Journal of Biotechnology 275 (2018) 13–16

Contents lists available at ScienceDirect

Journal of Biotechnology

journal homepage: www.elsevier.com/locate/jbiotec

Short Genome Communications

Genome sequence of two members of the chloroaromatic-degrading MT community: *Pseudomonas reinekei* MT1 and *Achromobacter xylosoxidans* MT3

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ARTICLE INFO

Keywords:
MT bacterial community
Genome sequence
Chloro- and nitro-aromatic compounds
Biodegradation

ABSTRACT

We describe the genome sequence of *Pseudomonas reinekei* MT1 and *Achromobacter xylosoxidans* MT3, the most abundant members of a bacterial community capable of degrading chloroaromatic compounds. The MT1 genome contains open reading frames encoding enzymes responsible for the catabolism of chlorosalicylate, methylsalicylate, chlorophenols, phenol, benzoate, *p*-coumarate, phenylalanine, and phenylacetate. On the other hand, the MT3 strain genome possesses no ORFs to metabolize chlorosalicylates; instead the bacterium is capable of metabolizing nitro-phenolic and phenolic compounds, which can be used as the only carbon and energy source by MT3. We also confirmed that MT3 displays the genetic machinery for the metabolism of chlorocathecols and chloromuconates, where the latter are toxic compounds secreted by MT1 when degrading chlorosalicylates. Altogether, this work will advance our fundamental understanding of bacterial interactions.

Anthropogenic activities have resulted in the generation and release of recalcitrant pollutant compounds into the environment, imposing large burdens on human health and ecological niches (Jeon et al., 2016). Among them, chloroaromatic compounds have been classified as one of the most toxic class of chemicals, their transformation and mineralization being difficult to achieve via conventional chemical methods (Adebusoye, 2016). Bacteria of the genus *Pseudomonas* are versatile microorganisms (Timmis, 2002)(Poblete-Castro et al., 2017), capable of metabolizing a wide array of halogenated aromatic compounds such as polychlorinated biphenyls and dioxins (Göbel et al., 2002). Some intermediates of the chloroaromatic and aromatic degradation pathway e.g. catechol and its derivatives, are highly toxic for many microorganisms (Schweigert et al., 2001)(Arora and Bae, 2014). One of the key enzymatic steps of the aerobic bacterial degradation of these compounds proceeds by the action of dioxygenases (Pieper, 2005) —enzymes that catalyze the oxidative *ortho*-cleavage ring of chlorocatechol to chloromuconate (Moiseeva et al., 2002)(Cámara et al., 2009). In nature, microbial degradation of aromatic compounds is a complex process of nutrient exchange (Bacosa et al., 2012)(Leewis et al., 2016), where the most abundant bacterial strain metabolizes the primary chloroaromatic compound. In the process, it secretes various intermediates such as chlorocatechol, chloromuconate, and dienelactones, of the highly specified aromatic degradation pathway, which are further used as carbon source by other members of the bacterial community (Schmidt et al., 1983)(Pelz et al., 1999). A bacterial consortium (MT community) capable of degrading 4- and 5-chlorosalicilate has served as a model system for studying bacterial interactions grown on pollutant compounds (Pelz et al., 1999)(Bobadilla Fazzini et al., 2009)(Pawelczyk et al., 2011). In this work the genomes of *Pseudomonas reinekei* MT1 and *Achromobacter xylosoxidans* MT3, the most abundant members of the community, were sequenced with the aim of revealing their gene content and metabolic potential, and thus to contribute to the understanding of bacterial collaboration.

DNA library construction and sequencing was carried out on an Illumina MiSeq platform (600 incorporated cycles; 2 × 300 bp). We designed a 490bp insert paired-end library to generate paired-end sequencing reads of 2 × 300bp, which resulted in 677 M reads for MT1, and 6.53 M reads for MT3 (totaling 1.82Gbp and 1.74Gbp, respectively). Next, raw data were filtered (> Q20) and merged (minimum overlapping of 20 bp) in PEAR version 0.9.8 (Zhang et al., 2013). Data

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https://doi.org/10.1016/j.jbiotec.2018.03.019
Received 25 January 2018; Received in revised form 21 March 2018; Accepted 27 March 2018
Available online 29 March 2018
0168-1656/

Table 1
Genome features of *Pseudomonas reinekei* MT1 and *Achromobacter xylosoxidans* MT3.

Feature	*P. reinekei* MT1	*A. xylosoxidans* MT3
Genome size (bp)	6,249,273	6,691,443
GC content (%)	60.50	67.56
rRNA	6	5
tRNA	61	54
Protein coding sequences (CDS)	5,729	6,264

were assembled using SPAdes version 3.7.0 with an auto cutoff coverage. Genome annotation was performed as in the NCBI Prokaryotic Genome Annotation Pipeline (Tatusova et al., 2016) and genomic sequences were deposited in GenBank as MSTQ00000000 and MSTP00000000 for *P. reinekei* MT1 and *A. xylosoxidans* MT3, respectively. During genome annotation, *A. xylosoxidans* MT3 was automatically reclassified as *A. denitrificans* MT3, sharing a 99% similarity with a *A. denitrificans* strain (GCA_001571365). However, a multi-locus phylogenetic analysis indicated that our genome is part of a clade of *A. xylosoxidans* members. This suggests that *A. denitrificans* (GCA_001571365) has been misidentified (Fig. S1). The NCBI record for *A. xylosoxidans* MT3 has been corrected.

The genome features of both strains are displayed in Table 1. *P. reinekei* MT1 has a total genome size of 6,249,273 bp and a G + C content of 60.5%, whereas *A. xylosoxidans* MT3 has a larger genome and higher G + C content than MT1 strain at 6,691,443 bp and 67.56%. To evaluate differences between strains, we then inspected the Cluster Orthologous Group (COG) profile of MT1 and MT3 (Fig. 1). The most abundant COG category for MT1 strain was “General function prediction only” (720) followed by “Transcription” (421). The same trend for COG categories was found for the MT3 strain (Fig. 1B). We next conducted a phylogenetic analysis (Fig. 2), where we collected a set of 30 orthologous genes from each genome in our dataset to be used as phylogenetic markers as implemented in AMPHORA2 (Wu and Scott, 2012). These genes were selected because they correspond to single-copy, non-recombining universal housekeeping genes, which ensures that phylogenetic inference is accurate. The selected genes were *dnaG, frr, infC, nusA, pgk, pyrG, rplA, rplB, rplC, rplD, rplE, rplF, rplK, rplL, rplN, rplP, rplS, rplT, rpmA, rpoB, rpsB, rpsC, rpsE, rpsI, rpsJ, rpsK, rpsM, rpsS, smpB*, and *tsf*. All nucleotide sequences were translation-aligned using TranslatorX and MAFFT (Abascal et al., 2010). The alignments were then concatenated using Seqotron version 1.0.1 (Fourment and Holmes, 2016) and the best partition scheme and substitution model were determined as in PartitionFinder2 (Lanfear et al., 2017). We searched for a distribution of probable trees using Bayesian Inference as implemented in MrBayes 3.2.5. We executed two separate runs of 20 million generations (4 chains each run; sampling every 1000 generations). We then summarized the tree distributions as the Maximum a Posteriori (MAP) estimate removing the first 5 thousand trees as burnin using the sumt command, and plotted the MAP estimate in FigTree (http://tree.bio.ed.ac.uk/software/figtree/; trees are mid-point rooted). Among the species representatives selected, MT1 closest relative was *Pseudomonas fluorescens* SBW25, both being sister taxa to a group of plant pathogenic species, and distantly related to *P. pseudoalcaligenes* and *P. stutzeri* (Fig. 2A). As described previously, we found MT3 to be closely related to *A. xylosoxidans* members, with *A. denitrificans* clade being basal to that of MT3 (Fig. 2B and Fig. S1).

Genome sequence analysis of *P. reinekei* MT1 shows open reading frames encoding enzymes responsible for the catabolism of chlorosalicylate, methylsalicylate, chlorophenols, phenol, benzoate, phenolbenzoate, *p*-coumarate, phenylalanine, and phenylacetate. MT1 has an incomplete glycolysis pathway trough the Embden–Meyerhof–Parnas pathway due to the lack of *pfk* encoding for the phosphofructokinase enzyme. This feature was also found in *A. xylosoxidans* MT3, so both strains could use the Entner–Doudoroff pathway to metabolize glucose, gluconate and other sugars and polyols as carbon sources. Genes encoding for enzymes responsible for the conversion of chlorosalicylate compounds were not found in *A. xylosoxidans* MT3. Instead, MT3 exhibits both a phenol and *p*-nitrophenol degradation pathway, where a phenol 2-monooxygenase P5 (encoded by *dmpP*) catalyzes the conversion of phenol into catechol and a 4-nitrophenol phosphatase, respectively (Fig. 3A). To fully corroborate our findings, we grew MT1 and MT3 strains in minimal medium supplemented with either benzoate, *p*-nitrophenol, phenol, or gluconate as the only carbon and energy source in aerobic shaking batch cultures. *A. xylosoxidans* MT3 metabolized all tested carbon substrates showing a yield of 0.5 and 0.3 ($g \cdot g^{-1}$) on phenol and *p*-nitrophenol, respectively (Fig. 3B). The MT1 strain was not able to mineralize *p*-nitrophenol, which is in agreement with our genome analysis and instead it showed a high biomass yield of 0.68 ($g \cdot g^{-1}$) on phenol. It is well reported that bacteria belonging to the genus *Pseudomonas* and *Achromobacter* are very efficient phenol- and *p*-nitrophenol-degrading microorganisms (Wan et al., 2007)(Shen et al., 2010), mineralizing high concentrations of the toxic compounds within less than 24 h. In regard to the catechol branch, both strains have genes encoding for enzymes belonging to the *ortho*- and *meta*-cleavage degradation pathway, where the first converts catechol into *cis-cis* muconate and the latest into 2-hydroxymuconic semialdehyde (Fig. 3A). Furthermore, *A. xylosoxidans* MT3 exhibits genes involved in the utilization of 4-Chlorocatechol and 3-Chloro-

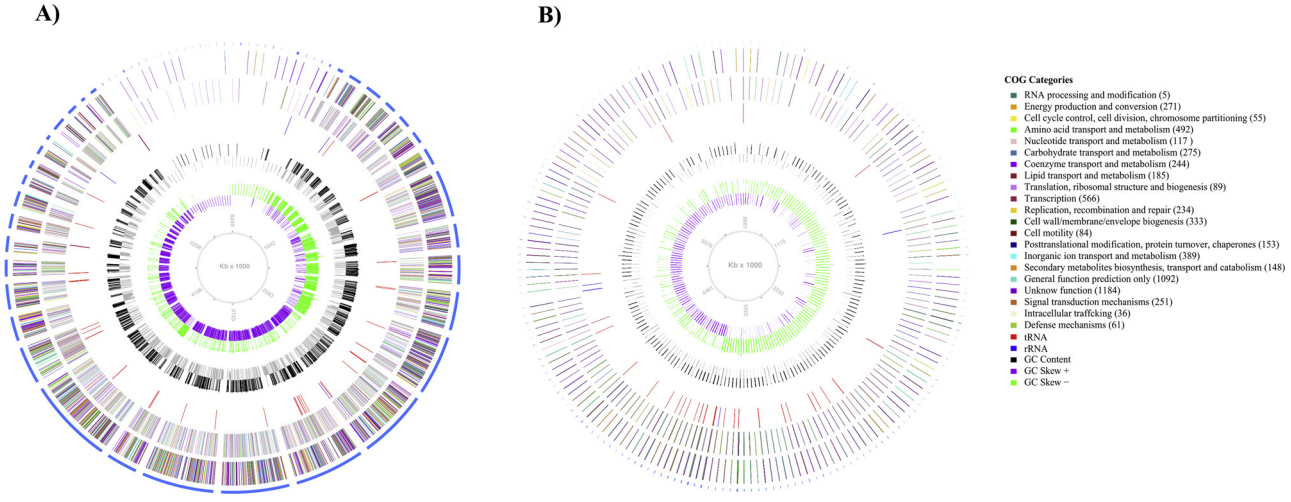

Fig. 1. Circular map of the A) *P. reinekei* MT1, and B) *A. xylosoxidans* MT3. Gene functions were annotated based on COG categories.

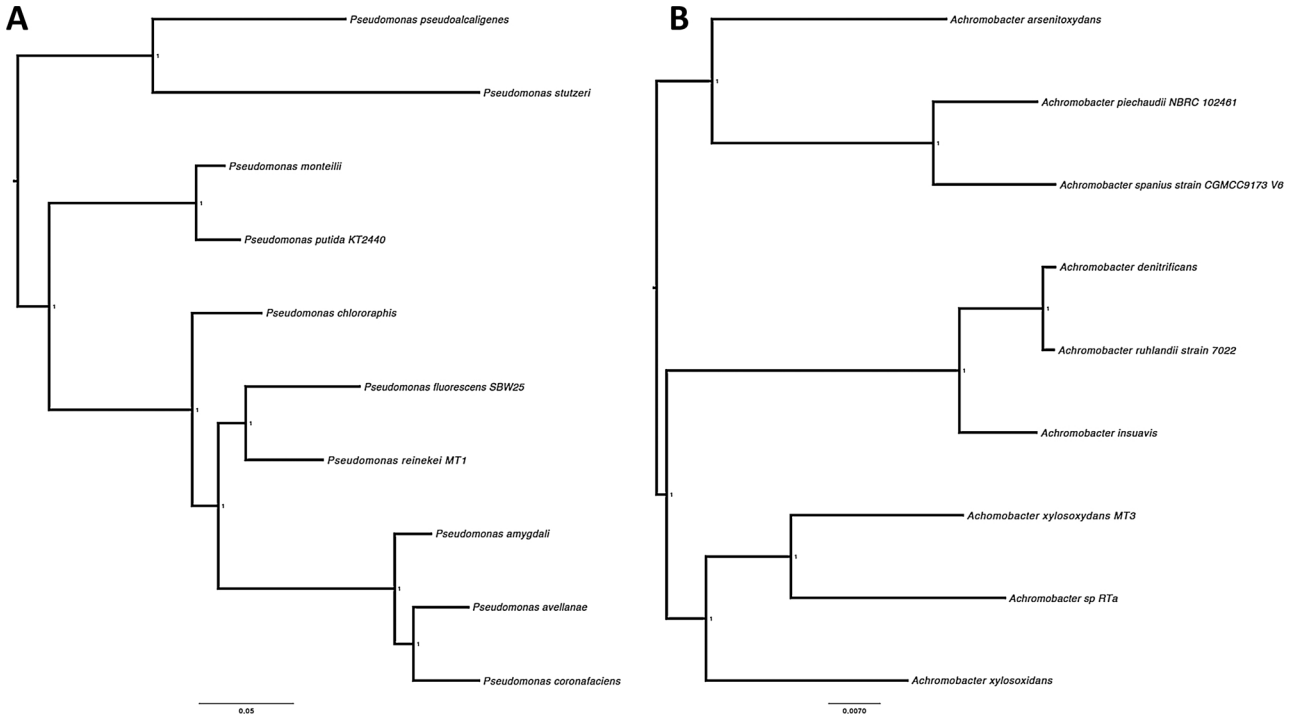

Fig. 2. Phylogenetic tree of different A) *Pseudomonas* species, and B) *Achromobacter* species.

cis,cis muconate, where the latter can be converted by muconate cycloisomerase to *cis*-Dienelactone, an enzyme that was not clear to be present in this bacterium. These findings could explain the role that *A. xylosoxidans* MT3 plays in detoxification when co-cultivated with *P. reinekei* MT1 on chlorosalicylates. The toxic intermediates 4-Chlorocatechol and 3-Chloro-*cis,cis* muconate produced by MT1 can be taken up by MT3, thus reducing the concentration levels and precursors for the synthesis of the dead-end compound protoanemonin in MT1, which is the main responsible chemical to challenge the fitness of the bacterial consortium. Dynamics population studies of the MT community growing on 4-chlorosalicylates showed that the presence of MT3 diminished protoanemonin level by 4-fold in the culture medium, increasing cell counts and viability for each member of the bacterial consortium (Fazzini et al., 2010). Nevertheless, there is still an open question concerning protoanemonin fate within the MT community, and whether MT3 has the ability to catabolize this highly toxic metabolite and quorum sensing inhibitor produced by MT1 (Blasco et al., 1995)(Bobadilla Fazzini et al., 2013)(Kalia, 2013). The muconate cycloisomerase found in *A. xylosoxidans* MT3 shares only 60.5% of identity compared to the one displayed by *P. reinekei* MT1. It is well reported that bacteria able to convert 3-Chloromuconate can either produce: protoanemonin, maleylacetate and/or cis-Dienelactone. The last two compounds, after several enzymatic steps, replenish the TCA cycle to sustain bacterial growth (Brückmann et al., 1998)(Nikodem et al., 2003). MT1 yields cis-Dielactone by direct dehalogenation of 3-Chloromuconate and Protoanemonin as a dead-end product from 4-chloromuconolactone, which is proposed to be catalyzed it by a *trans*-dienelactone hydrolase (*trans*-DLH) enzyme. In addition, the presence of both enzymes, muconate cycloisomerase and *trans*-DLH enzyme, has been shown to circumvent the formation of protoanemonin from 3-Chloromuconate, favoring the synthesis of maleylacetate in MT1 (Nikodem et al., 2003). We did not find open reading frames encoding enzymes belonging to the previously described enzyme-complex system when analyzing the genome of the MT3 strain, or enzymes to further

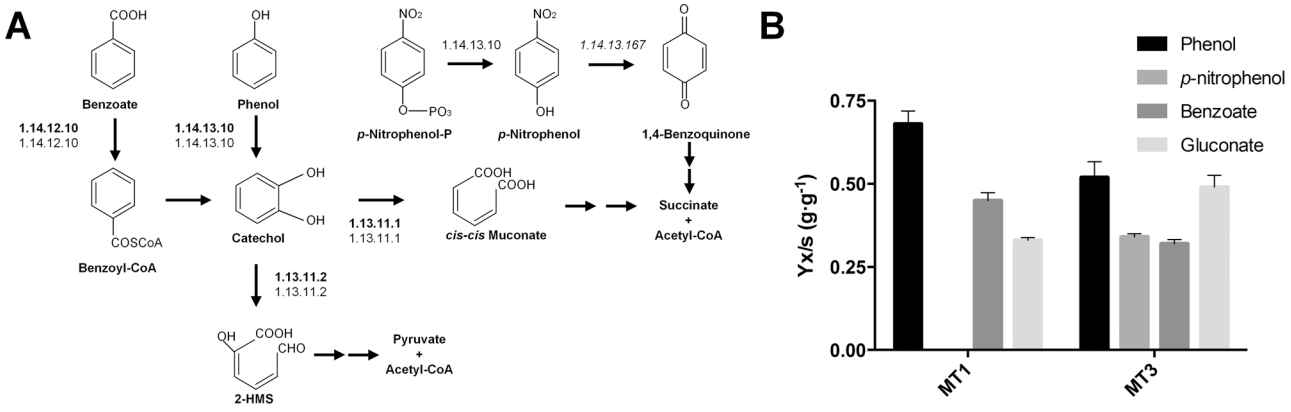

Fig. 3. A) Proposed metabolic degradation pathway of benzoate, phenol, and *p*-nitrophenol for *P. reinekei* MT1 (bold E.C. numbers) and *A. xylosoxidans* MT3 (lower E.C. numbers). B) Biomass yield on phenol, *p*-nitrophenol, benzoate, and gluconate by MT1 and MT3 strain at 1 mM, 1 mM, 5 mM, and 10 mM, concentrations, respectively. The strains were grown on (starting OD = 0.05) minimal medium (M9) consisting of 12.8 $g{\cdot}L^{-1}$ $Na_2HPO_4{\cdot}7H_2O$, 3 $g{\cdot}L^{-1}$ KH_2PO4, 0.5 $g{\cdot}L^{-1}$ NH_4Cl, 0.5 $g{\cdot}L^{-1}$ NaCl, supplemented with 0.12 $g{\cdot}L^{-1}$ of $MgSO_4{\cdot}7H_2O$, trace elements (6.0 $FeSO_4{\cdot}7H_2O$, 2.7 $CaCO_3$, 2.0 $ZnSO_4{\cdot}H2O$, 1.16 $MnSO_4{\cdot}H_2O$, 0.37 $CoSO_4{\cdot}7H_2O$, 0.33 $CuSO_4{\cdot}5H_2O$, 0.08 H_3BO_3) ($mg{\cdot}L^{-1}$) (filter-sterilized).

convert protoanemonin by MT1 strain. This is in agreement with *in vivo* experiments, where in steady-state as well as in dynamic pure and microbial community cultures, the produced protoanemonin by MT1 strain is not reincorporated to cells and further catabolized, remaining in the fermentation broth (Pelz et al., 1999)(Bobadilla Fazzini et al., 2009)(Fazzini et al., 2010).

Nucleotide sequence accession numbers. The genome assemblies described in this article have been deposited in GenBank under accession numbers MSTQ00000000 and MSTP00000000 for *P. reinekei* MT1 and *A. xylosoxidans* MT3, respectively.

Acknowledgements

This work was supported by grant from CONICYT Fondecyt de iniciación (No. 11150174). We would like to thank the George Washington University's high-performance computing facility, Colonial One, for providing data storage, support, and computing power for analyses (colonialone.gwu.edu/). ECN was funded by FONDECYT de iniciación11160905. Claudio Meneses thanks the support through FONDAP CRG15070009.

Appendix A. Supplementary data

Supplementary material related to this article can be found, in the online version, at doi:https://doi.org/10.1016/j.jbiotec.2018.03.019.

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