

Complete Genome Sequence of the Marine Carbazole-Degrading Bacterium *Erythrobacter* sp. Strain KY5

著者	Felipe Vejarano, Chiho Suzuki-Minakuchi, Yoshiyuki Ohtsubo, Masataka Tsuda, Kazunori Okada, Hideaki Nojiria
journal or publication title	Microbiology Resource Announcements
volume	7
number	8
page range	e00935-18
year	2018-08-30
URL	http://hdl.handle.net/10097/00125641

doi: 10.1128/MRA.00935-18



Complete Genome Sequence of the Marine Carbazole-Degrading Bacterium *Erythrobacter* sp. Strain KY5

Felipe Vejarano,^a Chiho Suzuki-Minakuchi,^{a,b} Yoshiyuki Ohtsubo,^c Masataka Tsuda,^c Kazunori Okada,^a  Hideaki Nojiri^{a,b}

^aBiotechnology Research Center, The University of Tokyo, Tokyo, Japan

^bCollaborative Research Institute for Innovative Microbiology, The University of Tokyo, Tokyo, Japan

^cGraduate School of Life Sciences, Tohoku University, Sendai, Japan

ABSTRACT We determined the complete genome sequence of *Erythrobacter* sp. strain KY5, a bacterium isolated from Tokyo Bay and capable of degrading carbazole. The genome consists of a 3.3-Mb circular chromosome that carries the gene clusters involved in carbazole degradation and biosynthesis of the photosynthetic apparatus of aerobic anoxygenic phototrophic bacteria.

In recent years, bacterial strains capable of degrading carbazole, a carcinogenic and mutagenic nitrogen-containing aromatic contaminant in fossil fuels (1–3), have been isolated from marine environments (4–8). While the nucleotide sequences of the carbazole-degradative *car* gene clusters have been determined, no complete genome sequences of these marine isolates have been obtained to date. Several *Erythrobacter* strains belong to marine aerobic anoxygenic phototrophic (AAP) bacteria, a ubiquitous group of proteobacteria that predominantly inhabit the euphotic zone in the oceans and are thought to contribute greatly to the carbon cycle in those habitats (9, 10). Recent reports of sequenced *Erythrobacter* sp. genomes have mainly provided information regarding their genome structure and the characteristics of their photosynthetic gene cluster (PGC) (11–16), while information about the presence or organization of xenobiotic catabolic genes is still scarce (12, 16), even though the capability to degrade such compounds has been reported in a few strains (5, 17). Here, we report the complete genome sequence of *Erythrobacter* sp. strain KY5, a Gram-negative AAP bacterium isolated from Tokyo Bay and capable of degrading carbazole.

Erythrobacter sp. KY5 was isolated from a mixed culture obtained from Tokyo Bay water samples, enriched in the presence of carbazole (0.5% [wt/vol]) in vitamin-supplemented (18), filter-sterilized seawater medium containing KH₂PO₄, yeast extract (10 mg/liter), and Fe-EDTA (3 mg/liter) (carbazole-containing seawater [CAR-SEA] medium). After carbazole depletion was observed, strain KY5 was isolated by spreading 100- μ l aliquots of the culture onto 8.5-cm plates containing the same enrichment medium solidified with 16 g/liter bacteriological agar. Degradation of the compound was evidenced by the formation of clear zones around the colonies and later confirmed upon culturing the strain in CAR-SEA medium.

Cultivation of strain KY5 in CAR-SEA medium at 30°C for 4 days was followed by DNA extraction using the Wizard genomic DNA purification kit (Promega) per the manufacturer's instructions. Whole-genome sequencing was performed using Illumina PCR-free and mate pair libraries in a MiSeq sequencer. Genome assembly was done using Newbler (454 Life Sciences) and finished using AceFileViewer (www.ige.tohoku.ac.jp/joho/gf/AceFileViewer.php) and GenoFinisher (http://www.ige.tohoku.ac.jp/joho/gf_e/GenoFinisher.php) for gap closing. Gene prediction and functional annotation were first done using the NCBI Prokaryotic Genome Annotation Pipeline (19) and the Microbial Genome Annotation Pipeline (20). Annotation results of the two pipelines were com-

Received 2 July 2018 Accepted 30 July 2018 Published 30 August 2018

Citation Vejarano F, Suzuki-Minakuchi C, Ohtsubo Y, Tsuda M, Okada K, Nojiri H. 2018. Complete genome sequence of the marine carbazole-degrading bacterium *Erythrobacter* sp. strain KY5. Microbiol Resour Announc 7:e00935-18. <https://doi.org/10.1128/MRA.00935-18>.

Editor Julia Maresca, University of Delaware

Copyright © 2018 Vejarano et al. This is an open-access article distributed under the terms of the Creative Commons Attribution 4.0 International license.

Address correspondence to Hideaki Nojiri, anojiri@mail.ecc.u-tokyo.ac.jp.

pared and checked for inconsistencies using GenomeMatcher (21), producing a curated annotation file that combined the results of both annotation pipelines.

The genome of strain KY5 consists of one 3,311,272-bp circular chromosome with an average G+C content of 60.77%. Functional annotation revealed a total of 3,080 coding sequences, 43 tRNA genes, and one copy of the rRNA genes. A 10.5-kbp *car* gene cluster was found with the configuration *carDAaCBaBbFE*, followed by a gene encoding a putative reductase of the initial carbazole degradation enzyme, carbazole 1,9a-dioxygenase, whose terminal oxygenase component is encoded by the *carAa* gene (22). Notably, 2.5 kbp upstream of the *car* gene cluster is an anthranilate-coenzyme A ligase ortholog, suggesting that this strain degrades carbazole through a coenzyme A intermediate and not catechol, which is consistent with the absence of anthranilate or catechol oxidoreductase genes. Strain KY5 also carries the conserved 39-kbp-long *Sphingomonadales* clade type III PGC (23) with genes for the biosynthesis of the photosynthetic apparatus components.

Data availability. The genome sequence of *Erythrobacter* sp. strain KY5 has been deposited in DDBJ/ENA/GenBank under the accession number CP021912.

REFERENCES

- Benedik MJ, Gibbs PR, Riddle RR, Willson RC. 1998. Microbial denitrogenation of fossil fuels. *Trends Biotechnol* 16:390–395. [https://doi.org/10.1016/S0167-7799\(98\)01237-2](https://doi.org/10.1016/S0167-7799(98)01237-2).
- Tsuda H, Hagiwara A, Shibata M, Ohshima M, Ito N. 1982. Carcinogenic effect of carbazole in the liver of (C57BL/6N x C3H/HeN)F1 mice. *J Natl Cancer Inst* 69:1383–1389.
- Jha AM, Singh AC, Bharti MK. 2002. Clastogenicity of carbazole in mouse bone marrow cells in vivo. *Mutat Res* 521:11–17. [https://doi.org/10.1016/S1383-5718\(02\)00210-3](https://doi.org/10.1016/S1383-5718(02)00210-3).
- Inoue K, Widada J, Nakai S, Endoh T, Urata M, Ashikawa Y, Shintani M, Saiki Y, Yoshida T, Habe H, Omori T, Nojiri H. 2004. Divergent structures of carbazole degradative *car* operons isolated from Gram-negative bacteria. *Biosci Biotechnol Biochem* 68:1467–1480. <https://doi.org/10.1271/bbb.68.1467>.
- Maeda R, Nagashima H, Widada J, Iwata K, Omori T. 2009. Novel marine carbazole-degrading bacteria. *FEMS Microbiol Lett* 292:203–209. <https://doi.org/10.1111/j.1574-6968.2009.01497.x>.
- Maeda R, Nagashima H, Zulkharnain AB, Iwata K, Omori T. 2009. Isolation and characterization of a *car* gene cluster from the naphthalene, phenanthrene, and carbazole-degrading marine isolate *Lysobacter* sp. strain OC7. *Curr Microbiol* 59:154–159. <https://doi.org/10.1007/s00284-009-9414-y>.
- Maeda R, Ishii T, Ito Y, Zulkharnain AB, Iwata K, Omori T. 2010. Isolation and characterization of the gene encoding the chloroplast-type ferredoxin component of carbazole 1,9a-dioxygenase from a putative *Kordiimonas* sp. *Biotechnol Lett* 32:1725–1731. <https://doi.org/10.1007/s10529-010-0358-x>.
- Ito Y, Maeda R, Iwata K, Omori T. 2011. Genetic characterisation of genes involved in the upper pathway of carbazole metabolism from the putative *Kordiimonas* sp. *Biotechnol Lett* 33:1859–1864. <https://doi.org/10.1007/s10529-011-0639-z>.
- Kolber ZS, Van Dover CL, Niederman RA, Falkowski PG. 2000. Bacterial photosynthesis in surface waters of the open ocean. *Nature* 407:177–179. <https://doi.org/10.1038/35025044>.
- Kolber ZS, Plumley FG, Lang AS, Beatty JT, Blankenship RE, VanDover CL, Vetricani C, Koblizek M, Rathgeber C, Falkowski PG. 2001. Contribution of aerobic photoheterotrophic bacteria to the carbon cycle in the ocean. *Science* 292:2492–2495. <https://doi.org/10.1126/science.1059707>.
- Wang Y, Zhang R, Zheng Q, Jiao N. 2014. Draft genome sequences of two marine phototrophic bacteria, *Erythrobacter longus* strain DSM 6997 and *Erythrobacter litoralis* strain DSM 8509. *Genome Announc* 2:e00677-14. <https://doi.org/10.1128/genomeA.00677-14>.
- Oh H-M, Giovannoni SJ, Ferreira S, Johnson J, Cho J-C. 2009. Complete genome sequence of *Erythrobacter litoralis* HTCC2594. *J Bacteriol* 191:2419–2420. <https://doi.org/10.1128/JB.00026-09>.
- Koblížek M, Janoušková J, Oborník M, Johnson JH, Ferreira S, Falkowski PG. 2011. Genome sequence of the marine photoheterotrophic bacterium *Erythrobacter* sp. strain NAP1. *J Bacteriol* 193:5881–5882. <https://doi.org/10.1128/JB.05845-11>.
- Zheng Q, Liu Y, Sun J, Jiao N. 2015. Genome sequence of aerobic anoxygenic phototrophic bacterium *Erythrobacter* sp. JL475, isolated from the South China Sea. *Mar Genomics* 21:15–16. <https://doi.org/10.1016/j.margen.2015.02.006>.
- Xu L, Wu Y-H, Zhou Y-G, Cheng H, Liu Q, Zhou P, Wu M, Cheng L, Xu X-W. 2017. Complete genome sequence of *Erythrobacter gangjinensis* CGMCC 1.15024T with two chromosomes. *Mar Genomics* 34:15–18. <https://doi.org/10.1016/j.margen.2017.02.004>.
- Yaakop AS, Chan CS, Kahar UM, Ee R, Chan K-G, Goh KM. 2015. Draft genome sequence of *Erythrobacter vulgaris* strain O1, a glycosyl hydrolase-producing bacterium. *Genome Announc* 3:1–2. <https://doi.org/10.1128/genomeA.00457-15>.
- Woo J-H, Hwang Y-O, Kang SG, Lee HS, Cho J-C, Kim S-J. 2007. Cloning and characterization of three epoxide hydrolases from a marine bacterium, *Erythrobacter litoralis* HTCC2594. *Appl Microbiol Biotechnol* 76:365–375. <https://doi.org/10.1007/s00253-007-1011-z>.
- Staley JT. 1968. *Prosthecomicrobium* and *Ancalomicrobium*: new prosthecate freshwater bacteria. *J Bacteriol* 95:1921–1942.
- Tatusova T, Dicuccio M, Badretdin A, Chetverin 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>.
- Sugawara H, Ohyama A, Mori H, Kurokawa K. 2009. Microbial Genome Annotation Pipeline (MiGAP) for diverse users, abstr S-001, p 1–2. *Abstr 20th Int Conf Genome Informatics, Kanagawa, Japan*.
- Ohtsubo Y, Ikeda-Ohtsubo W, Nagata Y, Tsuda M. 2008. GenomeMatcher: a graphical user interface for DNA sequence comparison. *BMC Bioinformatics* 9:376. <https://doi.org/10.1186/1471-2105-9-376>.
- Nojiri H. 2012. Structural and molecular genetic analyses of the bacterial carbazole degradation system. *Biosci Biotechnol Biochem* 76:1–18. <https://doi.org/10.1271/bbb.110620>.
- Zheng Q, Zhang R, Koblížek M, Boldareva EN, Yurkov V, Yan S, Jiao N. 2011. Diverse arrangement of photosynthetic gene clusters in aerobic anoxygenic phototrophic bacteria. *PLoS One* 6:e25050–e25057. <https://doi.org/10.1371/journal.pone.0025050>.