RESEARCH ARTICLE

Streptococcal taxonomy based on genome sequence analyses

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Abstract

The identification of the clinically relevant viridans streptococci group, at species level, is still problematic. The aim of this study was to extract taxonomic information from the complete genome sequences of 67 streptococci, comprising 19 species, by means of genomic analyses, multilocus sequence analysis (MLSA), average amino acid identity (AAI), genomic signatures, genome-to-genome distances (GGD) and codon usage bias. We then attempted to determine the usefulness of these genomic tools for species identification in streptococci. Our results showed that MLSA, AAI and GGD analyses are robust markers to identify streptococci at the species level, for instance, *S. pneumoniae*, *S. mitis*, and *S. oralis*. A *Streptococcus* species can be defined as a group of strains that share $\geq 95\%$ DNA similarity in MLSA and AAI, and $> 70\%$ DNA identity in GGD. This approach allows an advanced understanding of bacterial diversity.

Keywords

Comparative Genomic, Genomic Taxonomy, Streptococci species

This article is included in the **Phylogenetics** collection.

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Introduction

Bacteria are subjected to numerous forces driving their diversification. As a consequence, different strains of a single bacterial species sometimes have the ability to explore distinct niches, to be pathogenic or non-pathogenic and to present different metabolic pathways^{1,2}. In such a scenario, the identification of bacteria isolates to the species level is a hard task^{1,2}.

Currently, the genus *Streptococcus* comprises 99 recognized species, many of which are associated with disease in humans and animals (<http://www.bacterio.cict.fr/s/streptococcus.html>). The viridans group streptococci (VGS) encompass four phylogenetic clusters: Mitis, Mutans, Salivarius and Anginosus, which are part of the human microbiota, being isolated mainly from the oral cavity, gastrointestinal and genitourinary tracts³. The Mitis group currently includes the important pathogen *S. pneumoniae* and 12 other recognized species, *S. australis*, *S. cristatus* (formerly *S. crista*), *S. gordonii*, *S. infantis*, *S. mitis*, *S. oligofermentans*, *S. oralis*, *S. parasanguinis* (formerly *S. parasanguis*), *S. peroris*, *S. pseudopneumoniae*, *S. sanguinis* (formerly *S. sanguis*) and *S. sinensis*. The Anginosus group includes three recognized species, *S. anginosus*, *S. constellatus* (including two subspecies *S. constellatus* subsp. *constellatus* and *S. constellatus pharyngis*) and *S. intermedius*, and the Salivarius group includes *S. salivarius*, *S. vestibularis*, and *S. thermophilus*.

Currently, bacterial species are considered to be a group of strains (including the type strain) that are characterized by a certain degree of phenotypic consistency, showing > 70% DNA-DNA hybridization values and over 97% 16S rRNA sequence similarity^{4,5}. Identification of streptococci is based on the current taxonomic standards using a combination of 16S rRNA gene sequence analyses, DNA-DNA hybridization, serologic and phenotypic data; however, they have been strikingly resistant to satisfactory classification, reflected in frequently changing nomenclature^{6,7}. For instance, the 16S rRNA gene sequences of *S. mitis* and *S. oralis* are almost identical (> 99%) to *S. pneumoniae*, making the use of this information alone insufficient to distinguish these species⁸.

Recent studies have used whole genome analysis to determine the taxonomic relationships among bacterial species⁹⁻¹⁴. In order to determine the robustness of genomic markers in streptococci species delineation, we analyzed a collection of 67 complete genomes. The availability of whole genome sequences of several closely related species, for instance, *S. mitis* - *S. oralis* - *S. pneumoniae*, and *S. salivarius* - *S. thermophilus* - *S. vestibularis*, formed an ideal test case for the establishment of the genomic taxonomy of streptococci.

Material and methods

Genome sequence data

The genomic sequences of 67 streptococci that were publicly available for download by June 2nd, 2011 at the National Center for Biotechnology Information (NCBI) under the project accession number indicated in Table 1 were used in this study. The following analyses were performed according to Thompson *et al.* (2009)¹³ and are briefly described below.

16S rRNA gene sequence analysis and multilocus sequence analysis (MLSA)

The 16S rRNA gene sequences and the gene sequences used for MLSA were obtained from GenBank (<http://www.ncbi.nlm.nih.gov>). The MLSA approach was based on the concatenated sequences of five house-keeping genes (*aroE*, *ddl*, *gki*, *pheS* and *recA*)^{15,16}. The concatenated sequences were aligned with ClustalX program¹⁷. The phylogenetic inference was based on the neighbour-joining genetic distance method (NJ)¹⁸ using MEGA5¹⁹. Distance estimations were obtained according to the Kimura-2-parameter²⁰ for 16S rRNA gene and MLSA. The reliability of each tree topology was checked by 2000 bootstrap replications²¹.

Average amino acid identity (AAI)

The AAI of all conserved protein-coding genes was calculated as described previously²². Conserved protein-coding genes between a pair of genomes were determined by whole-genome pairwise sequence comparisons using the BLASTp algorithm²³. For these comparisons, all protein-coding sequences (CDSs) from one genome were searched against the genomic sequence of the other genome. The genetic relatedness between a pair of genomes was measured by the AAI of all conserved genes between the two genomes as computed by the BLAST algorithm. By this approach, a value of < 95% AAI of protein-coding genes indicates separate species.

Codon usage

Codon usage bias was calculated for each genome. The effective number of codons used in a sequence (N_c)²⁴ was calculated using CHIPS (<http://emboss.bioinformatics.nl/cgi-bin/emboss/chips>) with the default parameters.

Determination of dinucleotide relative abundance values and genomic dissimilarity

Mononucleotide and dinucleotide frequencies were calculated using COMPSEQ (<http://emboss.bioinformatics.nl/cgi-bin/emboss/compseq>) with default parameters. Dinucleotide relative abundances (p^*XY) were calculated using the equation $p^*XY = f_{XY}/f_Xf_Y$ where f_{XY} denotes the frequency of dinucleotide XY, and f_X and f_Y denote the frequencies of X and Y, respectively. The difference in genome signature between two sequences is expressed by the genomic dissimilarity (δ^*), which is the average absolute dinucleotide of relative abundance difference between two sequences, and were calculated using the equation: $\delta^*(f,g) = 1/16 \sum |p^*XY(f) - p^*XY(g)|$ (multiplied by 1000 for convenience), where the sum extends over all dinucleotides²⁵.

Genome-to-genome distances (GGD)

The genome distance was calculated using genome-to-genome distance calculator (GGDC)²⁶. Distances between a pair of genomes were determined by whole-genome pairwise sequence comparisons using BLAST²³. For these comparisons, algorithms were used to determine high-scoring segment pairs (HSPs) for inferring intergenomic distances for species delimitation. The corresponding distance threshold can be used for species delimitation²⁶.

Table 1. Genomic features of the streptococci. G+C content (%): guanine + cytosine content (%). No. of CDs: number of coding DNA sequence. *Nc*: effective number of codons.

Organism	GenBank accession no.	Genome size (nt)	G+C content (%)	No. of CDS	<i>Nc</i>
<i>S. agalactiae</i> A909	CP000114	2,127,839	35	1996	44.9
<i>S. agalactiae</i> NEM316	AL732656	2,211,485	35	2094	45.2
<i>S. agalactiae</i> 2603VR	AE009948	2,160,267	35	2124	45.1
<i>S. anginosus</i> F0211	AECT00000000	1,993,709	38	2035	50.6
<i>S. bovis</i> ATCC 700338	AEEL00000000	2,050,893	37	2088	44.5
<i>S. downei</i> F0415	AEKN00000000	2,239,421	43	2204	54.4
<i>S. dysgalactiae</i> subsp. <i>equisimilis</i> GGS-124	AP010935	2,106,340	39	2094	50.3
<i>S. equi</i> subsp. <i>equi</i> 4047	FM204883	2,253,793	41	2001	52.6
<i>S. equi</i> subsp. <i>zooepidemicus</i>	FM204884	2,149,868	41	1869	52.4
<i>S. equi</i> subsp. <i>zooepidemicus</i> MGCS10565	CP001129	2,024,171	41	1893	52.3
<i>S. gallolyticus</i> subsp. <i>gallolyticus</i> TX20005	AEEM00000000	2,214,091	37	2218	44.5
<i>S. gallolyticus</i> UCN34	FN597254	2,350,911	37	2223	44.4
<i>S. gordonii</i> str. <i>Challis</i> substr. CH1	CP000725	2,196,662	40	2051	52.4
<i>S. infantis</i> SK1302	AEDY00000000	1,792,252	39	2102	48.9
<i>S. infantarius</i> subsp. <i>infantarius</i> ATCC BAA-102	ABJK00000000	1,925,087	37	2051	44.0
<i>S. mitis</i> B6	FN568063	2,146,611	39	2004	50.4
<i>S. mitis</i> SK321	AEDT00000000	1,873,702	40	1757	49.8
<i>S. mutans</i> NN2025	AP010655	2,013,587	36	1895	46.4
<i>S. mutans</i> UA159	AE014133	2,030,921	36	1960	46.5
<i>S. oralis</i> ATCC 35037	AEDW00000000	1,884,712	41	1793	51.4
<i>S. parasanguinis</i> ATCC 15912	ADVN00000000	2,124,730	41	2035	52.8
<i>S. parasanguinis</i> F0405	AEKM00000000	2,050,302	41	1978	52.9
<i>S. pneumoniae</i> AP200	CP002121	2,130,580	39	2216	50.3
<i>S. pneumoniae</i> ATCC 700669	FM211187	2,221,315	39	1990	50.0
<i>S. pneumoniae</i> CGSP14	CP001033	2,209,198	39	2206	50.3
<i>S. pneumoniae</i> D39	CP000410	2,046,115	39	1914	49.8
<i>S. pneumoniae</i> G54	CP001015	2,078,953	39	2114	50.0
<i>S. pneumoniae</i> Hungary19A-6	CP000936	2,245,615	39	2155	50.2
<i>S. pneumoniae</i> INV104	FQ312030	2,142,122	39	1824	49.9
<i>S. pneumoniae</i> INV200	FQ312029	2,093,317	39	1930	50.0
<i>S. pneumoniae</i> JJA	CP000919	2,120,234	39	2123	50.2
<i>S. pneumoniae</i> OXC141	FQ312027	2,036,867	39	1824	49.9
<i>S. pneumoniae</i> P1031	CP000920	2,111,882	39	2073	50.1
<i>S. pneumoniae</i> R6	AE007317	2,038,615	39	2042	50.1
<i>S. pneumoniae</i> Taiwan19F-14	CP000921	2,112,148	39	2044	50.1
<i>S. pneumoniae</i> TCH843119A	CP001993	2,088,772	39	2275	50.4
<i>S. pneumoniae</i> TIGR4	AE005672	2,160,842	39	2105	50.0
<i>S. pneumoniae</i> 670-6B	CP002176	2,240,045	39	2352	50.4
<i>S. pneumoniae</i> 70585	CP000918	2,184,682	39	2202	50.1
<i>S. pseudoporcinus</i> SPIN 20026	AENS00000000	2,111,372	36	2030	48.6
<i>S. pyogenes</i> MGAS315	AE014074	1,900,521	38	1865	49.1
<i>S. pyogenes</i> MGAS2096	CP000261	1,860,355	38	1898	49.4
<i>S. pyogenes</i> MGAS5005	CP000017	1,838,554	38	1865	48.9
<i>S. pyogenes</i> MGAS6180	CP000056	1,897,573	38	1894	48.9
<i>S. pyogenes</i> MGAS8232	AE009949	1,895,017	38	1839	49.0
<i>S. pyogenes</i> MGAS9429	CP000259	1,836,467	38	1877	49.0
<i>S. pyogenes</i> MGAS10270	CP000260	1,928,252	38	1986	49.0
<i>S. pyogenes</i> MGAS10394	CP000003	1,899,877	38	1886	49.2
<i>S. pyogenes</i> MGAS10750	CP000262	1,937,111	38	1979	49.1
<i>S. pyogenes</i> M1 GAS	AE004092	1,852,441	38	1696	48.8
<i>S. pyogenes</i> NZ131	CP000829	1,815,785	38	1700	48.8

Organism	GenBank Accession no.	Genome size (nt)	G+C content (%)	No. of CDS	Nc
<i>S. pyogenes</i> SSI-1	BA000034	1,894,275	38	1859	49.1
<i>S. pyogenes</i> str. <i>Manfredo</i>	AM295007	1,841,271	38	1745	48.9
<i>S. salivarius</i> SK126	ACLO00000000	2,128,332	40	1992	47.0
<i>S. sanguinis</i> ATCC 49296	AEPO00000000	2,054,852	41	2013	51.7
<i>S. sanguinis</i> SK36	CP000387	2,388,435	43	2270	54.5
<i>S. sanguinis</i> VMC66	AEVH00000000	2,311,949	43	2260	54.5
<i>S. suis</i> BM407	FM252032	2,146,229	41	1932	52.0
<i>S. suis</i> GZ1	CP000837	2,038,034	41	1979	52.4
<i>S. suis</i> P17	AM946016	2,007,491	41	1824	51.9
<i>S. suis</i> SC84	FM252031	2,095,898	41	1898	52.0
<i>S. thermophilus</i> CNRZ1066	CP000024	1,796,226	39	1915	47.0
<i>S. thermophilus</i> LMD-9	CP000419	1,856,368	39	1709	46.8
<i>S. thermophilus</i> LMG 18311	CP000023	1,796,846	39	1888	46.9
<i>S. thermophilus</i> ND03	CP002340	1,831,949	39	1919	46.8
<i>S. uberis</i> 0140J	AM946015	1,852,352	36	1762	46.4
<i>S. vestibularis</i> F0396	AEKO00000000	2,022,289	39	1979	47.1

Results and discussion

In this work we compared complete genomes for 67 streptococci comprising 19 species to address their taxonomic position. A previous study with a small set of streptococci genomes (eight) and species (four), using a combination of several genomic analyses, showed the applicability of this approach in streptococci taxonomy⁹. Overall our analysis, using a large data set, showed that genomic taxonomy is an accurate approach to clearly define the streptococci species. The taxonomic resolution of the 16S rRNA, AAI, MLSA, GGD and codon usage analysis for streptococci species definition is summarized in Table 2.

General genomic features

The complete genome of the streptococci comprised a single chromosome. The estimated size of the genomes ranged from 1.7 Mb (*S. infantis*) to 2.3 Mb (*S. sanguinis*). The number of CDS varied from 1,700 (*S. pyogenes*) to 2,352 (*S. pneumoniae*) (Table 1). The average G+C content of streptococci genomes ranged from 35% to 43%. These species presented a variable interspecies genome size and G+C content, indicating heterogeneity within the genus *Streptococcus*. One of the reasons for this variability could be associated with the frequent occurrence of horizontal gene transfer events^{27–29}.

Phylogenetic reconstructions by 16S rRNA and MLSA

MLSA and 16S rRNA phylogenetic trees showed similar topologies (Figure 1). The MLSA was performed using five instead of the seven genes applied in the pneumococcus multilocus sequence typing (MLST) scheme (<http://spneumoniae.mlst.net/>)^{15,16}. Three genes, *aroE*, *ddl* and *gki*, are from the MLST scheme, and *pheS* and *recA* were included in this work. The concatenation of these genes (7741 bp) allowed an accurate delineation of the streptococci species considered here. The nucleotide sequence similarities were much lower for MLSA than 16S rRNA gene. A pairwise comparison of MLSA among the species revealed sequence similarity between 67% and 100%, while the 16S rRNA gene sequence similarities varied from 92% to 100%. At the intraspecies level, the similarity

values ranged from 95% to 100% for MLSA, and 99% to 100% for the 16S rRNA gene sequences. The closest species within the Mitis (*S. pneumoniae* - *S. oralis* - *S. mitis*) and Salivarius groups (*S. vestibularis* - *S. salivarius* - *S. thermophilus*) were clearly placed apart from each other by MLSA, while these species had almost identical 16S rRNA gene sequences ($\geq 99\%$ sequence similarity). A previously study showed that *recA* analysis is a valuable tool for proper identification of pneumococci in routine diagnostics, but limitations on discrimination of other members of the Mitis group were observed³⁰. *S. sanguinis* ATCC 49296 showed a much closer relationship with *S. oralis* ATCC 35037T (95% similarity) than to other *S. sanguinis* strains (77% similarity), suggesting it belongs to the species *S. oralis*. In addition, *S. bovis* ATCC 700338 was placed in the *S. gallolyticus* cluster with 98% MLSA sequence similarity. This work showed that MLSA, using this new combination of five concatenated genes (*aroE*, *ddl*, *gki*, *pheS* and *recA*), distinct from the *Streptococcus* MLST scheme, allowed a proper identification of most streptococci species, even within the VGS group.

Average amino acid identity (AAI)

The percentage of average amino acid identity (AAI) among streptococci species ranges from 68% to 94%, while within species it varies from 95% to 100%. The VGS species *S. pneumoniae*, *S. mitis* and *S. oralis* shared 89–93% AAI. The species *S. salivarius*, *S. thermophilus* and *S. vestibularis* showed a maximum AAI of 93%. *S. sanguinis* ATCC 49296 and *S. oralis* ATCC 35037 showed 96% identity and *S. bovis* ATCC 700338 and *S. gallolyticus* strains had 98% identity. These findings suggest that strains ATCC 49296 and ATCC 700338 belong to the species *S. oralis* and *S. gallolyticus*, respectively. According to our analyses the AAI and MLSA are the most useful genomic features for the elucidation of streptococci taxonomy.

Genome signature

The genomic dissimilarity values among streptococci were between 3 and 127, while the intraspecies values were between 0 and 17. Streptococci within the VGS group, for instance, *S. salivarius*,

Table 2. Taxonomic resolution of genomic analyses of streptococci species. MLSA: multilocus sequence analysis. AAI: amino acid identity. GGD: genome to genome distance. Nc: effective number of codons.

	16S rRNA (%)	MLSA (%)	AAI (%)	GGD (%)	Codon usage (Nc)
Intraspecies	≥99	≥95	≥95	>70	–
<i>S. pyogenes</i>	≥99	≥98	>97	>70	49
<i>S. agalactiae</i>	99	100	98	>70	45
<i>S. equi</i>	99	98	>96	>70	52
<i>S. suis</i>	100	100	100	>70	52
<i>S. pneumoniae</i>	99	≥97	>97	>70	50
<i>S. thermophilus</i>	99	100	>97	>70	47
Interspecies	≤99	<95	<95	<70	44–54
<i>S. thermophilus-salivarius-vestibularis</i>	99	<94	<92	<70	47
<i>S. pneumoniae-mitis-oralis</i>	>99	<94	<93	<70	50–51

Raw data: MLSA nucleotide sequences

2 Data Files

<http://dx.doi.org/10.6084/m9.figshare.157260>

Raw data: average amino acid identity

2 Data Files

<http://dx.doi.org/10.6084/m9.figshare.157261>

Raw data: genomic signatures

2 Data Files

<http://dx.doi.org/10.6084/m9.figshare.157262>

S. thermophilus and *S. vestibularis* species, showed dissimilarity values between 5 and 12 and *S. pneumoniae*, *S. mitis* and *S. oralis* species had dissimilarity values between 3 and 14. Thus, there was not a clear differentiation of these closely related species within the VGS group on the basis of the genomic dissimilarity values. This could be due to the extensive recombination and horizontal gene transfer events which occur between closely related streptococci species that share ecological niches^{12,30}.

On the other hand, species within the Pyogenic group had a distinct genomic signature, with values ranging from 13 to 85. However, genome signatures alone have significant limitations when used as phylogenetic markers for differentiating members of the VGS. The exact mechanisms that generate and maintain the genome signatures are complex, but possibly involve differences in species-specific compositional bias, i.e., G+C content, G+C and A+T skews, codon bias, and mutation bias^{32,33}.

Codon usage bias (Nc)

Nc values provide a meaningful measure of the extent of codon preference in a genome, values range between 20 (extremely biased

genome where one codon is used per amino acid) and 61 (all synonymous codons are used). Within the set of 67 complete streptococci genomes examined in this study, the Nc ranged from 44.0 to 54.5 (Table 1). For instance, *S. pneumoniae* - *S. oralis* - *S. mitis* species had Nc values of 50, 51 and 50, respectively. The Salivarius group (*S. vestibularis* - *S. salivarius* - *S. thermophilus*), and *S. bovis* ATCC 700338 - *S. gallolyticus* showed Nc values of 47 and 44.5, respectively. Overall, codon usage bias was very similar among the streptococci species investigated. However, *S. sanguinis* ATCC 49296 showed a much closer Nc value with the *S. oralis* ATCC 35037 (51.7 and 51.4, respectively) than other *S. sanguinis* strains (54.5), which was in agreement with the other analyses used in this study.

Genome distance analysis

The GGD was calculated only for closely related species that were not differentiated by 16S rRNA gene sequence analysis (Figure 1). Based on GGD analysis the species within the Mitis and Salivarius groups were identified as separate species, showing GGD values analogous to the < 70% discriminatory value used for DNA-DNA hybridization. Conversely, *S. bovis* ATCC 700338 and *S. gallolyticus* were identified as belonging to the same species by GGD.

S. bovis ATCC 700338 (biotype II) and *S. gallolyticus* as well as *S. sanguinis* ATCC 49296 and *S. oralis* ATCC 35037T were not separated and, therefore, according to this analysis would be classified as the same species, respectively. It was shown that *S. bovis* biotype I and II/2 isolates were, in fact, *S. gallolyticus*³⁴, and *S. sanguinis* ATCC 49296 was placed into *S. oralis* species by GGD analysis. A misidentification of *S. sanguinis* ATCC 49296 has already been shown by means of biochemical and serological properties by Narikawa and colleagues³⁵.

Another interesting result is that the *S. parasanguinis* ATCC 15912 and F0405 strains were found to be at the upper limits for definition as members of the same species based on different genomic analyses. For instance, they shared 95% AAI, 94% identity by MLSA, a value of 17 on the basis of genomic signature and < 70% similarity in GGD. Therefore, based on these genomic

Figure 1. Neighbor-joining tree based on 16S rRNA gene sequences and MLSA concatenated sequences of *Streptococcus*. The numbers at the nodes indicate the values of bootstrap statistics after 2000 replications, and values below 50% are not shown. Bars, 0.005% and 0.02% estimated sequence divergence.

markers, these *S. parasanguinis* strains could, in fact, be separate species. This data reflects the complexity of bacterial species delineation, since these organisms are all under a constant evolutionary process.

Conclusion

The delineation of closely related streptococci species was evident in this genomic study. Different methods produced different levels of taxonomic resolution. The methods with the higher resolution for species identification were MLSA and AAI, while closely related species had similar *Nc* values and genomic signatures. Based on the genomic analyses, a *Streptococcus* species can be defined as a group of strains that shares $\geq 95\%$ identity in MLSA and AAI, and $> 70\%$ identity in GGD. This definition may be useful to advance the taxonomy of *Streptococcus*. This approach allows an advanced understanding of bacterial diversity and identification.

Author contributions

CCT and VEE carried out the computational and genomic analyses and analyzed the results. All authors (ACPV, CCT, ELF, MAM and VEE) participated in discussing and writing the manuscript. All authors have agreed to the final contents of the article.

Competing interests

No relevant competing interests were disclosed.

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References

1. Gevers D, Cohan FM, Lawrence JG, *et al.*: **Opinion: Re-evaluating prokaryotic species.** *Nat Rev Microbiol.* 2005; 3(9): 733–9.
[PubMed Abstract](#) | [Publisher Full Text](#)
2. Cohan FM, Koeppel AF: **The origins of ecological diversity in prokaryotes.** *Curr Biol.* 2008; 18(21): R1024–34.
[PubMed Abstract](#) | [Publisher Full Text](#)
3. Alam S, Brailsford SR, Whitley RA, *et al.*: **PCR-Based methods for genotyping viridans group streptococci.** *J Clin Microbiol.* 1999; 37(9): 2772–6.
[PubMed Abstract](#) | [Free Full Text](#)
4. Stackebrandt E, Goebel BM: **Taxonomic Note: A place for DNA-DNA reassociation and 16S ribosomal-RNA sequence analysis in the present species definition in bacteriology.** *Int J Syst Bacteriol.* 1994; 44(4): 846–849.
[Publisher Full Text](#)
5. Wayne LG, Brenner DJ, Colwell RR, *et al.*: **Report of the ad hoc committee on reconciliation of approaches to bacterial systematics.** *Int J Syst Bacteriol.* 1987; 37(4): 463–464.
[Publisher Full Text](#)
6. Hoshino T, Fujiwara T, Kilian M: **Use of phylogenetic and phenotypic analyses to identify nonhemolytic streptococci isolated from bacteremic patients.** *J Clin Microbiol.* 2005; 43(12): 6073–85.
[PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#)
7. Kawamura Y, Hou XG, Sultana F, *et al.*: **Determination of 16S rRNA sequences of *Streptococcus mitis* and *Streptococcus gordonii* and phylogenetic relationships among members of the genus *Streptococcus*.** *Int J Syst Bacteriol.* 1995; 45(2): 406–8.
[PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#)
8. Suzuki N, Seki M, Nakano Y, *et al.*: **Discrimination of *Streptococcus pneumoniae* from viridans group streptococci by genomic subtractive hybridization.** *J Clin Microbiol.* 2005; 43(9): 4528–34.
[PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#)
9. Coenye T, Vandamme P: **Extracting phylogenetic information from whole-genome sequencing projects: the lactic acid bacteria as a test case.** *Microbiology.* 2003; 149(pt 12): 3507–17.
[PubMed Abstract](#) | [Publisher Full Text](#)
10. Coenye T, Gevers D, Van de Peer Y, *et al.*: **Towards a prokaryotic genomic taxonomy.** *FEMS Microbiol Rev.* 2005; 29(2): 147–67.
[PubMed Abstract](#) | [Publisher Full Text](#)
11. Richter SS, Heilmann KP, Dohm CL, *et al.*: **Accuracy of phenotypic methods for identification of *Streptococcus pneumoniae* isolates included in surveillance programs.** *J Clin Microbiol.* 2008; 46(7): 2184–8.
[PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#)
12. Croucher NJ, Harris SR, Fraser C, *et al.*: **Rapid pneumococcal evolution in response to clinical interventions.** *Science.* 2011; 331(6016): 430–4.
[PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#)
13. Thompson CC, Vicente ACP, Souza RC, *et al.*: **Genomic taxonomy of *Vibrios*.** *BMC Evol Biol.* 2009; 9: 258.
[PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#)
14. Thompson CC, Vieira NM, Vicente AC, *et al.*: **Towards a genome based taxonomy of *Mycoplasmas*.** *Infect Genet Evol.* 2011; 11(7): 1798–804.
[PubMed Abstract](#) | [Publisher Full Text](#)
15. Enright MC, Spratt BG: **A multilocus sequence typing scheme for *Streptococcus pneumoniae*: identification of clones associated with serious invasive disease.** *Microbiology.* 1998; 144(Pt 11): 3049–60.
[PubMed Abstract](#) | [Publisher Full Text](#)
16. Hanage WP, Fraser C, Spratt BG: **Sequences, sequence clusters and bacterial species.** *Philos Trans R Soc Lond B Biol Sci.* 2006; 361(1475): 1917–27.
[PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#)
17. Thompson JD, Gibson TJ, Plewniak F, *et al.*: **The CLUSTAL_X windows interface: flexible strategies for multiple sequence alignment aided by quality analysis tools.** *Nucleic Acids Res.* 1997; 25(24): 4876–82.
[PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#)
18. Saitou N, Nei M: **The neighbor-joining method: a new method for reconstructing phylogenetic trees.** *Mol Biol Evol.* 1987; 4(4): 406–25.
[PubMed Abstract](#)
19. Tamura K, Peterson D, Peterson N, *et al.*: **MEGA5: molecular evolutionary genetics analysis using maximum likelihood, evolutionary distance, and maximum parsimony methods.** *Mol Biol Evol.* 2011; 28(10): 2731–9.
[PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#)
20. Kimura M: **A simple method for estimating evolutionary rates of base substitutions through comparative studies of nucleotide sequences.** *J Mol Evol.* 1980; 16(2): 111–20.
[PubMed Abstract](#) | [Publisher Full Text](#)
21. Felsenstein J: **Confidence Limits on Phylogenies: An Approach Using the Bootstrap.** *Evolution.* 1985; 39(4): 783–791.
[Publisher Full Text](#)
22. Konstantinidis KT, Tiedje JM: **Towards a genome-based taxonomy for prokaryotes.** *J Bacteriol.* 2005; 187(18): 6258–64.
[PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#)
23. Altschul SF, Madden TL, Schäffer AA, *et al.*: **Gapped BLAST and PSI-BLAST: a new generation of protein database search programs.** *Nucleic Acids Res.* 1997; 25(17): 3389–402.
[PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#)

24. Wright F: **The 'effective number of codons' used in a gene.** *Gene*. 1990; **87**(1): 23–9.
[PubMed Abstract](#) | [Publisher Full Text](#)
25. Karlin S, Mrázek J, Campbell AM: **Compositional biases of bacterial genomes and evolutionary implications.** *J Bacteriol*. 1997; **179**(12): 3899–913.
[PubMed Abstract](#) | [Free Full Text](#)
26. Auch AF, Klenk HP, Göker M: **Standard operating procedure for calculating genome-to-genome distances based on high-scoring segment pairs.** *Stand Genomic Sci*. 2010; **2**(1): 142–8.
[PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#)
27. Zhang A, Yang M, Hu P, *et al.*: **Comparative genomic analysis of *Streptococcus suis* reveals significant genomic diversity among different serotypes.** *BMC genomics*. 2011; **12**: 523.
[PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#)
28. Bellanger X, Roberts AP, Morel C, *et al.*: **Conjugative transfer of the integrative conjugative elements ICES₁ and ICES₃ from *Streptococcus thermophilus*.** *J Bacteriol*. 2009; **191**(8): 2764–75.
[PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#)
29. Harvey RM, Stroeher UH, Ogunniyi AD, *et al.*: **A variable region within the genome of *Streptococcus pneumoniae* contributes to strain-strain variation in virulence.** *PLoS One*. 2011; **6**(5): e19650.
[PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#)
30. Zbinden A, Köhler N, Bloemberg GV: **recA-based PCR assay for accurate differentiation of *Streptococcus pneumoniae* from other viridans streptococci.** *J Clin Microbiol*. 2011; **49**(2): 523–7.
[PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#)
31. Donati C, Hiller NL, Tettelin H, *et al.*: **Structure and dynamics of the pan-genome of *Streptococcus pneumoniae* and closely related species.** *Genome Biol*. 2010; **11**(10): R107.
[PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#)
32. Karlin S: **Global dinucleotide signatures and analysis of genomic heterogeneity.** *Curr Opin Microbiol*. 1998; **1**(15): 598–610.
[PubMed Abstract](#) | [Publisher Full Text](#)
33. Foerstner KU, von Mering C, Hooper SD, *et al.*: **Environments shape the nucleotide composition of genomes.** *EMBO Rep*. 2005; **6**(12): 1208–13.
[PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#)
34. Devriese LA, Vandamme P, Pot B, *et al.*: **Differentiation between *Streptococcus gallolyticus* strains of human clinical and veterinary origins and *Streptococcus bovis* strains from the intestinal tracts of ruminants.** *J Clin Microbiol*. 1998; **36**(12): 3520–3.
[PubMed Abstract](#) | [Free Full Text](#)
35. Narikawa S, Suzuki Y, Takahashi M, *et al.*: ***Streptococcus oralis* previously identified as uncommon "*Streptococcus sanguis*" in Behçet's disease.** *Arch Oral Biol*. 1995; **40**(8): 685–90.
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Bruno Gomez-Gil

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The article is well written with an appropriate title and abstract. The methods are adequate for the aims of the study, but I would suggest that including the Average Nucleotide Identity (ANI) analysis as suggested by [Rosello-Mora et al. 2006](#), would certainly improve the manuscript. The online analysis can be found here <http://www.imedea.uib.es/jspecies/index.html>. The conclusions are adequate and the data sufficient to replicate all the analyses. The data are openly accessible at GenBank.

Competing Interests: No competing interests were disclosed.

I confirm that I have read this submission and believe that I have an appropriate level of expertise to confirm that it is of an acceptable scientific standard.

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Tomoo Sawabe

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- Title and abstract are good enough to attract readers in the scientific community.
- Genome based multi-gene sequence comparison is one of the promising tools to analyse

bacterial populations. To achieve the analysis for *Streptococcus*, the authors carefully designed massive data genome analysis. The results are strong enough and supported by the results of the analysis.

- The conclusion is clear that authors proposed a threshold value on the basis of genome-based MLSA in *Streptococcus* bacteria.

Competing Interests: No competing interests were disclosed.

I confirm that I have read this submission and believe that I have an appropriate level of expertise to confirm that it is of an acceptable scientific standard.

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