

Complete Genome Sequence of the 2,3-Butanediol-Producing *Klebsiella pneumoniae* Strain KCTC 2242

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Here we report the full genome sequence of *Klebsiella pneumoniae* KCTC 2242, consisting of a 5.26-Mb chromosome (57.6% GC%; 5,035 genes [4,923 encoding known proteins, 112 RNA genes]) and a 202-kb plasmid (50.2% GC%; 229 genes [229 encoding known proteins]).

2,3-Butanediol (2,3-BD) is a glycol widely used as a reagent in a number of chemical syntheses. In addition to its applications in plastics, solvent, and antifreeze preparations, it could also be converted to 1,3-butadiene (for synthetic rubber), diacetyl (flavoring agent), or methyl ethyl ketone (liquid fuel additive) or to precursors of polyurethane (8). Interest in microbial production of 2,3-butanediol has been increasing recently due to the extensive industrial applications of this product (3). This colorless and odorless liquid with a high boiling point and a low freezing point is a potential valuable fuel additive (5). *Klebsiella pneumoniae* KCTC 2242 is a Gram-negative, nonmotile, encapsulated, facultative anaerobic, rod-shaped bacterium. Although *K. pneumoniae* is a pathogenic microorganism, much research on 2,3-butanediol production is going on (2, 6). Whole-genome sequencing and transcriptome analysis were carried out not only to compare gene data with that of the already published genes of *K. pneumoniae* NTUH-K2044 (NCBI accession number [AP006725](#)), *K. pneumoniae* 342 (NCBI accession number [CP000964](#)), and *K. pneumoniae* MGH 78578 (NCBI accession number [CP000647](#)) but also to discover the characteristics and genomic level of *K. pneumoniae* KCTC 2242.

Genomic DNA isolated using an overnight culture of strain KCTC 2242 and a DNeasy blood and tissue kit (Qiagen) was sequenced by 454 GS FLX Titanium pyrosequencing (Roche), following the manufacturer's instructions, with 25× coverage (4). Then, the 501,755 reads generated, with a length of 129,696,091 bp, were assembled using a GS De Novo assembler (version 2.3; Roche). The N50 contig was 146,341 bp in length, and largest contig assembled was 358,873 bp. This assembly generated 67 large contigs (>500 bp), with a length of 5,394,883 bp. A total of 480,845 reads (95.83% of the total) were assembled into 10 scaffolds composed of 99 contigs, with a length of 5,426,912 bp. The N50 scaffold was 2,943,199 bp, and the average length of the scaffolds was 542,691 bp.

The order of scaffolds was determined by a similarity search among published references and confirmed by PCR size checks. Gaps both within and between scaffolds can be closed by long-range PCR (using MG *Taq*-HF DNA polymerase; Macrogen, Seoul, South Korea) and subsequent Sanger sequencing using an ABI 3730XL capillary sequencer. The sequences from ABI 3730XL sequencing and scaffolds were completely assembled into one cir-

cular genome by the use of Phred/Phrap/Consed software (1) and annotated with the Prokaryote Genomes Automatic Annotation Pipeline (PGAAP) (7).

The complete genome is composed of a circular chromosome of 5,259,571 bp (57.6% GC content), which includes 5,035 coding genes, 87 tRNA genes, and 25 rRNA genes. A total of 4,923 coding genes (97.77% of the total) have putative functions assigned on the basis of annotation. Plasmid pKCTC2242 contains 202,852 bp (50.2% GC content; 229 coding sequences [CDS]). A transcriptome experiment using KCTC 2242 and the Roche FLX system was performed to improve its genome annotation. Although small amounts of non-rRNA sequences were obtained from the transcriptome analysis, 6,195 reads (91%) of the 6,746 mapped non-rRNA reads were mapped to 1,169 predicted coding genes.

We found that acetoin production increases rapidly in the stationary phase during production of 2,3-butanediol. We also found that, after the 2,3-butanediol production is examined by transcriptome analysis, the expression level of acetoin reductase increases sharply. As described above, in producing 2,3-butanediol, it is necessary to block the route of succinic acid, lactic acid, and fumaric acid, as well as to inactivate acetoin reductase.

Nucleotide sequence accession numbers. The complete sequences of the *Klebsiella pneumoniae* KCTC 2242 main chromosome and of the *Klebsiella pneumoniae* KCTC 2242 pKCTC2242 plasmid have been deposited in NCBI GenBank (accession numbers [CP002910](#) and [CP002911](#), respectively).

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