

Genome Reports

Draft Genome Sequence of *Acetobacter oryzifermentans* AFY-4 Isolated from Rice Vinegar

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Acetobacter oryzifermentans AFY-4, a potent acetic acid producer, was isolated from traditional Korean rice vinegar. This study presents the draft genome sequence of *A. oryzifermentans* AFY-4. The genome of *A. oryzifermentans* AFY-4 is 3,222,280 bp in length, with a G+C content of 52.5%, including 2,962 protein-coding sequences (CDSs), 57 transfer RNA genes, and 15 ribosomal RNA genes. *A. oryzifermentans* AFY-4 exhibited high orthologous average nucleotide identity values with other *A. oryzifermentans* strains, including SLV-7, DmCS_004, and DM001. Pan- and core-genome analysis showed that the AFY-4 strain is more closely related to the SLV-7 strain than other *A. oryzifermentans* strains.

Keywords: *Acetobacter oryzifermentans*, genome, acetic acid bacteria, vinegar

Acetic acid bacteria are strictly aerobic, Gram-negative, and non-spore-forming bacteria belonging to the family *Acetobacteraceae* and typically exhibit optimal growth at pH 5–6.5, with the ability to grow at pH 3–4 [1]. *Acetobacter* is commonly found in alcoholic, acidic, sugary habitats, including traditional fermented foods and alcoholic beverages like vinegar, kombucha, sour beer, and water kefir [2, 3]. *Acetobacter oryzifermentans* was isolated from Korean traditional vinegar and proposed as a novel species due to its low average nucleotide identity (ANI) value (<91.0%) and *in silico* DNA-DNA hybridization (DDH) values (44.2%) with *A. pasteurianus*, despite its high 16S rRNA gene sequence similarity (>99.59%) to *A. pasteurianus* [4]. *A. oryzifermentans* AFY-4, known for its high acetic acid production and tolerance to low pH 3 [5], was previously

reported as *A. pasteurianus* AFY-4 based on 16S rRNA sequences. This study confirmed that the AFY-4 strain is *A. oryzifermentans* based on genome sequence and present draft genome information.

A. oryzifermentans AFY-4 was incubated in YCM broth (0.5% yeast extract, 1.0% CaCO₃, 2.5% mannitol, 3.0% ethanol) at 25 °C for 48 h. Genomic DNA was extracted using the FastDNA™ spin kit (MP Biomedicals, USA) according to the manufacturer's instructions. Size selection was performed using the BluePippin™ size selection system (Sage Science, USA) according to the PacBio 20-kb library protocol. The sequencing library was prepared using the SMRTbell template prep kit 1.0 (Pacific Biosciences, USA). It was sequenced using the PacBio RS II sequencer (Pacific Biosciences) based on a single molecule, real-time (SMRT) sequencing technology. The library was filtered for genome assembly using the hierarchical genome assembly process (HGAP, ver. 2) protocol with default parameter in SMRT analysis software (ver. 2.3.0) [6].

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A phylogenetic tree and heat map were constructed based on the unweighted pair group method with arithmetic mean (UPGMA), and orthologous ANI (OrthoANI)

were calculated from OAT software (ver. 0.93.1) [7]. EggNog (evolutionary genealogy of genes: non-supervised orthologous groups) classification was mapped to clus-

Table 1. Genome features of *A. oryzafermentans* AFY-4 and *Acetobacter* species.

Strain	Genome size (bp)	G+C content (%)	CDSs	rRNAs	tRNAs	GenBank Accession No.
<i>A. oryzafermentans</i> AFY-4	3,222,280	52.5	2,962	5, 5, 5 (5S, 16S, 23S)	57	JBFNUA000000000
<i>A. oryzafermentans</i> SLV-7 ^T	2,799,488	52.5	2,789	5, 5, 5 (5S, 16S, 23S)	57	CP011120
<i>A. oryzafermentans</i> DmCS_004 ¹⁾	2,843,884	52.5	2,682	1, 1, 1 (5S, 16S, 23S)	43	NZ_JOKL000000000
<i>A. oryzafermentans</i> DM001 ¹⁾	2,883,421	52.3	2,688	1, 1, 1 (5S, 16S, 23S)	43	NZ_AEUP000000000
<i>A. pasteurianus</i> Ab3	2,806,148	53.5	2,786	5, 5, 5 (5S, 16S, 23S)	58	CP012111
<i>A. oryzoeni</i> B6 ^T	2,744,393	53.0	2,912	5, 5, 5 (5S, 16S, 23S)	57	CP042808
<i>A. pomorum</i> SH	2,969,514	52.5	2,999	5, 5, 5 (5S, 16S, 23S)	56	CP023189
<i>A. tropicalis</i> BDGP1	3,988,649	55.5	3,713	5, 5, 5 (5S, 16S, 23S)	58	CP022699
<i>A. vaccinii</i> KACC 21233 ^T	2,814,618	59.5	2,732	5, 5, 5 (5S, 16S, 23S)	57	CP043506

T: Type strain

DmCS_004 and DM001 strains were reported as *A. pomorum* in GenBank. However, two strains were reclassified as *A. oryzafermentans* because these shared high ANI values (98.53-99.88%) with *A. oryzafermentans* [4].

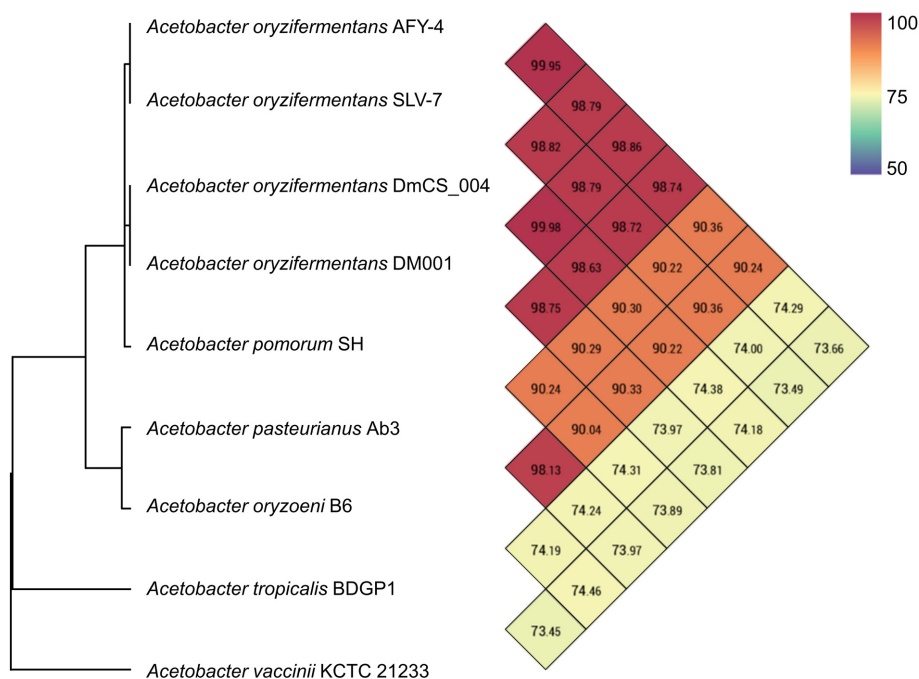


Fig. 1. OrthoANI analysis heat map based on the UPGMA tree of *A. oryzafermentans* AFY-4 and other *Acetobacter* spp.

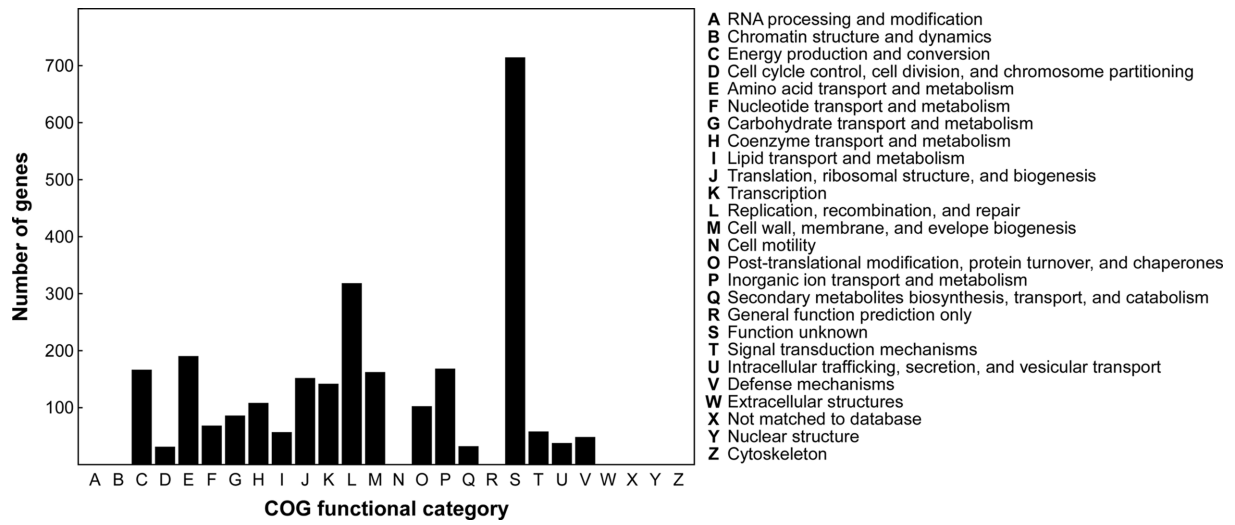


Fig. 2. Histogram of eggNog classification of *A. oryzafermentans* AFY-4 based on COG functional categories.

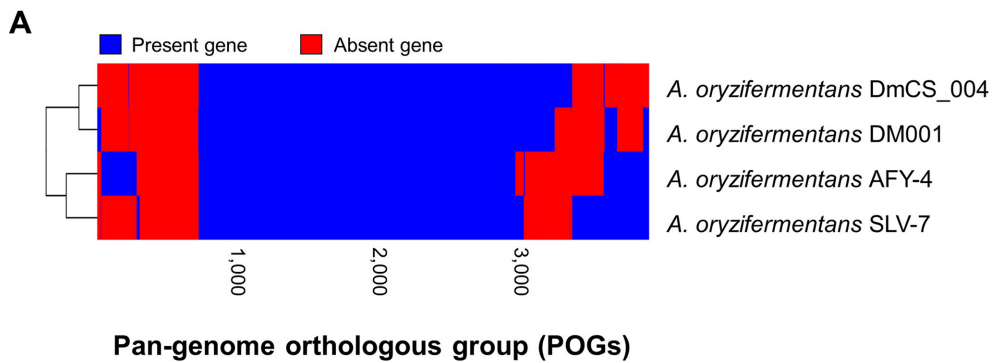


Fig. 3. Heat map based on gene content (presence/absence) of all CDSs among five *Acetobacter* species (A) and Venn diagram for specific and shared CDSs among four *A. oryzafermentans* strains (B).

ters of orthologous genes (COG) categories. Pan- and core-genome analysis was performed using all CDSs' gene presence and absence matrix and a Venn diagram [8] with the EzBioCloud comparative genomics database.

The total bases, mean length, and N_{50} value of the polymerase read in the draft genome of *A. oryzafermentans* AFY-4 were 3,220,280 bp, 941.5 bp, and 2,730,921 bp, respectively. The genomic information of *A. oryzafermentans* AFY-4 and other *Acetobacter* species are summarized in Table 1. The assembled draft genome of *A. oryzafermentans* AFY-4 consists of 5 contigs with a G+C content of 52.5% and includes 2,962 CDSs, 15 rRNA, and 57 tRNA genes. The numbers of rRNA and tRNA genes in *A. oryzafermentans* AFY-4 were like those in other *Acetobacter* strains.

The OrthoANI values were calculated based on comparisons with other *Acetobacter* genomes (Fig. 1). *A. oryzafermentans* AFY-4 exhibited a high OrthoANI value of 99.95% with *A. oryzafermentans* SLV-7, 98.75% with *A. pomorum* SH, and 90.24% with *A. pasteurianus* Ab3.

In eggNog analysis (Fig. 2), significant categories included genes related to replication, recombination, and repair (317 genes, 12.04%); amino acid transport and metabolism (189 genes, 7.18%); and inorganic ion transport and metabolism (169 genes, 6.42%), excluding genes of unknown function, which represented the largest group.

Gene presence and absence analysis revealed numerous gene insertions and deletions distributed across the genome of *A. oryzafermentans* AFY-4 compared to the other four strains (Fig. 3A). The AFY-4 strain harbors more than 200 genes not found in different strains. As shown in Fig. 3B, the Venn diagram indicates that 2,237 core-pan-genome orthologous groups (POGs) were identified among the four *A. oryzafermentans* strains (cut off=100%). Furthermore, the AFY-4 strain has 248 additional genes, including those for aspartate racemase, which are absent in the other strains. Both gene content (presence/absence) and Venn diagram results suggest that the AFY-4 strain is more closely related to the SLV-7 strain isolated from vinegar than the DmCS_004 [9] and DM001 [10] strains, isolated from the gut of *Drosophila melanogaster*. This geographic distribution of core genome evolution may be related to the 2,237 core-genome genes shared among the four *A. oryzafermentans* strains.

In conclusion, genomic analysis of *A. oryzafermentans* AFY-4 may provide invaluable information for future research on acetic acid production.

Nucleotide Sequence Accession Numbers

The draft *A. oryzafermentans* AFY-4 genome sequence has been deposited in the GenBank database under the accession number JBNUA000000000.

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Conflict of Interest

The authors have no financial conflicts of interest to declare.

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