JAST (Journal of Animal Science and Technology) TITLE PAGE

Upload this completed form to website with submission

ARTICLE INFORMATION	Fill in information in each box below
Article Type	Research Article
Article Title (within 20 words without abbreviations)	Complete genome sequence of *Corynebacterium* sp. SCR221107, encoding biosynthesis of vitamin B_{12} isolated from the rumen fluid of Holstein dairy cows
Running Title (within 10 words)	Complete genome sequence of *Corynebacterium* sp. SCR221107
Author	Keun Sik Baik, Sonny C. Ramos, Sang Hoon Na, Seon Ho Kim, A Rang Son, Michelle Miguel and Sang Suk Lee*
Affiliation	Ruminant Nutrition and Anaerobe Laboratory, Department of Animal Science and Technology, Sunchon National University, Suncheon 57922, Korea
ORCID (for more information, please visit https://orcid.org)	Keun Sik Baik (https://orcid.org/0000-0001-9087-9175) Sonny C Ramos (https://orcid.org/0000-0003-0257-9207) Sang Hoon Na (https://orcid.org/0000-0003-3581-5675) Seon Ho Kim (https://orcid.org/0000-0002-9350-1853) A Rang Son (https://orcid.org/0000-0002-3896-0950) Michelle Miguel (https://orcid.org/0000-0001-5364-3673) Sang Suk Lee (https://orcid.org/0000-0003-1540-7041)
Competing interests	No potential conflict of interest relevant to this article was reported.
Funding sources State funding sources (grants, funding sources, equipment, and supplies). Include name and number of grant if available.	This work was supported by Korea Institute of Planning and Evaluation for Technology in Food, Agriculture and Forestry (IPET) through Livestock Industrialization Technology Development Program, funded by Ministry of Agriculture, Food and Rural Affairs (321083-5).
Acknowledgements	Not applicable.
Availability of data and material	The complete genome of *Corynebacterium* sp. SCR221107 were deposited in National Center for Biotechnology GenBank under accession number CP115670.
Authors' contributions Please specify the authors' role using this form.	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, 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).

CORRESPONDING AUTHOR CONTACT INFORMATION

For the corresponding author (responsible for correspondence, proofreading, and reprints)	**Fill in information in each box below**
First name, middle initial, last name	Sang-Suk Lee*
Email address – this is where your proofs will be sent	rumen@scnu.ac.kr
Secondary Email address	rumen@scnu.ac.kr
Address	Ruminant Nutrition and Anaerobe Laboratory, Department of Animal Science and Technology, Sunchon National University, Suncheon 57922, Republic of Korea
Cell phone number	+82-10-2776-1187
Office phone number	+82-61-750-3237
Fax number	+82-61-750-3237

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 B_{12} 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 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 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.

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* [13, 14]. 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.

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

References

1. Nouioui I, Carro L, García-López M, Meier-Kolthoff JP, Woyke T, Kyrpides NC, et al. Genome-based taxonomic classification of the Phylum *Actinobacteria*. Front Microbiol. 2018;9:02007. https://doi.org/10.3389/fmicb.2018.02007

2. Dobinson HC, Anderson TP, Chambers ST, Doogue MP, Seaward L, Werno AM. Antimicrobial treatment options for granulomatous mastitis caused by *Corynebacterium* species. J Clin Microbiol. 2015;53:2895-9. https://doi:10.1128/jcm.00760-15

3. Cheleuitte-Nieves C, Gulvik CA, McQuiston JR, Humrighouse BW, Bell ME, Villarma A, et al. Genotypic differences between strains of the opportunistic pathogen *Corynebacterium bovis* isolated from humans, cows, and rodents. PLoS One. 2018;13:e0209231. https://doi: 10.1371/journal.pone.0209231

4. Al-Dilaimi A, Albersmeier A, Kalinowski J, Ruckert C. Complete genome sequence of *Corynebacterium vitaeruminis* DSM 20294^{T}, isolated from the cow rumen as a vitamin B producer. J Biotechnol. 2014;189:70-1. https://doi.org/10.1016/j.jbiotec.2014.08.036

5. Colombo M, Castilho NPA, Todorov SD, Nero LA. Beneficial and safety properties of a *Corynebacterium vitaeruminis* strain isolated from the cow rumen. Probiotics Antimicrob Proteins. 2017;9:157-62. https://doi: 10.1007/s12602-017-9263-0

6. Bechdel SI, Honeywell HE, Dutcher RA, Knutsen MH. Synthesis of vitamin B in the rumen of the cow. J Biol Chem. 1928;80:231-8. https://doi.org/10.1016/S0021-9258(18)83923-0

7. Chaisson MJ, Tesler G. Mapping single molecule sequencing reads using basic local alignment with successive refinement (BLASR): application and theory. BMC Bioinformatics. 2012;13:238. https://doi: 10.1186/1471-2105-13-238

8. Walker BJ, Abeel T, Shea T, Priest M, Abouelliel A, Sakthikumar S, et al. Pilon: an integrated tool for comprehensive microbial variant detection and genome assembly improvement. PLOS ONE. 2014;9:e112963. https://10.1371/journal.pone.0112963

9. Seemann T. Prokka: rapid prokaryotic genome annotation. Bioinformatics. 2014;30:2068-9. https://doi.org/10.1093/bioinformatics/btu153

10. Grissa I, Vergnaud G, Pourcel C. CRISPRFinder: a web tool to identify clustered regularly interspaced short palindromic repeats. Nucleic Acids Res. 2007;35 Suppl 2:W52-7. https://doi.org/10.1093/nar/gkm360

11. Florensa AF, Kaas RS, Clausen PTLC, Aytan-Aktug D, Aarestrup FM. ResFinder - an open online resource for identification of antimicrobial resistance genes in next-generation sequencing data and prediction of phenotypes from genotypes. Microb Genom. 2022;8:000748. https://doi.org/10.1099/mgen.0.000748

13. Kang D, Shi B, Erfe MC, Craft N, Li H. Vitamin B12 modulates the transcriptome of the skin microbiota in acne pathogenesis. Sci Transl Med. 2015;7:293ra103. https://doi:10.1126/scitranslmed.aab2009.

14. Peinado R dos S, Olivier DS, Eberle RJ, de Moraes FR, Amaral MS, Arni RK et al. Binding studies of a putative *C. pseudotuberculosis* target protein from vitamin B_{12} metabolism. Sci Rep. 2019;9:6350. http://doi: 10.1038/s41598-019-42935-y

Tables and Figures

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.

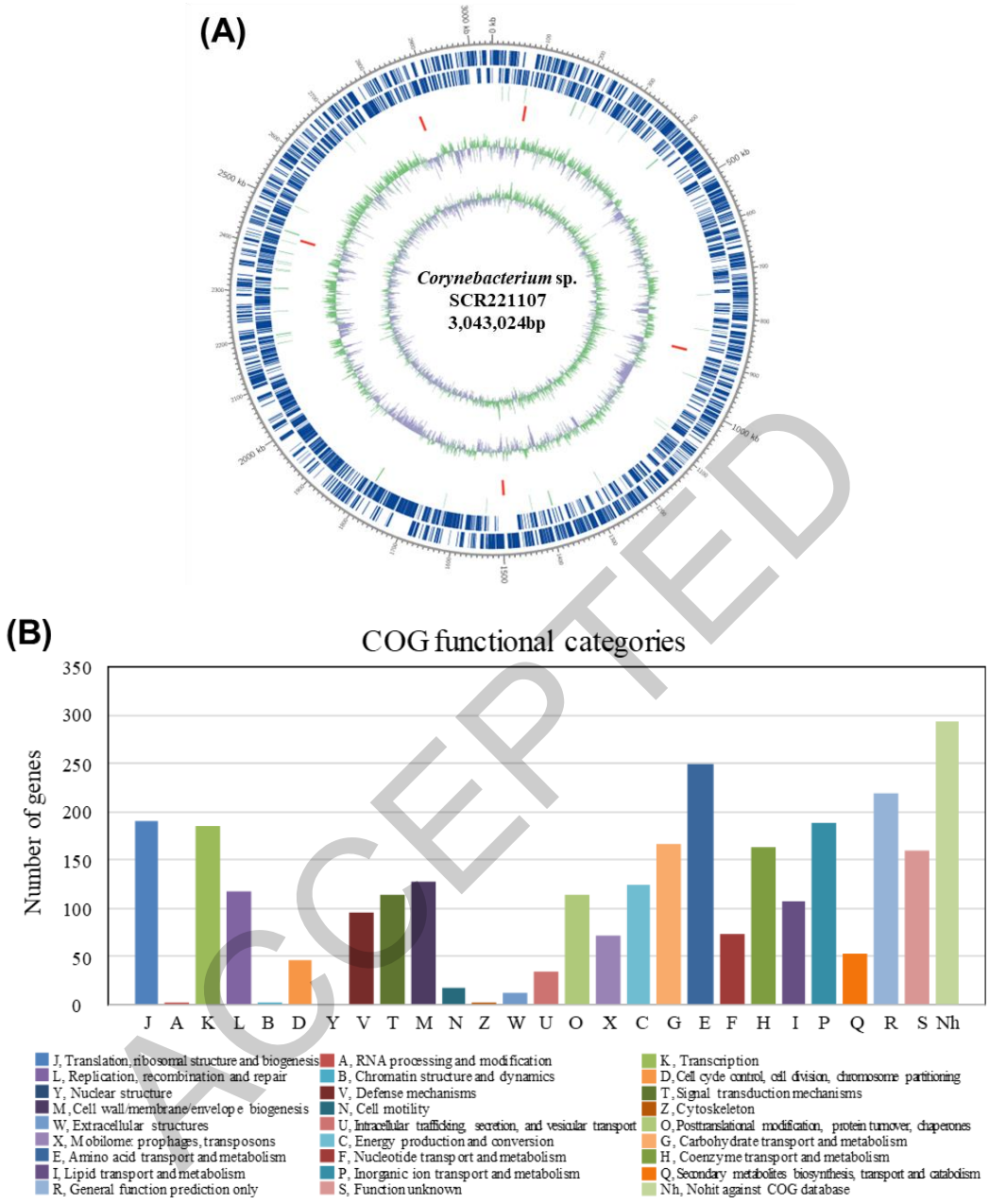

Figure 1. Genome map of *Corynebacterium* sp. SCR221107 (A) and the functional categorization of predicted coding sequences (B).

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.