Genome Sequence of the Acetogenic Bacterium *Moorella mulderi* DSM 14980^T

Genis Andrés Castillo Villamizar, Anja Poehlein

Genomic and Applied Microbiology & Göttingen Genomics Laboratory, Georg-August University Göttingen, Göttingen, Germany

Here, we report the draft genome sequence of *Moorella mulderi* DSM 14980^T, a thermophilic acetogenic bacterium, which is able to grow autotrophically on H_2 plus CO_2 using the Wood-Ljungdahl pathway. The genome consists of a circular chromosome (2.99 Mb).

The reduction of CO_2 mediated by acetogenic microorganisms is gaining more interest as a valuable tool for the generation of renewable energy and value-added chemicals (1–3). Thus, homoacetogenic bacteria that use the Wood-Ljungdahl pathway for the CO_2 fixation process have proven to be a main component in this research field (3–8). Among the numerous species of homoacetogens, three organisms have been relatively well studied (*Moorella thermoacetica*, *Acetobacterium woodii*, and *Clostridium ljungdahlii*) (9–13). However, several relevant species remain poorly studied, and the genetic information of many of them remains almost nonexistent or is very limited. Therefore, in this study, we report the draft genome sequence of *Moorella mulderi* DSM 14980^T a thermophilic homoacetogenic anaerobic bacterium originally isolated from a bioreactor with methanol as the energy source (14). Similar to *M. thermoacetica*, *M. mulderi* DSM 14980^T is able to grow on several substrates, including methanol, H_2-CO_2, pyruvate, and glucose. However, several differences have been reported. The optimal temperature of *M. mulderi* DSM 14980^T (65°C) is higher than the optimal temperature reported for *M. thermoacetica* (55 to 60°C). Moreover, in contrast to *M. thermoacetica*, *M. mulderi* DSM 14980^T is able to grow on lactate but cannot use nitrate as an electron acceptor (14).

The MasterPure complete DNA purification kit (Epicentre, Madison, WI, USA) was used to isolate the chromosomal DNA of *M. mulderi* DSM 14980^T. Isolated DNA was used to generate Illumina shotgun sequencing libraries. Sequencing was performed by employing a MiSeq system using MiSeq reagent kit version 3 (600 cycles), as recommended by the manufacturer (Illumina, San Diego, CA, USA), resulting in 2,785,408 paired-end reads (300 bp) that were trimmed using Trimmomatic 0.32 (15). *De novo* assembly performed with the SPAdes genome assembler software version 3.6.2 (16) resulted in 72 contigs (>500 bp) and an average coverage of 188.5-fold.

The genome of *M. mulderi* DSM 14980^T probably consists of a circular chromosome of (2.99 Mb) with an overall G+C content of 53.32%. Gene prediction and annotation were performed using Rapid Prokaryotic Genome Annotation (Prokka) (17). The genome harbored 3 rRNA genes, 52 tRNA genes, 2,240 protein-coding genes with predicted functions, and 859 genes coding for hypothetical proteins. The cluster of genes encoding enzymes of the methyl and carbonyl branches of the Wood-Ljungdahl pathway is conserved within acetogenic bacteria (18). Therefore, *M. mulderi* DSM 14980^T shows an arrangement identical to the pattern previously identified in *M. thermoacetica* strains ATCC 39073 and DSM 521^T (10, 18). The cluster is composed of eight genes (*acsFABCV*, *cooC*, and *acsDE*) encoding the subunits of the CO dehydrogenase–acetyl-coenzyme A (CoA) synthase complex. The genes encoding the two subunits of the methylene-THF reductase (*metVF*) are located four genes downstream of this cluster.

The genome analysis revealed that *M. mulderi* DSM 14980^T has a bigger genome size than *M. thermoacetica* DSM 521^T (2.52 Mb) and *M. thermoacetica* DSM 2955^T (2.62 Mb) (10, 19).

Nucleotide sequence accession numbers. This whole-genome shotgun project has been deposited at DDBJ/ENA/GenBank under the accession no. LTBC00000000. The version described in this paper is version LTBC01000000.

ACKNOWLEDGMENTS

FUNDING INFORMATION

REFERENCES

1. **Alissandratos A, Easton CJ.** 2015. Biocatalysis for the application of CO_2 as a chemical feedstock. Beilstein J Org Chem **11:**2370–2387. http://dx.doi.org/10.3762/bjoc.11.259.
2. **Drake HL, Gößner AS, Daniel SL.** 2008. Old acetogens, new light. Ann NY Acad Sci **1125:**100–128. http://dx.doi.org/10.1196/annals.1419.016.
3. **Hawkins AS, McTernan PM, Lian H, Kelly RM, Adams MWW.** 2013. Biological conversion of carbon dioxide and hydrogen into liquid fuels

and industrial chemicals. Curr Opin Biotechnol **24**:376–384. http://dx.doi.org/10.1016/j.copbio.2013.02.017.

4. **Bertsch J, Müller V**. 2015. Bioenergetic constraints for conversion of syngas to biofuels in acetogenic bacteria. Biotechnol Biofuels **8**:210. http://dx.doi.org/10.1186/s13068-015-0393-x.

5. **Tremblay P-L, Höglund D, Koza A, Bonde I, Zhang T**. 2015. Adaptation of the autotrophic acetogen *Sporomusa ovata* to methanol accelerates the conversion of CO_2 to organic products. Sci Rep **5**:16168. http://dx.doi.org/10.1038/srep16168.

6. **Fontaine FE, Peterson WH, McCoy E, Johnson MJ, Ritter GJ**. 1942. A new type of glucose fermentation by *Clostridium thermoaceticum*. J Bacteriol **43**:701–715.

7. **Andreesen JR, Schaupp A, Neurauter C, Brown A, Ljungdahl LG**. 1973. Fermentation of glucose, fructose, and xylose by *Clostridium thermoaceticum*: effect of metals on growth yield, enzymes, and the synthesis of acetate from CO_2. J Bacteriol **114**:743–751.

8. **Schaupp A, Ljungdahl LG**. 1974. Purification and properties of acetate kinase from *Clostridium thermoaceticum*. Arch Microbiol **100**:121–129. http://dx.doi.org/10.1007/BF00446312.

9. **Pierce E, Xie G, Barabote RD, Saunders E, Han CS, Detter JC, Richardson P, Brettin TS, Das A, Ljungdahl LG, Ragsdale SW**. 2008. The complete genome sequence of *Moorella thermoacetica* (f. *Clostridium thermoaceticum*). Environ Microbiol **10**:2550–2573.

10. **Poehlein A, Bengelsdorf FR, Esser C, Schiel-Bengelsdorf B, Daniel R, Dürre P**. 2015. Complete genome sequence of the type strain of the acetogenic bacterium *Moorella thermoacetica* DSM 521ᵀ. Genome Announc **3**(5):e01159-15. http://dx.doi.org/10.1128/genomeA.01159-15.

11. **Bertsch J, Müller V**. 2015. CO metabolism in the acetogen *Acetobacterium woodii*. Appl Environ Microbiol **81**:5949–5956. http://dx.doi.org/10.1128/AEM.01772-15.

12. **Younesi H, Najafpour G, Mohamed AR**. 2005. Ethanol and acetate production from synthesis gas via fermentation processes using anaerobic bacterium, *Clostridium ljungdahlii*. Biochem Eng J **27**:110–119. http://dx.doi.org/10.1016/j.bej.2005.08.015.

13. **Köpke M, Held C, Hujer S, Liesegang H, Wiezer A, Wollherr A, Ehrenreich A, Liebl W, Gottschalk G, Dürre P, Demain AL**. 2010. *Clostridium ljungdahlii* represents a microbial production platform based on syngas. Proc Natl Acad Sci USA **107**:13087–13092. http://dx.doi.org/10.1073/pnas.1004716107.

14. **Balk M, Weijma J, Friedrich MW, Stams AJM**. 2003. Methanol utilization by a novel thermophilic homoacetogenic bacterium, *Moorella mulderi* sp. nov., isolated from a bioreactor. Arch Microbiol **179**:315–320.

15. **Bolger AM, Lohse M, Usadel B**. 2014. Trimmomatic: a flexible trimmer for Illumina sequence data. Bioinformatics **30**:2114–2120.

16. **Bankevich A, Nurk S, Antipov D, Gurevich AA, Dvorkin M, Kulikov AS, Lesin VM, Nikolenko SI, Pham S, Prjibelski AD, Pyshkin AV, Sirotkin AV, Vyahhi N, Tesler G, Alekseyev MA, Pevzner PA**. 2012. SPAdes: a new genome assembly algorithm and its applications to single-cell sequencing. J Comput Biol **19**:455–477. http://dx.doi.org/10.1089/cmb.2012.0021.

17. **Seemann T**. 2014. Prokka: rapid prokaryotic genome annotation. Bioinformatics **30**:2068–2069. http://dx.doi.org/10.1093/bioinformatics/btu153.

18. **Poehlein A, Cebulla M, Ilg MM, Bengelsdorf FR, Schiel-Bengelsdorf B, Whited G, Andreesen JR, Gottschalk G, Daniel R, Dürre P**. 2015. The complete genome sequence of *Clostridium aceticum*: a missing link between Rnf- and cytochrome-containing autotrophic acetogens. mBio **6**:e01168-15. http://dx.doi.org/10.1128/mBio.01168-15.

19. **Bengelsdorf FR, Poehlein A, Esser C, Schiel-Bengelsdorf B, Daniel R, Dürre P**. 2015. Complete genome sequence of the acetogenic bacterium *Moorella thermoacetica* DSM 2955ᵀ. Genome Announc **3**:e01157-15. http://dx.doi.org/10.1128/genomeA.01157-15.