genomeA
Journals.ASM.org

Draft Genome Sequence of *Raoultella ornithinolytica* TNT, a Trinitrotoluene-Denitrating and Plant Growth-Promoting Strain Isolated from Explosive-Contaminated Soil

Sofie Thijs,[a] Jonathan Van Hamme,[b] Panagiotis Gkorezis,[a] Francois Rineau,[a] Nele Weyens,[a] Jaco Vangronsveld[a]

Hasselt University, Centre for Environmental Sciences, Agoralaan Building D, Diepenbeek, Belgium[a]; Thompson Rivers University, Department of Biological Sciences, Kamloops, British Columbia, Canada[b]

We report the draft genome of *Raoultella ornithinolytica* TNT, a Gram-negative bacterium of the *Enterobacteriaceae* isolated from military soil in Belgium. Strain TNT uses nitrite released from trinitrotoluene (TNT) for growth and is a potent plant growth promoter. An analysis of its 5.6-Mb draft genome will bring insights into TNT degradation-reinforcing bioremediation applications.

Received 2 May 2014 **Accepted** 13 May 2014 **Published** 29 May 2014

Citation Thijs S, Van Hamme J, Gkorezis P, Rineau F, Weyens N, Vangronsveld J. 2014. Draft genome sequence of *Raoultella ornithinolytica* TNT, a trinitrotoluene-denitrating and plant growth-promoting strain isolated from explosive-contaminated soil. Genome Announc. 2(3):e00491-14. doi:10.1128/genomeA.00491-14.

Address correspondence to Sofie Thijs, sofie.thijs@uhasselt.be.

Trinitrotoluene (TNT)-contaminated sites pose risks to human and environmental health, making remediation a priority. Detoxification by indigenous bacteria or use of TNT-transforming bacteria in bioaugmented rhizoremediation is preferred to invasive and costly excavation or incineration techniques.

A TNT-detoxifying strain was isolated from TNT-contaminated forest soil at a military site in Belgium. Identified as *Raoultella ornithinolytica* B6 by partial 16S rRNA gene sequencing and phenotypic profiling, the closest related partial 16S rRNA gene sequence (97.8%) was from strain B6 (GenBank accession no. CP004142) (1).

Here, genomic DNA was isolated using a DNeasy blood and tissue kit (Qiagen, Venlo, the Netherlands), treated with RNase I, and purified by phenol:chloroform extraction. Purified DNA was digested and sequencing adaptors ligated using an Ion Xpress Plus fragment library kit (Life Technologies Inc., Burlington, Ontario, Canada). Adaptor-ligated DNA was size selected (480 bp) on a 2% E-Gel SizeSelect agarose gel, and Agencourt AMPure XP beads (Beckman Coulter, Mississauga, Ontario, Canada) were used for purifications. An Ion library quantitation kit was used prior to amplification and enrichment with an Ion PGM Template OT2 400 kit on an Ion OneTouch 2 system. An Ion Sphere quality control kit was used prior to sequencing with an Ion PGM 400 sequencing kit and a single 316v2 Chip on an Ion Torrent PGM (Life Technologies, Inc., Carlsbad, CA).

In total, 2.49 million reads (mean length, 282 bases) generated 703 Mb of data, of which 938,910 reads were assembled using MIRA version 3.9.9 (2) into 39 contigs, giving a consensus length of 5,652,765 bp at 48× coverage. Mauve (3) was used to order the contigs and compare the genome with the closest related reference genome, that of *R. ornithinolytica* B6 (accession no. CP004142). The annotation was completed using the PGAP (NCBI) pipeline (4). The genome of *R. ornithinolytica* TNT consists of a single circular chromosome (55.5% G+C content), which includes 4,935 coding genes, 180 pseudogenes, 34 rRNAs (5S, 16S, 23S), 80 tRNAs, and 7 noncoding RNAs (ncRNAs).

In liquid cultures, strain TNT demonstrated rapid TNT transformation activity in minimal medium (113 mg liter^{-1} TNT, 0.1 mg liter^{-1} NH_4Cl, and 5 g liter^{-1} glucose). Analyses of the draft genome confirmed the presence of genes coding for multiple TNT-transforming enzymes, including nitroreductase A, nitroreductase B, and *N*-ethylmaleimide (NEM) reductase. The NEM product is similar to NemA in *Escherichia coli* and to XenB and PetN of *Enterobacter cloacae*, and it is possibly responsible for nitrite release from TNT (5–9). Strain TNT uses nitrite as the sole N source under aerobic conditions, and genes for a complete nitrite assimilation pathway are present. This strain produces indole, is Voges-Proskauer positive, utilizes glycerol, glucose, fructose, and sucrose as carbon sources, and grows at 20°C. Genes for plant growth-promoting characteristics are present, corroborating results from phenotypic tests: auxin biosynthesis, acetoin production, 1-aminocyclopropane-1-carboxylate deaminase activity, siderophore production, phosphorous solubilization, and chemotaxis. *R. ornithinolytica* TNT is a promising strain for rhizoremediation applications.

Nucleotide sequence accession numbers. This whole-genome shotgun project has been deposited at DDBJ/EMBL/GenBank under the accession no. JHQH00000000. The version described in this paper is version JHQH01000000.

ACKNOWLEDGMENTS

This work was supported by a Ph.D. grant to S.T. and a postdoc grant to N.W. from the Fund for Scientific Research-Flanders, Belgium (FWO-Vlaanderen), and the Methusalem project 08M03VGRJ.

REFERENCES

1. **Shin SH, Um Y, Beak JH, Kim S, Lee S, Oh MK, Kim YR, Lee J, Yang KS.** 2013. Complete genome sequence of *Raoultella ornithinolytica* strain B6, a 2,3-butanediol-producing bacterium isolated from oil-contaminated soil. Genome Announc. 1(3):e00395-13. http://dx.doi.org/10.1128/genomeA.00395-13.
2. **Chevreux B, Wetter T, Suhai S.** 1999. Genome sequence assembly using trace signals and additional sequence information, p 45–56. *In* Computer

science and biology. Proceedings of the German Conf. on Bioinformatics, GCB '99. GCB, Hannover, Germany.
3. **Rissman AI, Mau B, Biehl BS, Darling AE, Glasner JD, Perna NT.** 2009. Reordering contigs of draft genomes using the Mauve Aligner. Bioinformatics **25:**2071–2073. http://dx.doi.org/10.1093/bioinformatics/btp356.
4. **Angiuoli SV, Gussman A, Klimke W, Cochrane G, Field D, Garrity G, Kodira CD, Kyrpides N, Madupu R, Markowitz V, Tatusova T, Thomson N, White O.** 2008. Toward an online repository of Standard Operating Procedures (SOPs) for (meta)genomic annotation. Omics **12:**137–141. http://dx.doi.org/10.1089/omi.2008.0017.
5. **French CE, Nicklin S, Bruce NC.** 1998. Aerobic degradation of 2,4,6-trinitrotoluene by *Enterobacter cloacae* PB2 and by pentaerythritol tetranitrate reductase. Appl. Environ. Microbiol. **64:**2864–2868.
6. **Williams RE, Rathbone DA, Scrutton NS, Bruce NC.** 2004. Biotransformation of explosives by the old yellow enzyme family of flavoproteins. Appl. Environ. Microbiol. **70:**3566–3574. http://dx.doi.org/10.1128/AEM.70.6.3566-3574.2004.
7. **Wittich RM, Haïdour A, Van Dillewijn P, Ramos JL.** 2008. OYE flavoprotein reductases initiate the condensation of TNT-derived intermediates to secondary diarylamines and nitrite. Environ. Sci. Technol. **42:**734–739. http://dx.doi.org/10.1021/es071449w.
8. **van Dillewijn P, Wittich RM, Caballero A, Ramos JL.** 2008. Subfunctionality of hydride transferases of the old yellow enzyme family of flavoproteins of *Pseudomonas putida*. Appl. Environ. Microbiol. **74:**6703–6708. http://dx.doi.org/10.1128/AEM.00386-08.
9. **González-Pérez MM, van Dillewijn P, Wittich RM, Ramos JL.** 2007. *Escherichia coli* has multiple enzymes that attack TNT and release nitrogen for growth. Environ. Microbiol. **9:**1535–1540. http://dx.doi.org/10.1111/j.1462-2920.2007.01272.x.