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Complete genome sequence of *Corynebacterium* sp. SCR221107, encoding biosynthesis of vitamin B_{12} isolated from the rumen fluid of Holstein dairy cows

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Abstract

Corynebacterium sp. SCR221107 was isolated from the rumen fluid of healthy male Holstein dairy cows from a research farm at Suncheon, Jeollanam-do, Korea. *Corynebacterium* sp. SCR221107 is a functional probiotic candidate that produces vitamin B_{12}. All *Corynebacterium* sp. SCR221107 was sequenced using the PacBio RS II and Illumina HiSeq platforms and assembled *de novo*. The complete genome sequence of *Corynebacterium* sp. SCR221107 contained one circular chromosome (3,043,024 bp) with a guanine + cytosine (GC) content of 60.1%. Annotation analysis showed the presence of 2,639 protein-coding sequences, 15 rRNA genes, and 57 tRNA genes. Genome analysis found that *Corynebacterium* sp. SCR221107 encodes various genes associated with vitamin B12 synthesis and transport. The genomic information provided a detailed understanding of *Corynebacterium* sp. SCR221107, suggesting that this isolate may have potential probiotic applications.

Keywords: *Corynebacterium* sp., Holstein dairy cow, *De novo* assembly, Whole genome sequencing

Members of the genus *Corynebacterium* are Gram-positive, non-acid-fast, non-motile, straight to curved rod-shaped bacteria and are classified as members of the order *Mycobacteriales*, class *Actinomycetia*, and phylum *Actinobacteria* [1]. To date, the genus comprises 140 species and four subspecies with validly published names. *Corynebacterium* has been isolated from soil, food, and animals, including humans. Some strains of the genus of which are recognized as pathogens related to human and animal diseases [2,3]. However, *Corynebacterium vitaeruminis* has been studied for its beneficial functions and has been known to be non-pathogenic and non-virulent [4,5]. *C. vitaeruminis* as a bacterium that is capable of synthesizing vitamin B within the rumen of cows [6].

In this study, *Corynebacterium* sp. SCR221107 was isolated from the rumen fluid of a 1-year-old

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Competing interests
No potential conflict of interest relevant to this article was reported.

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Not applicable.

Availability of data and material
Upon reasonable request, the datasets of this study can be available from the corresponding author.

Authors' contributions
Conceptualization: Baik KS, Ramos SC, Lee SS.
Formal analysis: Baik KS, Na SH.
Methodology: Baik KS, Lee SS.
Validation: Kim SH, Lee SS.
Writing - original draft: Baik KS, Miguel M.
Writing - review & editing: Baik KS, Ramos SC, Na SH, Kim SH, Son AR, Miguel M, Lee SS.

Ethics approval and consent to participate
All animals used in this research were approved by the Sunchon National University (SCNU) Institutional Animal Care and Use Committee (SCNU-IACUC; approval number: SCNU-IACUC2022-11).

healthy male Holstein dairy cow in Suncheon, Jeollanam-do, Republic of Korea. The sample was incubated in an anaerobic atmosphere with 5% carbon dioxide, 5% hydrogen, and 90% nitrogen at 37℃ for 48 h on De Man, Rogosa and Sharpe (MRS) media. Genomic DNA was extracted from *Corynebacterium* sp. SCR221107 cell pellets using a Maxwell® Prokaryote SEV DNA Purification Kit (Promega, Madison, WI, USA), in line with the manufacturer's instructions. The genomic DNA obtained was sequenced commercially at Macrogen (Seoul, Korea) using the PacBio Sequel II system (Pacific Biosciences, Menlo Park, CA, USA) and the Illumina HiSeq platform. *De novo* assembly was performed using the Hierarchical Genome Assembly Process v3.0 (HGAP3) with default options within the SMRT Link v11.1 software. Read quality was confirmed by aligning shorter reads with longer reads using Basic Local Alignment with Successive Refinement v1 (BLASR) [7] and correcting errors using Pilon version 1.21 [8]. Genome annotation was performed using rapid prokaryotic genome annotation (Prokka) v1.14.6 [9] and the Basic Local Alignment Search Tool (BLAST+) v2.7.1+. Clustered regularly interspaced short palindromic repeats (CRISPR) were assessed using the CRISPR web server (http://crispr.i2bc.paris-saclay.fr) [10]. Resistance-related genes were analyzed using ResFinder 4.1 with a 90% threshold for gene identification [11].

A total of 159,928 reads with a mean subread length of 8,975 bases (N50) were obtained using PacBio sequencing, and 37,599,664 paired-end reads, totaling 5,677,549,264 base pair [bp], were obtained using Illumina sequencing. The genome statistics are presented in Table 1. The complete genome sequence of *Corynebacterium* sp. SCR221107 is composed of a single circular chromosome and does not contain plasmid DNA. The 3,043,024 bp genome with a G + C content of 60.1% contained 2,639 protein-coding sequences (CDS), 63 pseudogenes, and 72 RNA genes (15 rRNA genes, 57 tRNA genes, and three non-coding RNA genes), based on the NCBI Prokaryotic Genome Annotation Pipeline (PGAP) (Fig. 1). Furthermore, 2,639 CDSs were clustered into 20 Clusters of Orthologous Groups (COGs) of protein-based functional categories (Fig. 1B). Many genes were classified into functional categories for amino acid transport and metabolism (n = 249); translation, ribosomal structure, and biogenesis (n = 190); inorganic ion transport and metabolism (n = 189); general function prediction only (n = 219); transcription (n = 185); and coenzyme transport and metabolism (n = 163). One confirmed CRISPR region and two questionable CRISPR 9 regions (1 and 2) were also detected. This pattern was identified as the CRISPR-CAS II type. A search using ResFinder returned no hits for antibiotic resistance genes in *Corynebacterium* sp. SCR221107.

Table 1. Genome features of *Corynebacterium* sp. SCR221107

Property	Value
Average genome coverage	439×
Genome size (bp)	3,043,024
No. of contigs	1
GC content (%)	60.1
CDS	2,639
tRNA	54
rRNA (5S, 16S, 23S)	15 (5, 5, 5)
ncRNA	3
CRISPR arrays	1
GenBank accession no.	CP115670

bp, base pair; GC, guanine + cytosine; CDS, coding sequence; CRISPR, clustered regularly interspaced short palindromic repeats.

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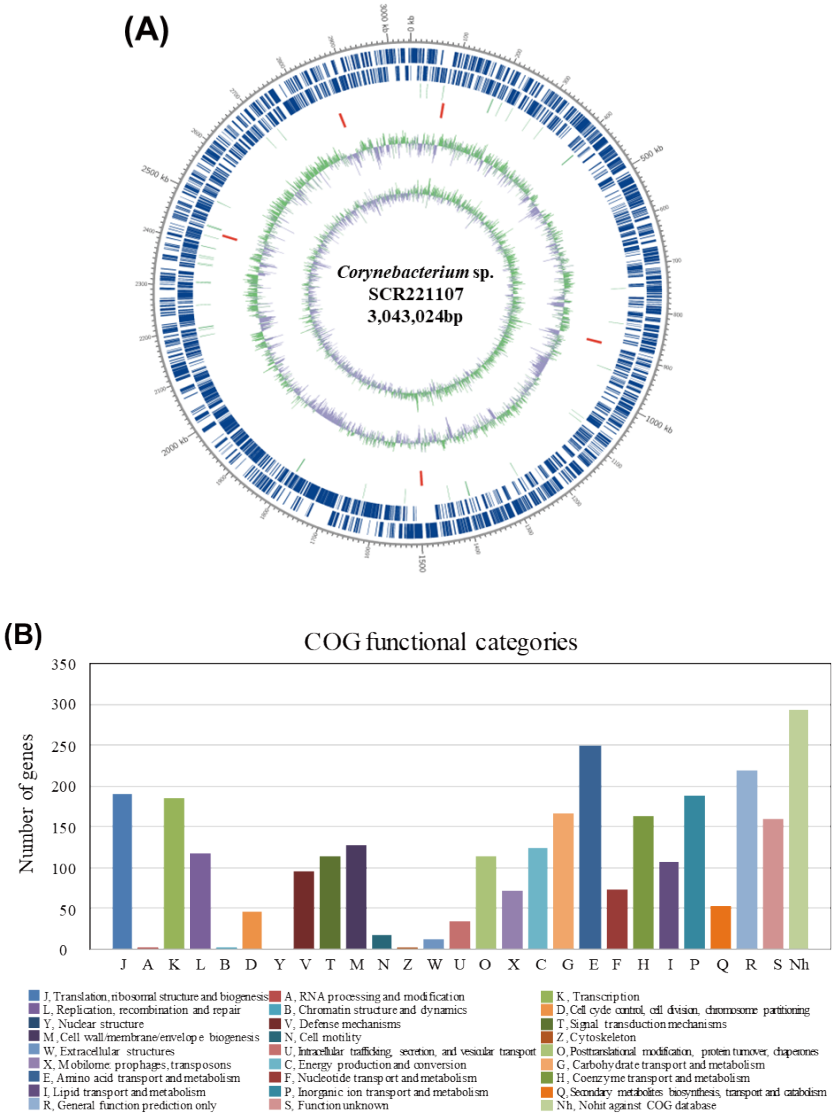

Fig. 1. Genome map of *Corynebacterium* sp. SCR221107 (A) and the functional categorization of predicted coding sequences (B). Marked characteristics are shown from the outside to the center: coding sequence (CDS) on the forward strand, CDS on the reverse strand, tRNA, rRNA, guanine + cytosine (GC) content, and GC skew. COG, Clusters of Orthologous Group.

Based on 16S rRNA gene sequence similarity data, it was found that the closest relatives of strain SCR221107 were *C. vitaeruminis* DSM 20294^T (98.5%) and *C. felinum* CCUG 39943^T (96.9%). As *Corynebacterium* sp. SCR221107 revealed close similarity with *C. vitaeruminis* DSM 20294^T, a known producer of B vitamin complex, the genomic analysis and annotation of coding regions unveiled a significant abundance of genes associated with vitamin biosynthesis. We identified cobalamin biosynthetic (vitamin B_{12}) and transport genes in *Corynebacterium* sp. SCR221107. In particular, *Corynebacterium* sp. SCR221107 possessed genes involved in the biosynthesis pathways of vitamin B_{12} such as *cobB, cobD, cobH, cobJ, cobK, cobL, cobM, cobN, cobQ, cobS, cobT, cobU, hemA, hemB, hemC, hemE, hemH, hemL, hemW,* and *hemY*, and transport genes such as *cbiM, cbiN,* and *cbiQ* [12,13]. The vitamin B12 gene clusters, which contain *hem-cob* operons, consisted of 20 genes responsible for various enzymatic transformations along the cobalamin (vitamin B_{12}) pathway. In addition, the genes/enzymes are involved in the oxygen-dependent pathway.

These results suggest that *Corynebacterium* sp. SCR221107 is a potential probiotic candidate capable of synthesizing vitamin B_{12}. The genomic data obtained from this study provides valuable insights into the biosynthetic pathways of vitamin B_{12} which might contribute for the development of vitamin B_{12}-enriched probiotics.

NUCLEOTIDE SEQUENCE ACCESSION NUMBER

The complete genome sequence of *Corynebacterium* sp. SCR221107 was deposited in the National Center for Biotechnology GenBank under the accession number CP115670.

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