

Genome resource of a 2'-deoxyuridine-degrading *Enterobacter* sp. strain C3 isolated from rice sheaths

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Data Note

Keywords: 2'-deoxyuridine, *Enterobacter* sp., rice sheath, fungal metabolite degradation

Posted Date: April 9th, 2026

DOI: <https://doi.org/10.21203/rs.3.rs-9350247/v1>

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Additional Declarations: The authors declare no competing interests.

Abstract

Enterobacter sp. strain C3 was isolated from rice sheaths by enrichment and activity-based screening for bacteria capable of degrading 2'-deoxyuridine (dU), a metabolite secreted by the rice blast fungus. Whole-genome sequencing and de novo assembly generated a curated chromosome-core draft genome consisting of 18 contigs, with six additional plasmid-like contigs identified. This genomic resource provides a basis for identifying genes involved in dU metabolism and for investigating how bacterial degradation of fungal metabolites may influence plant-microbe-pathogen interactions in rice-associated ecosystems.

Main text

Pyricularia oryzae (syn. *Magnaporthe oryzae*) is the causal fungus of rice blast, one of the most destructive diseases of rice worldwide, threatening food security and causing substantial economic losses (Skamnioti and Gurr 2009). Previous studies have identified 2'-deoxyuridine (dU), a metabolite secreted by the conidia of *P. oryzae*, as an infection-promoting metabolite. During compatible host-pathogen interactions, the accumulation of dU exacerbates fungal virulence and significantly increases lesion formation on rice leaves (Ando et al. 2011). To identify bacteria capable of degrading dU, an *in vitro* screening assay was conducted to isolate bacterial strains that metabolize exogenous dU.

Environmental samples, including bulk soil, rhizosphere soil, leaves, roots, and leaf sheaths, were collected and inoculated into minimal medium supplemented with dU (Table S1) as the sole carbon source. Cultures showing a significant reduction in dU concentration were serially diluted and plated onto Reasoner's 2A (R2A) agar to isolate single colonies (Reasoner and Geldreich 1985). Purified isolates were cryopreserved in glycerol at -80°C . To compare dU-degrading activity among isolates, activated cultures were standardized to the same optical density at 600 nm (OD_{600}) in R2A broth and inoculated into MM supplemented with 1 mM dU. After incubation at 28°C for 24 h, cultures were centrifuged at $20,000 \times g$ for 5 min, and 2- μL aliquots of the resulting cell-free supernatants were spotted onto aluminum-backed silica gel 60 F254 plates (Merck). Thin-layer chromatography was performed using chloroform:methanol (4:1, v/v) as the mobile phase. Plates were visualized under ultraviolet light to monitor dU depletion and the appearance of metabolic byproducts. The isolate exhibiting the highest activity was selected and designated strain C3 (Fig. 1). Taxonomic assignment based on 16S rRNA gene sequencing using the universal bacterial primers 10f_16S and 1541r_16S (Weisburg et al. 1991), followed by BLASTN analysis against the NCBI nucleotide database, identified strain C3 as a member of the genus *Enterobacter*.

To further purify the target bacterium, suspensions from the second enrichment cultures were sub-cultured onto MacConkey agar (Elazhary et al. 1973). This medium facilitates the preliminary differentiation of lactose-fermenting bacteria, allowing the target *Enterobacter* isolate to be recognized by its pink-to-red colonies (Fig. 1B). *Enterobacter* species are ubiquitous in agricultural ecosystems and participate in diverse plant-microbe interactions. Some members of this genus can degrade plant-

derived compounds such as kaempferol and agrochemicals such as chlorpyrifos and nicosulfuron (Singh et al. 2004; Xia et al. 2023; Xiao et al. 2025).

For whole-genome sequencing, strain C3 was cultured in Luria-Bertani (LB) broth at 28°C with shaking at 120 rpm. Bacterial cells were harvested at mid-exponential phase by centrifugation, and genomic DNA was extracted using the NucleoSpin Microbial DNA kit (Takara Bio, Japan) according to the manufacturer's instructions.

Library preparation and sequencing were performed essentially as described previously (Miura et al. 2024), with modifications. Whole-genome sequencing libraries were prepared with a MGIEasy Fast PCR-free FS Library Prep Set (MGI, China; Cat. 940-000021-00) and sequenced on a DNBSEQ-G400RS platform (MGI) using 2 × 150-bp paired-end reads. After adapter and quality trimming with fastp v1.1.0 (Chen et al. 2018), read quality was assessed using FastQC v0.12.1 and summarized with MultiQC v1.27. Paired-end reads were assembled *de novo* using SPAdes v4.2.0 with the --isolate option (Bankevich et al. 2012). Contigs shorter than 500 bp were excluded and assembly statistics were computed using QUAST v5.3.0 (Gurevich et al. 2013). Trimmed reads were mapped back to the length-filtered assembly (≥ 500 bp) using minimap2 v2.30-r1287 (Li 2018) and samtools (Danecek et al. 2021) to estimate per-contig mean depth and to assess assembly consistency; 99.71% of QC-passed reads mapped to the assembly, and 97.63% were properly paired. Taxonomic profiling of contigs was performed with Kraken2 v2.17.1 (Wood et al. 2019) using the PlusPF 16-GB database (k2_pluspf_16_GB_20260226), and contigs assigned to non-dominant taxa with markedly lower depth were flagged as potential contaminants. After curation, the chromosome-core assembly comprised 4,635,272 bp in 18 contigs (N50, 439,181 bp; GC content, 55.8%; largest contig, 1,558,469 bp). In addition, six plasmid-like contigs totaling 141,167 bp were identified. Genome completeness and contamination were estimated using CheckM2 v1.1.0 (Chklovski et al. 2023) on the chromosome-core assembly (with genes predicted by Prodigal v2.6.3; Hyatt et al. 2010), yielding 100.0% completeness and 0.0% contamination. A summary of the chromosome-core assembly and CDS annotation is provided in Table 1, and plasmid-like contigs with their CDS counts are listed in Table 2. Plasmid-like contigs were defined based on contig-wise taxonomic assignment (Kraken2) and depth distribution; some plasmid-like contigs showed taxonomic assignments different from the dominant taxon.

The draft genome sequence of *Enterobacter* sp. strain C3 has been deposited in DDBJ/EMBL/GenBank under the whole-genome shotgun (WGS) project BAAKFX000000000. The raw sequencing reads are available in the DDBJ Sequence Read Archive (DRA) under the accession number DRR932716, BioProject accession PRJDB40495.

Declarations

Acknowledgments

This work was supported by the Japan Society for the Promotion of Science (JSPS) through Grant-in-Aid for Scientific Research (C) (21K05595) to IS, and through Challenging Research (Exploratory) (22K19176) and Grant-in-Aid for Scientific Research (B) (23K26905) to DT. H.H. was supported by JST SPRING (JPMJSP2125) through the THERS Make New Standards Program for the Next Generation Researchers.

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Tables

Table 1 and 2 are available in the Supplementary Files section.

Figures

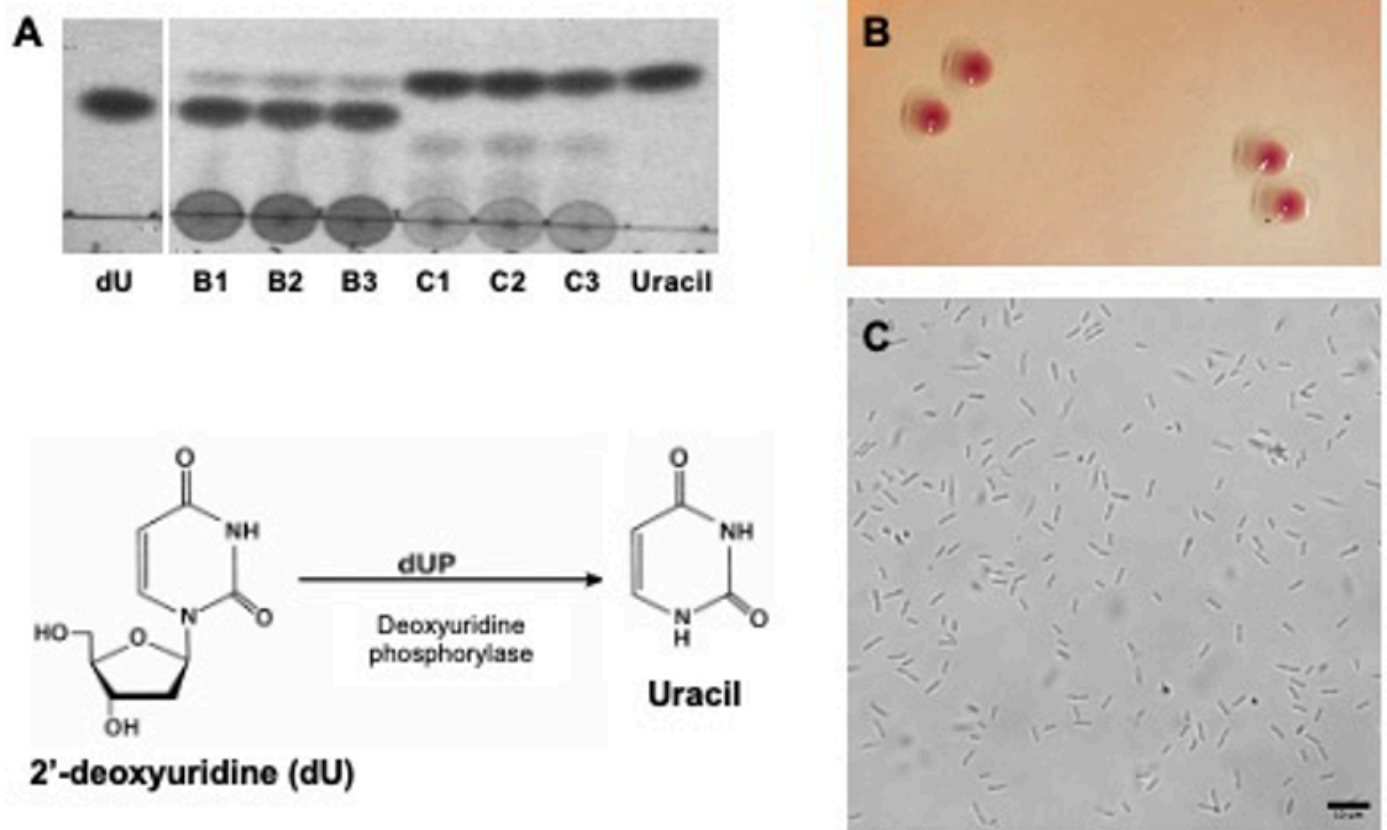


Figure 1

Phenotypic and biochemical characterization of *Enterobacter* sp. strain C3. **(A)** Thin-layer chromatography (TLC) analysis of dU-degrading activity following a 24-h incubation period. The lane labeled dU shows the substrate standard, and the lane labeled Uracil shows the product standard. Lanes B1–B3 represent three environmental isolates with weak dU-metabolizing activity, whereas lanes C1–C3 represent three independently purified colonies assigned to strain C3. In C1–C3, the dU signal was markedly reduced and an uracil-corresponding signal was detected, indicating efficient conversion of dU to uracil. The lower schematic illustrates the proposed phosphorolytic conversion of 2'-deoxyuridine to uracil by deoxyuridine phosphorylase (dUP). **(B)** Colony morphology of strain C3 on MacConkey agar, showing pink-to-red colonies indicative of lactose fermentation. **(C)** Light micrograph of strain C3 cells, showing a rod-shaped morphology. Bar = 10 μm .

Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- [TableS1MediumofdUMM.xlsx](#)
- [Table1chromosomecoresummaryv2.jpg](#)
- [Table2plasmidlikecontigsv2.jpg](#)