

Supplementary materials

Table S1 List of the complete *Streptococcus pyogenes* genomes used for the phylogenetic analysis Core-genome phylogeny was done using Parsnp [1]. SF370 was used as reference for the detection of mutations in *covR*, *covS*, *rocA*, *ropB*. FDAARGOS_149, MGAS8232 and STAB10015 were used as references to detect SNPs in the core-genome of the 3 groups of closely related isolates (ISR3 & ISR4, ISR7 & ISR8 and ISR9 & ISR11, respectively).

Strain	BioSample	BioProject	Assembly	Size (Mb)	GC%	Genes	Proteins	Release Date
SF370	SAMN02604089	PRJNA269	GCA_000006785.2	1.85243	38.5	1801	1693	10.04.01
MGAS8232	SAMN02603495	PRJNA286	GCA_000007285.1	1.89502	38.5	1920	1794	31.01.02
MGAS315	SAMN02603496	PRJNA311	GCA_000007425.1	1.90052	38.6	1923	1804	18.07.02
Manfredo	SAMEA1705956	PRJNA270	GCA_000009385.1	1.84127	38.6	1841	1720	05.02.07
SSI-1	-	PRJNA301	GCA_000011285.1	1.89427	38.6	1912	1808	04.03.03
MGAS10394	SAMN02603497	PRJNA12469	GCA_000011665.1	1.89988	38.7	1875	1738	03.08.04
MGAS5005	SAMN02603500	PRJNA13888	GCA_000011765.2	1.83856	38.5	1835	1724	05.08.05
MGAS6180	SAMN02603499	PRJNA13887	GCA_000012165.1	1.89757	38.4	1870	1748	04.08.05
MGAS9429	SAMN02603501	PRJNA16363	GCA_000013485.1	1.83647	38.5	1800	1680	04.05.06
MGAS10270	SAMN02603502	PRJNA16364	GCA_000013505.1	1.92825	38.4	1923	1778	04.05.06
MGAS2096	SAMN02603503	PRJNA16365	GCA_000013525.1	1.86035	38.7	1809	1609	04.05.06
MGAS10750	SAMN02603504	PRJNA16366	GCA_000013545.1	1.93711	38.3	1924	1770	04.05.06
NZ131	SAMN02604236	PRJNA20707	GCA_000018125.1	1.81578	38.6	1791	1626	16.10.08
Alab49	SAMN02603353	PRJNA73399	GCA_000230295.1	1.82731	38.6	1816	1699	07.10.11
MGAS15252	SAMN02603944	PRJNA49669	GCA_000250905.1	1.75083	38.5	1693	1587	01.03.12
MGAS1882	SAMN02603945	PRJNA66081	GCA_000250925.1	1.78103	38.5	1728	1624	01.03.12
A20	SAMN02603640	PRJNA175952	GCA_000307535.1	1.83728	38.5	1833	1724	22.10.12
M1 476	-	PRJDB141	GCA_000349925.2	1.83113	38.5	1820	1721	14.07.12

HSC5	SAMN02604317	PRJNA205050	GCA_000422045.1	1.81835	38.5	1806	1686	11.07.13
STAB902	SAMN03081524	PRJNA231895	GCA_000732425.1	1.89212	38.5	1912	1804	22.07.14
ATCC 19615	SAMN02905033	PRJNA244349	GCA_000743015.1	1.8448	38.5	1835	1702	22.08.14
M23ND	SAMN02800743	PRJNA248257	GCA_000756485.1	1.84648	38.6	1839	1719	22.09.14
7F7	SAMN02644974	PRJNA238516	GCA_000767505.1	1.70979	38.6	1653	1546	20.10.14
HKU360	SAMN02980885	PRJNA257934	GCA_000772185.1	1.94454	38.5	1916	1809	05.11.14
1E1	SAMN02649402	PRJNA238987	GCA_000772245.1	1.79615	38.5	1741	1614	05.11.14
AP1	SAMN02716635	PRJNA242701	GCA_000993765.1	1.90829	38.5	1930	1830	07.05.15
JRS4 (Wash.)	SAMN03610013	PRJNA283215	GCA_001014285.1	1.81197	38.6	1788	1667	28.05.15
D471	SAMN03610015	PRJNA283214	GCA_001014305.1	1.81197	38.6	1788	1667	28.05.15
NGAS743	SAMN02715744	PRJNA243328	GCA_001019635.1	1.91555	38.5	1924	1805	03.06.15
NGAS596	SAMN02715758	PRJNA243328	GCA_001019675.1	1.79131	38.5	1739	1617	03.06.15
NGAS327	SAMN02715759	PRJNA243328	GCA_001019695.1	1.70205	38.6	1644	1537	03.06.15
M28PF1	SAMN03733610	PRJNA284654	GCA_001020185.2	1.89698	38.4	1874	1752	05.06.15
5448	SAMN02866032	PRJNA252999	GCA_001021955.1	1.82952	38.5	1817	1725	08.06.15
STAB10015	SAMN03418291	PRJNA278400	GCA_001023495.1	1.95045	38.2	1917	1800	09.06.15
H293	SAMEA3865279	PRJEB1935	GCA_001039695.2	1.72625	38.6	1661	1539	13.05.14
HKU488	SAMN03846812	PRJNA289181	GCA_001051095.1	1.94341	38.5	1944	1832	09.07.15
NGAS322	SAMN03274509	PRJNA243328	GCA_001267805.1	1.95047	38.3	1929	1809	17.08.15
NGAS638	SAMN03274510	PRJNA243328	GCA_001267845.1	1.7914	38.6	1758	1650	17.08.15
MEW427	SAMN04419118	PRJNA308988	GCA_001535505.1	1.81445	38.5	1767	1554	25.01.16
MEW123	SAMN04419117	PRJNA308987	GCA_001535565.1	1.8787	38.3	1827	1653	25.01.16
JRS4 (Tokyo)	-	PRJDB114	GCA_001547715.1	1.81112	38.6	1785	1657	04.04.15
MTB313	SAMD00000328	PRJDB1654	GCA_001547815.1	1.74533	38.5	1843	1758	24.02.15
MTB314	SAMD00000332	PRJDB1668	GCA_001547835.1	1.74483	38.5	1722	1588	24.02.15
FDAARGOS_149	SAMN03996295	PRJNA231221	GCA_001559175.1	1.83964	38.5	1831	1720	11.02.16
AP53	SAMN04334485	PRJNA305806	GCA_001620285.1	1.86055	38.6	1862	1742	18.04.16
MGAS11027	SAMN03792044	PRJNA167314	GCA_001635895.1	1.78687	38.6	1738	1617	02.05.16
MGAS23530	SAMN03775331	PRJNA287028	GCA_001635935.1	1.70939	38.5	1640	1527	02.05.16

MGAS27061	SAMN03161558	PRJNA266333	GCA_001635975.1	1.74135	38.5	1675	1565	02.05.16
NS53	SAMN04625972	PRJNA317786	GCA_001638845.1	1.76512	38.4	1693	1587	06.05.16
STAB09014	SAMN03418290	PRJNA278400	GCA_001678885.1	1.86249	38.4	1823	1697	05.07.16
STAB901	SAMN03081523	PRJNA232121	GCA_000732385.1	1.79561	38.5	1812	1358	22.07.14

Table S2 Assemblies statistics determined using Quast version 3.1 [2], median coverage determined by mapping the trimmed reads on the assemblies and accession numbers

	ISR1	ISR2	ISR3	ISR4	ISR5	ISR6	ISR7	ISR8	ISR9	ISR10	ISR11
Number of contigs (>= 0 bp)	71	32	58	35	73	36	41	53	22	19	32
Number of contigs (>= 1000 bp)	29	19	23	17	30	26	26	33	18	16	21
Number of contigs (>= 10000 bp)	14	12	15	13	18	19	18	19	12	12	15
Number of contigs (>= 50000 bp)	12	12	11	10	11	11	13	13	10	10	10
Largest contig	516565	305309	751698	751702	278987	265863	251794	251794	504539	630053	529116
Total length (>= 0 bp)	1838723	1866975	1820544	1809116	1863500	1856594	1849575	1877415	1823476	1690061	1850892
Total length (>= 1000 bp)	1823729	1862425	1804961	1802965	1846068	1852149	1844325	1868156	1822707	1689494	1847894
Total length (>= 10000 bp)	1791557	1847827	1788337	1793215	1817829	1839380	1822960	1812159	1809795	1680544	1834982
Total length (>= 50000 bp)	1729935	1847827	1688855	1732523	1649245	1633436	1641065	1598590	1759057	1604391	1702270
N50	171252	225304	203845	221880	219677	206099	133991	134362	243478	163042	243478
L50	4	4	2	2	4	4	5	5	3	3	3
GC (%)	38.42	38.48	38.36	38.39	38.38	38.43	38.34	38.33	38.19	38.4	38.21
Median coverage	430	349	270	372	150	205	184	203	196	216	173
Contigs accession number	ERS 1420141	ERS 1420142	ERS 1420143	ERS 1420144	ERS 1420145	ERS 1420146	ERS 1420147	ERS 1420148	ERS 1420149	ERS 1420150	ERS 1420151

Table S3 Mutations detected in 4 different regulatory genes (*covR*, *covS*, *rocA*, *ropB*) and their effect on gene products

WT: Wild Type, nr: nucleotide replacement, del: deletion.

*mutations described by Ikebe et al. [3] °mutations described by Lynskey et al. [4]

Strain	<i>covR</i>	Effect	<i>covS</i>	Effect	<i>rocA</i>	Effect	<i>ropB/rgg</i>	Effect
<i>emm 1</i>								
ISR3	WT	-	nr	I332V	WT	-	WT	-
ISR4	WT	-	nr	I332V	WT	-	WT	-
<i>emm 22</i>								
ISR7	Nr	V128A	nr	-	nr	R259Q, V333A	WT	-
ISR8	Nr	V128A	nr	-	nr	R259Q, V333A	WT	-
<i>emm 28</i>								
ISR9	Nr	-	nr	E226G*	nr	R259Q, D396N, I404T	nr	-
ISR11	nr	-	Del 1bp (base 508)	Truncation at aa. 180*	nr	R259Q, D396N, I404T	nr	-
Other <i>emm</i> -types								
ISR1 - <i>emm 3</i>	Nr	-	nr	-	nr, 1bp del (base 1228)	I203N, L247F, R259Q, D396N, I404T, truncation at aa. 416°	nr	-
ISR2 - <i>emm 75</i>	WT	-	nr	S337L	nr	R259Q	nr	-
ISR5 - <i>emm 49</i>	Nr	-	nr	-	nr	R259Q	nr	-
ISR6 - <i>emm 5</i>	Nr	-	nr	K498N	nr	V47A, R259Q, D396N, I404T, P405S	nr	V169I
ISR10 - <i>emm 89</i>	Nr	-	nr	-	nr	R259Q	nr	-

Table S4 Virulence factor genes their products, extracted from the Virulence Factor Database [5]

Virulence factors	
<i>ccmA</i> : ABC transporter involved in cytochrome c biogenesis, ATPase component CcmA	<i>mf/spd</i> : Streptodornase
<i>cheA</i> : chemotaxis histidine kinase	<i>mf2</i> : deoxyribonuclease, phage-associated
<i>cpa</i> : fibronectin-binding protein, GAS pilus tip adhesin	<i>mf3</i> : deoxyribonuclease
<i>EF0818</i> : polysaccharide lyase, family 8 (Hyaluronidase)	<i>scpA</i> : C5a peptidase precursor
<i>emm</i> : M protein	<i>scpB</i> : streptococcal C5a peptidase
<i>fbaA</i> : protein F2 like fibronectin-binding protein	<i>sic</i> : streptococcal inhibitor of complement
<i>fbaB</i> : fibronectin-binding protein SfbII	<i>ska</i> : streptokinase A precursor
<i>fbp54</i> : fibronectin-binding protein Fbp54	<i>slo</i> : streptolysin O precursor
<i>fctA</i> : major pilin FctA	<i>smeZ</i> : streptococcal mitogenic exotoxin Z
<i>fctB</i> : minor pilin FctB	<i>spea</i> : streptococcal exotoxin A precursor, phage-associated
<i>grab</i> : protein G-related alpha 2M-binding protein	<i>speB</i> : pyrogenic exotoxin B
<i>hasA</i> : hyaluronate synthase	<i>spec</i> : streptococcal exotoxin C precursor, phage-associated
<i>hasB</i> : UDP-glucose 6-dehydrogenase	<i>speg</i> : streptococcal exotoxin G precursor
<i>hasC</i> : UDP-glucose pyrophosphorylase	<i>speh</i> : streptococcal exotoxin H precursor
<i>hylP</i> : hyaluronidase, phage-associated	<i>spei</i> : streptococcal exotoxin I precursor
<i>ideS/mac</i> : immunoglobulin G-degrading enzyme	<i>spej</i> : streptococcal exotoxin J precursor
<i>lepA</i> : signal peptidase I	<i>spek</i> : streptococcal exotoxin SpeK, phage-associated
<i>lmb</i> : Laminin-binding surface protein	<i>srtC1</i> : sortase

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3. Ikebe T, Ato M, Matsumura T, et al (2010) Highly frequent mutations in negative regulators of multiple virulence genes in group A streptococcal toxic shock syndrome isolates. *PLoS Pathog* 6:e1000832. doi: 10.1371/journal.ppat.1000832
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