

# Complete Genome Sequence of the Carbazole Degradar *Pseudomonas resinovorans* Strain CA10 (NBRC 106553)

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***Pseudomonas resinovorans* strain CA10 can grow on carbazole as its sole carbon and nitrogen source. Here, we report the complete nucleotide sequence of the CA10 genome (a 6,285,863-bp chromosome and a 198,965-bp plasmid). CA10 carries a larger number of genes that are potentially responsible for aromatic hydrocarbon metabolism than do other previously sequenced *Pseudomonas* spp.**

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*Pseudomonas resinovorans* strain CA10 (NBRC 106553) was isolated from activated sludge and can utilize carbazole, a nitrogen heteroaromatic compound, as its sole carbon, nitrogen, and energy source (1, 2). Strain CA10 possesses about 200 kb of the incompatibility group (Inc) P-7 plasmid pCAR1 (3, 4), whose full sequence was determined previously (5, 6). Carbazole is converted to catechol via anthranilate by plasmid-encoded Car and Ant enzymes (1), and the catechol is further degraded by the chromosomally encoded enzymes (1, 3, 7).

The genome of CA10 was determined using a combined strategy of GS FLX Titanium and HiSeq 1000 technologies. Two different types of libraries were constructed for sequencing: a standard library (600 to 900 bp) for GS FLX Titanium and a paired-end library (200 to 500 bp) for HiSeq 1000. A total of 117,465,953 base sequences (276,857 reads) were obtained with a depth of 18-fold genome coverage by the GS FLX Titanium system, and 487,867,241 base paired-end sequences (5,396,359 reads) were obtained with a depth of 75-fold genome coverage by the HiSeq 1000 system. The assembly was performed using Phred (8, 9) and Newbler v2.6 software (10). Ambiguities in the sequences were manually inspected and corrected using Illumina sequence reads. Closing of gaps was accomplished by (i) local assembly with the aid of Sanger reads of end sequences from fosmid libraries with inserts of 30 kb in pCC1FOS (Epicentre Biotechnologies, Madison, WI), (ii) filling with additional reads by primer walking, (iii) sequencing of fosmid clones with random insertions generated by the Template Generation System II kit (Finnzymes, Vantaa, Finland), and (iv) sequencing of PCR products using an ABI 3730 sequencer.

The genome of CA10 consists of a circular chromosome (6,285,863 bp; 65.6% G+C content) and a circular plasmid named pCAR1.3 (198,965 bp; 56.4% G+C content). pCAR1.3 is shorter than the previously reported pCAR1 and pCAR1.2 plasmids (GenBank/EMBL/DDBJ accession no. AB088420 and

AB474758, respectively), because one of the 1,261-bp DNA regions containing *carAa*, which was duplicated on pCAR1 and pCAR1.2 (i.e., these plasmids carry two copies of *carAa*), was deleted on pCAR1.3. Complete sequences of the CA10 genome were analyzed using MIGAP (<http://www.migap.org/>) for predicting protein-coding, tRNA, and rRNA genes. Their functional annotations were assigned using UniProt, InterPro, Hamap, and an in-house database composed of manually curated microbial genome sequences (S. Miyazawa, S. Isaki, N. Ichikawa, and N. Fujita, unpublished data). The CA10 chromosome has 5,652 coding sequences (CDSs), five rRNA operons, and 70 tRNA genes. The anthranilate-degradating genes *antABC* and *antR* were found not only on pCAR1.3 but also on the chromosome, as was predicted previously (11). Comparisons between CA10 and the other 14 previously sequenced *Pseudomonas* strains using the RAST server (<http://rast.nmpdr.org/>) (12) showed that CA10 carries a larger number of CDSs for metabolism of aromatic compounds (241 CDSs) than do the other strains (49 to 187 CDSs). This suggests that the CA10 may be suitable for the metabolism of various aromatic compounds as the original host of pCAR1.

**Nucleotide sequence accession numbers.** The nucleotide sequences of the CA10 chromosome and pCAR1.3 were deposited in the DDBJ/EMBL/GenBank databases under the accession no. [AP013068](https://www.ncbi.nlm.nih.gov/nuccore/AP013068) and [AP013069](https://www.ncbi.nlm.nih.gov/nuccore/AP013069), respectively.

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## REFERENCES

1. Nojiri H. 2012. Structural and molecular genetic analyses of the bacterial carbazole degradation system. *Biosci. Biotechnol. Biochem.* 76:1–18.

2. Ouchiyama N, Zhang Y, Omori T, Kodama T. 1993. Biodegradation of carbazole by *Pseudomonas* spp. CA06 and CA10. *Biosci. Biotechnol. Biochem.* 57:455–460.
3. Nojiri H, Sekiguchi H, Maeda K, Urata M, Nakai S, Yoshida T, Habe H, Omori T. 2001. Genetic characterization and evolutionary implications of a *car* gene cluster in the carbazole degrader *Pseudomonas* sp. strain CA10. *J. Bacteriol.* 183:3663–3679.
4. Shintani M, Yano H, Habe H, Omori T, Yamane H, Tsuda M, Nojiri H. 2006. Characterization of the replication, maintenance, and transfer features of the IncP-7 plasmid pCAR1, which carries genes involved in carbazole and dioxin degradation. *Appl. Environ. Microbiol.* 72:3206–3216.
5. Maeda K, Nojiri H, Shintani M, Yoshida T, Habe H, Omori T. 2003. Complete nucleotide sequence of carbazole/dioxin-degrading plasmid pCAR1 in *Pseudomonas resinovorans* strain CA10 indicates its mosaicism and the presence of large catabolic transposon Tn4676. *J. Mol. Biol.* 326: 21–33.
6. Takahashi Y, Shintani M, Yamane H, Nojiri H. 2009. The complete nucleotide sequence of pCAR2: pCAR2 and pCAR1 were structurally identical IncP-7 carbazole degradative plasmids. *Biosci. Biotechnol. Biochem.* 73:744–746.
7. Nojiri H, Maeda K, Sekiguchi H, Urata M, Shintani M, Yoshida T, Habe H, Omori T. 2002. Organization and transcriptional characterization of catechol degradation genes involved in carbazole degradation by *Pseudomonas resinovorans* strain CA10. *Biosci. Biotechnol. Biochem.* 66: 897–901.
8. Ewing B, Green P. 1998. Base-calling of automated sequencer traces using Phred. II. Error Probabilities. *Genome Res.* 8:186–194.
9. Ewing B, Hillier L, Wendl MC, Green P. 1998. Base-calling of automated sequencer traces using Phred. I. Accuracy assessment. *Genome Res.* 8:175–185.
10. Wijaya E, Frith MC, Suzuki Y, Horton P. 2009. Recount: expectation maximization based error correction tool for next generation sequencing data. *Genome Inform.* 23:189–201.
11. Urata M, Miyakoshi M, Kai S, Maeda K, Habe H, Omori T, Yamane H, Nojiri H. 2004. Transcriptional regulation of the *ant* operon, encoding two-component anthranilate 1,2-dioxygenase, on the carbazole-degradative plasmid pCAR1 of *Pseudomonas resinovorans* strain CA10. *J. Bacteriol.* 186:6815–6823.
12. Aziz RK, Bartels D, Best AA, DeJongh M, Disz T, Edwards RA, Formsma K, Gerdes S, Glass EM, Kubal M, Meyer F, Olsen GJ, Olson R, Osterman AL, Overbeek RA, McNeil LK, Paarmann D, Paczian T, Parrello B, Pusch GD, Reich C, Stevens R, Vassieva O, Vonstein V, Wilke A, Zagnitko O. 2008. The RAST server: rapid annotations using subsystems technology. *BMC Genomics* 9:75. doi:10.1186/1471-2164-9-75.