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Biological characteristics and whole-genome sequencing analysis of an alpha-hemolytic Streptococcus pyogenes strain

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Article

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Abstract

Background *Streptococcus pyogenes* is a major human pathogen. Recently, alpha-hemolytic isolates—unusual for this typically beta-hemolytic species—have emerged, but their virulence, antibiotic resistance, and evolution are poorly understood. This study used an alpha-hemolytic pyogenic streptococcus isolated from erysipelas skin secretions and used whole-genome sequencing, phenotypic testing, and bioinformatics to characterize its virulence, resistance, and phylogeny.

Methods A clinical isolate of alpha-hemolytic streptococcus was obtained from a tertiary care hospital. Species identification was conducted using matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF MS) and the VITEK® II Compact system. Antimicrobial susceptibility testing was performed using the disk diffusion

method and E-test strips. Whole-genome sequencing was carried out by Beijing Novogene Co., Ltd., followed by genome assembly, functional gene annotation, phylogenetic tree construction, multilocus sequence typing (MLST), and emm gene typing. Comprehensive bioinformatic analyses were conducted to identify antibiotic resistance genes and virulence-associated genes.

Results Both MALDI-TOF MS and biochemical assays confirmed the isolate as *Streptococcus pyogenes*. Antimicrobial susceptibility testing revealed resistance to macrolides and tetracycline, while sensitivity was maintained to penicillins, cephalosporins, and other tested agents. MLST assigned the strain to sequence type ST176, and emm typing identified it as emm58.7. Resistance genes including tet(M), mef(A), and msr(D) were detected. Additionally, multiple virulence factor-encoding genes—such as fbp54, hylA, hasA, hasB, hasC, ideS/mac, slo, sagA, speB, speC, speG, speJ, and smeZ—were identified in the genome.

Conclusion This S. pyogenes isolate exhibited atypical alpha-hemolysis rather than the classical beta-hemolytic phenotype on Columbia blood agar, resembling the hemolytic pattern of viridans group streptococci.The alpha-hemolytic phenotype may be attributed to a substitution at position 215 of the sagB gene, where serine (S) is replaced by proline (P), potentially disrupting the functional expression of streptolysin S (SLS) and thereby leading to alpha-hemolysis. However, this phenotypic variation did not compromise its accurate microbiological identification, virulence potential, or antimicrobial susceptibility profile. Due to morphological and hemolytic similarities with commensal flora, there is a risk of clinical underdetection. Integrating antigen detection, serological antibody testing, and molecular diagnostic methods can enhance the detection rate of such atypically presenting S. pyogenes strains, thereby reducing the likelihood of misdiagnosis or missed diagnosis.

Introduction

Streptococcus pyogenes(S. pyogenes)[1], also known as Group A Streptococcus (GAS), is a clinically significant pathogenic species within the genus Streptococcus. It is the primary causative agent of various suppurative infections and is commonly associated with pharyngitis, acute rheumatic fever, and

post-streptococcal glomerulonephritis. Additionally, it can lead to erysipelas, cellulitis, tonsillitis, lymphangitis, endocarditis, septic arthritis, sepsis, and scarlet fever. The extracellular toxins produced by this organism are frequently implicated in the development of streptococcal toxic shock syndrome. Following incubation at 35℃for 18 to 24 hours, *S. pyogenes* typically forms small colonies on blood agar plates, measuring 0.5–0.75 mm in diameter. These colonies are grayish-white, transparent or semi-transparent, with smooth, shiny, circular, and slightly raised surfaces, and usually exhibit beta-hemolysis. Alpha-hemolytic variants of *S. pyogenes* have been documented only rarely.The viridans group streptococci (VGS) are opportunistic pathogens commonly found as part of the normal microbiota in the human oral cavity, upper respiratory tract, and urogenital tract[24]. These organisms are frequently associated with subacute bacterial endocarditis and may lead to systemic infections in immunocompromised individuals. In neonates and those with impaired immune function, VGS can cause meningitis and severe suppurative complications, including brain and liver abscesses. In contrast to *S. pyogenes* or which penicillin remains the treatment of choice, VGS exhibit a relatively high rate of penicillin resistance, necessitating tailored antimicrobial therapy. In this report, we describe the isolation of an alpha-hemolytic strain of *S. pyogenes* from the clinical secretions of a patient diagnosed with erysipelas. The findings are presented below.

Methods

This alpha-hemolytic *S. pyogenes* strain was co-cultured with the reference strain *S. pyogenes* ATCC 19615 in a 35°C incubator supplemented with 5.0% CO_2 to exclude the possibility of atypical hemolysis resulting from environmental variables such as temperature, incubation duration, carbon dioxide concentration, and culture medium composition. Concurrently, the isolate was identified using MALDI-TOF MS (Beijing Yixin Bochuang) and the VITEK® II Compact system. Minimum inhibitory concentrations (MICs) of penicillin and ampicillin were determined by the E-test strip method, while inhibition zone diameters for other antimicrobial agents were assessed using the disk diffusion method for routine antimicrobial susceptibility testing. Whole-genome sequencing was conducted by Beijing Novogene Co., Ltd. Raw sequencing data were assembled into a draft genome using Shovill software, and contigs shorter than 250 bp were filtered to improve assembly quality. Gene annotation was performed through the BV-BRC (Bacterial and Viral Bioinformatics Resource Center | BV-BRC)platform. Multilocus sequence typing (MLST) was carried out using the Center for Genomic Epidemiology(Center for Genomic Epidemiology) platform, and emm gene typing was conducted by the U.S. Centers for Disease Control and Prevention (Streptococcus Lab | StrepLab | Blast-emm GAS Subtyping Request Form | CDC). The assembled sequences were submitted to the Virulence Factor Database (VFDB: Virulence Factor Database) for virulence gene identification and deposited in NCBI GenBank under an assigned accession number for public registration.Alignment of streptolysin S (SLS)-related genes sagA–I using BLAST in the NCBI database.

Results

Microbial culture and identification results

Following 24 hours of incubation at 35°C in a 5.0% CO_2 atmosphere, the reference strain *S. pyogenes* ATCC 19615 formed typical beta-hemolytic colonies. In contrast, the clinical isolate of *S. pyogenes* exhibited alpha-hemolysis, which persisted even after extending the incubation period to 72 hours. Species identification of this alpha-hemolytic isolate was performed using both MALDI-TOF MS and the Vitek II Compact system, with consistent results confirming it as *S. pyogenes*. Furthermore, the alpha-hemolytic phenotype remained stable after five consecutive subcultures.(Fig. 1)

Drug sensitivity results

This alpha-hemolytic *S. pyogenes* exhibits resistance to erythromycin and tetracycline, while demonstrating susceptibility to penicillin, ampicillin, cefepime, cefotaxime, clindamycin, levofloxacin, linezolid, and vancomycin. Notably, it remains susceptible to bacitracin.(Table 1)

Table 1
Drug sensitivity results

antimicrobial agent	RAD	MIC	susceptibility
penicillin		0.06	S
ampicillin		0.06	S
cefepime	32		S
cefotaxime	35		S
clindamycin	25		S
levofloxacin	22		S
linezolid	28		S
vancomycin	20		S
erythromycin	9		R
tetracycline	13		R

Whole genome sequence analysis

Gene annotation

The genome of the alpha-hemolytic *S. pyogenes* consists of 13 contigs, with a total length of 1,828,751 bp and an average G + C content of 38.31%. The genome was annotated using the RAST annotation server (RASTtk), which assigned it a unique genome identifier of 1314.4614. It belongs to the

superkingdom Bacteria and was annotated using translation table 11 (Bacterial, Archaeal and Plant Plastid). The taxonomic classification of this genome is as follows: cellular organisms > Bacteria > Terrabacteria group > Bacillota > Bacilli > Lactobacillales > Streptococcaceae > Streptococcus > Streptococcus pyogenes.

The genome contains 1,838 protein-coding sequences (CDS), 41 transfer RNA (tRNA) genes, and 5 ribosomal RNA (rRNA) genes. Among the predicted proteins, 282 were annotated as hypothetical proteins, while 1,556 were assigned functional roles. Of these functionally annotated proteins, 535 were assigned Enzyme Commission (EC) numbers, 438 were associated with Gene Ontology (GO) terms, and 363 were mapped to KEGG pathways. Additionally, PATRIC annotation classified the proteins into two types of protein families: 1,792 proteins were assigned to genus-specific protein families (PLFams), and 1,811 proteins belonged to cross-genus protein families (PGFams). The genomic sequence has been submitted to the NCBI database, and its GenBank assembly accession :GCA_054182765.1.

Genome circle diagram

A detailed circular genome diagram of the alpha-hemolytic *S. pyogenes* is shown in Fig. 2.

Phylogenetic analysis

The phylogenetic tree of the alpha-hemolytic *S. pyogenes* is shown in Fig. 3.

MLST typing results

The nucleotide sequence was submitted to the Center for Genomic Epidemiology for multilocus sequence typing (MLST). The allele numbers for the seven housekeeping genes (gki, gtr, murI, mutS, recP, xpt, yqiL) were determined as 3, 2, 1, 3, 1, 3, and 3, respectively. The resulting MLST profile was identified as *S. pyogenes* ST-176.

Emm typing results

The sequence was submitted to the U.S. CDC data comparison database (www2.cdc.gov/vaccines/biotech/strepblast.asp) for analysis. Based on a sequence homology of 99%, it was determined to belong to the same emm type. The emm type of this alpha-hemolytic *S. pyogenes* was identified as emm58.7.

Virulence factor results

In the VFDB database, this alpha-hemolytic *S. pyogenes* yielded 20 significant alignments.The detailed data are presented in Table 2

Table 2
Virulence factor results

Virulence gene	Identities(%)	Related virulence factor
hylA	98	hyaluronidase
hasA	97	Hyaluronic acid capsule
hasB	99	Hyaluronic acid capsule
hasC	99	Hyaluronic acid capsule
fbp54	97	Fibronectin binding proteins
sfbI/prtF1	98	Fibronectin binding proteins
sfbX	97	Fibronectin binding proteins
lmb	98	laminin-binding surface protein
grab	97	G-related α2macroglobulin-binding protein
slo	98	Streptolysin O
sagA	100	streptolysin S
scpA/scpB	97	streptococcal C5a peptidase
speB	99	Streptococcal pyrogenic exotoxin B
smeZ	95	Streptococcal pyrogenic exotoxins
speC	99	Streptococcal pyrogenic exotoxins
speG	99	Streptococcal pyrogenic exotoxins
speJ	97	Streptococcal pyrogenic exotoxins
mf/spd	99	Mitogenic factor
mf2	99	Mitogenic factor
ideS/mac	97	immunoglobulin G-degrading enzyme

Antimicrobial resistance(AMR) genes

A summary of the AMR genes annotated in this genome and their corresponding AMR mechanism is provided in Table 3

Table 3
AMR genes results

AMR Mechanism	Genes
Antibiotic target in susceptible species	Alr, Ddl, EF-G, EF-Tu, folA, Dfr, folP, gyrA, gyrB, Iso-tRNA, kasA, MurA, rpoB, rpoC, S10p, S12p
Antibiotic target protection protein	Msr(D), Tet(M)
Antibiotic target replacement protein	FabK
Efflux pump conferring antibiotic resistance	Mef(A)
Gene conferring resistance via absence	gidB
Protein altering cell wall charge conferring antibiotic resistance	PgsA
Regulator modulating expression of antibiotic resistance genes	LiaF, LiaR, LiaS

Alignment of streptolysin S (SLS)-related genes

The protein sequence alignment of the streptolysin S (SLS)-related gene sagA–I revealed that, apart from a few conservative substitutions, the amino acid at position 215 of sagB was substituted from serine (S) to proline (P).The sequence alignment results are shown in Fig. 4.

Discussion

S. pyogenes is the most pathogenic species within the genus Streptococcus and is responsible for approximately 90% of streptococcal infections. It harbors multiple virulence factors[2], which can be categorized into invasive structures, invasive enzymes and toxins. The invasive structures, collectively known as adhesins, include lipoteichoic acid, a component of the bacterial cell wall, and the M protein.Lipoteichoic acid is located beneath the outer layer of the M protein[3], contributing to the formation of a fimbrial-like structure that mediates binding to fibronectin on the surface of epithelial cells. This interaction represents the primary virulence factor enabling the bacterium to establish colonization on host surfaces such as the skin and respiratory mucosa.The M protein is the primary virulence factor of Streptococcus[4][5][6], exhibiting the capacity to resist phagocytic engulfment and to withstand intracellular killing within phagocytes. Furthermore, the M protein shares antigenic epitopes with host tissues such as the myocardium, glomerular basement membrane, and synovial membrane. During streptococcal infection, the host immune system generates antibodies directed against the M protein. These antibodies bind to the M protein, forming soluble immune complexes that may deposit in the aforementioned tissues. Due to molecular mimicry and cross-reactive antigens, these immune complexes can trigger inflammatory damage via type III or type II hypersensitivity reactions, thereby contributing to the development of post-streptococcal autoimmune disorders such as rheumatic fever and acute glomerulonephritis.The M protein is encoded by the emm gene, and emm typing is based on

sequence variation within a 180-base-pair region[7]. The emm typing of *S. pyogenes* exhibits notable variation depending on both the specimen source and geographical origin. Statistical analyses indicate that the emm58 type is more prevalent in regions including Serbia[9], Gabon[8], whereas it is less commonly observed in areas such as Spain[11],Brussels[13], Finland[12], and Adana[10]. A prior study conducted in Serbia identified 31 distinct emm types among 145 isolates, with emm58 accounting for 11% (16/145) of the total and predominantly isolated from skin specimens[9]. Similarly, a study in Hong Kong reported that emm58 was more frequently recovered from skin samples, with an isolation rate of approximately 10% (11/107)[14]. The *S. pyogenes* strain analyzed in this study belongs to the emm58.7 subtype and was also isolated from a skin specimen. Although the overall isolation frequency of emm58 strains is relatively low compared to other emm types, their potential to cause clinically significant infections remains substantial. Notably, this strain has been linked to multiple infections within the high-dependency unit of a level-I trauma center in India[15].

Invasive enzymes such as hyaluronidase, streptokinase, and streptodornase are collectively known as diffusion factors, which facilitate the dissemination of streptococci into surrounding tissues or through the lymphatic and circulatory systems via multiple mechanisms[2]. Hyaluronidase degrades hyaluronic acid in the interstitial matrix, thereby increasing tissue permeability; the experimental strain carries the hylA and hylP genes, each of which are associated with this enzymatic activity. Streptokinase acts by converting plasminogen into plasmin, leading to fibrinolysis, which dissolves blood clots and prevents plasma coagulation[16]. Streptodornase is a DNase that hydrolyzes highly viscous DNA present in pus, thereby reducing its viscosity and promoting drainage. Additionally, the immunoglobulin G-degrading enzyme encoded by the ideS/mac gene exhibits exceptionally high substrate specificity, enabling specific cleavage of IgG molecules[17].

Streptolysin and pyrogenic exotoxin are two major exotoxins produced by *S. pyogenes*. Streptolysin exhibits hemolytic activity, capable of lysing red blood cells as well as damaging white blood cells and platelets[18]. Based on oxygen sensitivity, it is classified into oxygen-labile streptolysin O (SLO) and oxygen-stable streptolysin S (SLS). SLO is highly antigenic and induces the host immune system to produce specific antibodies known as anti-streptolysin O (ASO or anti-O). In contrast, SLS lacks immunogenicity and mediates beta-hemolysis by promoting rapid chloride ion influx into red blood cells, leading to disruption of the anion-exchange protein band-3 and subsequent cell lysis. SLS is a potent polypeptide cytolysin synthesized by *S. pyogenes*[19]. Research has demonstrated that insertion mutations within any of the nine contiguous sagA–I genes essential for SLS biosynthesis disrupt its functional expression, resulting in alpha-hemolysis; however, insertions located upstream or downstream of this gene cluster do not impair SLS activity[20]. Furthermore, a previous study reported that a mutation in the sagC gene causing premature termination leads to loss of beta-hemolysis and is associated with severe soft tissue infections[21].BLAST comparisons of the sagA-I sequences among the test strains revealed that certain sequences exhibited conservative amino acid substitutions. Notably, a distinct substitution was observed at position 215 of sagB, where serine (S) was replaced by proline (P). According to the Conserved Domain Database (CDD) at NCBI, this residue is located within an unaligned region. It is hypothesized that this mutation may impact protein function through one of

two potential mechanisms: first, a structural regulatory mutation that alters protein conformational flexibility, thereby affecting substrate binding or signal transduction; second, a functional loss mutation leading to the disruption or absence of a critical functional domain[22].The precise genetic alterations in the sagA–I locus that affect SLS expression and lead to alpha-hemolysis require further investigation through expanded sample analyses and additional experimental validation.

Streptococcal pyrogenic exotoxin, commonly referred to as erythrogenic toxin or scarlet fever toxin, is produced by *S. pyogenes* strains lysogenized by bacteriophages. This exotoxin is a proteinaceous molecule and exists in three primary serotypes—A, B, and C—each exhibiting strong antigenicity and superantigenic activity[18]. Upon systemic absorption, these toxins can induce toxemic symptoms, including fever. The experimental strain under investigation harbors several superantigen-encoding genes, including speB, speC, speG, speJ, and smeZ, all of which contribute significantly to the pathogenesis of Streptococcus infection by facilitating bacterial colonization, proliferation, and dissemination within the host.

The standard treatment for *S. pyogenes* infections typically begins with beta-lactam antibiotics, particularly penicillin[23]. For patients with a documented history of penicillin allergy, alternative antibacterial agents such as first-generation cephalosporins, macrolides, lincosamides, vancomycin, or linezolid may be considered. However, in China, *S. pyogenes* exhibits a relatively high resistance rate to macrolides and clindamycin, rendering these agents generally unsuitable for empirical therapy. Alpha-hemolytic *S. pyogenes* isolates are resistant to erythromycin and tetracycline but remain susceptible to penicillin, cefepime, cefotaxime, clindamycin, levofloxacin, linezolid, and vancomycin. The presence of tetracycline resistance gene tet(M), macrolide resistance genes mef(A) and msr(D), is highly consistent with the observed antimicrobial susceptibility profile.

In conclusion, the absence of beta-hemolysis in *S. pyogenes* does not compromise the accuracy of microbial strain identification, assessment of virulence, or antimicrobial susceptibility testing. In this study, alpha-hemolytic *S. pyogenes* was isolated from sterile site specimens, confirming its clinical significance and ruling out contamination or false-negative results. During routine clinical microbiological examinations, initial screening for *S. pyogenes* in non-sterile sites—such as respiratory tract swabs and sputum—typically relies on the presence of a beta-hemolytic colony morphology[24]. However, this reliance may lead to oversight of infections caused by alpha-hemolytic variants, potentially resulting in erroneous negative reports. Evidence indicates that rapid antigen detection tests(RADT) targeting GAS, using throat swab samples, exhibit high sensitivity and specificity for diagnosing *S. pyogenes* infections[25]. Although certain non-pyogenes strains—such as Streptococcus dysgalactiae equlsimilis and Streptococcus anginosus—can express Lancefield group A antigens, the GAS RADT remains valuable for detecting alpha-hemolytic *S. pyogenes*[26][27]. A positive antigen result combined with observation of alpha-hemolysis on culture media enables accurate identification of these atypical isolates. Furthermore, MALDI-TOF mass spectrometry provides rapid and reliable identification of *S. pyogenes*[28]. Additionally, molecular methods—including loop-mediated isothermal amplification (LAMP), real-time fluorescent quantitative PCR, and metagenomic next-generation sequencing—play an

increasingly important role in identifying infections caused by alpha-hemolytic *S. pyogenes*[29][30]. These advanced techniques facilitate early pathogen detection, support timely and rational use of antimicrobial agents, and help prevent inappropriate antibiotic therapy.

Declarations

Ethics approval and consent to participate

This study was conducted in accordance with the Declaration of Helsinki. Ethics approval was obtained from the Ethics Committee of the 903rd Hospital of the Joint Logistic Support Force (Approval No. 2026081001/02/002C). This study used no personal identifying information (e.g., name, ID number, address, phone number), so individual informed consent was not required.

Consent for publication

Not applicable

Availability of data and materials

The datasets supporting the conclusions of this article are included within the article and its additional files. The sequence reads were deposited in the NCBI WGS under the project accession number PRJNA1389853.

Competing interests

The authors declare that they have no competing interests

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Authors' contributions

YM designed the study and wrote the main manuscript text;QW collected the isolates from clinical samples,conducted microbiological identification and drug sensitivity testing;MQ and YM did gene annotation and typing,performed the genomic analyses and created all figures ; all authors reviewed the manuscript.

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Figures

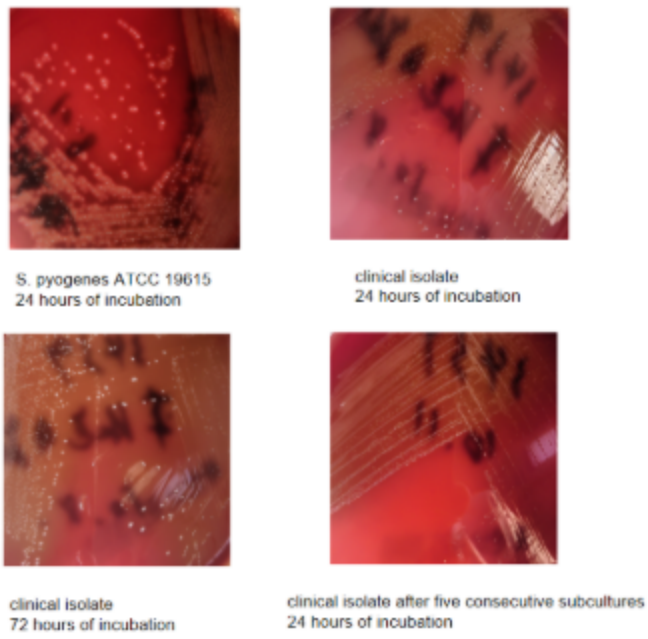


Figure 1

Microbial culture results

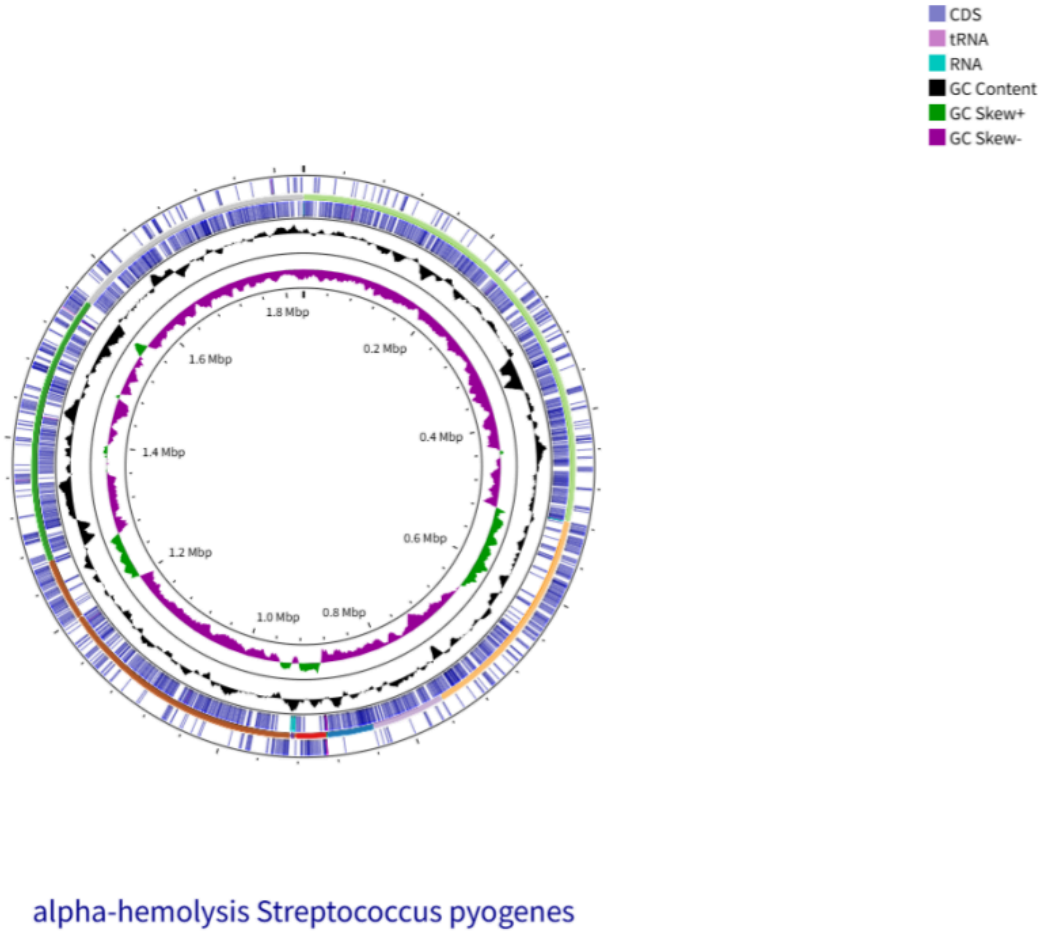


Figure 2

genome circular diagram

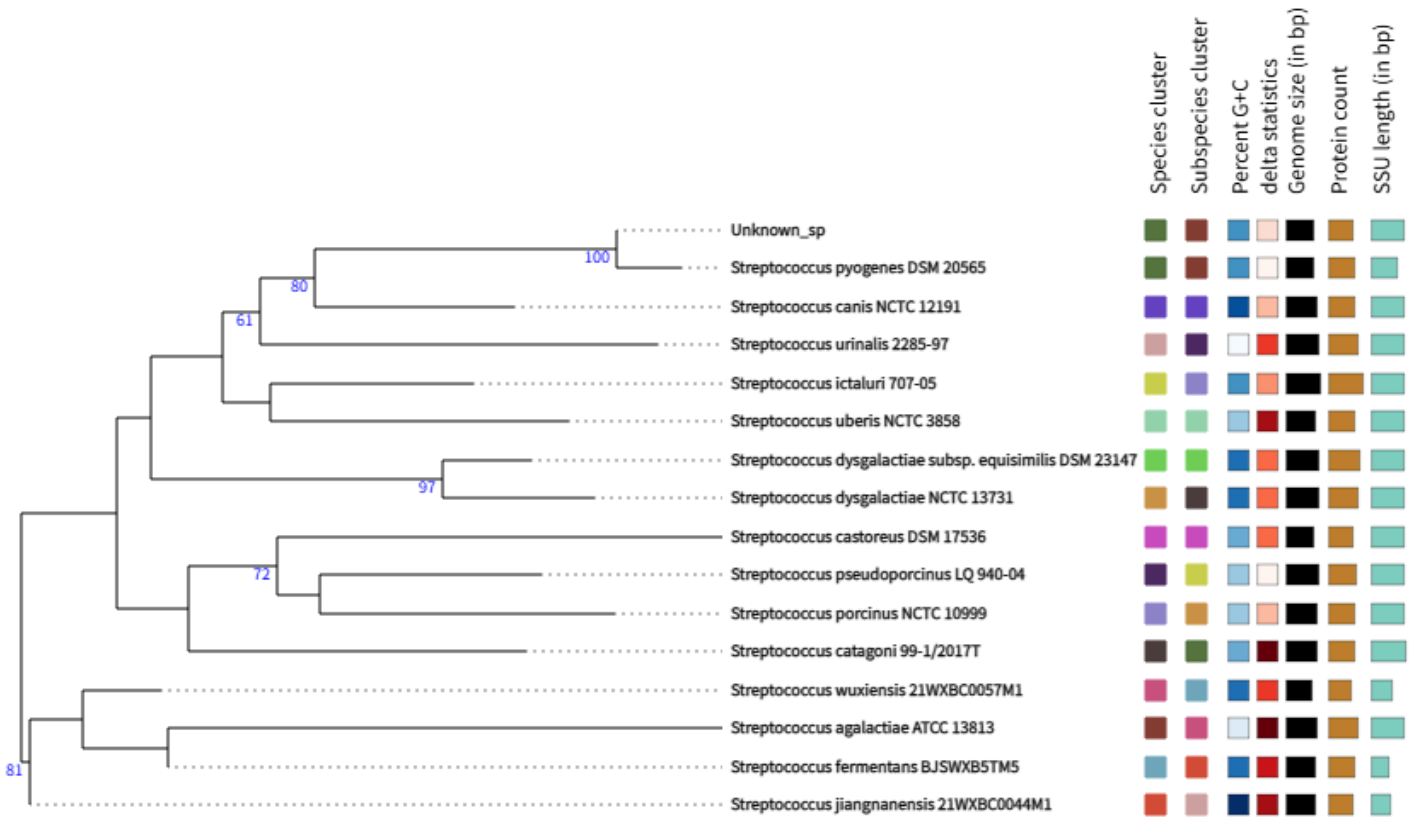


Figure 3

Phylogenetic tree

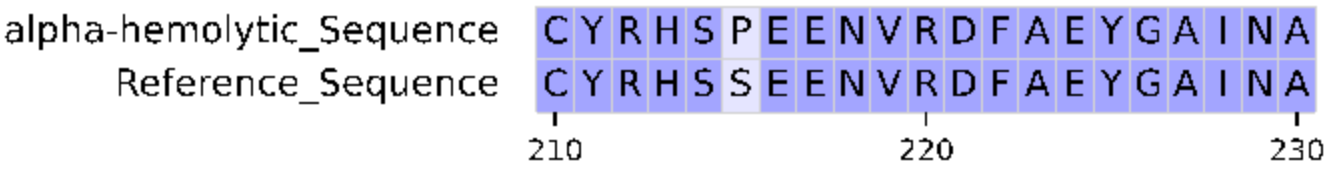


Figure 4

Alignment results