Genome analysis provides insights into microaerobic toluene-degradation pathway of Zoogloea oleivorans BucT

András Táncsics¹,² · Milán Farkas¹,² · Balázs Horváth³ · Gergely Maróti⁴ · Lauren M. Bradford⁵ · Tillmann Lueders⁵,⁶ · Balázs Kriszt¹,²

Received: 23 August 2019 / Revised: 24 September 2019 / Accepted: 3 October 2019 / Published online: 28 October 2019

© The Author(s) 2019

Abstract

Zoogloea oleivorans, capable of using toluene as a sole source of carbon and energy, was earlier found to be an active degrader under microaerobic conditions in aquifer samples. To uncover the genetic background of the ability of microaerobic toluene degradation in Z. oleivorans, the whole-genome sequence of the type strain BucT was revealed. Metatranscriptomic sequence reads, originated from a previous SIP study on microaerobic toluene degradation, were mapped on the genome. The genome (5.68 Mb) had a mean G + C content of 62.5%, 5005 protein coding gene sequences and 80 RNA genes. Annotation predicted that 66 genes were involved in the metabolism of aromatic compounds. Genome analysis revealed the presence of a cluster with genes coding for a multicomponent phenol-hydroxylase system and a complete catechol meta-cleavage pathway. Another cluster flanked by mobile-element protein coding genes coded a partial catechol meta-cleavage pathway including a subfamily I.2.C-type extradiol dioxygenase. Analysis of metatranscriptomic data of a microaerobic toluene-degrading enrichment, containing Z. oleivorans as an active-toluene degrader revealed that a toluene dioxygenase-like enzyme was responsible for the ring-hydroxylation, while enzymes of the partial catechol meta-cleavage pathway coding cluster were responsible for further degradation of the aromatic ring under microaerobic conditions. This further advances our understanding of aromatic hydrocarbon degradation between fully oxic and strictly anoxic conditions.

Keywords Zoogloea · Toluene degradation · Metatranscriptomics · Biodegradation

Introduction

At present, the genus Zoogloea (family Zoogloeaceae) contains five validly described species, which can be characterized as floc-forming, nitrogen-fixing bacteria. Members of the genus have been isolated from various habitats including activated sludge, soil or hydrocarbon contaminated groundwater (Xie and Yokota 2006; Shao et al. 2009; Farkas et al. 2015). Despite the fact that Zoogloea spp. play a crucial role in wastewater treatment by causing the flocculation of the activated sludge, limited genome sequence information is available regarding these bacteria. The first publicly available genome sequence was reported by Muller et al. (2017). Recent studies characterizing benzene- and toluene-degrading microbial communities have shown that Zoogloea genus-related bacteria could have an important role in the degradation of these contaminants in subsurface environments. Protein- and RNA-stable isotope probing (SIP) based analysis of an aerobic benzene-degrading microbial community revealed Zoogloea-related bacteria as predominant benzene-degraders (Jechalke

Communicated by Erko Stackebrandt.

András Táncsics
tancsics.andras@fh.szie.hu

¹ Regional University Center of Excellence in Environmental Industry, Szent István University, Gödöllő, Hungary
² Department of Environmental Safety and Ecotoxicology, Szent István University, Gödöllő, Hungary
³ SeqOmics Biotechnology Ltd., Mórahalom, Hungary
⁴ Institute of Plant Biology, Biological Research Centre, Hungarian Academy of Sciences, Szeged, Hungary
⁵ Institute of Groundwater Ecology, Helmholtz Zentrum München, Munich, Germany
⁶ Chair of Ecological Microbiology Bayreuth, Center of Ecology and Environmental Research (BayCEER), University of Bayreuth, Bayreuth, Germany
et al. 2013). Our previous DNA- and transcriptome-SIP studies have shown that Zoogloea oleivorans is a highly efficient toluene degrader under microaerobic conditions (Bradford et al. 2018; Táncsics et al. 2018). We hypothesized that Z. oleivorans was capable of degrading toluene under microaerobic conditions due to the fact that it harbours a catechol 2,3-dioxygenase (C23O) gene which encodes a subfamily I.2.C-type extradiol dioxygenase enzyme (Farkas et al. 2015). Kukor and Olsen (1996) suggested that this group of extradiol dioxygenases was adapted to environments with low-oxygen concentrations, hinting at their role in ring-cleavage reactions under hypoxic conditions. On the other hand, it is known that ring-cleaving dioxygenases belonging to the same subfamily may show different oxygen affinities, as was observed in the case of chlorocatechol 1,2-dioxygenases (Balcke et al. 2008). Case of chlorocatechol 1,2-dioxygenases may show different oxygen affinities, as was observed in the case of chlorocatechol 1,2-dioxygenases (Balcke et al. 2008), and comparative analysis of aerobic and microaerobic BTEX-degrading enrichment cultures (Benedek et al. 2018). In the present study, to uncover the genetic background of the ability of microaerobic toluene degradation in Z. oleivorans, the whole-genome sequence of the type strain BucT was revealed. In addition, metatranscriptomic (non-rRNA) sequence reads originated from our previous SIP study on microaerobic toluene degradation in aquifer samples with abundant Zoogloea spp. (Bradford et al. 2018) were mapped on the genome.

**Materials and methods**

Genomic DNA from Zoogloea oleivorans BucT was isolated using the DNeasy UltraClean Microbial Kit (Qiagen, Germany) according to the instructions of the manufacturer. The whole-genome sequencing was performed as described previously (Borsodi et al. 2019), briefly: Nextera Mate Pair Sample Preparation Kit (Illumina, USA) was used to generate mate-paired libraries according to the manufacturer’s protocol for gel-plus version with slight modifications. 13 µl of Mate-Paired Tagment Enzyme was used to produce a robust smear within the 7-11 kbp region. The 7-11 kbp DNA fraction was excised from the gel using the Zymoclean Large Fragment DNA Recovery kit (Zymo Research, USA) and the circularized DNA was sheared using Covaris S2. All quality measurements were performed on a TapeStation 2200 instrument (Agilent, USA). Final libraries were quantified using Qubit (ThermoFisher, USA) and sequenced on an Illumina MiSeq instrument using MiSeq Reagent Kit v2 (500 cycles) sequencing chemistry. De novo assembly and scaffolding were performed with CLC Genomics Workbench Tool v11 (Qiagen, Germany). The mate-paired reads were assembled into 107 contigs. Automatic annotation of the genome was performed by the NCBI Prokaryotic Genomes Automatic Annotation Pipeline (PGAP) v4.5 (Tatusova et al. 2016). The genome sequence of strain BucT has been deposited at the GenBank database under the WGS accession number SDKK00000000 (Bioproject: PRJNA516779; Biosample: SAMN10797634). Mapping of metatranscriptomic sequence reads (NCBI Gene Expression Omnibus accession number GSM3380032; sample name: 13CHunamp) on the de novo assembled genome of strain BucT was performed by CLC Genomics Workbench Tool v11 (Qiagen, Germany) using the following parameters: length fraction = 0.8; similarity fraction = 0.8. Phylogenetic tree was reconstructed using the maximum-likelihood algorithm using MEGA version 6.0. Tree topology and distances were evaluated by bootstrap analysis based on 1000 replicates. Graphical visualization of gene clusters was performed by using SnapGene v4.3.4.

**Results and discussion**

Strain BucT, the type strain of Zoogloea oleivorans, was isolated from a petroleum-hydrocarbon contaminated environment and was previously described by us as a new member of the genus Zoogloea (Farkas et al. 2015). As seen on the phylogenetic tree (Fig. 1), strain BucT represents a considerably distinct lineage of the genus Zoogloea, and is only distantly related to typical, activated sludge inhabiting Zoogloea species (e.g. Z. ramigera).

The whole-genome sequencing revealed that strain BucT has a 5,678,157 bp large genome with a G+C content of 62.5% and 5200 features (5005 protein coding genes). Annotation of the genome sequence identified that at least 66 genes affiliated with aromatic-hydrocarbon degradation. Prior to the genome sequence analysis, it was known that Z. oleivorans BucT harbours a catechol 2,3-dioxygenase gene, which encodes a subfamily I.2.C-type extradiol dioxygenase enzyme (Farkas et al. 2015). The genome sequence revealed that this gene is located in a gene cluster, which encodes only a partial meta-cleavage pathway (Fig. 2). This gene cluster is flanked by mobile-genetic elements (Tn3 family transposase upstream and an uncharacterized transposase downstream), and starts with a ferredoxin-coding gene, followed by the subfamily I.2.C-type C23O gene. The cluster contains 15 genes in a unique arrangement, which has not been observed before in the case of any cultured bacterium. However, this cluster was also found in the metagenome-assembled genome of an uncultivated Rhodoferax sp., capable of degrading sulfolane (Kasanke et al. 2019). The role of transposase mediated, partial meta-pathway coding gene clusters in chlorobenzene degradation was first observed and deeply studied in Pseudomonas putida GJ31 (Kunze et al. 2009). Since the partial meta-cleavage pathway coding gene cluster of strain BucT did not contain any gene encoding aromatic ring-hydroxylating dioxygenase (ARHD) enzyme, the involvement of another gene cluster in toluene degradation was assumable.

The genome sequence revealed that besides the subfamily I.2.C-type C23O gene, Z. oleivorans BucT harbours two
additional C23O genes with different length (930 and 936 bp, respectively), which are part of a phenol-degradation gene cluster (Fig. 3). Phylogenetic analysis of these genes showed that both of them encode subfamily I.2.A.-type extradiol dioxygenases (data not shown). The shorter C23O gene is located upstream of genes encoding a multicomponent phenol-hydroxylase system. It shares the highest similarity (between 80-85% at nucleotide level) with *Dechloromonas*,

![Maximum-likelihood tree based on 16S rRNA gene sequences showing the phylogenetic relationships between Zoogloea oleivorans Buc and related taxa including Thauera sp. DNT-1 (both highlighted with boldface type). Bootstrap values are shown at nodes as percentages of 1000 replicates; only values over 50% are shown. Bar, 0.02 changes per nucleotide position.](image1)

![Schematic representation of the partial meta-cleavage pathway coding gene cluster in the genome of Zoogloea oleivorans Buc, containing the subfamily I.2.C-type C23O gene. ORF 1: Tn3 family transposase; ORF 2: ferredoxin; ORF 3: I.2.C-type catechol 2,3-dioxygenase; ORF 4: heme-binding protein; ORF 5: 2-hydroxymuconic semialdehyde dehydrogenase; ORF 6: glutathione S-transferase; ORF 7: 2-hydroxymuconic semialdehyde hydrolase; ORF 8: 2-oxo-3-hexendioate decarboxylase; ORF 9: 2-oxo-3-hexendioate dehydrogenase; ORF 10: SDR family oxidoreductase; ORF 11: 4-hydroxy-2-oxovalerate aldolase; ORF 12: 2-oxo-3-hexendioate decarboxylase; ORF 13: 2-oxo-3-hexendioate decarboxylase; ORF 14: 2-oxacarboxylate tautomerase family protein; ORF 15: pyruvate carboxylase.](image2)

![Schematic representation of the multicomponent phenol-hydroxylase coding gene cluster in the genome of Zoogloea oleivorans Buc. ORF 1: sigma-54-dependent Fis family transcriptional regulator; ORF 2: oxidoreductase; ORF 3: aromatic ring-hydroxylating dioxygenase subunit alpha; ORF 4: DUF1302 domain-containing protein; ORF 5: DUF1329 domain-containing protein; ORF 6: YnfA family protein; ORF 7: ferredoxin; ORF 8: catechol 2,3-dioxygenase; ORF 9: phenol-hydroxylase component (DmpK); ORF 10: phenol-hydroxylase component (P1 oxygenase component, DmpL); ORF 11: phenol-hydroxylase component (P2 regulatory component, DmpM); ORF 12: phenol-hydroxylase component (P3 oxygenase component, DmpN); ORF 13: phenol-hydroxylase component (P4 oxygenase component, DmpO); ORF 14: phenol-hydroxylase component (DmpP); ORF 15: transcriptional repressor; ORF 16: XRE family transcriptional regulator; ORF 17: 2-hydroxymuconic semialdehyde dehydrogenase; ORF 18: 2-oxo-3-hexendioate decarboxylase; ORF 19: 2-oxo-3-hexendioate decarboxylase; ORF 20: 4-oxalocrotonate tautomerase; ORF 21: acetaldehyde-dehydrogenase (acylating); ORF 22: 4-hydroxy-2-oxovalerate aldolase; ORF 23: catechol 2,3-dioxygenase; ORF 24: SDR family oxidoreductase; ORF 25: 4-oxalocrotonate tautomerase.](image3)
Zoogloea, Thauera and Azoarcus-related C23O genes, which are also similarly located in phenol-degradation gene clusters. The second C23O gene is located close to the downstream end of the gene cluster and encodes a much more unique enzyme. Similar extradiol dioxygenase genes have been revealed so far only in the genome of Zoogloea sp. LCSB751, Azoarcus communis DSM12120 and Methyloverasitlis universalis EHg5. Further screening of the genome for genes encoding aromatic ring-hydroxylating enzymes revealed the presence of a cluster encoding a complete meta-cleavage pathway. This was a biphenyl-degradation gene cluster flanked by mobile-genetic elements and contained genes of a toluene dioxygenase-like enzyme and a 2,3-dihydroxybiphenyl 1,2-dioxygenase. In this cluster the ORF 2 coded the alpha subunit (458 aa), while ORF 3 coded the beta subunit (184 aa) of the toluene dioxygenase-like enzyme (Fig. 4) which exhibited homology to todC1 and todC2 proteins of Thauera sp. DNT-1, respectively (100% of the amino acids are identical). It was shown that this Thauera strain was able to degrade toluene under both aerobic and anaerobic conditions, and in the presence of oxygen it used a toluene-dioxygenase (tod) enzyme for initial activation of the aromatic ring (Shinoda et al. 2004). The tod genes encoding cluster was partially recovered by Shinoda et al. (2004) and we found that all of the revealed genes in this cluster were identical with the corresponding genes in the biphenyl-degradation gene cluster of Z. oleivorans. This observation together with the fact that the biphenyl-degradation gene cluster in the genome of strain BucT was flanked by mobile-genetic elements, suggest that this gene cluster could have been spread among members of the family Zoogloeaceae by horizontal gene transfer (HGT).

We have previously investigated a microaerobic, 13C-labelled toluene-degrading enrichment culture, in which Zoogloea oleivorans was an abundant toluene degrader, by RNA-stable isotope probing (Bradford et al. 2018). Metatranscriptomic data (non-rRNA sequence reads of the heavy RNA fraction) derived from this enrichment study were used to reveal which of the above-mentioned gene clusters of Z. oleivorans were involved in degradation. The partial meta-cleavage pathway encoding gene cluster was found to be expressed in the enrichment, especially the subfamily I.2.C-type C23O and the 2-hydroxymuconic semialdehyde dehydrogenase genes (86 and 88 gene reads in the metatranscriptome, respectively, and high-RPKM values).

![Fig. 4](https://example.com/fig4.png)

Fig. 4 Schematic representation of the toluene-dioxygenase coding gene cluster in a the genome of Zoogloea oleivorans BucT (ORF 1: GntR family transcriptional regulator; ORF 2: aromatic ring-hydroxylating dioxygenase subunit alpha; ORF 3: 3-phenylpropionate/cinnamic acid dioxygenase subunit beta; ORF 4: hypothetical protein; ORF 5: ferredoxin; ORF 6: pyridine nucleotide-disulphide oxidoreductase; ORF 7: cis-2,3-dihydrobiphenyl-2,3-diol dehydrogenase; ORF 8: 2,3-dihydroxybiphenyl 1,2-dioxygenase; ORF 9: 2-oxopent-4-enoate hydratase; ORF 10: acetaldehyde-dehydrogenase (acetylating); ORF 11: 4-hydroxy-2-oxovalerate aldolase; ORF 12: 2-hydroxy-6-oxo-6-phenylhexa-2,4-dienoate hydrolase; ORF 13: aromatic hydrocarbon degradation protein; ORF 14: alpha/beta fold hydrolase), and b in the genome of Thauera sp. DNT-1 (GenBank accession number: AB066264) (ORF 1 and ORF 2: terminal dioxygenase iron sulphur proteins (todC1 and todC2 proteins); ORF 3 ferredoxin; ORF 4: ferredoxin reductase; ORF 5: dehydrogenase). The percentages below the ORFs indicate the similarity of nucleotide sequences to the corresponding ORF of Zoogloea oleivorans BucT, which are depicted with the same colour.

© Springer
especially genes encoding the toluene-dioxygenase enzyme appeared highly expressed (115 reads altogether in the metatranscriptome). Accordingly, the formation of 3-methylcatechol through toluene-cis-dihydrodiol can be postulated. The phenomenon that a toluene-dioxygenase enzyme played a role in the hydroxylation of the aromatic ring under microaerobic conditions can be explained via previous observations. It has been shown for *Pseudomonas putida* F1, that the concentration of dissolved oxygen did not significantly affect the expression and longevity of toluene dioxygenase, and the strain could also grow on toluene under microaerobic conditions (Costura and Alvarez 2000). The above-mentioned *Thauera* sp. strain DNT-1 was also able to degrade toluene aerobically when only trace amount of oxygen was present in the environment (Shinoda et al. 2004). On the other hand, ring monooxygenation is usually the predominant activation mechanism of toluene degradation under microaerobic conditions, instead of dioxygenation. Thus it has been observed for toluene-degrading chemostat cultures, that *Burkholderia* (formerly *Pseudomonas*) *cepacia* strain G4, which uses a monooxygenation mechanism for toluene activation, outcompeted *Pseudomonas putida* strain F1 (using dioxygenation) under oxygen limitation (Duetz et al. 1994). A predominance of ring monooxygenation was also observed in hypoxic, toluene-degrading constructed wetlands, linked to members of the *Burkholderiaceae* and *Comamonadaceae* (Martinez-Lavanchy et al. 2015).

In summary, results of the present study provide evidence that under microaerobic conditions a toluene dioxygenase-like enzyme of *Zoogloea oleivorans* was involved in the initial activation (aromatic ring-hydroxylation) of toluene, while the subfamily I.2.C-type extradiol dioxygenase catalysed the ring-cleavage reaction. The gene clusters encoding the tod-like and the subfamily I.2.C-type extradiol dioxygenase enzymes were flanked by mobile-genetic elements, suggesting that these gene clusters were acquired by strain Buc<sup>T</sup> through HGT events. Thus, the capacity of microaerobic toluene degradation seems like a mosaic encoded in the genome of *Zoogloea oleivorans* Buc<sup>T</sup>.

**Acknowledgements** Open access funding provided by Szent István University (SZIE). This research was supported by the Higher Education Institutional Excellence Program (NKFH-1159-6/2019) awarded by the Ministry for Innovation and Technology of Hungary within the framework of water related researches of Szent István University. Lauren M. Bradford was funded by the European Research Council (ERC) under the European Union’s Seventh Framework Program (FP7/2007-2013), grant agreement 616644 (POLLOX) to Tillmann Lueders.

**Open Access** This article is distributed under the terms of the Creative Commons Attribution 4.0 International License (http://creativecommons.org/licenses/by/4.0/), which permits unrestricted use, distribution, and reproduction in any medium, provided you give appropriate credit to the original author(s) and the source, provide a link to the Creative Commons license, and indicate if changes were made.

**References**

Balcke GU, Wegener S, Kiesel B, Benndorf D, Schlömann M, Vogt C (2008) Kinetics of chlorobenzene biodegradation under reduced oxygen levels. Biodegrad 19:507–518

Benedek T, Szentygyőrgyi F, Szabó I, Kriszt B, Révész F, Radó J, Maróti G, Táncsics A (2018) Aerobic and oxygen-limited enrichment of BTEX-degrading biofilm bacteria: dominance of *Malkia* versus *Acidovorax* species. Environ Sci Pollut Res Int 25:32178–32195

Borsodi AK, Aszalós JM, Bibari P, Nagy I, Schumann P, Spröer C, Kovács AL, Bóka K, Övári M, Szili-Kovács T, Tóth E (2019) *Anaerobacillus alkaliphilus* sp. nov., a novel alkaliphilic and moderately halophilic bacterium. Int J Syst Evol Microbiol 69:631–637

Bradford LM, Vestergaard G, Táncsics A, Zhu B, Schloter M, Lueders T (2018) Transcriptome-stable isolate probing provides targeted functional and taxonomic insights into microaerobic pollutant-degrading aquifer microbiota. Front Microbiol 9:2696

Costura RK, Alvarez PJJ (2000) Expression and longevity of toluene dioxygenase in *Pseudomonas putida* F1 induced at different dissolved oxygen concentrations. Water Res 34:3014–3018

Duetz WA, de Jong C, Williams PA, van Andel JG (1994) Competition in chemostat culture between *Pseudomonas* strains that use different pathways for the degradation of toluene. Appl Environ Microbiol 60:2858–2863

Farkas M, Táncsics A, Kriszt B, Benedek T, Tóth EM, Kéki Z, Veres PG, Szoboszlay S (2015) *Zoogloea* oleivorans sp. nov., a flocc-forming, petroleum hydrocarbon-degrading bacterium isolated from biofilm. Int J Syst Evol Microbiol 65:274–279

Jechalke S, Franchini AG, Bastida F, Bombach P, Rosell M, Seifert J, von Bergen M, Vogt C, Richnow HH (2013) Analysis of structure, function, and activity of a benzene-degrading microbial community. FEMS Microbiol Ecol 85:14–26

Kasanke CP, Collins RE, Leigh MB (2019) Identification and characterization of a dominant sulfonate-degrading *Rhodolflexa* sp. via stable isotope probing combined with metagenomics. Sci Rep 9:3121

Kukor JJ, Olsen RH (1996) Catechol 2,3-dioxygenases functional in oxygen-limited (hypoxic) environments. Appl Environ Microbiol 62:1728–1740

Kunze M, Zerlin KF, Retzlaff A, Pohl JO, Schmidt E, Janssen DB, Vilchez-Vargas R, Pieper DH, Reineke W (2009) Degradation of chloroaromatics by *Pseudomonas putida* GJ31: assembled route for chlorobenzene degradation encoded by clusters on plasmid pKW1 and the chromosome. Microbiology 155:4069–4083

Martinez-Lavanchy PM, Chen Z, Lünsmann V, Marin-Cevada V, Vilches-Vargas R, Pieper DH, Reiche N, Kappelmeyer U, Imparato V, Junca H, Nijenhuis I, Müller JA, Kuschk P, Heipieper HJ (2015) Microbial toluene removal in hypoxic model constructed wetlands occurs predominantly via the ring monooxygenation pathway. Appl Environ Microbiol 81:6241–6252

Muller EEL, Narayanasamy S, Zeimes M, Laczny CC, Lebrun LA, Herold M, Hicks ND, Gillece JD, Schupp JM, Keim P, Wilmes P (2017) First draft genome sequence of a strain belonging to the *Anaerobacillus* genus and its gene expression in situ. Stand Genomic Sci 12:64

Shao Y, Chung BS, Lee SS, Park W, Lee SS, Jeon CO (2009) *Zoogloea caeni* sp. nov., a flocc-forming bacterium isolated from activated sludge. Int J Syst Evol Microbiol 59:526–530

Shinoda Y, Sakai Y, Uenishi H, Uchihashi Y, Hiraishi A, Yukawa H, Yurimoto H, Kato N (2004) Aerobic and anaerobic biodegradation by a newly isolated denitrifying bacterium, *Thauera* sp. strain DNT-1. Appl Environ Microbiol 70:1385–1392
Táncsics A, Szalay AR, Farkas M, Benedek T, Szoboslay S, Szabó I, Lueders T (2018) Stable isotope probing of hypoxic toluene degradation at the Siklós aquifer reveals prominent role of Rhodocyclaceae. FEMS Microbiol Ecol 94:fiy088
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
Xie CH, Yokota A (2006) Zoogloea oryzae sp. nov., a nitrogen-fixing bacterium isolated from rice paddy soil, and reclassification of the strain ATCC 19623 as Crabtreella saccharophila gen. nov., sp. nov. Int J Syst Evol Microbiol 56:619–624

Publisher’s Note Springer Nature remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.