



## Genome Sequences of Two Copper-Resistant *Escherichia coli* Strains Isolated from Copper-Fed Pigs

## Freja L. Lüthje,<sup>a</sup> Henrik Hasman,<sup>b</sup> Frank M. Aarestrup,<sup>b</sup> Hend A. Alwathnani,<sup>c</sup> Christopher Rensing<sup>a,d</sup>

Department of Plant and Environmental Science, University of Copenhagen, Frederiksberg, Denmark<sup>a</sup>; National Food Institute, Technical University of Denmark, Lyngby, Denmark<sup>b</sup>; Department of Botany and Microbiology, King Saud University, Riyadh, Saudi Arabia<sup>c</sup>; Institute of Urban Environment, Chinese Academy of Sciences, Xiamen, China<sup>d</sup>

The draft genome sequences of two copper-resistant *Escherichia coli* strains were determined. These had been isolated from copper-fed pigs and contained additional putative operons conferring copper and other metal and metalloid resistances.

Received 12 November 2014 Accepted 19 November 2014 Published 24 December 2014

Citation Lüthje FL, Hasman H, Aarestrup FM, Alwathnani HA, Rensing C. 2014. Genome sequences of two copper-resistant *Escherichia coli* strains isolated from copper-fed pigs. Genome Announc. 2(6):01341-14. doi:10.1128/genomeA.01341-14.

**Copyright** © 2014 Lüthje et al. This is an open-access article distributed under the terms of the Creative Commons Attribution 3.0 Unported license. Address correspondence to Christopher Rensing, chres@life.ku.dk.

There is a growing concern that agricultural practices such as animal feedlots contribute to the increasing dissemination of antibiotic- and metal-resistance determinants (1). Previous work has identified an additional copper-resistance determinant named *pco* (for plasmid copper resistance) on a plasmid in *E. coli* strains from copper-fed pigs (2, 3).

Two strains of copper-resistant *E. coli* (77-3009-5, 77-30253-3) were isolated from copper-fed pigs as part of the Danish Integrated Antimicrobial Resistance Monitoring (DANMAP) surveillance program (4). The isolates were collected from healthy animals at or just prior to slaughter in 2003. Genomic DNA (gDNA) was purified from the isolates using the Easy-DNA extraction kit (Invitrogen) and DNA concentrations were determined using the Qubit dsDNA BR assay kit (Invitrogen). The isolates were sequenced on the MiSeq platform (Illumina). For sequencing on the MiSeq, chromosomal DNA of the isolates was used to create genomic libraries using the Nextera XT DNA sample preparation kit (Illumina, cat. no. FC-131-1024) and sequenced using version  $3, 2 \times 300$  bp chemistry on the Illumina MiSeq platform.

*E. coli* strain 77-3009-5 had an estimated 5,332,861 bp on 375 contigs with the longest contig being 194,538 bp and the smallest being 94 bp. The coverage was 63-fold, and the  $N_{50}$  was 79,648 bp. The GC content was 50.4% and a total of 5,279 coding sequences were predicted using the Rapid Annotation using Subsystem Technology (RAST) server. *E. coli* strain 77-30253-3 was approximately 5,369,161 bp on 790 contigs. The longest contig was 211,389 bp, the smallest 101, and the GC content was 50.7%. It had an  $N_{50}$  of 58,415 bp; 5,294 coding sequences were predicted by RAST; and the coverage was 130-fold.

Both *E. coli* strains have genomes substantially larger than the wild-type strain *E. coli* K12 (app. 4,600,000 bp). Both strains contain the previously characterized chromosomal genes encoding proteins involved in copper homeostasis (5). In addition, *E. coli* 77-3009-5 contains a copper-resistance island with nearby Tn7-related genes. This mobile island contains two determinants: the *pco* determinant, known to give additional copper resistance (6, 7), and the *sil* determinant, which was previously shown to confer

silver resistance (8). This 20-gene island is conserved in many strains of pathogenic *E. coli*, *Salmonella enterica*, *Klebsiella pneumoniae*, and *Enterobacter cloacae* (9). The genes were in the following order: *pcoE-pcoS-pcoR-pcoD-pcoC-pcoB-pcoA-pcoE-endopeptidase-hypothetical protein-silP-copG-silA-silB-silF-silC-silR-silS-silE-putative exported protein*. *E. coli* 77-3009-5 also carried an operon conferring tellurium resistance (10) and another conferring mercury resistance (11). These functions were most likely encoded on Inc-F plasmids in *E. coli* 77-3009-5, based on the presence of the different *tra* genes. *E. coli* 77-30253-3 lacks the 20-gene copper resistance island but instead has genes responsible for synthesis and handling of yersiniabactin that protects against copper toxicity (12).

Nucleotide sequence accession numbers. The genomes of *E. coli* 77-3009-5 and 77-30253-3 were deposited at NCBI GenBank under the accession numbers JRPP00000000 and JRQF00000000, respectively. The versions described in this paper are versions JRPP01000000 and JRQF01000000.

## **ACKNOWLEDGMENTS**

This work was supported by the Center for Environmental and Agricultural Microbiology (CREAM) funded by the Villum Kann Rasmussen Foundation.

## REFERENCES

- Berg J, Thorsen MK, Holm PE, Jensen J, Nybroe O, Brandt KK. 2010. Cu exposure under field conditions coselects for antibiotic resistance as determined by a novel cultivation-independent bacterial community tolerance assay 44:8724–8728. http://dx.doi.org/10.1021/es101798r.
- Tetaz TJ, Luke RKJ. 1983. Plasmid-controlled resistance to copper in Escherichia coli. 154:1263–1268.
- Williams JR, Morgan AG, Rouch DA, Brown NL, Lee BT, Williams JR, Morgan AG, Rouch DA, Brown NL. 1993. Copper-resistant enteric bacteria from United Kingdom and Australian piggeries. Appl. Environ. Microbiol. 59:2531–2537.
- 4 Danish Integrated Antimicrobial Resistance Monitoring. 2005. DANMAP 2004—use of antimicrobial agents and the occurrence of antimicrobial resistance in bacteria from food animals, foods and humans in Denmark. DANMAP, Copenhagen.
- 5. Outten FW, Huffman DL, Hale JA, O'Halloran TV. 2001. The indepen-

- dent *cue* and *cus*Systems confer copper tolerance during aerobic and anaerobic growth in *Escherichia coli*. J. Biol. Chem. **276**:30670 –30677. http://dx.doi.org/10.1074/jbc.M104122200.
- 6. Brown NL, Barrett SR, Camakaris J, Lee BTO. 1995. Molecular genetics and transport analysis of the copper-resistance determinant (*pco*) from *Escherichia coli* plasmid pRJ1004. Mol.Microbiol.17:1153–1166.
- 7. Lee SM, Grass G, Rensing C, Barrett SR, Yates CJD, Stoyanov JV, Brown NL. 2002. The Pco proteins are involved in periplasmic copper handling in *Escherichia coli*. Biochem. Biophys. Res. Commun. 295: 616–620. http://dx.doi.org/10.1016/S0006-291X(02)00726-X.
- 8. Gupta A, Phung LT, Taylor DE, Silver S. 2001. Diversity of silver resistance genes in IncH incompatibility group plasmids. Microbiology 147:3393–3402.
- Kremer AN, Hoffmann H. 2012. Subtractive hybridization yields a silver resistance determinant unique to nosocomial pathogens in the *Enterobacter cloacae* complex. J. Clin. Microbiol. 50:3249–3257. http://dx.doi.org/ 10.1128/JCM.00885-12.
- 10. Taylor DE. 1999. Bacterial tellurite resistance. Trends Microbiol. 7:111–115. http://dx.doi.org/10.1016/S0966-842X(99)01454-7.
- Summers AO. 1986. Organization, expression, and evolution of genes for mercury resistance. Annu. Rev. Microbiol. 40:607–634. http://dx.doi.org/ 10.1146/annurev.mi.40.100186.003135.
- 12. Chaturvedi KS, Hung CS, Crowley JR, Stapleton AE, Henderson JP. 2012. The siderophore yersiniabactin binds copper to protect pathogens during infection. Nat. Chem. Biol. 8:731–736. http://dx.doi.org/10.1038/nchembio.1020.