



Complete Genome Sequence of Aneurinibacillus migulanus E1, a Gramicidin S- and D-Phenylalanyl-L-Propyl Diketopiperazine-Deficient Mutant

💿 Lassaad Belbahri,^{a,b} Faizah N. Alenezi,^{b,c} Lenka Luptakova,^{a,b,d} Mostafa E. Rateb,^e Steve Woodward^{b,c}

Laboratory of Soil Biology, University of Neuchatel, Neuchâtel, Switzerland^a; NextBiotech, Agareb, Tunisia^b; University of Aberdeen, Institute of Biological and Environment Science, Aberdeen, Scotland, United Kingdom^c; University of Veterinary Med & Pharmacy, Institute of Biology, Zoology & Radiobiology, Department of Biology and Genetics, Košice, Slovakia^d; School of Science & Sport, University of West of Scotland, Paisley, United Kingdom^e

L.B. and F.N.A. contributed equally to this article.

We report here the complete genome sequence of the *Aneurinibacillus migulanus* E1 mutant deficient in gramicidin S (GS) and D-phenylalanyl-L-propyl diketopiperazine (DKP) formation. The genome consists of a circular chromosome (6,301,904 bp, 43.20% G+C content) without any plasmid. The complete genome sequence enables further investigation of the biosynthetic mechanism and the biological function of gramicidin S.

Received 26 October 2015 Accepted 27 October 2015 Published 17 December 2015

Citation Belbahri L, Alenezi FN, Luptakova L, Rateb ME, Woodward S. 2015. Complete genome sequence of Aneurinibacillus migulanus E1, a gramicidin S- and D-phenylalanyl-L-propyl diketopiperazine-deficient mutant. Genome Announc 3(6):e01441-15. doi:10.1128/genomeA.01441-15.

Copyright © 2015 Belbahri et al. This is an open-access article distributed under the terms of the Creative Commons Attribution 3.0 Unported license.

Address correspondence to Lassaad Belbahri, lassaad.belbahri@unine.ch.

Gramicidin S (GS) is a cationic cyclic decapeptide with the primary structure $[cyclo-(Val-Orn-Leu-D-Phe-Pro)_2]$ (1). GS is an extremely powerful antibiotic drug against a broad spectrum of both Gram-negative and Gram-positive bacteria and against several pathogenic fungi (2–5). GS-deficient mutants in *Aneurinibacillus migulanus* have been classified into five different categories. The mutant E1 belongs to the fifth group that contains both a phenylalanine-activating enzyme and a complex of proline-, valine-, ornithine-, and leucine-activating enzymes similar to those of the wild-type strain (6). E1 is unable to synthetize GS or D-phenylalanyl-L-propyl diketopiperazine (DKP).

The genome of the A. migulanus E1 mutant was sequenced by the Illumina HiSeq 2000 platform (2 \times 125 bp), and the sequencing coverage was $100 \times$. After sequencing, the reads were assembled using CLC Genomics Workbench 7.0.3 (CLC bio). Annotation was performed using the NCBI Prokaryotic Genomes Automatic Annotation Pipeline (PGAAP) version 2.9 (7). The whole genome is represented by a circular chromosome of 6,301,904 bp with no plasmid. The G+C content was around 43.20%, as reported for other Aneurinibacillus species (2, 3). The genome contains 5,672 coding sequences (CDSs), 11 rRNA operons, and 95 tRNA genes. Using anti-SMASH 3.0, 12 gene clusters for secondary metabolites have been predicted in the genome of the E1 A. migulanus mutant strain (8). Nonribosomal peptides, terpenes, polyketides, siderophores, bacteriocins, microcins, lasso peptides, and arylpolyene are among the predicted secondary metabolites. This suggests an important structural diversity of E1 secondary metabolites. The availability of the genome sequence of the E1 mutant is supposed to help identify mutations in this gramicidin S-deficient mutant and therefore to understand the genetic regulation of gramicidin S biosynthesis in *A. migulanus*.

Nucleotide sequence accession number. The sequence of the E1 chromosome has been deposited in GenBank under accession the no. LIXL00000000. The version described in this paper is the first version.

ACKNOWLEDGMENTS

This work was supported by the European Union's Seventh Framework Programme grant 245268 (ISEFOR; to L.B.). Further support came from the SwissBOL project (the Swiss Federal Office for the Environment, to L.B.) and the Sciex-Scientific Exchange Programme NMS.CH (to L.L. and L.B.).

REFERENCES

- Marahiel MA, Danders W, Krause M, Kleinkauf H. 1979. Biological role of gramicidin S in spore functions. Studies on gramicidin-S-negative mutants of *Bacillus brevis* ATCC 9999. Eur J Biochem 99:49–55. http:// dx.doi.org/10.1111/j.1432-1033.1979.tb13229.x.
- Alenezi FN, Weitz HJ, Belbahri L, Nidhal J, Luptakova L, Jaspars M, Woodward S. 2015. Draft genome sequence of *Aneurinibacillus migulanus* NCTC 7096. Genome Announc 3(2):e00234-15. http://dx.doi.org/ 10.1128/genomeA.00234-15.
- Alenezi FN, Weitz HJ, Belbahri L, Ben Rebah H, Luptakova L, Jaspars M, Woodward S. 2015. Draft genome sequence of *Aneurinibacillus migulanus* strain Nagano. Genome Announc 3(2):e00232-15. http://dx.doi.org/ 10.1128/genomeA.00232-15.
- Alenezi FN, Fraser S, Bełka M, Doğmuş TH, Hečkova Z, Oskay F, Belbahri L, Woodward S. 2015. Biological control of Dothistroma needle blight on pine with *Aneurinibacillus migulanus*. Forest Pathol, in press. http://dx.doi.org/10.1111/efp.12237.
- Alenezi FN, Rekik I, Bełka M, Ibrahim AF, Luptakova L, Jaspers M, Woodward S, Belbahri L. 2015. Strain-level diversity of secondary metabolism in the biocontrol species *Aneurinibacillus migulanus*. Microbiol Res 182:116–124. http://dx.doi.org/10.1016/j.micres.2015.10.007.

- 6. Shimura K, Iwaki M, Kanda M, Hori K, Kaji E, Hasegawa S, Saito Y. 1974. On the enzyme system obtained from some mutants of *Bacillus brevis* deficient in gramicidin S formation. Biochim Biophys Acta **338**:577–587. http://dx.doi.org/10.1016/0304-4165(74)90321-3.
- 7. Angiuoli SV, Gussman A, Klimke W, Cochrane G, Field D, Garrity GM, 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.

 Weber T, Blin K, Duddela S, Krug D, Kim HU, Bruccoleri R, Lee SY, Fischbach MA, Müller R, Wohlleben W, Breitling R, Takano E, Medema MH. 2015. antiSMASH 3.0—a comprehensive resource for the genome mining of biosynthetic gene clusters. Nucleic Acids Res 43:W237–W243. http://dx.doi.org/10.1093/nar/gkv437.