## High-efficiency microbial saccharification with novel xylan-saccharifying Bacteria

Lignocellulosic biomass is attracting attention as the world's most abundant renewable resource, and its effective utilization is being sought. However, the development of an efficient and cost-effective technology for biomass saccharification is a challenge, and JIRCAS has developed the "microbial saccharification method," which does not rely on commercial cellulose saccharification enzymes (cellulases), but only on the cultivation of saccharifying microorganisms (see FY2022 Research Highlights: Technology development to saccharify cellulose “only by cultivating microorganisms” without using cellulase enzymes).

Some agricultural waste biomass contains not only cellulose but also a large amount of xylan. Since xylan inhibits the saccharification of cellulase enzyme, it is necessary to search for xylan-saccharifying microorganisms that can be incorporated into microbial saccharification methods and to develop saccharification technology using such microorganisms. We screened microorganisms that efficiently saccharify xylan at 60°C under anaerobic conditions using xylan as the sole carbon source and obtained DA-C8 bacteria. The characteristics of the DA-C8 bacteria isolated in this study are reported.

We have screened microorganisms that efficiently saccharify xylan from Ishigaki Island compost at 60°C under anaerobic conditions using a medium containing xylan as the sole carbon source and obtained DA-C8 bacteria. This bacterium belongs to the same phylogenetic lineage as the known *Xylanibacillus composti*, but based on genetic, chemotaxonomic, and phylogenetic analyses (including digital DNA-DNA hybridization), average amino acid sequence identity values, and major polar lipid composition, a new genus and species, *Insulambacter and I. thermoxylanivorax*, were proposed (Fig. 1). *I. thermoxylanivorax* DA-C8 not only can completely saccharify xylan (Fig. 2) but also hemicelluloses other than xylan, such as arabinoxylan and galactan. It also grows over a wide temperature (37–60°C; optimum temperature: 55°C) and pH range (4–11; optimum pH: 9). In a microbial saccharification test using oil palm fiber (EFB), which contains relatively high amounts of xylan, the saccharification capacity of *Clostridium thermocellum* alone was 24.7% and 13.2% for *I. thermoxylanivorax* DA-C8, which has a high cellulose saccharification capacity. When *I. thermoxylanivorax* DA-C8 and *C. thermocellum* were co-cultured, the saccharification efficiency was 58.1%, showing an extremely high saccharification efficiency. This is a 2- to 4-fold increase in saccharification efficiency compared to each alone (Fig. 3). *I. thermoxylanivorax* DA-C8 has been deposited as a reference strain at the RIKEN BioResource Center (JCM 34211T) and the German Microbial Cell Culture Collection Center (DSM 111723T) and is available for distribution.

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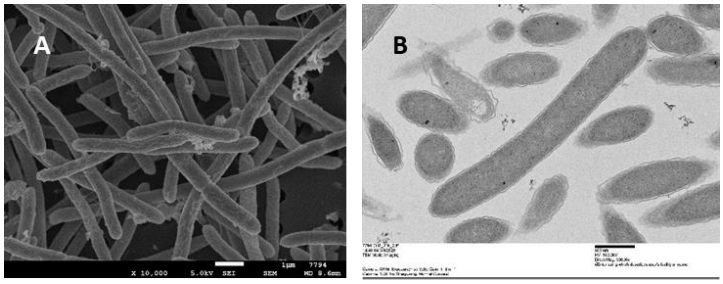


**Fig. 1. Cell morphology of *I. thermoxylanivorax* strain DA-C8 cultured in xylose carbon source medium**

A. Scanning electron microscopy image of *I. thermoxylanivorax* DA-C8. White scale bar is 1 μm;
B. Transmission electron microscopy image of a thin section of *I. thermoxylanivorax* DA-C8 strain.

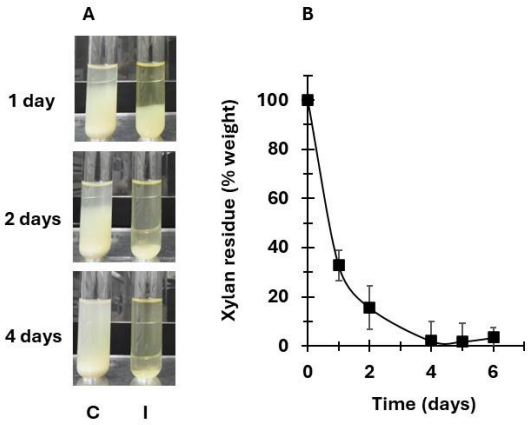


**Fig. 2. Xylan saccharification capacity of *I. thermoxylanivorax* DA-C8**

A. Xylan saccharification by *I. thermoxylanivorax* DA-C8 in 1% xylan carbon source medium. Days 1, 2, and 4 after inoculation with *I. thermoxylanivorax* DA-C8. C: uninoculated, I: inoculated with *I. thermoxylanivorax* DA-C8;
B. Residual percentage of xylan over time in 1% xylan carbon source medium

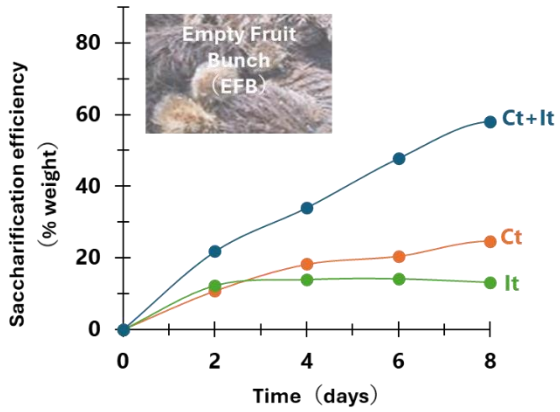


**Fig. 3. Microbial saccharification by co-culture of *I. thermoxylanivorax* DA-C8 and *C. thermocellum***

Microbial saccharification was measured in a medium containing 1% EFB fiber. Comparison of saccharification capacity of *C. thermocellum* alone (Ct), *I. thermoxylanivorax* DA-C8 alone (It), and *C. thermocellum* in co-culture with *I. thermoxylanivorax* DA-C8 (Ct+It), which has high cellulose saccharification capacity used in the microbial saccharification method.

References: 1) Chhe et al. (2021) Characterization of a thermophilic facultatively anaerobic bacterium *Paenibacillus* sp. strain DA-C8 that exhibits xylan degradation under anaerobic conditions. *J. Biotechnol.* 342: 64–71. https://doi.org/10.1016/j.jbiotec.2021.10.008 © Elsevier B.V. 2021
2) Chhe et al. (2023) *Int J Syst Evol Microbiol.* 73(3): 005724. © The Author(s) 2023