Complete Genome Sequence of *Streptococcus dysgalactiae* subsp. *equisimilis* 167 Carrying Lancefield Group C Antigen and Comparative Genomics of *S. dysgalactiae* subsp. *equisimilis* Strains

Shinya Watanabe, Teruo Kirikae, and Tohru Miyoshi-Akiyama*

Department of Infectious Diseases, National Center for Global Health and Medicine, Shinjuku-ku, Tokyo, Japan

*Corresponding author: E-mail: takiyam@ri.ncgm.go.jp.

Accepted: July 31, 2013

Data deposition: The nucleotide sequence of the chromosome of SDSE 167 has been deposited in the DNA Database of Japan under accession AP012976.

Abstract

Streptococcus dysgalactiae subsp. *equisimilis* (SDSE) is an emerging human pathogen that causes life-threatening invasive infections such as streptococcal toxic shock syndrome. Recent epidemiological studies reveal that invasive SDSE infections have been increasing in Asia, Europe, and the United States. Almost all SDSE carry Lancefield group G or C antigen. We have determined the complete genome sequence of a human group C SDSE 167 strain. A comparison of its sequence with that of four SDSE strains, three in Lancefield group G and one in Lancefield group A, showed approximately 90% coverage. Most regions showing little or no homology were located in the prophages. There was no evidence of massive rearrangement in the genome of SDSE 167. Bayesian phylogeny using entire genome sequences showed that the most recent common ancestor of the five SDSE strains appeared 446 years ago. Interestingly, we found that SDSE 167 harbors sugar metabolizing enzymes in a unique region and streptodornase in the phage region, which presumably contribute to the degradation of host tissues and the prompted *covRS* mutation, respectively. A comparison of these five SDSE strains, which differ in Lancefield group antigens, revealed a gene cluster presumably responsible for the synthesis of the antigenic determinant. These results may provide the basis for molecular epidemiological research of SDSE.

Key words: *Streptococcus dysgalactiae* subsp. *equisimilis*, Lancefield group C, complete genome sequence, Bayesian phylogeny.

Introduction

Streptococcus dysgalactiae subsp. *equisimilis* (SDSE) belongs to Lancefield group G or C (or, more rarely, A) streptococci (Vandamme et al. 1996; Takahashi et al. 2011). Although previously considered much less pathogenic in humans than group A (GAS: *Streptococcus pyogenes*) and group B streptococci, SDSE has been increasingly reported to cause invasive infections, such as sepsis and streptococcal toxic shock-like syndrome (Sylvetsky et al. 2002; Cohen-Poradosu et al. 2004; Liao et al. 2008). Recent epidemiological studies have shown that SDSE contributes significantly to the burden of invasive infections caused by β-hemolytic streptococci (Takahashi et al. 2011).

Genome analyses of SDSE and targeted microarray analyses of GAS virulence genes in 58 SDSE strains isolated from infected humans have shown that clinically isolated SDSE strains have many of the important virulence factors present in GAS, including streptolysin S (SLS), streptolysin O (SLO), streptokinase, and antiphagocytic surface M proteins (Davies et al. 2007). Several important GAS virulence factors, however, are missing from the SDSE genome, including SpeB, a chromosomally encoded cysteine protease, and a hyaluronic acid capsule. Moreover, SDSE lacks superantigenic activity because its *speG* gene, encoding a superantigen homolog, does not show superantigenic activity against human peripheral mononuclear cells (Zhao et al. 2007).

In this study, we determined, for the first time, the complete genome sequence of a group C SDSE 167 strain isolated from a human patient and shown, using a mouse model, to be the most virulent strain. The 167 genome was compared

with the complete genome sequences of four SDSE strains, three in Lancefield group G and one in Lancefield group A.

Materials and Methods

Bacterial Strains and Virulence in Mice (or Pathogenicity against Mice)

SDSE 167 strain was isolated from a patient with an invasive infection in Japan; the other completely sequenced strains described are listed in table 1. SDSE was cultured in 5% sheep blood agar or brain–heart infusion medium at 37 °C under 5% CO_2 as described (Miyoshi-Akiyama et al. 2003a). The virulence of these SDSE strains listed in supplementary table S1, Supplementary Material online, was compared using a mouse i.p. infection model (Miyoshi-Akiyama et al. 2003b). Protocols of all animal experiments were approved by the ethical committee of the National Center for Global Health and Medicine based on the "Basic Guidelines for Proper Conduct of Animal Testing and Related Activities in the Research Institutions under the Jurisdiction of the Ministry of Health, Labour and Welfare (MHLW) of Japan."

Preparation of Genomic DNA and Genome Sequencing

Streptococci were lysed as described (Miyoshi-Akiyama et al. 2003a), and genomic DNA was purified using DNeasy Blood & Tissue kits (QIAGEN). An 8-kb pair-end library of the SDSE 167 genome was prepared and sequenced using GS junior according to the manufacturer's instruction (Roche). This generated 230,950 reads, covering 41,119,010 bp (19.8-fold coverage), which were assembled into scaffolds and contigs. Gap filling was performed by conventional Sanger sequencing of the polymerase chain reaction (PCR) fragments based on brute force PCR among the contigs and scaffolds. The nucleotide sequence of the chromosome of SDSE 167 has been deposited in the DNA Database of Japan under accession no. AP012976.

In Silico Analyses

MetaGeneAnnotator was used for primary CDS extraction (Noguchi et al. 2008), with initial functional assignment and manual correction performed by in silico molecular cloning (in silico biology, inc.). Prophage regions and clustered regularly interspaced short palindromic repeats (CRIPSRs) were identified by Prophage Finder (Bose and Barber 2006) and CRISPRFinder (Grissa et al. 2007), respectively.

Phylogenetic Analyses

Whole-genome sequences were aligned with MAFFT (Katoh and Standley 2013). The evolutionary model (simple HYK) was chosen based on the results obtained with jModelTest 2.1.2 (Darriba et al. 2012) and convergence of the tree during preliminary phylogenetic analyses. A post-probable phylogenetic tree was constructed from genome sequence alignment with BEAST (Drummond and Rambaut 2007). BEAST was also used to estimate time from the most recent appearance of a common ancestor. The sequence alignments used are available from the corresponding author upon request.

Table 1
SDSE Strains Completely Sequenced

Strain	167	AC-2713	ATCC 12394	GGS_124	RE378
Length (nt)	2,076,397	2,179,445	2,159,491	2,106,340	2,151,145
G + C%	39.57	39.52	39.5	39.58	39.49
CDS	2,223	221	2,056	2,094	1,877
rRNA operon	5	5	5	5	5
tRNA	56	57	57	57	56
emm	*stL839*	*stG485.0*	*stG166b.0*	*stg480.0*	*stg6792*
Lancefield	C	A	G	G	G
Acc. no.	AP012976	NC_019042	NC_017567	NC_012891	NC_018712
Isolation year	2003	1999	1939	2006	2007
Locus tag of putative enzyme cluster synthesizing Lancefield antigen determinant	SDSE167_0822 to SDSE167_0826	SDSE_0797 to SDSE_0799	SDE12394_04095 to 04110	SDEG_0759 to SDEG_0762	GGS_0731 to GGS_0734
The Blast hits	Group C SDSE, *Streptococcus equi* subsp. *equi, Streptococcus equi* subsp. *zooepidemicus*	Group A SDSE, *S. pyogenes*	Group G SDSE	Group G SDSE	Group G SDSE
Reference	This study	Suzuki et al. 2011	Brandt et al. 1999	Shimomura et al. 2011	Yoshida et al. 2011

NOTE.—Overview of SDSE strains used for comparisons with the 167 genome.

PCR Analysis

Conventional PCR to analyze the distribution of genes identified in this study was performed using TAKARA LATaq according to the manufacturer's instruction (TAKARA BIO Inc.). Primers used to amplify the corresponding genes are listed in supplementary table S2, Supplementary Material online.

Results and Discussion

SDSE 167, carrying Lancefield group C antigen, was isolated from an invasively infected human patient in 2003. We found that it was the most virulent SDSE strain isolated with an LD_{50} of 9.6×10^5 CFU/mouse in our SDSE collection having LD_{50} values ranging from 9.6×10^5 to 4.5×10^7 CFU/mouse (supplementary table S1, Supplementary Material online).

The SDSE 167 genome consists of a single circular chromosome of 2,076,397 bp with an average GC content of 39.57% (fig. 1 and table 1). The chromosome was shown to contain a total of 2223 protein-encoding genes, and 56 tRNA genes for all amino acids. In addition, the chromosome harbors two prophage-like elements (fig. 1 and table 2). To analyze evolutionary relationship of SDSE 167 with other SDSE strains, whole genome data of the five SDSE strains listed in table 1, including 167, were compared. Genome coverage analysis using the Blast algorithm indicated that approximately 90% of genome is shared among the five SDSE strains (supplementary table S3, Supplementary Material online).

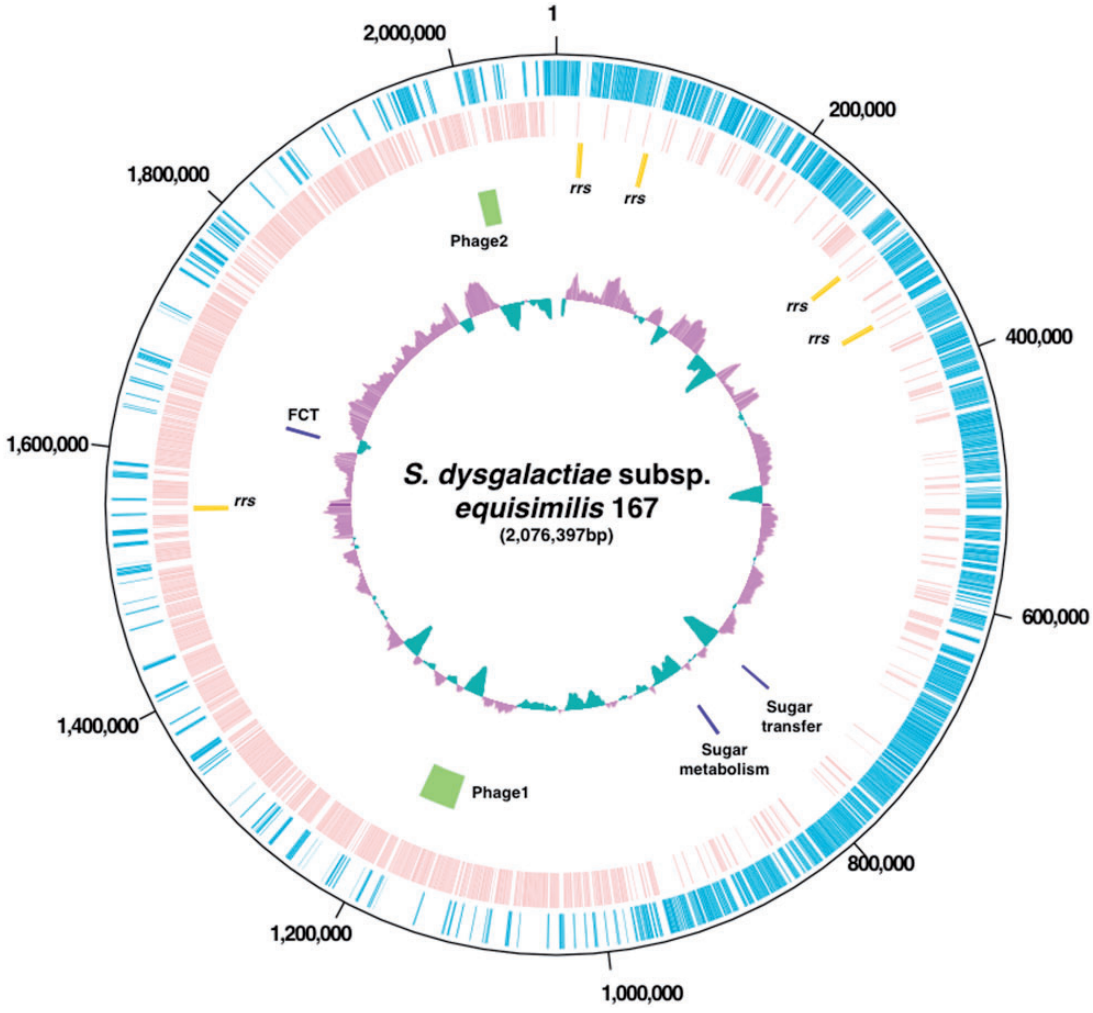

Fig. 1.—Circular representation of the genome of *Streptococcus dysgalactiae* subsp. *equisimilis* strain 167. Circle 1 (outermost circle) indicates the distances from the putative origin of replication. Circles 2 and 3 show annotated CDS encoded by the forward (light blue) and reverse (pink) chromosomal strands, respectively. Circle 4 shows the *rrs* operons. Circle 5 shows prophages (green). Circle 6 shows unique regions found in 167 other than in phages, including regions encoding enzymes involved in sugar transfer and sugar metabolism and the FCT region. Circle 7 (innermost circle) shows the G + C content with more and less than average (0.40) in purple and brown, respectively.

Table 2
Distribution of Prophages among SDSE Strains and Genes Carried by Each Prophage

Strain	Phage No.	Length	Best Hit of Blast	Comment
167	Φ1	37,972	Streptodornase (Sdn) Putative cell wall hydrolase, lysin Putative holin Putative hyaluronidase Head maturation protease Site-specific recombinase Putative transcriptional activator Putative C5 methylase MarMP1 Single-strand binding protein Putative replisome organizer	
	Φ2	18,822		Defective
AC-2713	Φ1	10,880	gp44 clamp loader subunit	Defective
	Φ2	36,966	Site-specific recombinase Putative cell wall hydrolase, lysin Holin Putative platelet-binding protein ClpP-like protease Putative portal protein Putative DNA methylase Transferase Putative helicase Putative DNA polymerase A domain	
	Φ3	5,854	DNA cytosine methylase Transcriptional regulator Putative repressor protein	Defective
	Φ4	38,710	Putative helicase Site-specific recombinase Putative cell wall hydrolase, lysin Amidase Holin PblB Putative DNA methylase Endonuclease of the HNH family with predicted DNA-binding module at C-terminus Transferase Putative helicase Phi APSE P51-like protein Putative DNA polymerase A domain	
	Φ5	14,532		Defective
	Φ6	5,920	Csp Putative DNA polymerase III delta prime subunit	Defective
ATCC12394	Φ1	10,872	gp44 clamp loader subunit	Defective
	Φ2	11,328	DNA polymerase accessory protein	Defective
	Φ3	11,990	IMPB Putative methyltransferase-endonuclease	Defective
	Φ4	28,611	Putative DNA polymerase III delta prime subunit	

(continued)

Table 2 Continued

Strain	Phage No.	Length	Best Hit of Blast	Comment
GGS_124	Φ1	10,897	gp44 clamp loader subunit	Defective
	Φ2	18,298	Putative DNA binding protein Putative DNA methylase	Defective
	Φ3	41,484	Streptodornase Cell wall hydrolase Putative holin protein Putative hyaluronidase Putative platelet-binding protein Putative human platelet-binding Putative endodeoxyribonuclease Putative recombinase	Defective
	Φ4	10,774	Site-specific recombinase	Defective
	Φ5	35,572	Putative *N*-acetylmuramoyl-L-alanine amidase Putative holin Putative endodeoxyribonuclease Putative platelet-binding protein Recombination protein	
	Φ6	19,428	Putative DNA polymerase III delta prime subunit	Defective
RE378	Φ1	8,852	Putative helicase	Defective

NOTE.—Prophage regions of the SDSE strains were identified using Prophage Finder (Bose and Barber 2006). Proteins encoded by the prophage were analyzed by the Blast algorithm, with the best hits listed. Only the Blast results not categorized as "hypothetical protein" are listed in the table.

Genome rearrangement analysis showed no evidence of massive recombination among these SDSE strains (fig. 2*a*). Some regions showing diversity are located in the prophage regions, as omitting the prophage regions from analysis resulted in decreased rearrangements (fig. 2*b*). The relative stability of the whole genomes of the SDSE strains allowed alignment and analysis of the phylogenetic evolution of SDSE using whole-genome sequences. This phylogenetic analysis using BEAST, which is designed to reconstruct evolutionary history over time from sampled DNA sequences using a post-probabilistic approach (Drummond and Rambaut 2007), indicated that the genetic distance of 167 is relatively far from the others, suggesting that the most recent common ancestor of all five SDSE strains appeared about 446 years ago (fig. 3*a*). Essentially the same results were obtained from analysis of core genome sequences, after omitting the phage regions (fig. 3*b*).

We compared prophage regions among the SDSE strains (table 2). SDSE 167 harbors two prophages, with a gene encoding streptodornase (*sdzD*), which presumably contribute to the prompted *covRS* mutation in vivo responsible for conversion of GAS into more virulent phenotype (Walker et al. 2007) found in prophage 1. The *sdzD* gene was shared among the SDSE isolates (supplementary table S2, Supplementary Material online). A comprehensive homology search of prophages found in SDSE showed that some of the prophages are shared, whereas the two prophages in 167 showed relatively low identity values, indicating that these prophages are essentially unique (supplementary table S4, Supplementary Material online). We also analyzed the genomes for the presence of clustered regularly interspaced short palindromic repeats (CRISPR) and CRISPR-associated sequence proteins (CASs) (Bhaya et al. 2011; Wiedenheft et al. 2012). CRISPR relies on small RNAs for sequence-specific detection and cleaving of foreign nucleic acids, including bacteriophages and plasmids. All SDSE strains sequenced previously harbor at least one probable CRISPR with more than 20 spacers. In contrast, newly sequenced 167 does not harbor any probable CRISPR or CAS. Thus, presumably there is no interference by the CRISPR system in 167, and SDSE 167 may be prone to infection by phages. Analysis of a larger number of group C SDSE isolates is necessary to determine whether the absence of CRISPR interference is common to these isolates (table 3).

Analysis of unique regions other than prophage regions in SDSE 167 showed that SDSE 167 harbors two unique gene clusters, which are not found in the other four SDSE strains. One cluster encodes glycosyl transferase and membrane proteins (table 1, locus_tag: SDSE167_0822 to SDSE167_0826). The finding, that this cluster is surrounded by other carbohydrate modifying enzymes, such as α-(1,2)-rhamnosyltransferase, and α-L-Rhaalpha-1,3-L-rhamnosyltransferase

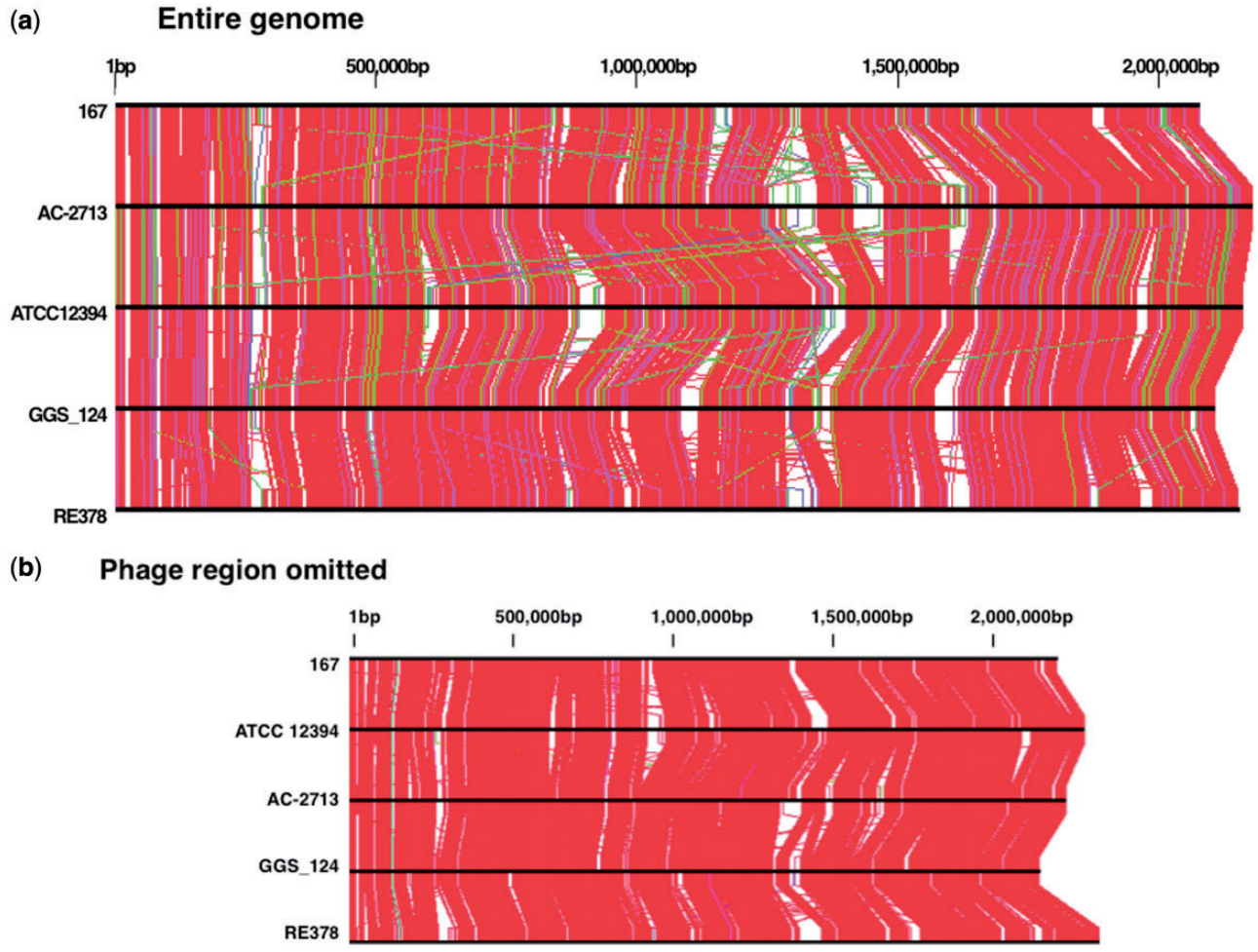

Fig. 2.—Genome rearrangement maps of *S. dysgalactiae* subsp. *equisimilis* 167 with four other SDSE strains. Genome rearrangement maps were prepared with entire genomes (*a*) or after omitting the phage regions (*b*) using in silico molecular cloning. Sequences were aligned from the predicted replication origin of each genome. Red and green represent the highest and lowest identity of nucleotide sequences, respectively.

(SDSE167_0813 to 0829). This region shows 65% identity with six genes of *Streptococcus mutans* (*rgpA* through *rgpF*), whose disruption results in a loss of serotype-specific antigenicity, specified by the glucose side chains of rhamnose–glucose polysaccharide from the cell wall (Yamashita et al. 1998), suggesting that this region may be involved in the synthesis of Lancefield group C antigen. Blast analyses of the cluster as well as the corresponding region of the other SDSE strains, three in Lancefield group G and one in Lancefield group A, indicated that these clusters showed identity with those of bacteria carrying group C, G, and A antigens, respectively (table 1). PCR analysis using the regions-specific primers showed that corresponding regions of each putative group antigen were carried by the SDSE isolates (supplementary table S1, Supplementary Material online). Although phenotypic analysis is necessary to elucidate the functional roles of these clusters, their sequences may be used in place of serotyping to identify Lancefield group antigens.

The other unique region found in SDSE 167 is located at SDSE167_0904 to SDSE167_0915. This region did not show significant homology with genome of *Streptococcus pyogenes* except for first 1 kb region, which encodes two hypothetical proteins only at nucleic acid level. The remaining region shows weak identity with the PTS system, galactitol-specific IIC component of *Enterococcus faecium* NRRL B-2354 and ribulose-phosphate 3-epimerase of *Streptococcus agalactiae* 09mas018883. This region contains sugar metabolizing enzymes, including tagatose-6-phosphate kinase, phosphoenolpyruvate-dependent sugar, the phosphotransferase system, the phosphotransferase system protein, the PTS system galactitol-specific IIC component, PTS system galactitol-specific IIC component, predicted protein, class II aldolase/adducin, and allulose-6-phosphate 3-epimerase. Our recent microarray results suggested that, upon injection into mouse peritoneal

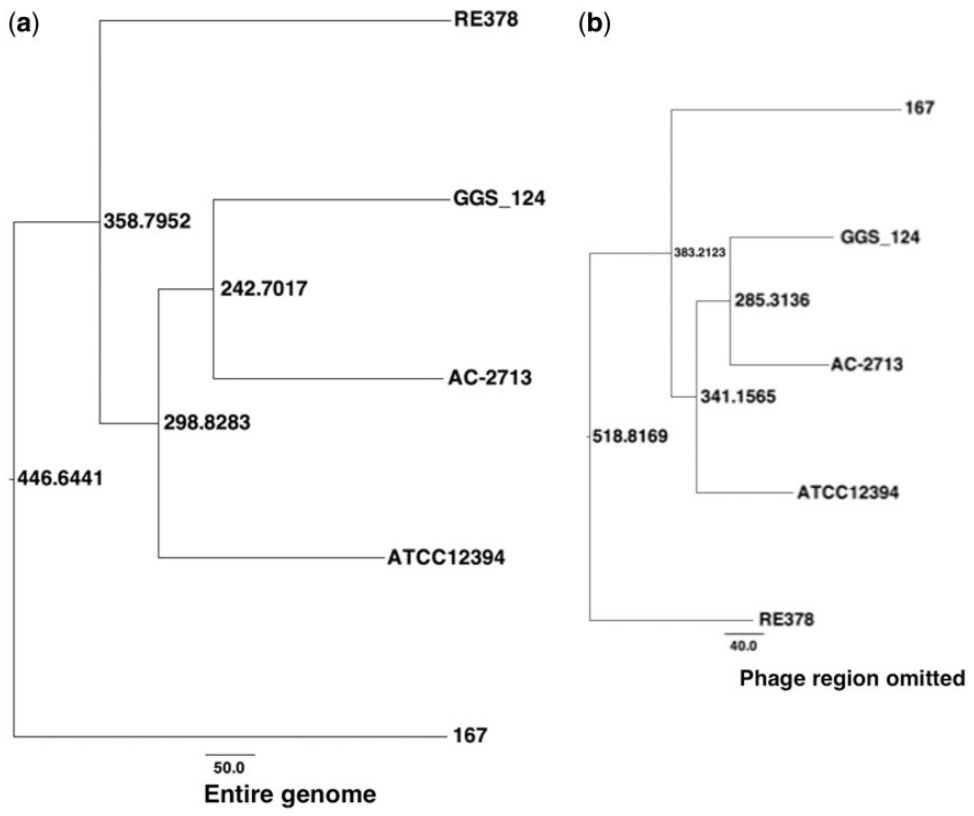

Fig. 3.—Bayesian phylogenetic trees of SDSE. Phylogenetic trees were prepared with entire genomes (*a*) or after omitting the phage regions (*b*) using BEAST (Drummond and Rambaut 2007) and visualized using FigTree. Estimated ages of subclades are shown as the median values. ESS values of all parameters in BEAST are more than 200. The posterior probability value for each branch was 1.

Table 3
Distribution of CRISPR and Cas Genes among the SDSE Strains

Strain	CRISPR ID	No. of Spacers	Cas Genes
167	tmp_1_Possible CRISPR_1	1	No hits
	tmp_1_Possible CRISPR_2	1	No hits
AC-2713	tmp_1_Probable CRISPR_2	19	Csn1
	tmp_1_Probable CRISPR_3	14	Cas3
	tmp_1_Possible CRISPR_1	1	Csn1
ATCC12394	tmp_1_Probable CRISPR_2	25	Csn1
	tmp_1_Probable CRISPR_3	29	Cas3
	tmp_1_Possible CRISPR_1	2	No hits
	tmp_1_Possible CRISPR_4	1	No hits
GGS_124	tmp_1_Probable CRISPR_2	18	Csn1
	tmp_1_Possible CRISPR_1	1	No hits
	tmp_1_Possible CRISPR_3	1	No hits
RE378	tmp_1_Probable CRISPR_2	7	Csn1
	tmp_1_Probable CRISPR_3	13	Cas3
	tmp_1_Possible CRISPR_1	1	No hits
	tmp_1_Possible CRISPR_4	1	No hits

Note.—CRISPR and Cas genes among the SDSE strains were identified using CRISPRfinder (Grissa et al. 2007). Possible CRISPR, probable CRISPR, number of spacers, and type of Cas are presented.

cavities, SDSE degrades host tissue polysaccharides by secreting poly/oligosaccharide lyases, while simultaneously using the Entner–Doudoroff pathway to metabolize acquired carbohydrates (Watanabe et al. 2013), and this region was found in 167 among the SDSE isolates (supplementary table S1, Supplementary Material online). Thus, this unique region containing sugar metabolizing enzymes may contribute to the higher virulence of SDSE 167.

In conclusion, we determined, for the first time, the complete genome sequence of a group C SDSE strain 167 and compared it with the genome sequences of other SDSE strains. Our results may provide insight into the pathogenic mechanism of SDSE and may form the basis of molecular epidemiological research on these highly virulent bacteria.

Supplementary Material

Supplementary tables S1–S4 are available at *Genome Biology and Evolution* online (http://www.gbe.oxfordjournals.org/).

Acknowledgments

The authors thank Mrs. Komiya and Mrs. Sakurai for excellent work in the genome analysis. This work was partly supported

by JSPS KAKENHI Grant Number 24390109 (to T.M.A.) and 25860330 (to S.W.) and by a Grant for International Health Research (23A-301) from the Ministry of Health, Labour and Welfare of Japan (to T.K.).

Literature Cited

Bhaya D, Davison M, Barrangou R. 2011. CRISPR-Cas systems in bacteria and archaea: versatile small RNAs for adaptive defense and regulation. Annu Rev Genet. 45:273–297.

Bose M, Barber RD. 2006. Prophage Finder: a prophage loci prediction tool for prokaryotic genome sequences. In Silico Biol. 6:223–227.

Brandt CM, Haase G, Schnitzler N, Zbinden R, Lütticken R. 1999. Characterization of blood culture isolates of *Streptococcus dysgalactiae* subsp. equisimilis possessing Lancefield's group A antigen. J Clin Microbiol. 37:4194–4197.

Cohen-Poradosu R, et al. 2004. Group G streptococcal bacteremia in Jerusalem. Emerg Infect Dis. 10:1455–1460.

Darriba D, Taboada GL, Doallo R, Posada D. 2012. jModelTest 2: more models, new heuristics and parallel computing. Nat Methods. 9:772.

Davies MR, et al. 2007. Virulence profiling of *Streptococcus dysgalactiae* subspecies *equisimilis* isolated from infected humans reveals 2 distinct genetic lineages that do not segregate with their phenotypes or propensity to cause diseases. Clin Infect Dis. 44:1442–1454.

Drummond AJ, Rambaut A. 2007. BEAST: Bayesian evolutionary analysis by sampling trees. BMC Evol Biol. 7:214.

Grissa I, Vergnaud G, Pourcel C. 2007. CRISPRFinder: a web tool to identify clustered regularly interspaced short palindromic repeats. Nucleic Acids Res. 35:W52–W57.

Katoh K, Standley DM. 2013. MAFFT multiple sequence alignment software version 7: improvements in performance and usability. Mol Biol Evol. 30:772–780.

Liao CH, Liu LC, Huang YT, Teng LJ, Hsueh PR. 2008. Bacteremia caused by group G streptococci, Taiwan. Emerg Infect Dis. 14:837–840.

Miyoshi-Akiyama T, et al. 2003a. Streptococcus dysgalactiae-derived mitogen (SDM), a novel bacterial superantigen: characterization of its biological activity and predicted tertiary structure. Mol Microbiol. 47: 1589–1599.

Miyoshi-Akiyama T, et al. 2003b. Quantitative and qualitative comparison of virulence traits, including murine lethality, among different M types of group A streptococci. J Infect Dis. 187:1876–1887.

Noguchi H, Taniguchi T, Itoh T. 2008. MetaGeneAnnotator: detecting species-specific patterns of ribosomal binding site for precise gene prediction in anonymous prokaryotic and phage genomes. DNA Res. 15:387–396.

Shimomura Y, et al. 2011. Complete genome sequencing and analysis of a Lancefield group G *Streptococcus dysgalactiae* subsp. *equisimilis* strain causing streptococcal toxic shock syndrome (STSS). BMC Genomics 12:17.

Suzuki H, et al. 2011. Comparative genomic analysis of the *Streptococcus dysgalactiae* species group: gene content, molecular adaptation, and promoter evolution. Genome Biol Evol. 3:168–185.

Sylvetsky N, Raveh D, Schlesinger Y, Rudensky B, Yinnon AM. 2002. Bacteremia due to beta-hemolytic Streptococcus group G: increasing incidence and clinical characteristics of patients. Am J Med. 112: 622–626.

Takahashi T, Ubukata K, Watanabe H. 2011. Invasive infection caused by *Streptococcus dysgalactiae* subsp. *equisimilis*: characteristics of strains and clinical features. J Infect Chemother. 17:1–10.

Vandamme P, Pot B, Falsen E, Kersters K, Devriese LA. 1996. Taxonomic study of lancefield streptococcal groups C, G, and L (*Streptococcus dysgalactiae*) and proposal of *S. dysgalactiae* subsp. *equisimilis* subsp. nov. Int J Syst Bacteriol. 46:774–781.

Walker MJ, et al. 2007. DNase Sda1 provides selection pressure for a switch to invasive group A streptococcal infection. Nat Med. 13: 981–985.

Watanabe S, Shimomura Y, Ubukata K, Kiriae T, Miyoshi-Akiyama T. 2013. Concomitant regulation of host tissue-destroying virulence factors and carbohydrate metabolism during invasive diseases induced by group G streptococci. J Infect Dis. Advance Access published July 30, 2013, doi: 10.1093/infdis/jit353.

Wiedenheft B, Sternberg SH, Doudna JA. 2012. RNA-guided genetic silencing systems in bacteria and archaea. Nature 482:331–338.

Yamashita Y, Tsukioka Y, Tomihisa K, Nakano Y, Koga T. 1998. Genes involved in cell wall localization and side chain formation of rhamnose-glucose polysaccharide in *Streptococcus mutans*. J Bacteriol. 180: 5803–5807.

Yoshida H, et al. 2011. A CD46 transgenic mouse model for studying the histopathology of arthritis caused by subcutaneous infection with *Streptococcus dysgalactiae* subspecies *equisimilis*. J Med Microbiol. 60:1860–1868.

Zhao J, et al. 2007. Cloning, expression, and characterization of the superantigen streptococcal pyrogenic exotoxin G from *Streptococcus dysgalactiae*. Infect Immun. 75:1721–1729.

Associate editor: Bill Martin