



Streptococcus ruminicola sp. nov., new species of the *Streptococcus bovis*/*Streptococcus equinus* complex (SBSEC) isolated from the rumen of Korean domestic ruminants

Seon Young Park¹ · Neak Muhammad^{2,3} · Song-Gun Kim^{2,3} · Hyemin Kwon^{4,6} · Seongwon Seo¹ · Ji Hyung Kim⁵

Received: 11 August 2022 / Revised: 30 August 2022 / Accepted: 12 September 2022

© The Author(s), under exclusive licence to Springer-Verlag GmbH Germany, part of Springer Nature 2022

Abstract

A total of three Gram-positive, and oxidase and catalase-negative facultative anaerobic non-motile bacteria were isolated from the rumen fluid of cows and goats and these strains were designated CNU_G2^T, CNU_77-61, and CNU_G3. They grew at 20–45 °C, pH 6.5–7, and 0–6.5% NaCl (w/v). The G + C contents (%) of the three isolates were 37.9, 37.8 and 37.8, respectively. Phylogenomic analysis indicated that these strains were distinct from other *Streptococcus* species. The average nucleotide identity between the isolates and the closest strain *S. infantarius* subsp. *infantarius* ATCC BAA-102^T was 94.0–94.5%, while the digital DNA–DNA hybridization (dDDH) values between the isolates and the aforementioned related strain were 58.2–61.4%, respectively. Fatty acid analysis revealed higher proportions of C_{16:0} (> 28%) in all three isolates, while the proportion of C_{18:0} was higher in CNU_G2^T (25.8%); however, it was less than 12% in all the representing strains used in the study. The C_{14:0} composition of strains CNU_77-61 (22.1%) and CNU_G3 (24.1%) was higher than that of type strains of CNU_G2^T (8.1%). Based on the morphological, biochemical, and molecular phylogenetic features of the three novel isolates, they represent a novel species of the genus *Streptococcus*, for which we propose as *Streptococcus ruminicola* sp. nov. The type strain is CNU_G2^T (= KCTC 43308^T = GDMCC 1.2785^T).

Keywords SBSEC · Rumen fluid · South Korea · Novel species · Polyphasic taxonomy · Genome

Communicated by Erko Stackebrandt.

Seon Young Park and Neak Muhammad contributed equally to this work.

✉ Ji Hyung Kim
kzh81@gachon.ac.kr

Abbreviations

SBSEC	<i>Streptococcus bovis</i> / <i>Streptococcus equinus</i> Complex
BHIA	Brain heart infusion agar
ANI	Average nucleotide identity
GGDC	Genome-to-genome distance

Introduction

The genus *Streptococcus* encompass lactic acid-producing bacteria belonging to the order *Lactobacillales* and family *Streptococcaceae*. It is composed of 113 species and 31 subspecies (<https://psn.dsmz.de/genus/streptococcus>) (Parte et al. 2020). *Streptococcus bovis*/*Streptococcus equinus* complex (SBSEC) contains Gram-positive, non-enterococcal group D *Streptococcus* species that grow well under facultative anaerobic conditions and easily adapts to various environmental conditions (Nagaraja and Titgemeyer 2007) SBSEC is a group of commensal bacteria commonly found in the gastrointestinal tract (GI) of humans and animals, particularly ruminants, such as cows, goats, sheep, and camels (Jans et al. 2015). This complex

¹ Division of Animal and Dairy Sciences, College of Agriculture and Life Science, Chungnam National University, Daejeon 34134, Korea

² Biological Resource Center/Korean Collection for Type Cultures (KCTC), Korea Research Institute of Bioscience and Biotechnology, Jeonbuk 56212, Korea

³ University of Science and Technology (UST), Daejeon 34113, Korea

⁴ Infectious Disease Research Center, Korea Research Institute of Bioscience and Biotechnology, Daejeon 34141, Korea

⁵ Department of Food Science and Biotechnology, College of Bio-Nano Technology, Gachon University, Seongnam 13120, Korea

⁶ Department of Microbiology and Molecular Biology, College of Bioscience and Biotechnology, Chungnam National University, Daejeon 34134, Korea

can be divided into three biotypes based on their ability to ferment mannitol: mannitol-fermenting *Streptococci* (biotype I) non-mannitol-fermenting *Streptococci* (biotype II), while biotype II is further divided into two subtypes (II/1 and II/2) based on their ability to degrade bile-esculin and use it in subsequent biochemical reactions (Facklam 2002; Beck et al. 2008). The taxonomy of SBSEC has undergone several changes over the past 20 years in accordance with newly described species or subspecies that were originally grouped in the *S. bovis* family (Parker and Ball 1976; Jans et al. 2015; Dekker and Lau 2016). The classification of SBSEC is still undergoing revision and is often evaluated by biochemical tests such as the analytical profile index (API) system for species identification (Pompilio et al. 2019). However, the emergence of DNA-based identification methods has led to their widespread adoption in this field. Molecular methods, such as average nucleotide identity (ANI), digital DNA–DNA hybridization (dDDH) and 16S rRNA sequence analysis, have allowed for the reliable differentiation of subspecies within the SBSEC group (Schlegel et al. 2003). Partial *rpoB*, *sodA*, *groEL*, and *gyrB* gene sequencing within the SBSEC group has been described as the most reliable tool for the identification of seven species (or subspecies), including *S. equinus*, *S. lutetiensis* (previously *S. infantarius* subsp. *coli*), *S. infantarius* subsp. *infantarius*, *S. alactolyticus*, *S. gallolyticus* subsp. *pasteurianus*, *S. gallolyticus* subsp. *macedonicus*, and *S. gallolyticus* subsp. *gallolyticus* (Pompilio et al. 2019) making up these complexes. *S. bovis* and *S. equinus* are synonymized, indicating they are used to describe the same bacterial species (Herrera et al. 2009; Jans et al. 2015). Recently, proteomic-based matrix-assisted laser desorption ionization time-of-flight analysis (MALDI-TOF) and whole-genome sequencing have been applied to discriminate between clinical isolates and novel isolates belonging to the SBSEC group (Hinse et al. 2011; Pompilio et al. 2019). In our previous study (Park et al. 2021), several genomes of SBSEC strains (strain CNU_G2, CNU_77-61, and CNU_G3) isolated from domestic ruminants were clearly differentiated from the available genomes of the other type strains of SBSEC, although phylogenetic analysis of the *sodA* gene-based comparison indicated that the three isolates could be classified into two species (*S. equinus* and *S. lutetiensis*). In this study, we propose a novel species, named *Streptococcus ruminicola* sp. nov., which was isolated from Korean domestic ruminants, and characterized it based on a taxonomic approach including morphological, physiological, and genomic differences compared with type strains of SBSEC.

Materials and methods

Isolation and culture conditions

Three strains of *S. ruminicola* sp. nov. were isolated from rumen fluid samples collected from bovine (Hanwoo, *Bos taurus taurus*; CNU_G2^T and CNU_G3) and caprine (Korean native goat, *Capra aegagrus hircus*; CNU_77-61) individuals housed in the Center for Animal Science Research, Chungnam National University, Korea (36°22′41.4″ N 126°56′49.2″ E). The samples were collected using a rumen cannula and cultured on selective agar media as previously described (Park et al. 2021). Pure cultures of individual colonies and enrichment were performed using brain heart infusion (BHI) agar (BHIA; Difco) by incubation at 37 °C for 24 h under aerobic conditions. Frozen stocks of three isolates were stored in BHI broth (BHIB; Difco) supplemented with 10% glycerol at – 80 °C. All three isolates CNU_G2^T, CNU_77-61, and CNU_G3 were deposited in the Korean Collection for Type Cultures (KCTC 43,308, KCTC 43,306, and KCTC 43,307) and Guangdong Microbial Culture Collection Center (GDMCC 1.2785, GDMCC 1.2784, and GDMCC 1.2786).

Physiological and biochemical analysis

Morphological features of CNU_G2^T, CNU_77-61, and CNU_G3 were observed by Gram staining followed by light microscopy (DM2500, Leica, Germany) (Fig. S1). To determine the growth features, bacteria were cultured at 37 °C for 24 h under aerobic conditions in BHI broth and observed a range of eight temperatures (4, 10, 15, 20, 25, 37, 45, and 60 °C), pH (4.5, 5, 6, 6.5, 7, 7.5, 8, 9, and 10), and NaCl concentrations (eight concentrations, 0–7%, in increments of 0.5%) (w/v). All three isolates grew at 20–45 °C, at pH 6–9, and to tolerate the presence of 0–6.5% NaCl. Biochemical characterization was performed using the oxidase reagent, ID color catalase, and API 20 STREP (bioMérieux Inc., Marcy l'Étoile, France), according to the manufacturer's instructions. For comparison, the type strains of SBSEC were tested in parallel with isolates.

Chemotaxonomic analysis

For the analysis of cellular fatty acids, the isolates were cultured at 37 °C for 24 h under aerobic conditions in BHIB. To obtain optimal results, bacterial colonies were selected from the third quadrant in the late-log phase. The fatty acids of the strains were extracted in four steps: saponification, methylation, extraction, and base washing, according to the standard protocol in the Microbial Identification System

(MIDI, Hewlett-Packard, USA). The fatty acids of the isolates were analyzed using a gas chromatograph (6890 N with 7683 autosampler, Agilent Technologies Inc., CA, USA) and identified using the TSBA6 6.10 library (Sasser 1990). The cellular fatty acid analysis of strains CNU_G2^T, CNU_77-61, and CNU_G3 were compared with those of the type strains of SBSEC.

Whole-genome sequence analysis

The genomic DNA of the three isolates was extracted and sequenced using a hybrid approach with the HiSeq X-10 platform (Illumina, San Diego, CA, USA) and the PacBio RS II system (Pacific Biosciences, Menlo Park, CA, USA), as previously described (14 A phylogenetic tree based on the genome of three isolates and 16 *Streptococcus* type strains, including SBSEC available in the GenBank database, was constructed using the Type (Strain) Genome Server (TYGS; <https://tygs.dsmz.de/>) (Meier-Kolthoff et al. 2022). The tree was inferred using FastME (ver. 2.1.6.1) (Lefort et al. 2015) from the genome BLAST distance phylogeny (GBDP), and branch lengths were scaled in terms of the GBDP distance formula D4 (Meier-Kolthoff et al. 2013). The average nucleotide identity values between the three isolates and the type strains of SBSEC were calculated using the JSpecies software (<http://www.imedeia.uib.es/jspecies>) with default settings. The dDDH values were calculated using the Genome-to-Genome Distance Calculator using the recommended BLAST + alignment with distance formula 2 (ver. 3.0, <https://ggdc.dsmz.de/>) (Meier-Kolthoff et al. 2022). Heat maps based on the ANI and dDDH values were generated using R (ver. 4.1.2) with the package heatmap (Kolde R and Kolde MR 2015).

Phylogenetic analysis

Phylogenetic analyses of strains CNU_G2^T, CNU_77-61, and CNU_G3 were performed to discriminate the species of SBSEC based on the single genes: the 16S rRNA gene, *gyrB* (DNA gyrase subunit B), *groEL* (60 kDa heat-shock protein), and *rpoB* (DNA-directed RNA polymerase subunit beta) (Glazunova et al. 2009). The 16S rRNA and housekeeping gene sequences from the genomes of the strains CNU_G2^T, CNU_77-61, and CNU_G3 were compared to those of other *Streptococcus* type strains, including the SBSEC available in GenBank, using BLAST searches (www.ncbi.nlm.nih.gov/BLAST). Multiple sequence alignments for each strain were performed using Clustal X (ver. 2.1) (Larkin et al. 2007) and BioEdit (ver. 7.1.0.3) (Hall 1999). To construct phylogenetic trees, the datasets were phylogenetically analyzed using MEGA-X (ver. 10.0) (Kumar et al. 2018) with the maximum likelihood method. The genetic distances were calculated using the Jukes–Cantor model (Chor et al. 2006),

and the level of the clade was assessed by bootstrap analysis based on 1000 replications.

Results and discussion

Phenotypic characterization

All three isolates were Gram-stain positive, non-motile, oxidase-negative, catalase-negative, and grew at 20–45 °C, at pH 6–9, and to tolerate the presence of 0–6.5% NaCl. For comparison with the API 20 STREP system, the type strains of SBSEC were tested in parallel with isolates (Table 1). The three isolates were positive for leucine aminopeptidase activity and acidification of starch, glycogen, lactose, and raffinose, which demonstrates a difference from other previously reported SBSEC type strains. In particular, the three isolates could not hydrolyze esculin, which revealed discrimination with respect to the classification of SBSEC biotypes, as previously described (Facklam 2002; Beck et al. 2008).

Chemotaxonomy characterization

The major fatty acids (> 28%) in all three isolates and existing strains were C_{16:0}. The C_{14:0} compositions of strains CNU_77-61 (22.1%) and CNU_G3 (24.1%) were higher than those of type strains of CNU_G2^T (8.1%), whereas the proportion of C_{18:0} was only higher in CNU_G2^T (25.8%) and in *S. gallolyticus* subsp. *pastuerianus* NEM 1202^T (28.1%), but < 12% in all the remaining existing strains. The fatty acid profile essentially resembled that of the closely related *Streptococcus* type strains, while small differences were observed in the percentages of some components that are tabulated in (Table S1).

Genome characterization

The CNU_G2^T and CNU_G3 genomes respectively comprised 1,960,491 and 1,864,202 bp, with 37.9 and 37.8% G + C content, and both possessed a single plasmid (86,459 and 41,625 bp with 31.8 and 34.0%), encoding 1958 and 1790 CDS, 21 and 21 rRNAs, 70 and 69 tRNAs. The genome of strain CNU_77-61 size was 1,917,833 bp with 37.8% G + C content, consisting of 1815 protein-coding sequences (CDS), 21 rRNAs, and 70 tRNAs. The genomes of strains CNU_G2^T, CNU_77-61, and CNU_G3 were deposited in DDBJ/ENA/GenBank under accession numbers listed in Table S2.

Phylogenetic characterization

The taxonomic position, based on the genomes of the novel strains CNU_G2^T, CNU_77-61, and CNU_G3, indicated

Table 1 Differential phenotype characteristics of strains CNU_G2^T, CNU_77-61, CNU_G3, and the type strains of SBSEC

Phenotype characteristic	Strains										
	1	2	3	4	5	6	7	8	9	10	11
Temperature range (°C)	20–45	20–45	20–45	20–45	20–45	20–45	20–45	20–45	20–45	20–45	30–45
pH range	6–9	6–9	6–9	5–9	6–9	5–9	6–9	5–9	5–9	5–9	5–9
pH optimum	6.5	7	6.5	7	6.5	7	6.5	6.5	6.5	6.5	7
Salt tolerance%	0–6.5	0–6.5	0–6.5	0–4	0–4	0–6.5	0–6.5	0–6.5	0–6.5	0–6.5	0–4
Enzyme activity											
Esculin	–	–	–	–	+	–	+	+	+	–	+
α-galactosidase	–	–	–	+	–	–	–	–	–	–	+
β-galactosidase	–	–	–	–	–	–	–	+	–	+	–
Leucine aminopeptidase	+	+	+	+	–	–	+	+	–	–	+
Acidification											
Starch	+	+	+	+	–	+	+	–	+	–	–
Glycogen	+	+	+	–	–	–	+	–	+	–	–
Inulin	–	–	–	–	–	–	+	–	–	–	–
Lactose	+	+	+	+	–	+	+	+	+	+	–
Mannitol	–	–	–	–	–	–	–	–	+	–	+
Raffinose	+	+	+	+	–	+	+	+	+	+	+
Trehalose	+	–	+	–	+	–	–	+	+	–	–

Strains: 1 CNU_G2^T, 2 CNU_77-61, 3 CNU_G3, 4 *S. lutetiensis* NCTC 13,774^T (Poyart et al. 2002), 5 *S. infantarius* subsp. *infantarius* ATCC BAA-102^T, 6 *S. bovis* ATCC 33317^T (Schlegel et al. 2000), 7 *S. equinus* ATCC 9812^T, 8 *S. gallolyticus* subsp. *pastuerianus* NEM 1202^T (Poyart et al. 2002), 9 *S. gallolyticus* subsp. *gallolyticus* DSM 16831^T (Schlegel et al. 2003), 10 *S. gallolyticus* subsp. *macedonicus* DSM 15879^T (Tsakalidou et al. 1998), 11 *S. alactolyticus* ATCC 43,077^T, + positive, – negative, ND no data available. According to API 20 STREP test, all strains were positive for the following: Gram-stain and acetoin production, also all strains were negative for the following: hydrolysis of hippuric acid, pyrrolidonyl arylamidase, β-glucuronidase, alkaline phosphatase, arginine dihydrolase, ribose acidification, arabinose acidification and sorbitol acidification

that they were closely related to *S. infantarius* subsp. *infantarius* ATCC BAA-102^T among SBSEC but separated from other SBSEC clades (Fig. 1). In ANI analysis, the genomic similarity of strain CNU_G2^T was demonstrated to be highly similar to those of CNU_77-61 and CNU_G3, with 96.8 and 96.7%, and these values were separated from *S. lutetiensis* NCTC 13774^T and *S. infantarius* subsp. *infantarius* ATCC BAA-102^T with 93.1 and 94.0% (Fig. 2a). Based on the dDDH analysis, the three isolates were clustered together, *S. lutetiensis* NCTC 13774^T and *S. infantarius* subsp. *infantarius* ATCC BAA-102^T but indicated low similarity values of 52.2–61.4%, which confirmed the results of ANI (Fig. 2b). For species delineation, the proposed cutoff thresholds of ANI and dDDH values were 95 and 70%, respectively (Richter and Rosselló-Móra 2009; Meier-Kolthoff et al. 2013). Thus, the values of ANI and dDDH of the isolated strains were below the standard cutoff values, further indicating that the isolates represent a novel *Streptococcus* species.

The nucleotide similarity of the 16S rRNA sequences revealed that each strain in this study was highly similar (> 99%) to the *S. infantarius* subsp. *infantarius* ATCC BAA-102^T, *S. lutetiensis* NCTC 13774^T, *S. bovis* ATCC 33317^T, and *S. equinus* ATCC 9812^T (Table S2), which clustered with the result of the phylogenetic tree (Fig. S2). The 16S rRNA

sequence comparison was unusable for discrimination between closely related species (or subspecies), as previously reported (Herrera et al. 2009; Jans et al. 2015). Thus, phylogenetic analyses, based on the four housekeeping genes, were performed to clarify the taxonomic assessment of SBSEC and the three isolates (Fig. S2). The *groEL*, *rpoB*, and *gyrB* gene trees indicated that these novel strains were clustered together with *S. infantarius* subsp. *infantarius* ATCC BAA-102^T but clearly separated from the other SBSEC branches. Additionally, multi-gene phylogenetic analysis was performed based on a concatenated alignment of *groEL*, *gyrB*, and *rpoB* gene sequences from SBSEC, which supports more robust relationships for classification and identification than can be obtained using a single gene sequence (Fig. S3). The three strains were phylogenetically closest to *S. infantarius* subsp. *infantarius* ATCC BAA-102^T, each of which clustered in substantially distinct clades, consistent based on the genome phylogenetic analysis.

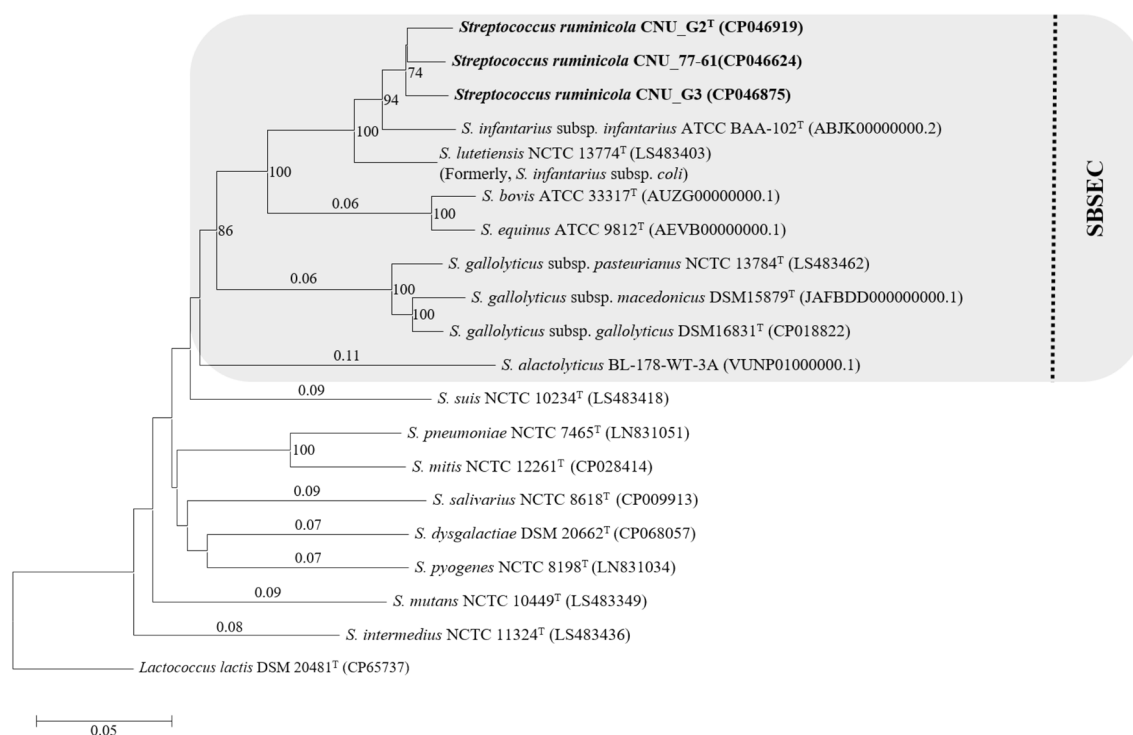


Fig. 1 Phylogenetic tree based on genome sequences of *Streptococcus ruminicola* CNU_G2^T, CNU_77-61, CNU_G3, and sixteen representative type strains of *Streptococcus* species and subspecies including SBSEC by Type (Strain) Genome Server (TYGS). The tree was constructed using the Genome-BLAST Distance Phylogeny (GBDP)

with branch support of formulas D4, inferred from sum of all identities found in high-scoring pairs (HSPs) divided by total genome length. The three representative genomes used in this study are emphasized in bold

Conclusion

Based on the results of phenotypic, phylogenetic, and genotypic analyses, strains CNU_G2^T, CNU_77-61, and CNU_G3 represent a novel species in the genus *Streptococcus*, for which the name *Streptococcus ruminicola* sp. nov. were proposed. The type strain used was CNU_G2^T (= KCTC 43308^T = GDMCC 1.2785^T).

Description of *Streptococcus ruminicola* sp. nov.

Streptococcus ruminicola [ru.mi.ni'co.la. L. neut. n. *rumen* (*gen. ruminis*), first stomach of ruminants, rumen; L. masc./fem. n. suff. *-cola*, inhabitant, dweller; from L. masc./fem. n. *incola*, dweller; N.L. masc./fem. n. *ruminicola*, inhabitant of the rumen].

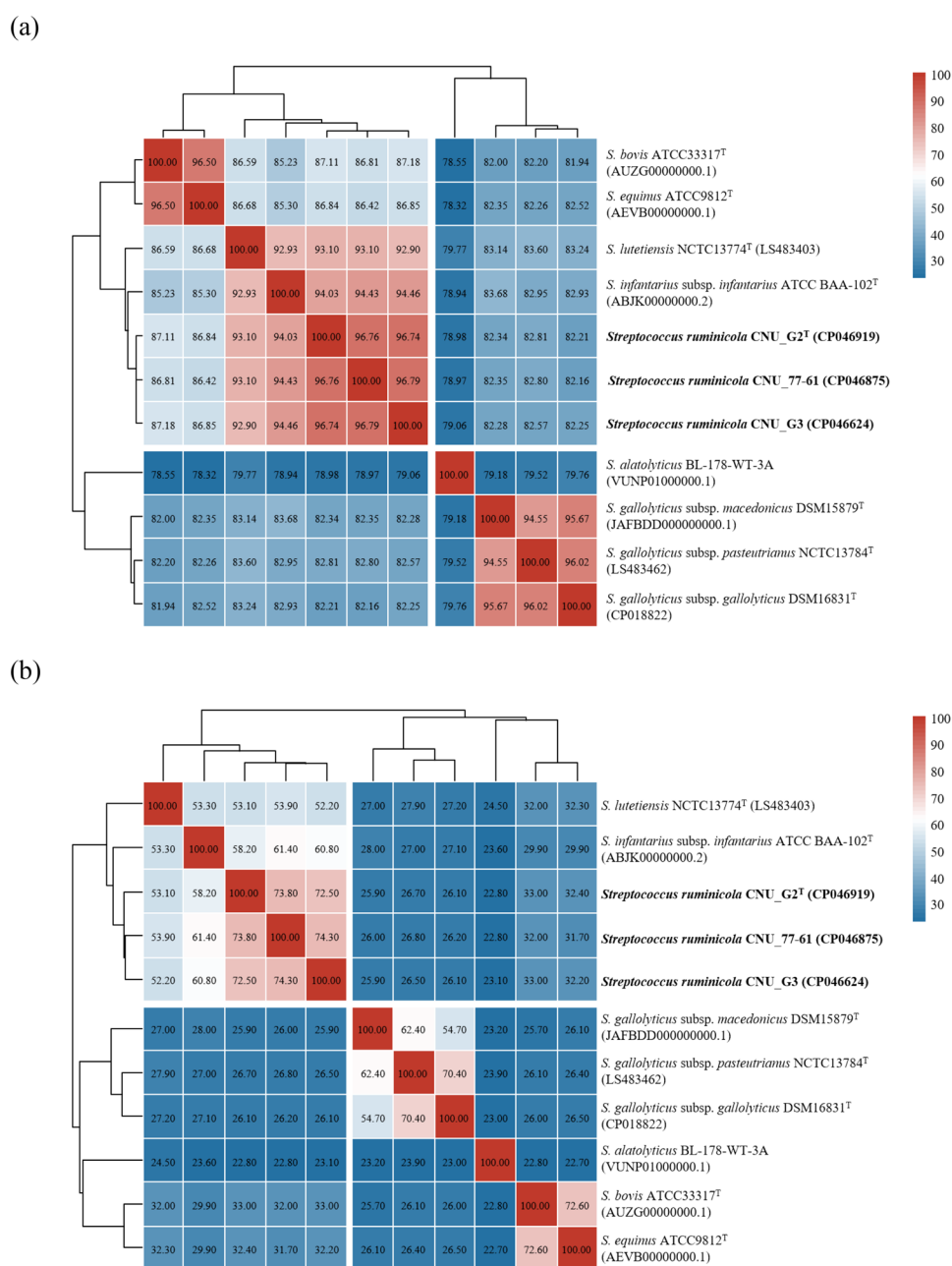
Cells are Gram-positive, non-motile, oxidase- and catalase-negative, and grow facultative anaerobic condition on BHIA for 24 h at 37 °C. Growth can be observed at 20–45 °C in BHI broth, at pH 6–9 (optimum pH 6.5–7.0), and 0–6.5% (w/v) of NaCl. In the API 20 STREP test, cells are positive for acetoin production (Voges–Proskauer test) and enzymes β-glucosidase and leucine arylamidase, but negative for hippuric acid production and the

enzymes alkaline phosphatase, arginine dihydrolase, α-galactosidase, β-glucuronidase, β-galactosidase, and pyrrolidonyl arylamidase. Cells produce acids from glycogen, lactose, raffinose, starch, and trehalose, whereas no acids are produced from L-arabinose, D-mannitol, inulin, D-ribose, and D-sorbitol. The predominant cellular fatty acids are C_{16:0} and C_{18:0}. The genomic DNA G + C content is 37.9%.

The type strain CNU_G2^T (= KCTC 43308^T = GDMCC 1.2785^T) was isolated from the rumen fluid of native Korean cattle (Hanwoo, *Bos taurus taurus*) reared at the Center for Animal Science Research, Chungnam National University, Korea. The GenBank/EMBL/DDBJ accession numbers for the 16S rRNA gene and whole-genome sequence of the strain CNU_G2^T is MN075410 and CP046919, respectively.

Additional strains are CNU_77-61 (= KCTC 43,306 = GDMCC 1.2784) and CNU_G3 (= KCTC 43,307 = GDMCC 1.2786) which are different from the type strain in the following properties: acid production from trehalose, fatty acids composition, and genome size. The 16S rRNA gene of the CNU_77-61 and CNU_G3 were deposited in the GenBank under the MN075482 and MN075411, and those for the whole-genome were CP046875 and CP046624, respectively.

Fig. 2 Heat maps representing the degree of similarity of *Streptococcus ruminicola* CNU_G2^T, CNU_77-61, CNU_G3, and the SBSEC genome based on **a** the average nucleotide identity and **b** digital DNA–DNA hybridization (dDDH). The three representative genomes used in this study are emphasized in bold. Color intensity indicates ANI and dDDH values. Squares corresponding to values of less than 95 and 70% are colored in blue and values of more than 95 and 70% are indicated with red squares



Supplementary Information The online version contains supplementary material available at <https://doi.org/10.1007/s00203-022-03255-4>.

Author contributions Conceptualization, SYP, SS and JHK; data curation, SYP, NM and HK; formal analysis, SYP, NM and HK; funding acquisition, SGK. and JHK; investigation, SYP and SGK; methodology, SYP, NM, and HK; project administration, JHK; resources, SYP and SS; supervision, SS and JHK; writing-original draft, SYP, NM, and JHK; and writing-review and editing, SYP, SGK and JHK. All authors have read and agreed to the published version of the manuscript.

Funding This work was supported by the Korea Research Institute of Bioscience and Biotechnology (KRIBB) Research Initiative Program (KGM5232221), the National Research Foundation of Korea

(NRF-2020R111A2068827), and the Development of technology for biomaterialization of marine fisheries byproducts of Korea Institute of Marine Science & Technology Promotion (KIMST) funded by the Ministry of Oceans and Fisheries (KIMST-20220128).

Declarations

Conflict of interest The author(s) declare that there are no conflict of interest.

Ethical approval This study was conducted at the Center for Animal Science Research, Chungnam National University, Korea. The animal use and protocols for this experiment were reviewed and approved by the Chungnam National University Animal Research Ethics Commit-

tee (IACUC approval Nos. CNU-00455 (April, 2014) and CNU-01021 (April, 2018)).

References

- Beck M, Frodl R, Funke G (2008) Comprehensive study of strains previously designated *Streptococcus bovis* consecutively isolated from human blood cultures and emended description of *Streptococcus gallolyticus* and *Streptococcus infantarius* subsp. *coli*. J Clin Microbiol 46:2966–2972. <https://doi.org/10.1128/JCM.00078-08>
- Benahmed FH et al (2014) Draft genome sequences of *Streptococcus bovis* strains ATCC 33317 and JB1. Genome Announc 2:e01012-01014. <https://doi.org/10.1128/genomeA.01012-01014>
- Chor B, Hendy MD, Snir S (2006) Maximum likelihood Jukes–Cantor triplets: analytic solutions. Mol Biol Evol 23:626–632. <https://doi.org/10.1093/molbev/msj069>
- Dekker JP, Lau AF (2016) An update on the *Streptococcus bovis* group: classification, identification, and disease associations. J Clin Microbiol 54:1694–1699. <https://doi.org/10.1128/JCM.02977-15>
- Facklam R (2002) What happened to the streptococci: overview of taxonomic and nomenclature changes. Clin Microbiol Rev 15:613–630. <https://doi.org/10.1128/CMR.15.4.613-630.2002>
- Glazunova OO, Raoult D, Roux V (2009) Partial sequence comparison of the *rpoB*, *sodA*, *groEL* and *gyrB* genes within the genus *Streptococcus*. Int J Syst Evol Microbiol 59:2317–2322. <https://doi.org/10.1099/ijs.0.005488-0>
- Hall T (1999) BioEdit: a user-friendly biological sequence alignment editor and analysis program for Windows 95/98/NT. Nucleic Acids Symp 95–98
- Herrera P, Kwon YM, Riche SC (2009) Ecology and pathogenicity of gastrointestinal *Streptococcus bovis*. Anaerobe 15:44–54. <https://doi.org/10.1016/j.anaerobe.2008.11.003>
- Hinse D et al (2011) Differentiation of species of the *Streptococcus bovis/equinus*-complex by MALDI-TOF mass spectrometry in comparison to *sodA* sequence analyses. Syst Appl Microbiol 34:52–57. <https://doi.org/10.1016/j.syapm.2010.11.010>
- Jans C, Meile L, Lacroix C, Stevens MJ (2015) Genomics, evolution, and molecular epidemiology of the *Streptococcus bovis/Streptococcus equinus* complex (SBSEC). Infect Genet Evol 33:419–436. <https://doi.org/10.1016/j.meegid.2014.09.017>
- Jin D et al (2013) Dynamics of fecal microbial communities in children with diarrhea of unknown etiology and genomic analysis of associated *Streptococcus lutetiensis*. BMC Microbiol 13:1–12. <https://doi.org/10.1186/1471-2180-13-141>
- Kolde R, Kolde MR (2015) Package ‘pheatmap.’ R Package 1:790
- Kumar S, Stecher G, Li M, Knyaz C, Tamura K (2018) MEGA X: molecular evolutionary genetics analysis across computing platforms. Mol Biol Evol 35:1547. <https://doi.org/10.1093/molbev/msy096>
- Larkin MA et al (2007) Clustal W and Clustal X version 2.0. Bioinformatics 23:2947–2948. <https://doi.org/10.1093/bioinformatics/btm404>
- Lefort V, Desper R, Gascuel O (2015) FastME 2.0: a comprehensive, accurate, and fast distance-based phylogeny inference program. Mol Biol Evol 32:2798–2800. <https://doi.org/10.1093/molbev/msv150>
- Meier-Kolthoff JP, Auch AF, Klenk H-P, Göker M (2013) Genome sequence-based species delimitation with confidence intervals and improved distance functions. BMC Bioinformatics 14:1–14. <https://doi.org/10.1186/1471-2105-14-60>
- Meier-Kolthoff JP, Carbasse JS, Peinado-Olarte RL, Göker M (2022) TYGS and LPSN: a database tandem for fast and reliable genome-based classification and nomenclature of prokaryotes. Nucleic Acids Res 50:D801–D807. <https://doi.org/10.1093/nar/gkab902>
- Nagaraja T, Titgemeyer E (2007) Ruminal acidosis in beef cattle: the current microbiological and nutritional outlook. J Dairy Sci 90:E17–E38
- Park SY et al (2021) Diversity and antimicrobial resistance in the *Streptococcus bovis/Streptococcus equinus* complex (SBSEC) isolated from Korean domestic ruminants. Microorganisms 9:98. <https://doi.org/10.7845/kjm.2020.0048>
- Parker MT, Ball LC (1976) Streptococci and aerococci associated with systemic infection in man. J Med Microbiol 9:275–302. <https://doi.org/10.1099/00222615-9-3-275>
- Parte AC, Carbasse JS, Meier-Kolthoff JP, Reimer LC, Göker M (2020) List of prokaryotic names with standing in nomenclature (LPSN) moves to the DSMZ. International J Dairy Sci 70:5607. <https://doi.org/10.1099/ijsem.0.004332>
- Pompilio A, Di Bonaventura G, Gherardi G (2019) An overview on *Streptococcus bovis/Streptococcus equinus* complex isolates: identification to the species/subspecies level and antibiotic resistance. Int J Mol Sci 20:480. <https://doi.org/10.3390/ijms20030480>
- Poyart C, Quesne G, Trieu-Cuot P (2002) Taxonomic dissection of the *Streptococcus bovis* group by analysis of manganese-dependent superoxide dismutase gene (*sodA*) sequences: reclassification of *Streptococcus infantarius* subsp. *coli* as *Streptococcus lutetiensis* sp. nov. and of *Streptococcus bovis* biotype II.2 as *Streptococcus pasteurianus* sp. nov. Int J Syst Evol Microbiol 52:1247–1255. <https://doi.org/10.1099/00207713-52-4-1247>
- Richter M, Rosselló-Móra R (2009) Shifting the genomic gold standard for the prokaryotic species definition. Proc Natl Acad Sci U S A 106:19126–19131. <https://doi.org/10.1073/pnas.0906412106>
- Sasser M (1990) Identification of bacteria by gas chromatography of cellular fatty acids. MIDI Technical Note MIDI Inc, Newark
- Schlegel L, Grimont F, Collins MD, Regnault B, Grimont P, Bouvet A (2000) *Streptococcus infantarius* sp. nov., *Streptococcus infantarius* subsp. *infantarius* subsp. nov. and *Streptococcus infantarius* subsp. *coli* subsp. nov., isolated from humans and food. Schlegel 50:1425–1434. <https://doi.org/10.1099/00207713-50-4-1425>
- Schlegel L, Grimont F, Ageron E, Grimont PA, Bouvet A (2003) Reappraisal of the taxonomy of the *Streptococcus bovis/Streptococcus equinus* complex and related species: description of *Streptococcus gallolyticus* subsp. *gallolyticus* subsp. nov., *S. gallolyticus* subsp. *macedonicus* subsp. nov. and *S. gallolyticus* subsp. *pasteurianus* subsp. nov. Int J Syst Evol Microbiol 53:631–645. <https://doi.org/10.1099/ijs.0.02361-0>
- Tsakalidou E et al (1998) Identification of streptococci from Greek Kasser cheese and description of *Streptococcus macedonicus* sp. nov. J Syst Evol Microbiol 48:519–527. <https://doi.org/10.1099/00207713-48-2-519>

Publisher's Note Springer Nature remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.

Springer Nature or its licensor holds exclusive rights to this article under a publishing agreement with the author(s) or other rightsholder(s); author self-archiving of the accepted manuscript version of this article is solely governed by the terms of such publishing agreement and applicable law.