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 (Genome announcement)
Article Title (within 20 words without abbreviations)	Complete genome sequence of *Treponema pedis* GNW45 isolated from dairy cattle with active bovine digital dermatitis in Korea
Running Title (within 10 words)	Complete genome sequence of *Treponema pedis* GNW45
Author	Hector Espiritu, Lovelia Mamuad, Edeneil Jerome Valete, Sang suk Lee, Yong il Cho
Affiliation	Department of Animal Science and Technology,
ORCID (for more information, please visit https://orcid.org)	Hector Espiritu (0000-0001-9051-1995) Lovelia Mamuad (0000-0002-1866-0897) Edeneil Jerome Valete (0000-0003-0885-0627) Myung hwan Jung (0000-0001-8124-8945) Sang suk Lee (0000-0003-1540-7041) Yong il Cho (0000-0001-7756-3416)
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 the National Research Foundation of Korea (NRF), Project No. 2021R1I1A3043691
Acknowledgements	Not applicable.
Availability of data and material	This Whole Genome Shotgun project for *Treponema pedis* GNW45 (KCTC 15381BP) has been deposited at DDBJ/ENA/GenBank under the accession CP061839.
Authors' contributions Please specify the authors' role using this form.	Conceptualization: Lee SS, Cho YI Data curation: Espiritu H Formal analysis: Espiritu H, Jung M Methodology: Espiritu H, Mamuad L, Valete EJ Software: Espiritu H, Jung M Validation: Espiritu H, Lee SS, Cho YI Investigation: Espiritu H, Mamuad L Writing - original draft: Espiritu H, Cho YI Writing - review & editing: Espiritu H, Mamuad L, Valete EJ, Jung M, Lee SS, Cho YI
Ethics approval and consent to participate	This article does not require IRB/IACUC approval because there are no human and animal participants.

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	Yong il Cho
Email address – this is where your proofs will be sent	ycho@scnu.ac.kr
Secondary Email address	
Address	Department of Animal Science and Technology, Sunchon National University, Suncheon, Jeonnam, 57922, Republic of Korea
Cell phone number	+8210-5589-2662
Office phone number	+82-61-750-3234
Fax number	+82-61-750-3234

Abstract

Treponema pedis (*T. pedis*), a fastidious anaerobic spirochete, is one of the main pathogens involved in the development and progression of bovine digital dermatitis (BDD), a lameness-causing hoof infection in cattle. Here, the complete genome sequencing of *T. pedis* GNW45 isolated from a dairy cow infected with BDD, was presented. Libraries for long and short reads were sequenced using PacBioRSII and Illimuna HiSeqXTen platforms, respectively. De-novo assembly was done using the long reads, producing a circular contig, by which the short reads were aligned to generate a more accurate genome sequence. The genome has a total size of 3,077,465 base pairs, with 36.84% guanine-cytosine content. A total of 2,749 protein-coding sequences, seven ribosomal RNA's, and 45 transfer RNA's were annotated. Functional analysis revealed genes associated with pathogenicity and survivability in the complex pathobiome of BDD. This study provided novel insights into the survival and pathogenic mechanisms of *T. pedis* GNW45.

Keywords: Bovine digital dermatitis, *Treponema pedis*, lameness, complete genome, dairy cattle

Bovine digital dermatitis (BDD) is a highly contagious disease affecting the hoof of cattle, characterized by the development of ulcerative lesions and chronic hyperkeratosis [1]. The disease causes significant lameness, resulting in substantial losses for both dairy and beef producers due to decreased efficiency in milk production and average daily gain [2]. BDD mainly affects the interdigital area of intensively managed cattle, especially high-yielding dairy cows, and spreads through direct contact and contaminated environments [3]. BDD is a polybacterial disease, and previous research showed that multiple *Treponema* species are involved, with *T. medium*, *T. pedis*, and *T. phagedenis* considered as the core species responsible for the development and progression of the disease [4]. The present study focused on the whole genome sequencing (WGS) of *T. pedis* GNW45 isolated from BDD in Korea. This analysis could provide insights into the virulence factors and pathogenic mechanisms underlying the disease.

Treponema pedis GNW45 was isolated from a Holstein-Friesian cow suffering from BDD in a farm in Hwaseong, Gyeonggi province. Under anaerobic conditions, GNW45 was isolated and purified following the protocol described in a previous study [5]. DNA of GNW45 was purified and was analyzed for quality inspection and WGS. Sequencing and assembly were performed as previously described [6]. In brief, a library for long-read sequencing was prepared using the PacBio 20 kb SMRTbell™ kit for the PacBio RSII platform (Pacific Biosciences, USA), generating 188,880 total subreads. Concurrently, a short-read library was prepared using the TruSeqNano DNA Kit for the Illumina HiSeqXTen platform,

which produced 8,990,700 filtered short reads. De-novo assembly was performed by mapping the PacBio RSII single-pass reads to seed reads using the Hierarchical Genome Assembly Process (HGAP3). A single circular contig was generated after utilizing the PacBio long reads. To improve accuracy and quality, sequence compensation and error correction were performed by aligning the Illumina reads to the pre-assembled genome using Pilon v1.21. Annotation was performed using Prokka v1.12b. Functional annotation of protein-coding sequences (CDS) was carried out using EggNOG v5.0 and mapped in circular presentation using Proksee server.

The genome of *T. pedis* GNW45 is 3,077,465 bp long, and is composed of 2,783 genes. The sequencing and annotation statistics is presented in Table 1, and the circular representation of the genome properties and the summary of functional annotation is shown in Figure 1A and 1B, respectively. The genus *Treponema* is composed of a diverse number of species from a variety of niches, and can play as either commensal or pathogen, or both [7]. Other studies have isolated *T. pedis* from infections in animals other than BDD, like necrotic skin ulcer and gingiva in pigs, and hoof canker in horses, demonstrating that it could thrive in an environment with a diverse microbial community [7], contributing to progression of polybacterial diseases. However, the role of this bacterium in the complex bacterial community of BDD is still not clear, hence we highlighted in this report some of the important properties of this bacterium that could potentially contribute to its pathogenicity.

The helical morphology of *T. pedis* is regulated by multiple flagellar-motility associated proteins, enabling its chemotactic response to specific chemical signals through various chemotaxis proteins. These mechanisms involve 58 genes for flagellar biosynthesis, 97 genes for chemotaxis, and four genes for motility. Furthermore, the presence of chemoreceptors, such as methyl-accepting chemotaxis proteins (MCPs), assists in bacterial adherence to the host, in conjunction with motility. Additionally, genes for fibronectin-binding protein (*yloA*), dentilisin (*prtP*, *prcA*, *prcB*), major surface protein (Msp), serine proteases (*htrA*, *clpP*), and internalins (*inlA*, *inlJ*), have been identified to have roles in adhesion, host invasion, and immune evasion.

T. pedis has genetic adaptations for metabolic competition, including 59 Rearrangement hotspots (RHS) and YD-repeat proteins that inhibit neighboring cell growth, enabling competitive exclusion. [8]. Beyond competitive adaptations, the BDD microbiome possesses genetic elements that may facilitate cooperative metabolic interactions with other microbes. Regulatory riboswitches and genes involved in Vitamin B12 and porphyrin biosynthesis (*cob*'s) and transport have been identified. Porphyrin has been demonstrated to induce a proinflammatory host response in other skin infections. [9]. Furthermore, the presence of a Type 2C CRISPR-Cas system has been detected, with the repeat region encompassing a total of 141 spacers, revealing its previous exposure to foreign genetic elements which may reflect the strain's environmental history and potential virulence [10].

The WGS of *T. pedis* GNW45 from BDD revealed genes for motility and chemotaxis, and competitive and cooperative interactions. It also possesses host adhesion-related genes, a CRISPR-Cas

system, several proteases, and porphyrin biosynthesis genes, suggesting its ability to adhere, invade, and modulate the inflammatory immune response of the host. These findings enhance our understanding of pathogenicity of *T. pedis* in BDD and its complex interactions within the microbial ecosystem, highlighting its virulence mechanisms for infection and colonization.

References

1. Mamuad LL, Joo B, Al S, Espiritu HM, Jeong S, Kim W, et al. *Treponema* spp ., the dominant pathogen in the lesion of bovine digital dermatitis and its characterization in dairy cattle. Vet Microbiol [Internet]. Elsevier; 2020;245:108696. Available from: https://doi.org/10.1016/j.vetmic.2020.108696

2. Espiritu HM, Mamuad LL, Kim S, Jin S, Lee S, Kwon S, et al. Microbiome Shift, Diversity, and Overabundance of Opportunistic Pathogens in Bovine Digital Dermatitis Revealed by 16S rRNA Amplicon Sequencing. Animals. 2020;10:1798.

3. Evans NJ, Murray RD, Carter SD. Bovine digital dermatitis: Current concepts from laboratory to farm. Vet J. 2016;211:3–13.

4. Staton GJ, Clegg SR, Ainsworth S, Armstrong S, Carter SD, Radford AD, et al. Dissecting the molecular diversity and commonality of bovine and human treponemes identifies key survival and adhesion mechanisms. PLoS Pathog [Internet]. Public Library of Science; 2021 [cited 2021 Jun 21];17:e1009464. Available from: https://doi.org/10.1371/journal.ppat.1009464

5. Espiritu HM, Mamuad LL, Jin S, Kim S, Kwon S, Lee S, et al. Genotypic and Phenotypic Characterization of *Treponema phagedenis* from Bovine Digital Dermatitis. Microorganisms. 2020;8:1520.

6. Espiritu HM, Mamuad LL, Jin S, Kim S, Lee S, Cho Y. High quality genome sequence of *Treponema phagedenis* KS1 isolated from bovine digital dermatitis. J Anim Sci Technol. 2020;62:948–51.

7. Svartström O, Mushtaq M, Pringle M, Segerman B. Genome-wide relatedness of *Treponema pedis*, from gingiva and necrotic skin lesions of pigs, with the human oral pathogen Treponema denticola. PLoS One. 2013;8.

8. Koskiniemi S, Lamoureux JG, Nikolakakis KC, De Roodenbeke CTK, Kaplan MD, Low DA, et al. Rhs proteins from diverse bacteria mediate intercellular competition. Proc Natl Acad Sci U S A [Internet]. National Academy of Sciences; 2013 [cited 2022 Oct 11];110:7032–7. Available from: https://www.pnas.org/doi/abs/10.1073/pnas.1300627110

9. 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 [Internet]. American Association for the Advancement of Science; 2015 [cited 2022 Oct 25];7. Available from: https://www.science.org/doi/10.1126/scitranslmed.aab2009

10. Louwen R, Staals RHJ, Endtz HP, van Baarlen P, van der Oost J. The Role of CRISPR-Cas Systems in Virulence of Pathogenic Bacteria. Microbiol Mol Biol Rev. 2014;78:74–88.

Tables and Figures

Table 1. General properties of the complete genome sequence of *Treponema pedis* GNW45.

Property	Value
Genome size (bp)	3,077,465
GC content (%)	36.84
Genes (Total)	2,783
CDSs (with protein)	2,749
rRNAs	3, 2, 2 (5S, 16S, 23S)
tRNA	45
ncRNA	4
CRISPR-Cas system	1 (Type 2C)
Pseudogene (CDS without protein)	120
Protein with COG (EggNOG DB) match	2,597

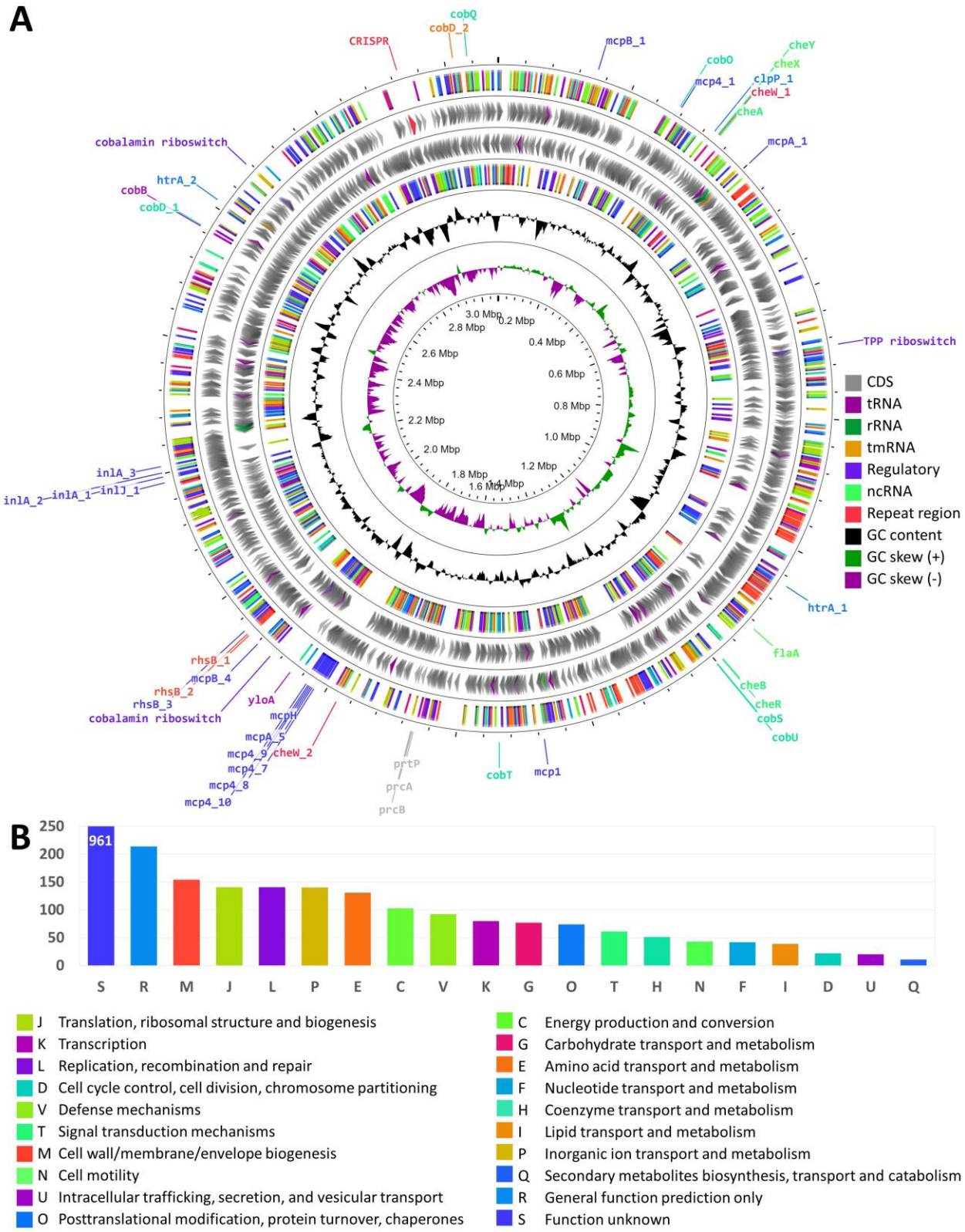

Figure 1. Structural and functional properties of the high-quality de-novo assembled genome of *Treponema pedis* GNW45 showing the genes highlighted in this study. Circular representation of the

assembled genome shown in (A) composed of six track rings. From the outside: the functional annotation of the coding sequences (CDS) based on the Cluster of Orthologous Genes (COG) on the forward strand. Second and third ring: structural annotation including CDS, transfer RNA, ribosomal RNA, transfer-messenger RNA, regulatory genes, non-coding RNA, and repeat region of the forward and the reverse strand, respectively. Fourth ring: the functional annotation of the CDS based on COG on the strand reverse strand. Fifth and sixth track ring shows the guanine-cytosine (GC) content and the GC skew, respectively. (B) represents the count of COGs based on their functional category, as mapped in (A).