



## Draft Genome Sequence of Se(IV)-Reducing Bacterium Pseudomonas migulae ES3-33

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*Pseudomonas migulae* ES3-33 is a Gram-negative strain that strongly reduces Se(IV) and was isolated from a selenium mining area in Enshi, southwest China. Here we present the draft genome of this strain containing potential genes involved in selenite reduction and a large number of genes encoding resistances to copper and antibiotics.

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The genus *Pseudomonas* is known to perform diverse tasks, including plant growth promotion, environmental bioremediation, and biological control of various pathogens (1–3). Due to these beneficial properties, the genus *Pseudomonas* has attracted increased scientific interest in recent years. Due to the similarities of the 16S rRNA gene sequences, *Pseudomonas migulae* has been assigned into the *Pseudomonas fluorescens* group (4).

The strain of *Pseudomonas migulae* ES3-33 was isolated from soil samples (soil Se of >20 mg/kg) in an Se mine area in Enshi, southwest China. The 16S rRNA sequences of *Pseudomonas migulae* ES3-33 revealed that it belonged to the species *Pseudomonas migulae*, with sequence similarities of 99.56% using the EzTaxon server (5, 6). Chromosomal DNA was purified from isolates by using a Gentra Puregene Yeast/Bac.Kit (Qiagen). The draft genome sequence of *Pseudomonas migulae* ES3-33 was determined by the Illumina MiSeq 2x250PE platform to generate a paired-end library. *De novo* assembly was performed with SPAdes 3.5.0, resulting in 162 contigs (>200 bp). A total of 5,591 open reading frames (ORFS) were predicted by the RAST server (7, 8) and annotated using the information from GenBank and RAST (9).

The size of the draft genome sequence is 6,075,381 bp, with an average GC content of 59.7%, and the longest contig size assembled is 389,672 bp. The genome consists of 5,404 protein-coding sequences that were assigned predicted functions, 58 tRNA genes, and 10 rRNA genes.

*Pseudomonas migulae* ES3-33 can rapidly reduce selenite to red selenium nanoparticles and is highly selenite resistant, with an MIC of 150 mM. The genome was analyzed and shown to contain potential selenite reductases. Two glutathione reductases (GR), a thioredoxin reductase (THxR), an NADH:flavin oxidoreductase (OYE family), and two nitrite reductases which have previously been reported to reduce selenite were identified on the genome (10–15). An increased presence of copper resistance determinants was observed, possibly due to contamination of the Se mine with other metals. The genome encodes multiple proteins potentially conferring copper resistance, such as the *copABCD* operon also

found in *Pseudomonas putida* PNL-MK25 (16), three putative *cus* systems (17), copper-sensing two-component systems (18, 19), two blue copper oxidases, and multiple Cu(I)-translocating P-type ATPases. At least one of the determinants was a mobile element flanked by the Tn7 transposon system.

In addition, *Pseudomonas migulae* ES3-33 also contains genes encoding drug resistance (fluoroquinolones, penicillin, cephalosporin), antibiotic resistance (streptothricin), and other virulence proteins, including multidrug resistance tripartite systems, streptothricin acetyltransferase, fluoroquinolones resistance protein,  $\beta$ -lactamase, class C, and other penicillin-binding proteins.

Multiple virulence genes of *Pseudomonas migulae* ES3-33 might improve our fundamental understanding of multidrug, heavy and transition metal resistance mechanisms, and this knowledge may be applicable to bioremediation.

**Nucleotide sequence accession numbers.** This whole-genome shotgun project has been deposited at DDBJ/EMBL/GenBank under the accession no. JZRI00000000. The version described in this paper is version JZRI01000000.

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## REFERENCES

- Haas D, Défago G. 2005. Biological control of soil-borne pathogens by fluorescent *pseudomonads*. Nat Rev Microbiol 3:307–319. http:// dx.doi.org/10.1038/nrmicro1129.
- O'Mahony MM, Dobson AD, Barnes JD, Singleton I. 2006. The use of ozone in the remediation of polycyclic aromatic hydrocarbon contaminated soil. Chemosphere 63:307–314. http://dx.doi.org/10.1016/ j.chemosphere.2005.07.018.
- Yen KM, Karl MR, Blatt LM, Simon MJ, Winter RB, Fausset PR, Lu HS, Harcourt AA, Chen KK. 1991. Cloning and characterization of a *Pseudomonas mendocina* KR1 gene cluster encoding toluene-4monooxygenase. J Bacteriol 173:5315–5327.

- 4. Anzai Y, Kim H, Park JY, Wakabayashi H, Oyaizu H. 2000. Phylogenetic affiliation of the *pseudomonads* based on 16S rRNA sequence. Int J Syst Evol Microbiol 50:1563–1589. http://dx.doi.org/10.1099/00207713-50-4 -1563.
- 5. Verhille S, Baïda N, Dabboussi F, Hamze M, Izard D, Leclerc H. 1999. *Pseudomonas gessardii* sp. nov. and *Pseudomonas migulae* sp. nov., two new species isolated from natural mineral waters. Int J Syst Bacteriol **49**: 1559–1572. http://dx.doi.org/10.1099/00207713-49-4-1559.
- Kim OS, Cho YJ, Lee K, Yoon SH, Kim M, Na H, Park SC, Jeon YS, Lee JH, Yi H, Won S, Chun J. 2012. Introducing EzTaxon-e: a prokaryotic 16S rRNA gene sequence database with phylotypes that represent uncultured species. Int J Syst Evol Microbiol 62:716–721. http://dx.doi.org/ 10.1099/ijs.0.038075-0.
- 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.
- 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:D206–214. http://dx.doi.org/10.1093/nar/gkt1226.
- Song JY, Jeong H, Yu DS, Fischbach MA, Park HS, Kim JJ, Seo JS, Jensen SE, Oh TK, Lee KJ, Kim JF. 2010. Draft genome sequence of *Streptomyces clavuligerus* NRRL 3585, a producer of diverse secondary metabolites. J Bacteriol 192:6317–6318. http://dx.doi.org/10.1128/ JB.00859-10.
- Hunter WJ. 2014. Pseudomonas seleniipraecipitans proteins potentially involved in selenite reduction. Curr Microbiol 69:69-74. http:// dx.doi.org/10.1007/s00284-014-0555-2.

- DeMoll-Decker H, Macy JM. 1993. The periplasmic nitrite reductase of *Thauera selenatis* may catalyze the reduction of selenite to elemental selenium. Arch Microbiol 160:241–247.
- Hunter WJ. 2014. A *Rhizobium selenitireducens* protein showing selenite reductase activity. Curr Microbiol 68:311–316. http://dx.doi.org/10.1007/ s00284-013-0474-7.
- Kessi J. 2006. Enzymic systems proposed to be involved in the dissimilatory reduction of selenite in the purple non-sulfur bacteria *Rhodospirillum rubrum* and *Rhodobacter capsulatus*. Microbiology 152:731–743. http:// dx.doi.org/10.1099/mic.0.28240-0.
- Ma J, Kobayashi DY, Yee N. 2007. Chemical kinetic and molecular genetic study of selenium oxyanion reduction by *Enterobacter cloacae* SLD1a-1. Environ Sci Technol 41:7795–7801. http://dx.doi.org/10.1021/ es0712672.
- Basaglia M, Toffanin A, Baldan E, Bottegal M, Shapleigh JP, Casella S. 2007. Selenite-reducing capacity of the copper-containing nitrite reductase of *Rhizobium sullae*. FEMS Microbiol Lett 269:124–130. http:// dx.doi.org/10.1111/j.1574-6968.2006.00617.x.
- Adaikkalam V, Swarup S. 2005. Characterization of *copABCD* operon from a copper-sensitive *Pseudomonas putida* strain. Can J Microbiol 51: 209–216. http://dx.doi.org/10.1139/w04-135.
- Outten FW, Huffman DL, Hale JA, O'Halloran TV. 2001. The independent *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.
- Barton MD, Petronio M, Giarrizzo JG, Bowling BV, Barton HA. 2013. The genome of *Pseudomonas fluorescens* strain r124 demonstrates phenotypic adaptation to the mineral environment. J Bacteriol 195:4793–4803. http://dx.doi.org/10.1128/JB.00825-13.
- Zhang XX, Rainey PB. 2008. Regulation of copper homeostasis in *Pseudomonas fluorescens* SBW25. Environ Microbiol 10:3284–3294. http://dx.doi.org/10.1111/j.1462-2920.2008.01720.x.