Streptococcus xiaochunlingii sp. nov. E24 Isolated From the Oropharynx of Healthy Chinese Children

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A Gram-positive, α-hemolytic, catalase-negative, facultative anaerobic and non-motile coccus was isolated from throat swabs taken from the oropharynx of healthy children. The genome was shown to be 1,950,659 bp long and contained 42.03 mol% G + C bases with 1,942 protein-coding and 53 RNA coding genes. The results of 16S rRNA gene sequencing strongly suggested that the strain is a member of the Streptococcus genus, with 98.04, 98.11, and 97.34% similarities to Streptococcus australis ATCC 700641T, Streptococcus rubneri LMG 27207T and Streptococcus parasanguinis ATCC 15912T, respectively. A sodA gene comparison exhibited a sequence identity of 92.6% with the closest strain Streptococcus australis ATCC 700641T. In silico DNA-DNA hybridization showed a highest DNA similarity value of 52% with Streptococcus australis ATCC 700641T. Comparing 18 biochemical traits, the similarities of the Streptococcus strain E24 were 72% with Streptococcus rubneri LMG 27207T, 78% with Streptococcus australis ATCC 700641T and 44% with Streptococcus parasanguinis ATCC 15912T. We suggest that based on the genotypic and phenotypic results that the strain E24 is a novel species of the Streptococcus genus and propose the name Streptococcus xiaochunlingii sp. nov. E24.

Keywords: Streptococcus, phenotypic analyses, 16S rRNA, novel species, genome sequencing

INTRODUCTION

The microbiota of the upper respiratory tract has been denoted as gatekeepers to respiratory health, since it prevent pathogens from overgrowing and disseminating toward the lungs (Man et al., 2017; Tsang et al., 2019). In the first week after birth, the upper respiratory microbiota of infants develop from a mixed toward a Streptococcus viridans-predominated bacterial community, followed by niche differentiation to Staphylococcus aureus and other bacteria frequently leading to a composition of Moraxella spp., Dolosigranulum spp., and Streptococcus spp. (Bosch et al., 2016). In a previous study, we isolated Streptococcus strain E24 as one of 4 antagonistic strains of infants develop from a mixed toward a Streptococcus viridans-predominated bacterial community, followed by niche differentiation to Staphylococcus aureus and other bacteria frequently leading to a composition of Moraxella spp., Dolosigranulum spp., and Streptococcus spp. (Bosch et al., 2016). In a previous study, we isolated Streptococcus strain E24 as one of 4 antagonistic strains which had bacteriostatic effects on Klebsiella pneumoniae, Proteus vulgaris, Enterobacter cloacae, and Acinetobacter Baumannii (Li et al., 2019).

In recent decades, due to improved phenotypic and molecular identification methods and with the development of the new taxonogenomics concept (Fournier et al., 2015), the genus Streptococcus has been significantly expanded with the addition of many newly discovered species (Huch et al., 2013; Ricaboni et al., 2017); more than 110 species are now recognized within the
genus *Streptococcus*. With 16S rRNA gene sequence analyses revealed that *Streptococcus* species were clustered into six groups comprising *S. anginosus*, *S. mitis*, *S. mutans*, *S. bovis*, *S. pyogenes*, and *S. salivarius* (Kawamura et al., 1995). Here we present the results of taxonogenomics concept analyses of a novel strain termed *Streptococcus* strain E24.

**MATERIALS AND METHODS**

**Collection of Bacterial Samples**

Bacterial colonies from throat swabs of healthy children were cultivated aerobically for 24 h at 37°C on agar plates enriched with sheep blood (5%) (Becton Dickinson, Heidelberg, Germany). During streptococci isolation a single, circular, unpigmented and α-hemolytic colony of 0.5–1.0 mm diameter emerged, which was purified by repeated streaking and finally stored at −80°C. The study was conducted in accordance with the “Declaration of Helsinki” guidelines and approved by the Ethics Committee of Shenyang Medical College (approval number: No. 2015052902). Written informed consent was obtained from the legal representatives of the participating children.

**16S rRNA sodA Gene Analyses**

The nucleic acids of the strain were extracted for gene amplification and cloning of the 16S rRNA gene was accomplished by using a commercial DNA Purification Kit (Wizard Genomic DNA Purification Kit, Promega, Madison, WI, United States) and the 16S rRNA universal primers 27F/1492R (5′-AGAGTTTGATCMTGGCTCAG-3′ and 5′-GGYTACCTTGTTACGACTT-3′, respectively). The sequence obtained by sequencing was aligned using Basic Local Alignment Search Tool (BLAST) of NCBI. We collected 16S rRNA gene sequences of all known *Streptococcus* species from the NCBI database (Pontigo et al., 2015). Phylogenetic trees were constructed with two different algorithms, namely neighbor-joining (Saitou and Nei, 1987) and maximum-likelihood (Guindon and Gascuel, 2003) by close-neighbor-interchange (CNI) (search level = 2, random additions = 100) using MEGA version 7.0 (Kumar et al., 2016) and the reliability of the nodes was estimated by bootstrap analysis (1000 replications) (Felsenstein, 1985). Additionally, we analyzed the sodA gene since it has been proposed for phylogenetic characterization especially for differentiating streptococci (Poyart et al., 1998) with the primers forward: 5′-CCITAYCITAYGAGCIYTIGARCC-3′ and reverse: 5′-ARRTARTAIGCRTGYTCCCAIACRTC-3′ leading to a 430 bp fragment.

**Biochemical and Phenotypic Characterizations**

Isolate growths were examined in aerobic, anaerobic and microaerophilic atmospheres, with or without 5% CO2 and at 4, 15, 22, 30, 35, 37 and 42°C using nutrient agar containing sheep blood (5%, Solarbio, Beijing, China) (Facklam and Elliott, 1995). The nutrient agar consisted of peptone 10.0 g/L, beef powder 3.0 g/L sodium chloride 5.0 g/L, agar 15.0 g/L with pH of 7.3 ± 0.1 (Qingdao Hope Bio-Technology Co., Ltd., Qingdao, China). Cell motility and chain building was evaluated with the aid of a Leica DM1000 light microscope (Leica Microsystems, France). Specimens were also examined after the classical Gram staining (Austrian, 1960). The susceptibility of each isolates to salt was tested by growing the strains in the presence of 2.5, 3.5, 4.5 or 6.5 g/L NaCl. Moreover, nine different pHs were tested: 4.0, 5.0, 5.5, 6.0, 6.5, 7.0, 7.5, 8.0, and 9.0 (Supplementary Table 1).

Cell sizes were calculated from images captured on a scanning electron microscope. Bacteria were fixed in glutaraldehyde phosphate (2.5%) buffer overnight at 4°C. They were then washed twice with phosphate buffer (PBS), fixed for 30 min in a 1% osmium tetroxide solution (SPI supplies Inc., West Chester, PA, United States) and then washed 3 times with PBS before being dehydrated with 50, 70, 80, 90, 95 (twice), and 100% ethanol (three times), respectively (5 min per ethanol concentration). After natural drying, each sample was subjected to ion sputtering and then the gold was sprayed onto the surface and then viewed on a Hitachi 3400N scanning electron microscope (Hitachi High Technologies, Japan).

Sporulation was tested with *Streptococcus* strain E24 solutions (concentration: 1 × 10^6 cfu/mL) divided into two groups, from which one group was heated to 80–90°C for 20 min and the other served as control. Then 50 µl solutions of each group were applied on nutrient agar plates containing 5% sheep blood. The strains were incubated under normal conditions at 37°C overnight to observe the bacterial colony formation. No colony growth was observed on the plate of the heat treatment group on the next day.

**Biochemical Assays**

Isolates were characterized with Rapid ID32 Strep, API ZYM and API 50CH systems (bioMérieux, Marcy-l’Étoile, France) as well as catalase (Becton Dickinson, Franklin Lakes, NJ, United States) and oxidase (Qingdao Hope Bio-Technology Co., Ltd., Qingdao, China) assays carried out separately.

**Susceptibility to Antibiotics**

The antibiotic susceptibility of the strain *Streptococcus* strain E24 was determined using disk diffusion (Le Page et al., 2015) on Mueller-Hinton E agar (bioMérieux, Marcy-l’Étoile, France) consisting of casamino acid 17.5 g/L, beef extract 2.0 g/L, soluble starch 1.5 g/L Agar 17.0 g/L at pH 7.2 ± 0.2 (25°C) (Qingdao Hope Bio-Technology Co., Ltd., Qingdao, China). The antimicrobial agents tested were: vancomycin, ceftriaxone, cefepime, cefotaxime, Rina Thiazole Amine and chloramphenicol (all at 30 µg); ampicillin and penicillin (10 µg); and clindamycin (2 µg).

**Analysis of Fatty Acids in Bacteria**

Fatty acid methyl esters (FAMEs) were analyzed using a gas chromatography/mass spectrometry [see (Dione et al., 2016) and (Sasser, 1990)]. FAMEs were prepared from a sample consisting of 40 mg of bacterial biomass, separated using an MS column.
(Elite 5) and examined with a mass spectrometry (Clarus 500-SQ 8 S, PerkinElmer, France) followed by appropriate searches of spectral databases.

**Extraction of DNA and Sequencing of the Genome**

Genomic DNA was extracted using the SDS methodology (Lim et al., 2016). Agarose gel electrophoresis (HE-120, Tanon Science & Technology Inc., Shanghai, China) was used to detect the DNA, which was analyzed and quantified with a Qubit 2.0 Fluorometer (Life Technologies, Carlsbad, CA, United States). 1 μg samples were used as preparations of DNA and sequence libraries were constructed using a NEBNext Ultra DNA Library Prep Kit for Illumina (New England Biolabs Inc., Ipswich, MA, United States), with appropriate index codes. Samples were sonicated to sizes of about 350 bp and fragments end-polished, A-tailed and ligated for subsequent amplification by PCR. The PCR products were purified and libraries evaluated to determine the distribution of sizes using real-time PCR (Agilent 2100 Bioanalyzer, Agilent Technologies, Palo Alto, CA, United States). Beijing Novogene Bioinformatics Technology Co., Ltd sequenced the genome (Illumina NovaSeq PE150, Illumina Inc., San Diego, United States).

**Genome Sequencing Assembly and Annotation**

After preprocessing, appropriate data were gathered using SOAP (version 2.04) (Li et al., 2008, 2010), SPAdes (Bankevich et al., 2012) and AbYSS (Simpson et al., 2009) assembly software, and finally integrated using CISA software. The preliminary assembly results were optimized and holes filled with GapCloser (version 1.12) to obtain the final assembly result. Fragments below 500 bp were filtered out. Encoded genes were predicted using GeneMarkS software. The protein-coding sequences produced with BLAST were annotated by comparison with the NR, GO, KEGG, COG, PFAM, TCDB, and Swiss-Prot protein databases.

**Genome Comparison**

Average nucleotide identity (ANI) was used to assess genomic similarity and to define species with values of 95%. The ANI analysis was performed with OAT (Yoon et al., 2017) and DNA-DNA hybridization (DDH) was used to assess the genomic similarity and to define species with 70% hybridization values (Tindall et al., 2010). The Genome-to-Genome Distance Calculator (GGDC) (Meier-Kolthoff et al., 2013) was employed to conduct a digital DNA-DNA hybridization (dDDH) analysis. The adjacent to the *Streptococcus* strain E24 located strains were selected based on BLAST alignment and the phylogenetic tree constructed from 16S rRNA gene results data.

**RESULTS**

**Phylogenetic Analysis**

The newly determined 16S rRNA (Figures 1A,B) isolate gene sequence was aligned with sequences of other *Streptococcus* species retrieved from GenBank. Strain *Streptococcus* strain E24 shared 98.04, 98.11, and 97.34 % 16S rRNA gene sequence similarity with *S. australis* ATCC 700641T, *S. rubneri* LMG 27207T and *S. parasanguinis* ATCC 15912T, respectively. The housekeeping gene sodA (Figures 1C,D) of *Streptococcus* strain E24 shared 94.94, 92.74, and 90.80% similarities with *S. australis* ATCC 700641T, *S. timonensis* Marseille-P2915T and *S. infantis* ATCC 700779T, respectively. Figures 1A,C represent phylogenetic trees constructed with the neighbor-joining and Figures 1B,D phylogenetic trees constructed with the maximum-likelihood method. Taken together, the phylogenetic analyses demonstrated that *Streptococcus* strain E24 represents a novel species within the genus *Streptococcus*. The 16S rRNA gene sequences of *Streptococcus* strain E24 is recorded in the NCBI database (accession no. MN592637).

**Phenotypic Characteristics**

Morphological features were determined using cells cultured aerobically at 37°C on enriched nutrition agar (vide supra) for 24 h, when the bacteria became punctate, gray and formed α-hemolytic colonies having edges that were undulated. Gram staining was performed using the classical Gram stain procedure (Austrian, 1960) and light microscopy images showed Gram-positive cocci (Figure 2A). Cells had an average diameter of about 5 μm as viewed by an electron microscopy (Figure 2B). Salt tolerance was determined by the growth of the strains in the presence of 2.5 g/L. The isolate was found to be unable to grow at or below 22°C but could grow at up to 42°C, with 37°C being the optimal temperature. The cells were facultative anaerobe, and the pH for optimal growth was 7 (Table 1 and Supplementary Table 1).

**Biochemical Characterization and FAME Profile**

Catalase and oxidase tested negative and *Streptococcus* strain E24 did not hydrolyse arginine, aesculin or starch except hippurate and was negative for esterase, leucin arylamidase, valin arylamidase and β-D-galactosidase, but β-D-glucuronidase, β-D-glucosidase and alkaline phosphatase produced positive results. *Streptococcus* strain E24 does not produce acid from glycerol, erythritol, d- or l-arabinose, d-ribose, d- or l-xyllose, d-adenitol, methyl β-D-xyloside, d-fructose-l-sorbose, l-rhamnose, dulcitol, d-mannitol, methyl α-D-mannopyranoside, methyl α-D-glucopyranoside, amygdalin, arbutin, aesculin, salicin, cellobiose, d-maltose, trehalose, inulin, melezitose, starch, glycogen, xyitol, gentiobiose, d-raffinose, d-melibiose, d-sorbitol, d-lyxose, d-tagatose, d- or l-fucos, d- or l-arabitol or 2- or 5-ketogluconate. Utilization of d-lactose is variable. *Streptococcus* strain E24 was capable of producing acid from D-galactose, D-glucose, D-mannose, inositol, N-acetylglucosamine and succrose. Different biochemical characteristics between the novel *Streptococcus* strain E24 (strain number 1) and other members of the *Streptococcus* genus are shown in Table 2. Hexadecanoic acid (16:0), (9Z)-9-octadecenoic acid (18:1 w9c), octadecanoic acid (18:0) and (11Z)-11-octadecenoic acid (18:1 w7c) comprised 62% of all fatty acids (Table 3).
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FIGURE 1 | Phylogenetic trees of Streptococcus strain E24 and closely related members of the Streptococcal species groups. (A) Neighbor-joining and (B) maximum-likelihood 16S rRNA gene sequences comparisons, (C) neighbor-joining and (D) maximum-likelihood sodA gene sequences comparisons. Sequences of Enterococcus faecalis and Lactococcus lactis subsp. lactis were used as out-groups. Bootstrap values ≥ 50 are indicated and the bars indicate 0.01 and 0.05 substitutions per site.

FIGURE 2 | Microscopic imaging of Streptococcus strain E24 (A) Gram stained coccus under light microscope 10 x 100 magnification, scale bar = 10 µm. (B) Transmission electron microscope image (operating voltage of 30.0 kV), scale bar = 5.0 µm.

Antibiotic Susceptibility
Antibiotic susceptibility tests of Streptococcus strain E24 revealed susceptibility to vancomycin, cefepime, Rina thiazole amine, penicillin, chloramphenicol and clindamycin, but resistance to ceftriaxone, ampicillin, and cefotaxime.

Genome Properties
A draft of the genome of Streptococcus strain E24 revealed that it was comprised of 1,950,659 bp with a 42.03% G + C content (Figure 3). It is comprised of five scaffolds (five contigs). Among the 1,994 genes predicted, 1,942 encoded proteins and 52 RNAs (3 genes were 5S rRNA, 1 gene 16S rRNA, 1 gene 23S rRNA and 47 genes tRNA). Of these, 1,525 genes (78.52%) were specified with putative functions according to COG or NR BLAST analysis. Twenty-two genes were ORFs (1.13%) and the remaining genes were responsible for hypothetical proteins. Table 4 shows the COG functional categories of the sorted genes. The sequence of the genome was recorded in the NCBI database (accession no. VFSG0000000).

Comparisons of Genomes
The genome sequence length of Streptococcus strain E24 (1,950 Mb) was less than S. pseudopneumoniae ATCC BAA-960T.
### TABLE 1 | Classification and general features of Streptococcus strain E24.

| Property         | Result |
|------------------|--------|
| α-Hemolytic      | +      |
| Gram stain       | Positive |
| Cell shape       | Cocci |
| Motility         | Non-motile |
| Sporulation      | Non-spore forming |
| Temperature range | 22–42°C |
| Optimum temperature | 37°C |
| Habitat          | Child respiratory tract |
| Oxygen requirement | Facultative aerobic |
| Salinity         | 0–2.5 g/L |
| Optimum salinity | 0 g/L |
| pH optimum       | 7      |

Streptococcus strain E24 has been deposited in the Korean Agricultural Culture Collection (deposition no. KACC 21425) and Guangdong Microbial Culture Collection Center (deposition no. GDMMC 1.1634). The genome sequence and 16S rRNA gene sequence were recorded in the NCBI database (accession no. VFSG00000000; accession no. MN592637).

### TABLE 2 | Differential characteristics of Streptococcus strain E24 in comparison with other related organisms.

| Strains                  | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 |
|--------------------------|---|---|---|---|---|---|---|---|---|
| Hemolysis                | α | β | α | α | α | α | α | α | α |
| Arginine (Voges Proskauer) | - | - | - | - | - | - | - | - | - |
| Hydrolysis of:           |   |   |   |   |   |   |   |   |   |
| Arginine                 | - | - | + | - | - | - | v | + | + |
| Aesculin                 | - | - | - | + | v | - | - | v | - |
| Hippurate                | + | - | - | - | - | - | - | - | - |
| Starch                   | - | - | - | + | v | - | - | v | - |
| Production of:           |   |   |   |   |   |   |   |   |   |
| α-D-galactosidase        | + | - | - | - | - | - | - | - | - |
| β-D-galactosidase        | - | - | - | + | + | - | + | + | + |
| β-D-glucosidase          | + | - | - | - | - | - | - | v | - |
| Acid from:               |   |   |   |   |   |   |   |   |   |
| D-lactose                | v | + | + | + | + | + | + | + | + |
| D-mannitol               | - | - | - | - | - | - | v | - | - |
| D-melibiose              | - | - | - | + | v | - | - | v | - |
| D-raffinose              | - | + | - | v | - | - | v | + | + |
| D-ribose                 | - | - | - | - | v | - | - | v | - |
| D-sorbitol               | - | - | - | - | - | - | v | - | - |
| D-succrose               | + | + | - | + | v | - | - | v | - |
| D-tagatose               | - | - | - | - | - | - | v | - | - |
| D-trehalose              | - | - | - | - | - | - | - | v | - |

### TABLE 3 | Fatty acid profiles of Streptococcus strain E24.

| Fatty acid | Percent (%) |
|------------|-------------|
| 16:00      | 26.03       |
| 18:1 w9c   | 13.47       |
| 18:00      | 11.88       |
| Summed feature 8 (18:1 w7c) | 11.01 |
| Summed feature 5 (18:2 w6,9c/18:0 ante) | 7.9 |
| 14:00      | 7.32        |
| Summed feature 3 (16:1 w7c/16:1 w6c) | 6.82 |
| 16:1 w9c   | 6.46        |
| 12:00      | 2.15        |
| 16:1 w5c   | 1.03        |
| 17:00      | 0.91        |
| 17:1 iso w5c | 0.76 |
| 20:4 w6,9,12,15c | 0.75 |
| 15:00      | 0.73        |
| 17:1 w8c   | 0.56        |
| 17:1 anteiso w9c | 0.51 |
| 19:0 iso   | 0.5         |
| 17:0 iso   | 0.46        |
| 17:0 anteiso | 0.46 |
| 13:0 2OH   | 0.29        |

DISCUSSION

The phenotypic analysis obtained from API strips (Table 2) revealed, that the Streptococcus strain E24 was the only one which hydrolyzed hippurate and there were essential differences between the nine analyzed streptococci, which has been described also for other Streptococcus strains in which the probability that a strain was correctly identified
by nine phenotypic tests was 90–100% (Raemy et al., 2013). The similarities of the 16S rRNA gene sequences from *Streptococcus* strain E24 with the closest related streptococci were 98.04, 98.11, and 97.34% for *Streptococcus australis* ATCC 700641<sup>T</sup>, *Streptococcus rubneri* LMG 27207<sup>T</sup> and *Streptococcus parasanguinis* ATCC 15912<sup>T</sup>, whereas the sodA gene comparison exhibited a sequence identity of 92.6% with the closest strain *Streptococcus australis* ATCC 700641<sup>T</sup>. These similarities are in line with another newly identified *Streptococcus* strain exhibiting 98.7 and 92.6% similarities of 16S rRNA and rpoB gene sequences between the *Streptococcus timonensis* sp. nov. and the *Streptococcus infantis* strain JCM 10157<sup>T</sup> (Ricaboni et al., 2017). There were also essential differences of G + C contents and genome sequence lengths between *Streptococcus* strain E24 and eight other streptococci, but also within the 8 streptococci species, which has been described also for other *Streptococcus* strains (Thompson et al., 2013) and attributed to frequent occurrence of horizontal gene transfers (Bellanger et al., 2009; Harvey et al., 2011; Zhang et al., 2011). Most obviously, the dDDH analysis revealed 24 to 52% similarities between *Streptococcus* strain E24 and 11 comparison strains and the similarities between the 12 different streptococci strains was between 23.5 and 57.8%, which is less than the proposed limit of 70% similarity for discrimination of *Streptococcus* species (Auch et al., 2010; Thompson et al., 2013).

In summary, based on the genotypic, phylogenetic and phenotypic results, it is concluded that the *Streptococcus* strain E24 is a novel species of the genus *Streptococcus*, which is distinct from its closest phylogenetic neighbors *Streptococcus australis* ATCC 700641<sup>T</sup>, *Streptococcus rubneri* LMG 27207<sup>T</sup> and *Streptococcus parasanguinis* ATCC 15912<sup>T</sup> and for which the name *Streptococcus xiaochunlingii* sp. nov. E24 is proposed.

**DESCRIPTION OF *Streptococcus xiaochunlingii* sp. nov. E24**

*Streptococcus xiaochunlingii* (xiao’chun’ lingi.i. N.L. gen masc. named after Professor Xiao Chunling, who is the leader of the laboratory in which the strain has been isolated).

*Streptococcus* strain E24 is a non-motile, non-spore-forming, facultative anaerobic and Gram-positive coccus, isolated from...
a temperature growth range of 22 to 42°C. After 48 h of aerobic incubation on 5% sheep’s blood–enriched nutrition agar, colonies are pinpoint, grayish and α-hemolytic, with undulated edges and with a diameter of 0.5 to 1 mm. Cells are roughly round with a 0.5 μm diameter. Cells did not hydrolyse arginine, asesculin or starch except hippurate and were negative in tests for esterase, leucin arylamidase, valin arylamidase and β-D-galactosidase. Positive for β-D-glucuronidase, β-D-glucosidase and alkaline phosphatase. Does not produce acid from glycerol, erythritol, d- or l-arabinose, d-ribose, d- or l-xyllose, d-adenitol, methyl β-D-xyllose, d-fructose-l-sorbose, l-rhamnose, dulcitol, d-mannitol, methyl α-D-mannopyranoside, methyl α-D-glucopyranoside, amygdalin, arbutin, asesculin, salicin, cellobiose, d-maltose, trehalose, inulin, melezitose, starch, glycogen, xylitol, gentiobiose, d-lyxose, d-raffinose, d-tagatose, d- or l-fucose, d- or l-arabitol, d-sorbitol, d-melibiose or 2- or 5- ketoglucuronate. Utilization of d-lactose is variable. They were capable of producing acid from D-galactose, D-glucose, d-mannose, inositol, N-acetylglucosamine and sucrose. Catalase and oxidase tested negative. The genome was shown to be 1,950,659 bp long and contained 42.03 mol% G + C bases with 1,942 protein-coding and 53 RNA coding genes. The results of 16S rRNA gene sequencing strongly suggested that the strain was a member of the Streptococcus genus, with 98.04, 98.11, and 97.34% similarities to Streptococcus australis ATCC 700641T, Streptococcus rubneri LMG 27207T and Streptococcus parasanguis ATCC 15912T, respectively. A sodA gene comparison exhibited a sequence identity of 92.6% with the closest strain Streptococcus australis ATCC 700641T. In silico DNA–DNA hybridization showed a closest DNA similarity value of 52% with Streptococcus australis ATCC 700641T. Streptococcus strain E24 has been deposited in the Korean Agricultural Culture Collection (deposition no. KACC 21425) and Guangdong Microbial Culture Collection Center (deposition no. GDMCC 1.1634). The genome and 16S rRNA gene sequences are recorded in the NCBI database (accession no. VFSG0000000; accession no. MN592637).

### Table 4: Number of genes associated with 26 general COGs functional categories.

| Code | Value | % of total | Description |
|------|-------|------------|-------------|
| A    | 0     | 0          | RNA processing and modification |
| B    | 0     | 0          | Chromatin structure and dynamics |
| C    | 49    | 3.21       | Energy production and conversion |
| D    | 24    | 1.57       | Cell cycle control, cell division, chromosome partitioning |
| E    | 144   | 9.44       | Amino acid transport and metabolism |
| F    | 71    | 4.66       | Nucleotide transport and metabolism |
| G    | 128   | 8.39       | Carbohydrate transport and metabolism |
| H    | 65    | 4.26       | Coenzyme transport and metabolism |
| I    | 44    | 2.89       | Lipid transport and metabolism |
| J    | 190   | 1.25       | Translation, ribosomal structure and biogenesis |
| K    | 109   | 7.15       | Transcription |
| L    | 85    | 5.57       | Replication, recombination and repair |
| M    | 103   | 6.75       | Cell wall/membrane/envelope biogenesis |
| N    | 9     | 0.59       | Cell motility |
| O    | 64    | 4.20       | Posttranslational modification, protein turnover, chaperones |
| P    | 64    | 4.20       | Inorganic ion transport and metabolism |
| Q    | 19    | 1.25       | Secondary metabolites biosynthesis, transport and catabolism |
| R    | 117   | 7.67       | General function prediction only |
| S    | 91    | 5.97       | Function unknown |
| T    | 67    | 4.39       | Signal transduction mechanisms |
| U    | 18    | 1.18       | Intracellular trafficking, secretion, and vesicular transport |
| V    | 49    | 3.21       | Defense mechanisms |
| W    | 2     | 0.13       | Extracellular structures |
| X    | 13    | 0.85       | Mobiome: prophages, transposons |
| Y    | 0     | 0          | Nuclear structure |
| Z    | 0     | 0          | Cytoskeleton |

COGs, clusters of orthologous groups. Total based on the number of protein-coding genes found in the genome.

### Table 5: Pairwise comparison of Streptococcus strain E24 with nine other Streptococcal species using genome-to-genome distances and formula 2 (DDH estimates based on identities/high-scoring segment pair length).

| Strains: 1. Streptococcus strain E24; 2. S. australis (Wilcox et al., 2001); 3. S. rubneri (Handley et al., 1991; Wilcox et al., 2001; Huch et al., 2013); 4. S. parasanguis (Whiley et al., 1990; Wilcox et al., 2001); 5. S. oralis subsp. Turginus (Zbinden et al., 2012); 6. S. imvisans (Ricaboni et al., 2017); 7. S. infantis (Kawamura et al., 1998; Whiley and Hardie, 2009); 8. S. oralis subsp. Dentisani (Jensen et al., 2016); 9. S. oralis subsp. Oralis (Jensen et al., 2016); 10. S. pseudopneumoniae (Arbique et al., 2004; Zbinden et al., 2012); 11. S. mitis (Whiley and Hardie, 2009); 12. S. pneumoniae (Whiley and Hardie, 2009). | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 1 | 100% | 52% | 37.5% | 26.3% | 24.20% | 29% | 27.6% | 24% | 24.8% | 25.8% | 25% | 25.5% |
| 2 | 100% | 40.3% | 27.40% | 24.00% | 28.10% | 28.70% | 24.00% | 24.80% | 24.90% | 25.60% | 26.00% |
| 3 | 100% | 26.00% | 24.70% | 29.30% | 27.40% | 24.70% | 24.60% | 25.00% | 25.6% | 26.10% |
| 4 | 100% | 24.50% | 26.00% | 26.50% | 25.70% | 23.50% | 24.80% | 24.60% | 24.60% | 25.60% |
| 5 | 100% | 25.20% | 26.30% | 25.50% | 24.70% | 24.60% | 25.60% | 23.50% | 24.80% | 25.60% |
| 6 | 100% | 26.00% | 25.50% | 25.70% | 26.20% | 24.80% | 25.60% | 24.70% | 25.60% |
| 7 | 100% | 24.80% | 26.30% | 26.70% | 26.30% | 25.60% | 24.80% | 26.00% | 25.80% |
| 8 | 100% | 44.90% | 31.30% | 31.30% | 31.30% | 31.30% | 31.10% | 31.30% | 31.30% |
| 9 | 100% | 32.30% | 31.70% | 31.80% | 31.80% | 31.80% | 31.80% | 31.80% | 31.80% |
| 10 | 100% | 48.20% | 57.80% | 57.80% | 57.80% | 57.80% | 57.80% | 57.80% | 57.80% |
| 11 | 100% | 47.20% | 57.80% | 57.80% | 57.80% | 57.80% | 57.80% | 57.80% | 57.80% |
| 12 | 100% | 57.80% | 57.80% | 57.80% | 57.80% | 57.80% | 57.80% | 57.80% | 57.80% |

According to the data, strain E24 is most similar to Streptococcus australis, with 98.11% similarity, followed by Streptococcus rubneri with 97.34% similarity. The lowest similarity was observed with Streptococcus parasanguis, with 92.6% similarity.
DATA AVAILABILITY STATEMENT

The datasets presented in this study can be found in online repositories. The names of the repository/repositories and accession numbers can be found below: https://www.ncbi.nlm.nih.gov/, VFSG00000000; https://www.ncbi.nlm.nih.gov/, MN592637.

ETHICS STATEMENT

The studies involving human participants were reviewed and approved by Ethics Committee of Shenyang Medical College (approval number: No. 2015052902). Written informed consent to participate in this study was provided by the participants’ legal guardian/next of kin.

AUTHOR CONTRIBUTIONS

XL and YZ were responsible for the conception and design of the study, drafted, commented, and revised the manuscript. XL, YZ, YS, HQ, and DL were in charge of statistical analysis. All authors were responsible for data acquisition and analysis, read and approved the final version of this manuscript.

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SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fmicb.2020.563213/full#supplementary-material
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**Conflict of Interest:** The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.