

SUPPLEMENTARY ONLINE DATA

EndoS₂ is a unique and conserved enzyme of serotype M49 group A *Streptococcus* that hydrolyses N-linked glycans on IgG and α₁-acid glycoprotein

Jonathan SJÖGREN^{*1,2}, Weston B. STRUWE^{†3}, Eoin F. J. COSGRAVE^{†4}, Pauline M. RUDD[†], Martin STERVANDER[‡], Maria ALLHORN^{*2}, Andrew HOLLANDS^{§||2}, Victor NIZET^{*||¶2} and Mattias COLLIN^{*2}

^{*}Department of Clinical Sciences, Division of Infection Medicine, Lund University, Biomedical Center B14, SE-22184 Lund, Sweden, [†]The National Institute for Bioprocess Research and Training, NIBRT, University College Dublin, Fosters Avenue, Mount Merrion, Blackrock, Co. Dublin, Ireland, [‡]Molecular Ecology and Evolution Laboratory, Department of Biology, Lund University, Ecology Building, SE-223 62 Lund, Sweden, [§]School of Biological Sciences, University of Wollongong, Wollongong, NSW 2522, Australia, ^{||}Department of Pediatrics, University of California San Diego, 9500 Gilman Drive, Mail Code 0687, La Jolla, CA 92093, U.S.A., and [¶]Skaggs School of Pharmacy and Pharmaceutical Sciences, University of California San Diego, 9500 Gilman Drive, Mail Code 0687, La Jolla, CA 92093, U.S.A.

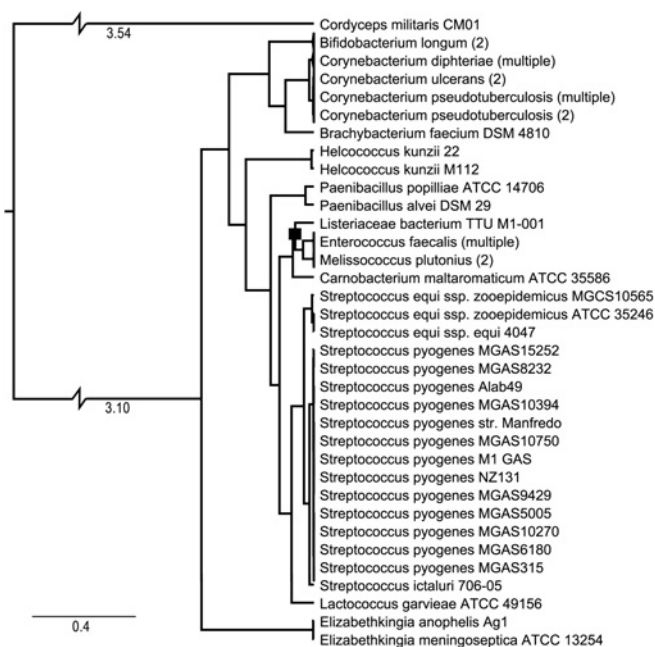


Figure S1 Phylogenetic reconstruction of 16S rRNA sequences, for inference of taxonomic relationship between taxa of the EndoS-like protein phylogeny, based on 1576 nucleotides

All internal nodes were supported by a Bayesian posterior probability (PP) of 0.99–1.0 except for the grouping of the Listeriaceae family bacterium, highlighted with a black squares signifying PP < 0.80. The scale bar indicates genetic distance. Note that the branches connecting the ingroup and outgroup are truncated owing to the large taxonomic difference (genetic distances stated below branches). For accession numbers, see Table S3.

¹ To whom correspondence should be addressed (email jonathan.sjogren@med.lu.se).

² Patents for the use of EndoS₂ have been applied for by Genovis AB. Jonathan Sjögren, Andrew Hollands, Victor Nizet, Maria Allhorn and Mattias Collin are listed as inventors on the application that is pending.

³ Present address: Chemistry Research Laboratory, Department of Chemistry, University of Oxford, Oxford OX1 3TA, U.K.

⁴ Present address: Waters Corporation, 34 Maple Street, Milford, MA 01757, U.S.A.

The sequences of *Streptococcus pyogenes* ndoS₂ will appear in the DDBJ, EMBL, GenBank® and GSDB Nucleotide Sequence Databases under accession numbers KC155346 (strain 3487-05), KC155348 (strain AP49), KC155347 (strain ACN49), KC155349 (strain AW1) and KC155350 (strain AW2).

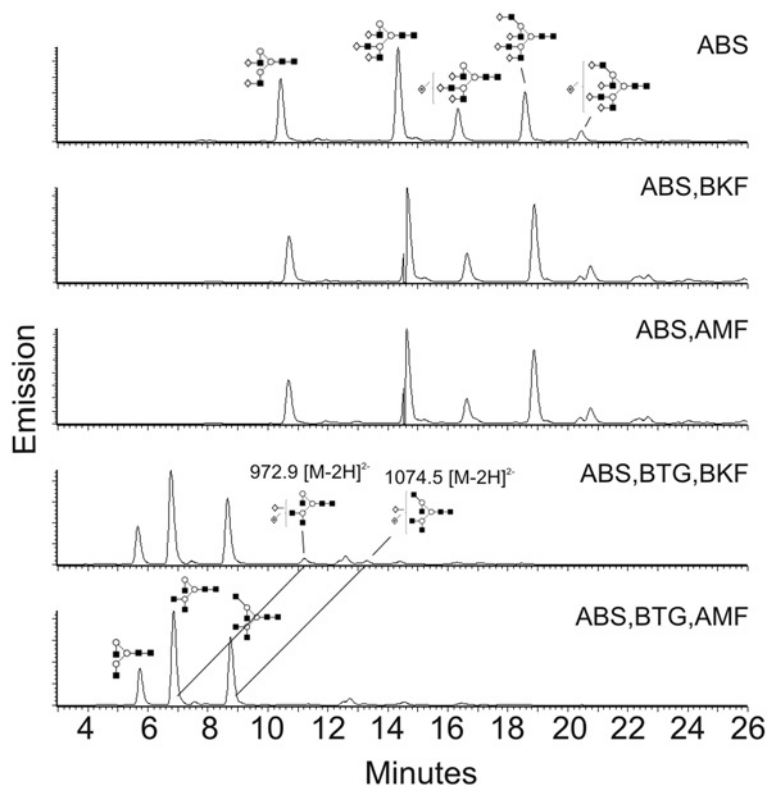


Figure S2 HILIC-FLD-MS and exoglycosidase sequencing of AGP N-glycans

The fucose linkage position was confirmed as $\alpha(1,3)$ to galactose residues on tri- and tetra-antennary glycans and were detected as $[M - 2H]^{2-}$ ions 972.9 and 1074.5.

Table S1 *E. coli* and group A streptococcal strains used in the present study

Strains of *E. coli* and *S. pyogenes* with accession numbers. Also indicated is the GenBank® accession number for *ndaS2* sequences. N/A, not applicable.

Strain	Source/accession number	<i>ndaS2</i> accession number
<i>E. coli</i>		
Top10	Invitrogen	N/A
BL21 pLysS	Invitrogen	N/A
GAS (serotype)		
NZ131 (M49)	ASM1812v1	AC161688
3487-05 (M49)	<i>S. pyogenes</i> , serotype M49	KC155346
ACN49 (M49)	Strain 3274-98 from Center for Disease Control and prevention. A gift from Actinova	KC155347
AP49 (M49)	<i>S. pyogenes</i> strain collection, Institute of Hygiene and Epidemiology, Prague, Czech Republic	KC155348
AW1 (M49)	Nephritis strain H 9449 B from the collection of L. Wannamaker	KC155349
AW2 (M49)	Strain H 5424 from the collection of L. Wannamaker	KC155350

Table S2 Plasmids and primers used in the present study

Plasmids and primers used for recombinant expression and sequencing.

(a) Plasmids

Name	Source
pGEX-5X-3	GE Healthcare
pGEX-ndoS2	The present study
pGEX-ndoS2(E-L)	The present study
pGEX-ndoS	[1]
pCR2.1	Invitrogen

(b) Primers

Name	Sequence (5'→3')
ndoS2-out-R	GCGCCACTTTCTGGTAGTCTAAC
Seq38-R	TTGGGACCTCAGCCATAG
Seq42-R	GGATTGTTAGGGTCGTATTCC
Seq54-R	TTTAGCCCTTTTGCCAG
Seq15-F	CCTGAAGAAGATGCTCGTG
Seq17-F	TGATGACCGAAGACAAACG
Seq24-F	GAGACGAAGAAGCATTTC
Seq28-F	TCACTTGGGGACAACTAAC
ndoS2-F-BamHI	CTGTAAGGATCCAGGAGAAGACTG
ndoS2-R-XhoI	GAAACCTCGAGTCTTTGTAATCGTAGGACTT
ndoS2(E-L)-F-BamHI	CTAGATATTGATATTCCTCACGAATTTACGAAC
ndoS2(E-L)-R-XhoI	GTTTCGTAATTCGTGAAGAATATCAATATCTAG

Table S3 Accession numbers for EndoS-like protein and 16S rRNA sequencesFor the EndoS-like protein dataset, outgroup sequences are indicated with an asterisk. For the EndoS₂ sequences of the present study, see Table S1.

Taxa	Strain	GenBank® accession number	
		EndoS-like protein	16S rRNA
<i>Actinomyces neuii</i>	BVS029A5	ZP_11040533	
<i>Beauveria bassiana</i>	ARSEF 2860	EJP67240	
<i>Bifidobacterium longum</i> subsp. <i>infantis</i>	157F	YP_004209228	AP010890
<i>Bifidobacterium longum</i> subsp. <i>infantis</i>	ATCC 15697	YP_002323900	AP010889
<i>Bifidobacterium longum</i> subsp. <i>longum</i>	44B	EIJ32132	
<i>Bifidobacterium longum</i> subsp. <i>longum</i>	1-6B	EIJ25186	
<i>Brachy bacterium faecium</i>	DSM 4810	YP_003153977; YP_003155571	X91032
<i>Carnobacterium maltaromaticum</i>	ATCC 35586	ZP_10279268	JF749289
<i>Carnobacterium maltaromaticum</i>	LMA28	YP_006993899	
<i>Chryseobacterium meningosepticum</i>	ATCC 13254	AJ704541	
<i>Cordyceps militaris</i>	CM01	EGX89767	HQ585075
<i>Corynebacterium diphtheriae</i>	241	YP_005126330	CP003207
<i>Corynebacterium diphtheriae</i>	BH8	YP_005161243	CP003209
<i>Corynebacterium diphtheriae</i>	C7 (β)	YP_005163607	CP003210
<i>Corynebacterium diphtheriae</i>	CDCE 8392	YP_005134619	CP003211
<i>Corynebacterium diphtheriae</i>	INCA 402	YP_005128539	CP003208
<i>Corynebacterium diphtheriae</i>	PW8	YP_005143699	CP003216
<i>Corynebacterium diphtheriae</i>	31A	YP_005158899	
<i>Corynebacterium diphtheriae</i> bv. <i>intermedius</i> str.	NCTC 5011	EIK55328	
<i>Corynebacterium pseudotuberculosis</i>	31	YP_006214375	CP003421
<i>Corynebacterium pseudotuberculosis</i>	258	YP_006353466	CP003540
<i>Corynebacterium pseudotuberculosis</i>	Cp162	YP_006437976	CP003652
<i>Corynebacterium pseudotuberculosis</i>	FRC41	YP_003784295	CP002097
<i>Corynebacterium pseudotuberculosis</i>	PAT10	YP_005691331	CP002924
<i>Corynebacterium pseudotuberculosis</i>	X81907		
<i>Corynebacterium pseudotuberculosis</i>	316	YP_005304514	CP003077
<i>Corynebacterium pseudotuberculosis</i>	CIP 52.97	YP_005695446	
<i>Corynebacterium ulcerans</i>	809	YP_005711595	CP002790
<i>Corynebacterium ulcerans</i>	BR-AD22	YP_004630749	CP002791
<i>Elizabethkingia anophelis</i>	Ag1	ZP_09415888	EF426425
<i>Elizabethkingia meningoseptica</i>	EBA2	FLAME	
<i>Enterococcus faecalis</i>	ATCC 29200	ZP_04439322	ACHK01000081
<i>Enterococcus faecalis</i>	OG1RF	CP002621	
<i>Enterococcus faecalis</i>	V583	NP_813917	
<i>Enterococcus faecalis</i>	HER1044	AAR20477	
<i>Enterococcus faecalis</i>	DS5	ZP_05561082	
<i>Enterococcus faecalis</i>	ERV85	EJV36587	
<i>Enterococcus faecalis</i>	PC1.1	ZP_06746878	
<i>Enterococcus faecalis</i>	R508	EJV39420	
<i>Enterococcus faecalis</i>	R712	ZP_06630726	
<i>Enterococcus faecalis</i>	T1	ZP_05422239	
<i>Enterococcus faecalis</i>	T2	ZP_05425204	
<i>Enterococcus faecalis</i>	T3	ZP_05502539	
<i>Enterococcus faecalis</i>	T8	ZP_05560447	
<i>Enterococcus faecalis</i>	TX0012	EFT95260; EFT95516; EFT95520	
<i>Enterococcus faecalis</i>	TX0104	ZP_03950001	
<i>Enterococcus faecalis</i>	TX0109	ZP_07567140	
<i>Enterococcus faecalis</i>	TX0309B	EFU85828	
<i>Enterococcus faecalis</i>	TX0470	ZP_07760464	
<i>Enterococcus faecalis</i>	TX0855	ZP_07555326	
<i>Enterococcus faecalis</i>	TX0860	ZP_07559314	
<i>Enterococcus faecalis</i>	TX1322	ZP_04435748	
<i>Enterococcus faecalis</i>	TX1341	EFU12187	
<i>Enterococcus faecalis</i>	TX1342	EFU15396	
<i>Enterococcus faecalis</i>	TX1346	EFU16620	
<i>Enterococcus faecalis</i>	TX1467	EGG58918	
<i>Enterococcus faecalis</i>	TX2137	EFT38634	
<i>Enterococcus faecalis</i>	TX4244	EFT91060	
<i>Enterococcus gallinarum</i>	EG2	ZP_05648160	
<i>Enterococcus saccharolyticus</i>	30_1	ZP_09110633	
<i>Helcococcus kunzii</i>	ATCC 51366	ZP_09737336; ZP_09738693	
<i>Helcococcus kunzii</i>	22	NR_029237	
<i>Helcococcus kunzii</i>	M112	JN861738	
<i>Lactobacillus zeae</i>	KCTC 3804	ZP_09453558	
<i>Lactococcus garvieae</i>	ATCC 49156	YP_004779109	AP009332
Listeriaceae family bacterium	TTU M1-001	ZP_09890591	JQ287762
<i>Melissococcus plutonius</i>	ATCC 35311	YP_004455741	AP012200

Table S3 Continued

Taxa	Strain	GenBank® accession number	
		EndoS-like protein	16S rRNA
<i>Melissococcus plutonius</i>	DAT561	YP_005320150	AP012282
<i>Paenibacillus alvei</i>	DSM 29	ZP_10866627	AJ320491
<i>Paenibacillus dendritiformis</i>	C454	ZP_09678120	
<i>Paenibacillus popilliae</i>	ATCC 14706	GAC41484	AB073198
<i>Streptococcus canis</i>	FSL Z3-227	ZP_10274531	
<i>Streptococcus equi</i> subsp. <i>equi</i>	4047	YP_002745815	FM204883
<i>Streptococcus equi</i> subsp. <i>zooepidemicus</i>	MGCS10565	YP_002122753	CP001129
<i>Streptococcus equi</i> subsp. <i>zooepidemicus</i>	ATCC 35246	AEJ24585	CP002904
<i>Streptococcus equi</i> subsp. <i>zooepidemicus</i>	H70	YP_002745127	
<i>Streptococcus ictaluri</i>	707-05	ZP_09127116; ZP_09126970*	
<i>Streptococcus ictaluri</i>	706-05	DQ462420	
<i>Streptococcus iniae</i>	9117	ZP_11067943	
<i>Streptococcus pyogenes</i>	Alab49	YP_006072662	CP003068
<i>Streptococcus pyogenes</i>	M1 GAS	NP_269818	AE006615
<i>Streptococcus pyogenes</i>	MGAS315	NP_665372	AE014074
<i>Streptococcus pyogenes</i>	MGAS5005	YP_282903	CP000017
<i>Streptococcus pyogenes</i>	MGAS6180	YP_280992	CP000056
<i>Streptococcus pyogenes</i>	MGAS8232	NP_607886	AE009954
<i>Streptococcus pyogenes</i>	MGAS9429	YP_597275	CP000259
<i>Streptococcus pyogenes</i>	MGAS10270	YP_599216	CP000260
<i>Streptococcus pyogenes</i>	MGAS10394	YP_060848	CP000003
<i>Streptococcus pyogenes</i>	MGAS10750	YP_603093	CP000262
<i>Streptococcus pyogenes</i>	MGAS15252	YP_005389405	CP003116
<i>Streptococcus pyogenes</i>	str. Manfredo	YP_001127900	AM295007
<i>Streptococcus pyogenes</i>	NZ131	YP_002286383	CP000829
<i>Streptococcus pyogenes</i>	ATCC 10782	ZP_07460044	
<i>Streptococcus pyogenes</i>	HKU QMH11M0907901	EIK41346	
<i>Streptococcus pyogenes</i>	M49 591	ZP_00365754	
<i>Streptococcus pyogenes</i>	CS101	U78969_1*	
<i>Streptococcus</i> sp. group C	C116	ADC53484	
<i>Trichosporon asahii</i> var. <i>asahii</i>	CBS 2479	EJT46459	

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- Collin, M. and Olsén, A. (2001) EndoS, a novel secreted protein from *Streptococcus pyogenes* with endoglycosidase activity on human IgG. *EMBO J.* **20**, 3046–3055

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