AMERICAN SOCIETY FOR MICROBIOLOGY | Microbiology Resource Announcements

| Environmental Microbiology | Announcement

Draft genome sequence of furan-degrading *Rhodococcus erythropolis* strain FUR100

Steffen Helbich,[1] Christine Woiski,[1] Daniel Dobslaw,[1] Thomas Gerl,[1] Yevhen Vainshtein,[2] Kai Sohn,[2] Karl-Heinrich Engesser[1]

AUTHOR AFFILIATIONS See affiliation list on p. 2.

ABSTRACT *Rhodococcus erythropolis* FUR100 was isolated from a mixture of soil and activated sludge. It can use furan as a sole source of carbon and energy. Its draft genome sequence may provide insight into the genetics of furan catabolism.

KEYWORDS furan, *Rhodococcus erythropolis*, microbial degradation

Furan is an aromatic five-membered heterocyclic compound containing one oxygen atom. It is a precursor used in the synthesis of fine chemicals (1). In rat and human liver, furan is transformed by P450 monooxygenases to the carcinogenic cis-2-butene-1,4-dial, possibly via the unstable intermediate 2,3-epoxy-2,3-dihydrofuran (2–4). Bacterial aerobic metabolism of furan derivatives furfural, 2-furoic acid, 5-hydroxymethylfurfural, and dibenzo[b,d]furan is well researched (5–7) but not of the heterocycle itself. *Rhodococcus erythropolis* FUR100 can use furan as a sole source of carbon and energy. The strain was obtained by enrichment in 100 mL liquid mineral salt medium (8), inoculated with a mixture of 10 g soil sample and 10 mL activated sludge from a wastewater treatment plant (Stuttgart-Büsnau, Germany, 48.75191 N 9.08968 E), supplied with 5 mM furan, and incubated at 30°C, 250 rpm, in a baffled flask (500 mL), sealed gas tight with a polytetrafluoroethylene septum. After 7 days, 1 mL of suspension was transferred to fresh medium supplied with furan and incubated until a visible increase in turbidity was observed. This step was repeated a second time. A dilution of a 1 mL sample was plated on mineral salts medium plates and incubated at 30°C in a desiccator supplied with a 0.001% furan atmosphere. Single colonies were picked and streaked onto fresh plates to obtain pure cultures. These pure cultures were tested again for growth in liquid culture to exclude the use of agar as a carbon source. One of the isolated strains, FUR100, was further examined.

DNA was obtained from a fresh liquid culture (same conditions as isolation procedure). Cells were harvested after 4 days, washed twice with PBS (4°C, 10,000 × *g*, 10 min), and resuspended in 5 mL PBS. Cell pellets were created by pipetting droplets (~100 µL) directly into liquid nitrogen and disrupted using a Mixer Mill MM 200 (Retsch). DNA was isolated with the QIAamp DNA Mini Kit (Qiagen) according to the manufacturer's protocol. The sequencing library was prepared from 50 ng of input material using the Nextera DNA Library Preparation Kit (Illumina) according to the manufacturer's protocol and sequenced on an Illumina HiSeq2500 platform in paired-end mode for 150 cycles. A sequencing depth of 5,866,912 reads was generated. Sample integrity and sequencing library quality were ensured by using a Fragment Analyzer (Agilent). Raw sequencing reads were demultiplexed with the bcl2fastq software (Illumina, 2015). Default parameters were used for all software unless otherwise specified. Adapter trimming and low-quality read removal were performed using BBDuk (BBMap v34.41 http://sourceforge.net/projects/bbmap/) with the parameters: trimpolyg = 10 hdist = 1 mink = 12 maxns = 10 minlen = 50 trimq = 20 qtrim = t ktrim = r k = 28. The quality of the

Editor J. Cameron Thrash, University of Southern California, USA

Address correspondence to Karl-Heinrich Engesser, karl-heinrich.engesser@iswa.uni-stuttgart.de.

The authors declare no conflict of interest.

See the funding table on p. 2.

Received 17 November 2023
Accepted 8 January 2024
Published 24 January 2024

sequencing was controlled with FastQC (http://www.bioinformatics.babraham.ac.uk/projects/fastqc/). The genome was *de novo* assembled using ABySS 2.0.2 (9), followed by an evaluation with Quast v5.0.2 (10). The genome was annotated by NCBI using PGAP (11). It has a size of 7,667,056 bp with a GC content of 62.5% and a coverage of 215.0×. It is split into 105 contigs with an N_{50} of 145,273 bp. There are 7,186 predicted genes of which 6,992 are protein coding. The taxonomy of strain FUR100 was determined using the TYGS webserver (v389), closest match being *Rhodococcus erythropolis* JCM 3201^T (dDDH d_4 88.5%, confidence interval 86.0%–90.5%) (12, 13).

ACKNOWLEDGMENTS

We thank Christian Grumaz and Karoline Glanz at Fraunhofer IGB (Stuttgart, Germany) for library preparation and sequencing.

AUTHOR AFFILIATIONS

[1]Department of Biological Waste Air Purification, University of Stuttgart, Institute for Sanitary Engineering, Water Quality and Solid Waste Management, Stuttgart, Germany
[2]Fraunhofer Institute for Interfacial Engineering and Biotechnology, Stuttgart, Germany

AUTHOR ORCIDs

Steffen Helbich http://orcid.org/0000-0002-2740-270X
Christine Woiski http://orcid.org/0000-0003-2797-3331
Daniel Dobslaw http://orcid.org/0000-0002-7865-2880
Yevhen Vainshtein https://orcid.org/0000-0001-7944-8892
Kai Sohn http://orcid.org/0000-0002-6682-6409
Karl-Heinrich Engesser http://orcid.org/0000-0001-7273-2962

FUNDING

Funder	Grant(s)	Author(s)
Federal State of Baden-Wuerttemberg, Germany	BWPLUS BWBÖ 17008	Daniel Dobslaw
German Research Foundation	Open Access Publication Funding/2023-2024/University of Stuttgart (512689491)	Karl-Heinrich Engesser

AUTHOR CONTRIBUTIONS

Steffen Helbich, Conceptualization, Investigation, Writing – original draft, Writing – review and editing | Christine Woiski, Investigation, Methodology, Writing – review and editing | Daniel Dobslaw, Conceptualization, Project administration, Resources, Supervision, Writing – review and editing | Thomas Gerl, Investigation, Methodology | Yevhen Vainshtein, Data curation, Writing – original draft, Writing – review and editing | Kai Sohn, Data curation | Karl-Heinrich Engesser, Resources, Supervision, Writing – review and editing

DATA AVAILABILITY

The draft genome sequence of *Rhodococcus erythropolis* FUR100 has been deposited in GenBank under BioProject accession PRJNA680546, BioSample accession number SAMN16882220, assembly accession GCA_016019085.1, and SRA accession SRR26192813.

REFERENCES

1. Hoydonckx HE, van Rhijn WM, van Rhijn W, de Vos DE, Jacobs PA. 2000. Furfural and derivatives, p 733. In Ullmann's encyclopedia of industrial chemistry. Vol. 3. Wiley.
2. Chen LJ, Hecht SS, Peterson LA. 1995. Identification of cis-2-butene-1,4-dial as a microsomal metabolite of furan. Chem Res Toxicol 8:903–906. https://doi.org/10.1021/tx00049a001
3. Guengerich FP. 2003. Cytochrome P450 oxidations in the generation of reactive electrophiles: epoxidation and related reactions. Arch Biochem Biophys 409:59–71. https://doi.org/10.1016/s0003-9861(02)00415-0
4. Peterson LA. 2013. Reactive metabolites in the biotransformation of molecules containing a furan ring. Chem Res Toxicol 26:6–25. https://doi.org/10.1021/tx3003824
5. Wierckx N, Koopman F, Ruijssenaars HJ, de Winde JH. 2011. Microbial degradation of furanic compounds: biochemistry, genetics, and impact. Appl Microbiol Biotechnol 92:1095–1105. https://doi.org/10.1007/s00253-011-3632-5
6. Schmid A, Rothe B, Altenbuchner J, Ludwig W, Engesser K-H. 1997. Characterization of three distinct extradiol dioxygenases involved in mineralization of dibenzofuran by *Terrabacter* sp. strain DPO360. J Bacteriol 179:53–62. https://doi.org/10.1128/jb.179.1.53-62.1997
7. Armengaud J, Happe B, Timmis KN. 1998. Genetic analysis of dioxin dioxygenase of *Sphingomonas sp.* strain RW1: catabolic genes dispersed on the genome. J Bacteriol 180:3954–3966. https://doi.org/10.1128/JB.180.15.3954-3966.1998
8. Helbich S, Barrantes I, Dos Anjos Borges LG, Pieper DH, Vainshtein Y, Sohn K, Engesser K-H. 2023. The 2-methylpropene degradation pathway in *Mycobacteriaceae* family strains. Environ Microbiol 25:2163–2181. https://doi.org/10.1111/1462-2920.16449
9. Jackman SD, Vandervalk BP, Mohamadi H, Chu J, Yeo S, Hammond SA, Jahesh G, Khan H, Coombe L, Warren RL, Birol I. 2017. ABySS 2.0: resource-efficient assembly of large genomes using a bloom filter. Genome Res 27:768–777. https://doi.org/10.1101/gr.214346.116
10. Gurevich A, Saveliev V, Vyahhi N, Tesler G. 2013. QUAST: quality assessment tool for genome assemblies. Bioinform 29:1072–1075. https://doi.org/10.1093/bioinformatics/btt086
11. Tatusova T, DiCuccio M, Badretdin A, Chetvernin V, Nawrocki EP, Zaslavsky L, Lomsadze A, Pruitt KD, Borodovsky M, Ostell J. 2016. NCBI prokaryotic genome annotation pipeline. Nucleic Acids Res 44:6614–6624. https://doi.org/10.1093/nar/gkw569
12. Meier-Kolthoff JP, Göker M. 2019. TYGS is an automated high-throughput platform for state-of-the-art genome-based taxonomy. Nat Commun 10:2182. https://doi.org/10.1038/s41467-019-10210-3
13. Meier-Kolthoff JP, Carbasse JS, Peinado-Olarte RL, Göker M. 2022. TYGS and LPSN: a database tandem for fast and reliable genome-based classification and nomenclature of prokaryotes. Nucleic Acids Res 50:D801–D807. https://doi.org/10.1093/nar/gkab902