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Draft Genome Sequences of Three β -Lactam-Catabolizing Soil *Proteobacteria*

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

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

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 β -lactams as their carbon sources. The genomes encode multiple β -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° 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 ~10⁸ 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 β -lactamases or

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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. β -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 β -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 β -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.

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