



Genome Announcements

Complete genome sequence of a phthalic acid esters degrading *Mycobacterium* sp. YC-RL4

Lei Ren¹, Shuanghu Fan¹, Junhuan Wang, Nahurira Ruth, Cheng Qiao, Yang Jia, Yanchun Yan*^{*}

Chinese Academy of Agricultural Sciences, Graduate School, No.12 Zhongguancun South Street, Beijing, China

ARTICLE INFO

Article history:

Received 29 May 2016

Accepted 14 September 2016

Available online 3 June 2017

Associate Editor: John McCulloch

Keywords:

Genome Sequence

Mycobacterium sp.

Biodegradation

Phthalic acid esters

ABSTRACT

Mycobacterium sp. YC-RL4 is capable of utilizing a broad range of phthalic acid esters (PAEs) as sole source of carbon and energy for growth. The preliminary studies demonstrated its high degrading efficiency and good performance during the bioprocess with environmental samples. Here, we present the complete genome of *Mycobacterium* sp. YC-RL4, which consists of one circular chromosome (5,801,417 bp) and one plasmid (252,568 bp). The genomic analysis and gene annotation were performed and many potential genes responsible for the biodegradation of PAEs were identified from the genome. These results may advance the investigation of bioremediation of PAEs-contaminated environments by strain YC-RL4.

© 2017 Sociedade Brasileira de Microbiologia. Published by Elsevier Editora Ltda. This is an open access article under the CC BY-NC-ND license (<http://creativecommons.org/licenses/by-nc-nd/4.0/>).

Plasticizers have been used as additives in plastic or polymeric materials to improve their flexibility, transparency, durability, and longevity. PAEs are among the most widely used plasticizers. These PAEs do not physically bind to the polymer matrix of products and can easily migrate to the environment. The detection of PAEs in soil, water, and air has been widely reported. The toxicity evaluation of PAEs indicated their developmental and reproductive toxicity to human and animals.^{1,2} PAEs are listed as top-priority environmental pollutants by the United States Environment Protection Agency (US EPA) and the European Union.³ Great efforts have been made to eliminate PAEs from the environment and biodegradation is among the most widely investigated methods.

Various PAEs-degrading bacteria have isolated and characterized, including genera *Gordonia*, *Pseudomonas*, *Burkholderia*, and *Bacillus*.^{4–8} Although PAEs-degrading isolates and degrading pathway were widely reported, the knowledge of degrading related molecular mechanism is limited. Previously, we isolated *Mycobacterium* sp. YC-RL4 from petroleum contaminated soil, which was capable of utilizing several kinds of PAEs as sole source of carbon and energy for growth.⁹ Here, we report the complete genome sequence of *Mycobacterium* sp. YC-RL4 and we hope the genomic information would advance our understanding of PAEs degrading mechanism, which may provide new gene resources for biotechnology and gene engineering.

* Corresponding author. Tel.: +86 10 82109685; fax: +86 10 82106609.

E-mail: yanyanchun@caas.cn (Y. Yan).

¹ These authors equally contributed to this work and should be considered as co-first author.

<http://dx.doi.org/10.1016/j.bjm.2016.09.022>

1517-8382/© 2017 Sociedade Brasileira de Microbiologia. Published by Elsevier Editora Ltda. This is an open access article under the CC BY-NC-ND license (<http://creativecommons.org/licenses/by-nc-nd/4.0/>).

Table 1 – General genome features of *Mycobacterium* sp. YC-RL4.

Features	Chromosome	pMYC1
Length (bp)	5,801,417	252,568
G + C content (%)	67.48	65.7
Total number of genes	5532	249
Protein coding genes	5385	249
tRNAs	47	0
rRNA genes	6	0

The genomic DNA of strain YC-RL4 was extracted using the Bacterial Genomic DNA Extraction kit (Takara, Japan), and the quality and quantity were evaluated by Bioanalyzer 2100 (Agilent) before sequencing. The genome of strain YC-RL4 was sequenced using Single Molecular, Real-Time (SMRT) technology with the PacBio RS II platform. After quality control, a total length of 1,550,468,925 bp data was obtained with 256-fold average coverage. All reads were de novo assembled using MHAP (v8.0).^{10,11} The assembled genome was annotated by NCBI Prokaryotic Genome Annotation Pipeline.¹² Protein coding sequences, tRNA and rRNA were identified.

The generated genome sequences revealed strain YC-RL4 comprises 6,053,985 bp, which were finally assembled into one circular chromosome (5,801,417 bp) with an average G + C content of 67.48% and one plasmid (pMYC01, 252,568 bp) with a average G + C content of 65.7%. In total, 5781 genes were predicted, including 5634 protein coding sequences, 47 tRNAs, and 6 rRNA genes. All the genomic information and annotated results were presented in Table 1.

Microorganisms can evolve different strategies to fit the environment. We analyzed the potential biodegradation related genes. Several genes and gene clusters located in the genome and plasmid that may contribute to the degradation of PAEs were identified. The biodegradation of PAEs was always initiated by hydrolyzing of two ester bonds (Ren et al., 2016). The generated phthalic acid (PA) was further utilized by ring cleavage. One esterase gene in the genome sequence responsible for the hydrolyzation of monoalkyl phthalates (MAPs) to PA (MAPs was the intermediate of PAEs catabolism) was identified.^{13,14} In addition, complete benzoate metabolism pathway was identified and located in genome sequence, which may be involved in the metabolism of PA. Some aromatic compounds catabolism related genes were also annotated in the genome. Meanwhile, annotation of pathway was performed by assigning predicted genes to Kyoto Encyclopedia of Genes and Genomes (KEGG) database¹⁵ and 2206 CDSSs were involved in 117pathways. The genome information of strain YC-RL4 would be useful for elucidating the molecular mechanism of PAEs metabolism and provides new approach for the bioremediation of PAEs contaminated environments.

Strain and nucleotide sequence accession numbers

This strain has been deposited in China General Microbiological Culture Collection Center (CGMCC) with deposit number as

CGMCC No. 10993. The chromosome and plasmid sequences of *Mycobacterium* sp. YC-RL4 were deposited in GenBank under the accession numbers CP015596 and CP015597, respectively.

Conflicts of interest

The authors declare no conflicts of interest.

Acknowledgements

This work was supported by the National Natural Science Foundation of China (31540067, 31170119), and Basic Research Fund of CAAS (0042014006, 0042012003 and 0042011006).

REFERENCES

- Blom A, Ekman E, Johannsson A, Norrgren L, Pesonen M. Effects of xenoestrogenic environmental pollutants on the proliferation of a human breast cancer cell line (MCF-7). *Arch Environ Contam Toxicol.* 1998;34:306–310.
- Jobling S, Reynolds T, White R, Parker MG, Sumpter JP. A variety of environmentally persistent chemicals, including some phthalate plasticizers, are weakly estrogenic. *Environ Health Perspect.* 1995;103:582–587.
- Wang JL, Liu P, Qian Y. Microbial-degradation of di-n-butyl phthalate. *Chemosphere.* 1995;31:4051–4056.
- Chang HK, Zylstra GJ. Novel organization of the genes for phthalate degradation from *Burkholderia cepacia* DBO1. *J Bacteriol.* 1998;180:6529–6537.
- Jin D. Biodegradation of di-n-butyl phthalate by *Rhodococcus* sp. JDC-11 and molecular detection of 3,4-phthalate dioxygenase gene. *J Microbiol Biotechnol.* 2010;20: 1440–1445.
- Jin D, Bai Z, Chang D, et al. Biodegradation of di-n-butyl phthalate by an isolated *Gordonia* sp. strain QH-11: genetic identification and degradation kinetics. *J Hazard Mater.* 2012;221–222:80–85.
- Xu X, Li H, Gu J. Biodegradation of an endocrine-disrupting chemical di-n-butyl phthalate ester by *Pseudomonas fluorescens* B-1. *Int Biodeterior Biodegradation.* 2005;55: 9–15.
- Quan CS, Liu Q, Tian WJ, Kikuchi J, Fan SD. Biodegradation of an endocrine-disrupting chemical, di-2-ethylhexyl phthalate, by *Bacillus subtilis* No 66. *Appl Microbiol Biotechnol.* 2005;66:702–710.
- Ren L, Jia Y, Ruth N, et al. Biodegradation of phthalic acid esters by a newly isolated *Mycobacterium* sp. YC-RL4 and the bioprocess with environmental samples. *Environ Sci Pollut Res.* 2016, <http://dx.doi.org/10.1007/s11356-016-6829-4>.
- Berlin K, Koren S, Chin C, Drake JP, Landolin JM, Phillippy AM. Assembling large genomes with single-molecule sequencing and locality-sensitive hashing. *Nat Biotechnol.* 2015;33:623–630.
- Chin C, Alexander DH, Marks P, et al. Nonhybrid, finished microbial genome assemblies from long-read SMRT sequencing data. *Nat Methods.* 2013;10: 563–569.
- Pruitt KD, Tatusova T, Brown GR, Maglott DR. NCBI Reference Sequences (RefSeq): current status, new features and genome annotation policy. *Nucleic Acids Res.* 2012;40:D130–D135.

13. Hara H, Stewart GR, Mohn WW. Involvement of a novel ABC transporter and monoalkyl phthalate ester hydrolase in phthalate ester catabolism by *Rhodococcus jostii* RHA1. *Appl Environ Microbiol.* 2010;76:1516–1523.
14. Nishioka T, Iwata M, Imaoka T, et al. A mono-2-ethylhexyl phthalate hydrolase from a *Gordonia* sp. that is able to dissimilate di-2-ethylhexyl phthalate. *Appl Environ Microbiol.* 2006;72:2394–2399.
15. Kanehisa M, Araki M, Goto S, et al. KEGG for linking genomes to life and the environment. *Nucleic Acids Res.* 2008;36:D480–D484 [database issue].