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Complete Genome Sequence of a Carbon Monoxide-Utilizing Acetogen, *Eubacterium limosum* KIST612[▽]

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***Eubacterium limosum* KIST612 is an anaerobic acetogenic bacterium that uses CO as the sole carbon/energy source and produces acetate, butyrate, and ethanol. To evaluate its potential as a syngas microbial catalyst, we have sequenced the complete 4.3-Mb genome of *E. limosum* KIST612.**

Synthesis gas (syngas) (H_2, CO_2, and CO) has been highlighted for use as a potential feedstock for the production of biofuels and valuable chemicals (9, 16). *Eubacterium limosum* KIST612 isolated from an anaerobic digester has been considered a microbial syngas catalyst due to its rapid growth under high CO pressure (>1 atm) and production of acetate and butyrate and ethanol from CO (5–7). To understand its physiological properties (e.g., a high tolerance to CO and production of ethanol) and provide metabolic engineering principles, we attempted to obtain the complete genome sequence information for this microorganism.

The genome of *E. limosum* KIST612 was sequenced by a combination of Illumina Genome Analyzer IIx (GAIIx) and Roche 454 GS FLX (454 GS FLX) platforms. We obtained two libraries of 643,326 single-end (SE) reads and 291,735 paired-end (PE) reads containing 3-kb inserts from 454 GS FLX. The third genomic library of 35,235,888 PE reads containing 400-bp inserts was obtained from GAIIx. To combine these three libraries (454 GS FLX SE and PE and GAIIx PE) into a single procedure, we first assembled GAIIx PE reads into 296 contigs (4,635,997 bases) by the ABySS 1.20 assembler (15) and split into overlapping ~1.5-kb fake reads (45,221 reads). We merged these fake reads with 454 SE and PE reads (total 935,061 reads) and assembled into 9 scaffolds (34 contigs) by the Newbler gsAssembler 2.3 (454 Life Sciences, Branford, CT). We determined the actual order of 9 scaffolds in a single contig with a series of PCRs based on a permutation table of scaffolds. The genome was finished by filling gaps with sequencing and primer walking of PCR products using an ABI 3730 capillary sequencer (Applied Biosystems, CA).

The complete genome of *E. limosum* KIST612 consisted of 4,276,902 bp in a single circular chromosome with an average G+C content of 47.5%. Approximately 91% of the nucleotides were predicted as 4,516 protein-coding regions by the union of Glimmer (8), GeneMarkS (3), and Prodigal (10). The predicted proteins were annotated by BLAST (1) and the RAST server (2). Seventy-eight percent (3,541) of the open reading frames were annotated with known proteins. Five copies of the 16S-23S-5S rRNA operon and a separate 5S rRNA locus were predicted by RNAmmer 1.2 (12), and the 58 tRNA genes were identified by tRNAscan-SE 1.23 (13).

Metabolic pathway analysis revealed that *E. limosum* KIST612 uses the Wood-Ljungdahl pathway to fix CO (or CO_2) and converts it into acetyl coenzyme A (acetyl-CoA), like other syngas-utilizing acetogens such as *Moorella thermoacetica* (14), *Clostridium ljungdahlii* (11), and *Clostridium carboxidivorans* strain $P7^T$ (4). *E. limosum* KIST612 also contains 10 genes annotated as subunits of hydrogenases that may provide reducing equivalents for CO_2 reduction to organic carbons. The genome of *E. limosum* KIST612 contains genes that encode key enzymes that convert acetyl-CoA into potential bioenergy-compatible acids/alcohols (acetate, butyrate, and ethanol). In addition to these genes, key genes for growing on syngas can be a platform of synthetic biology to construct carbon fixation pathways for the production of biofuels or chemicals from syngas.

Nucleotide sequence accession number. The complete genome sequence of *E. limosum* KIST612 has been deposited in NCBI GenBank under accession number CP002273.

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