, TMG Biosciences, LLC, Austin, Texas, USA. † Deceased.  $\beta$ -lactamase-like features in the genomes of ABC07, ABC08, and ABC10, respectively. The top three functional categories identified for each strain were carbohydrates, amino acids and derivatives, cofactors, vitamins, prosthetic groups, and pigments for ABC07; carbohydrates, metabolism of aromatic compounds, and amino acids and derivatives for ABC08; and carbohydrates, metabolism of aromatic compounds, and amino acids and amino acids and derivatives for ABC10.

Analysis of genomes from three antibiotic-catabolizing bacteria has revealed the presence of multiple  $\beta$ -lactamase genes in each organism and suggests a conserved role for the metabolism of aromatic carbon sources and amino acids. Antibiotic inactivation is confirmed in these strains phenotypically (9) and, here, by genotype, and it may represent the first step in  $\beta$ -lactam catabolism. The release of these genomes should significantly aid in the identification of antibiotic catabolism pathways.

Accession number(s). This whole-genome shotgun project has been deposited at DDBJ/ENA/GenBank under the accession numbers NGUS00000000, NGUR00000000, and NGUQ00000000 for strains ABC07, ABC08, and ABC10, respectively. The versions described in this paper are the first versions, NGUS01000000, NGUR01000000, and NGUQ01000000, respectively.

#### **ACKNOWLEDGMENTS**

This work is supported in part by awards to G.D. through the Edward Mallinckrodt, Jr Foundation (Scholar Award) and from the NIH Director's New Innovator Award (http://commonfund.nih.gov/newinnovator/), the National Institute of Diabetes and Digestive and Kidney Diseases (NIDDK [http://www.niddk.nih.gov]), the National Institute of General Medical Sciences (NIGMS [http://www.nigms.nih.gov]), and the National Institute of Allergy and Infectious Diseases (NIAID [https://www.niaid.nih.gov]) of the National Institutes of Health (NIH) under award numbers DP2DK098089, R01GM099538, and R01Al123394, respectively. T.S.C. received support from a National Institute of Child Health and Development training grant through award no. T32 HD049305 (Kelle H. Moley, principal investigator). K.J.F. received support from the NIGMS Cell and Molecular Biology Training Grant (GM 007067), the NHGRI Genome Analysis Training Program (T32 HG000045), and the NSF as a graduate research fellow (award number DGE-1143954). M.K.G. received support as a Spencer T. Olin Fellow at Washington University and from the NSF as a graduate research fellow (DGE-1143954). Sequencing through the U.S. Army Edgewood Chemical Biological Center was supported in part through funding provided by the Transformational Medical Technologies Initiative of the Defense Threat Reduction Agency, U.S. Department of Defense.

The content is solely the responsibility of the authors and does not necessarily represent the official views of the funding agencies.

We are thankful to Robert Potter of the Dantas lab for general helpful discussions regarding the manuscript.

#### REFERENCES

- Crofts TS, Gasparrini AJ, Dantas G. 2017. Next-generation approaches to understand and combat the antibiotic resistome. Nat Rev Microbiol 15:422–434. https://doi.org/10.1038/nrmicro.2017.28.
- Pramer D, Starkey RL. 1951. Decomposition of streptomycin. Science 113:127. https://doi.org/10.1126/science.113.2927.127.
- Kameda Y, Kimura Y, Toyoura E, Omori T. 1961. A method for isolating bacteria capable of producing 6-aminopenicillanic acid from benzylpenillin. Nature 191:1122–1123. https://doi.org/10.1038/1911122a0.
- Abd-El-Malek Y, Monib M, Hazem A. 1961. Chloramphenicol, a simultaneous carbon and nitrogen source for a *Streptomyes* sp. from Egyptian soil. Nature 189:775–776. https://doi.org/10.1038/189775a0.
- Johnsen J. 1977. Utilization of benzylpenicillin as carbon, nitrogen and energy source by a *Pseudomonas fluorescens* strain. Arch Microbiol 115:271–275. https://doi.org/10.1007/BF00446452.

- Johnsen J. 1981. Presence of beta-lactamase and penicillin acylase in a *Pseudomonas* sp. utilizing benzylpenicillin as a carbon source. J Gen Appl Microbiol 27:499–503. https://doi.org/10.2323/jgam.27.499.
- Beckman W, Lessie TG. 1979. Response of *Pseudomonas cepacia* to beta-lactam antibiotics: utilization of penicillin G as the carbon source. J Bacteriol 140:1126–1128.
- Barnhill AE, Weeks KE, Xiong N, Day TA, Carlson SA. 2010. Identification of multiresistant *Salmonella* isolates capable of subsisting on antibiotics. Appl Environ Microbiol 76:2678–2680. https://doi.org/10.1128/AEM .02516-09.
- Dantas G, Sommer MOA, Oluwasegun RD, Church GM. 2008. Bacteria subsisting on antibiotics. Science 320:100–103. https://doi.org/10.1126/ science.1155157.
- 10. Xin Z, Fengwei T, Gang W, Xiaoming L, Qiuxiang Z, Hao Z, Wei C. 2012.

Isolation, identification and characterization of human intestinal bacteria with the ability to utilize chloramphenicol as the sole source of carbon and energy. FEMS Microbiol Ecol 82:703–712. https://doi.org/10.1111/j .1574-6941.2012.01440.x.

 Arkin AP, Stevens RL, Cottingham RW, Maslov S, Henry CS, Dehal P, Ware D, Perez F, Harris NL, Canon S, Sneddon MW, Henderson ML, Riehl WJ, Gunter D, Murphy-Olson D, Chan S, Kamimura RT, Brettin TS, Meyer F, Chivian D, Weston DJ, Glass EM, Davison BH, Kumari S, Allen BH, Baumohl J, Best AA, Bowen B, Brenner SE, Bun CC, Chandonia J-M, Chia J-M, Colasanti R, Conrad N, Davis JJ, DeJongh M, Devoid S, Dietrich E, Drake MM, Dubchak I, Edirisinghe JN, Fang G, Faria JP, Frybarger PM, Gerlach W, Gerstein M, Gurtowski J, Haun HL, He F, Jain R, et al. 2016. The DOE Systems Biology KnowledgeBase (KBase). bioRxiv. https://doi.org/10.1101/096354.

 Overbeek R, Olson R, Pusch GD, Olsen GJ, Davis JJ, Disz T, Edwards RA, Gerdes S, Parrello B, Shukla M, Vonstein V, Wattam AR, Xia F, Stevens R. 2014. 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.