

# Genome Sequence of *Citrobacter* sp. Strain A1, a Dye-Degrading Bacterium

Gieik Far Chan,<sup>a,b</sup> Han Ming Gan,<sup>c</sup> and Noor Aini Abdul Rashid<sup>a,b</sup>

Nanoporous Materials for Biological Application Research Group (NAMBAR), Sustainability Research Alliance, Universiti Teknologi Malaysia, Johor, Malaysia<sup>a</sup>; Department of Biological Sciences, Faculty of Biosciences and Bioengineering, Universiti Teknologi Malaysia, Johor, Malaysia<sup>b</sup>; and ScienceVision SB, Setia Alam, Seksyen U13, Shah Alam, Selangor, Malaysia<sup>c</sup>

***Citrobacter* sp. strain A1, isolated from a sewage oxidation pond, is a facultative aerobe and mesophilic dye-degrading bacterium. This organism degrades azo dyes efficiently via azo reduction and desulfonation, followed by the successive biotransformation of dye intermediates under an aerobic environment. Here we report the draft genome sequence of *Citrobacter* sp. A1.**

*Citrobacter* sp. is a ubiquitous Gram-negative enteric coccobacillus from the family of *Enterobacteriaceae*. Despite the pathogenicity of *Citrobacter* sp., biotechnological significance of the bacterium was reported mainly in dye decolorization, heavy metal precipitation, and biohydrogen production (1, 6, 7, 10). A local strain of *Citrobacter* sp., designated strain A1, was isolated from a sewage oxidation pond in the vicinity of Universiti Teknologi Malaysia in 1997 (N. A. A. Rashid, A. R. H. M. Yusoff, R. Ahmad, S. Misran, G. F. Chan, and P. Murugaiya, presented at the Symposium Kimia Analisis, Kuala Terengganu, Terengganu, Malaysia, 22 July 1999). Based on a carbon source utilization test (Biolog) and 16S rRNA gene sequences, strain A1 was closely related to *Citrobacter freundii* (2).

Strain A1 shows great potential in the decolorization of a broad range of azo dyes under microaerophilic conditions at 45°C. Strain A1 was found to possess a flavin reductase which is involved in the reduction of azo bond in various dyes (2, 11). Furthermore, the potential of strain A1 to operate as a bacterial consortium with other bacteria was explored. The syntropic interaction between strain A1, *Enterococcus casseliflavus* C1, and *Enterobacter cloacae* L17 enhanced the biodegradation of azo dye (3). Under microaerophilic conditions, the decolorization of amaranth by the consortium led to the production of hydrazo intermediate, followed by symmetric reductive cleavage to form aromatic amines, namely, 1-aminonaphthalene-4-sulfonic acid and 1-aminonaphthalene-2-hydroxy-3,6-disulfonic acid. The successive biotransformation of these dye intermediates via reductive deamination and desulfonation may be carried out by strain A1 (3). Further aerobic incubation led to the further catabolism of the dye intermediates to benzoyl-coenzyme A (CoA), protocatechuate, salicylate, gentisate, catechol, and cinnamic acid, which can be channeled into the beta-ketoadipate pathway (3). In addition, strain A1 can produce slimy extracellular polymeric substance (EPS) during azo dye decolorization which may serve as a protection for the bacterial consortium against harmful chemicals in the environment. In order to gain further insight into the versatility of strain A1 for future biotechnological applications, the genome of this bacterium was sequenced.

The draft genome sequence of *Citrobacter* sp. A1 was determined using Genome Analyzer IIx (100-bp paired-end reads). The paired-end reads were assembled *de novo* into 74 contigs (121× coverage) using CLC Genomics Workbench 4.8 (CLC bio, Denmark). The  $N_{50}$  length is 227,071 bp, and the longest contig is 719,223 bp. The open reading frames (ORFs), tRNAs, and rRNAs were determined using

Prodigal 2.60, RNAMmer 1.2, and tRNAscan-SE 1.3, respectively (5, 8, 9). Subsequent annotation was performed using Blast2GO (4). The draft genome sequence contains 5,096,012 bp with an average GC content of 51.81%. A total of 4,853 ORFs, 63 tRNAs, and 4 rRNAs were identified.

*Citrobacter* sp. A1 possesses genes for azo reduction, deamination, and desulfonation, as well as for the degradation of benzoate, catechol, gentisate, and protocatechuate. In addition, the draft genome of the bacterium reveals its potential in heavy metal reduction, nitrate reduction, sulfate assimilation, quorum sensing, and biofilm formation. This reflects on the catabolic versatility of *Citrobacter* sp. A1, which holds promise in the biodegradation of various xenobiotics and the bioremediation of heavy metals.

**Nucleotide sequence accession numbers.** This whole-genome shotgun project of *Citrobacter* sp. A1 has been deposited at DDBJ/EMBL/GenBank under the accession number AKTT00000000. The version described in this paper is the first version, AKTT01000000.

## ACKNOWLEDGMENTS

This work was supported by the Research University Grant Scheme (2011 to 2012, no. 01H61) provided by Universiti Teknologi Malaysia, Malaysia.

## REFERENCES

1. An S-Y, et al. 2002. Decolorization of triphenylmethane and azo dyes by *Citrobacter* sp. *Biotechnol. Lett.* 24:1037–1040.
2. Chan GF. 2005. Molecular and enzymatic studies on the decolourization of azo dyes by *Citrobacter freundii* A1. Ph.D. thesis. Universiti Teknologi Malaysia, Johor, Malaysia.
3. Chan GF, et al. 2012. Communal microaerophilic-aerobic biodegradation of Amaranth by novel NAR-2 bacterial consortium. *Bioresour. Technol.* 105:48–59.
4. Conesa A, et al. 2005. Blast2GO: a universal tool for annotation, visualization and analysis in functional genomics research. *Bioinformatics* 21:3674–3676.
5. Hyatt D, et al. 2010. Prodigal: prokaryotic gene recognition and translation initiation site identification. *BMC Bioinformatics* 11:119.
6. Jeong BC, Hawes C, Bonthron KM, Macaskie LE. 1997. Localization of

Received 18 July 2012 Accepted 27 July 2012

Address correspondence to Noor Aini Abdul Rashid, nooraini\_nar@utm.my.

Copyright © 2012, American Society for Microbiology. All Rights Reserved.

doi:10.1128/JB.01285-12

- enzymatically enhanced heavy metal accumulation by *Citrobacter* sp. and metal accumulation *in vitro* by liposomes containing entrapped enzyme. *Microbiology* 143:2497–2507.
7. **Khalid A, Arshad M, Crowley DE.** 2009. Biodegradation potential of pure and mixed bacterial cultures for removal of 4-nitroaniline from textile dye wastewater. *Water Res.* 43:1110–1116.
  8. **Lagesen K, et al.** 2007. RNAmmer: consistent and rapid annotation of ribosomal RNA genes. *Nucleic Acids Res.* 35:3100–3108.
  9. **Lowe TM, Eddy SR.** 1997. tRNA-scan-SE: a program for improved detection of transfer RNA genes in genomic sequence. *Nucleic Acids Res.* 25:955–964.
  10. **Vatsala TM, Mohan Raj S, Manimaran A.** 2008. A pilot-scale study of biohydrogen production from distillery effluent using defined bacterial co-culture. *Int. J. Hydrogen Energy* 33:5404–5415.
  11. **Wahab MFA.** 2007. Analysis of *Citrobacter freundii* A1 whole cell and its recombinant flavin reductase biodegradation of azo dyes using spectrophotometric and voltametric techniques. M.Sc. thesis, Universiti Teknologi Malaysia, Johor, Malaysia.