

Genome Sequence of *Enterococcus* sp. Strain C1, an Azo Dye Decolorizer

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***Enterococcus* sp. strain C1 is a facultative anaerobe which was coisolated with *Citrobacter* sp. strain A1 from a sewage oxidation pond. Strain C1 could degrade azo dyes very efficiently via azo reduction and desulfonation in a microaerophilic environment. Here the draft genome sequence of *Enterococcus* sp. C1 is reported.**

Enterococci are classified as lactic acid bacteria, which have been well reported as bacteriocin producers (6). Though low in pathogenicity, enterococci have been associated with human infections and cases of antibiotic resistance (6, 12). In addition, enterococci are enteric bacteria commonly found in aquatic environments and are useful as a fecal indicator for water quality monitoring (1). Among the enterococci, *Enterococcus faecalis* has been reported to possess aerobic azoreductase for biodegradation of azo dyes (4, 11).

A local strain of *Enterococcus* sp., designated C1, was coisolated with *Citrobacter* sp. strain A1 from the sewage oxidation pond at Universiti Teknologi Malaysia (2). Strain C1 is a Gram-positive facultative anaerobic diplococcus that is closely related to *E. casseliflavus* based on the 16S rRNA gene characterization (2). Strain C1 could completely decolorize 0.1 g liter⁻¹ of a broad range of azo dyes under microaerophilic conditions at 45°C within 2 h (2, 3). Further study showed that strain C1 expressed an enzyme with activity similar to that of azoreductase which could contribute to the decolorization of azo dyes (9). Based on the mass spectral data, the microaerophilic decolorization of amaranth by the bacterium led to symmetric reductive cleavage to form aromatic amines of 1-aminonaphthalene-4-sulfonic acid and 1-aminonaphthalene-2-hydroxy-3,6-disulfonic acid. The subsequent catabolism of azo dye intermediates could also be performed by strain C1 via reductive deamination and desulfonation (3). In order to gain further insight on the catabolic potential of strain C1, the genome of the bacterium was sequenced.

The genome of *Enterococcus* sp. C1 was sequenced using Genome Analyzer Ix and 100-bp paired-end reads. The paired-end reads were assembled *de novo* into 36 contigs (272× coverage) using CLC Genomics Workbench 4.8 (CLC bio, Denmark). The N50 length is 394,574 bp, and the longest contig is 660,758 bp. The draft genome sequence contains 3,644,928 bp with GC content of 42.47%. Data pertaining to the rRNAs, tRNAs, and open reading frames (ORFs) were determined using RNAmmer 1.2, tRNAscan-SE 1.3, and Prodigal 2.60, respectively (7, 8, 10). A total of 3,377 ORFs, 47 tRNAs, and 3 rRNAs were identified. The draft genome of strain C1 was annotated using Blast2GO (5).

Strain C1 possesses genes that code for flavin mononucleotide (FMN)-dependent NADH azoreductase, copper amine oxidase, and sulfatase that could be involved in catabolism of azo dyes. The draft genome of strain C1 reveals the presence of regulatory systems which may be involved in the transcriptional control of biodegradation pathways of aromatic pollutants. These regulatory

proteins include members of the GntR, xre, MarR, IclR, AraC, FNR, and LysR families of transcriptional regulators. Uniquely, the draft genome of the bacterium also reveals the presence of phage elements which are not commonly found among enterococci. Since phages are important drivers of microbial evolution, further study in this direction should be important in the understanding of genetic diversity of *Enterococcus*. The molecular identification and characterization of these phages in strain C1 may enable subsequent genetic engineering of phages which can endow other enterococci with the ability to degrade azo dyes, thus improving the overall bioremediation capability of the microbial community in a bioreactor. Hence, this suggests the marvelous potential of *Enterococcus* sp. C1 in bioremediation applications.

Nucleotide sequence accession numbers. This *Enterococcus* sp. C1 whole-genome shotgun project has been deposited at DDBJ/EMBL/GenBank under accession number AKKS00000000. The version described here is the first version, AKKS01000000.

ACKNOWLEDGMENT

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

REFERENCES

1. Badgley BD, Nayak BS, Harwood VJ. 2010. The importance of sediment and submerged aquatic vegetation as potential habitats for persistent strains of enterococci in a subtropical watershed. *Water Res.* 44:5857–5866.
2. Chan GF, Rashid NAA, Koay LL, Chang SY, Tan WL. 2011. Identification and optimization of novel NAR-1 bacterial consortium for the biodegradation of Orange II. *Insight Biotechnol.* 1:7–16.
3. Chan GF, et al. 2012. Communal microaerophilic-aerobic biodegradation of Amaranth by novel NAR-2 bacterial consortium. *Bioresour. Technol.* 105:48–59.
4. Chen H, Wang R-F, Cerniglia CE. 2004. Molecular cloning, overexpression, purification, and characterization of an aerobic FMN-dependent azoreductase from *Enterococcus faecalis*. *Protein Expr. Purif.* 34:302–310.
5. Conesa A, et al. 2005. Blast2GO: a universal tool for annotation, visualization and analysis in functional genomics research. *Bioinformatics* 21: 3674–3676.
6. Foulquié Moreno MR, Sarantinopoulos P, Tsakalidou E, De Vuyst L.

Received 30 July 2012 Accepted 6 August 2012

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doi:10.1128/JB.01372-12

2006. The role and application of enterococci in food and health. *Int. J. Food Microbiol.* **106**:1–24.
7. Hyatt D, et al. 2010. Prodigal: prokaryotic gene recognition and translation initiation site identification. *BMC Bioinformatics* **11**:119. doi: 10.1186/1471-2105-11-119.
 8. Lagesen K, et al. 2007. RNAmmer: consistent and rapid annotation of ribosomal RNA genes. *Nucleic Acids Res.* **35**:3100–3108.
 9. Lee SZ. 2003. Development of an enzyme assay for decolorization of azo dyes by bacterium C1. Universiti Teknologi, Malaysia.
 10. 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.
 11. Mate MS, Pathade G. 2012. Biodegradation of C.I. Reactive Red 195 by *Enterococcus faecalis* strain YZ66. *World J. Microb. Biot.* **28**:815–826.
 12. Peters J, Mac K, Wichmann-Schauer H, Klein G, Ellerbroek L. 2003. Species distribution and antibiotic resistance patterns of enterococci isolated from food of animal origin in Germany. *Int. J. Food Microbiol.* **88**:311–314.