

Figure S1. Schematic representation of the membrane topology of synthases predicted from *M. agalactiae*, *M. mycoides* subsp. *mycoides*, *M. capricolum* subsp. *capripneumoniae* and *M. mycoides* subsp. *capri*. Strains and locus tags are indicated. The DxD motif is shown in red and the Q/RXXRW-like motif in blue.

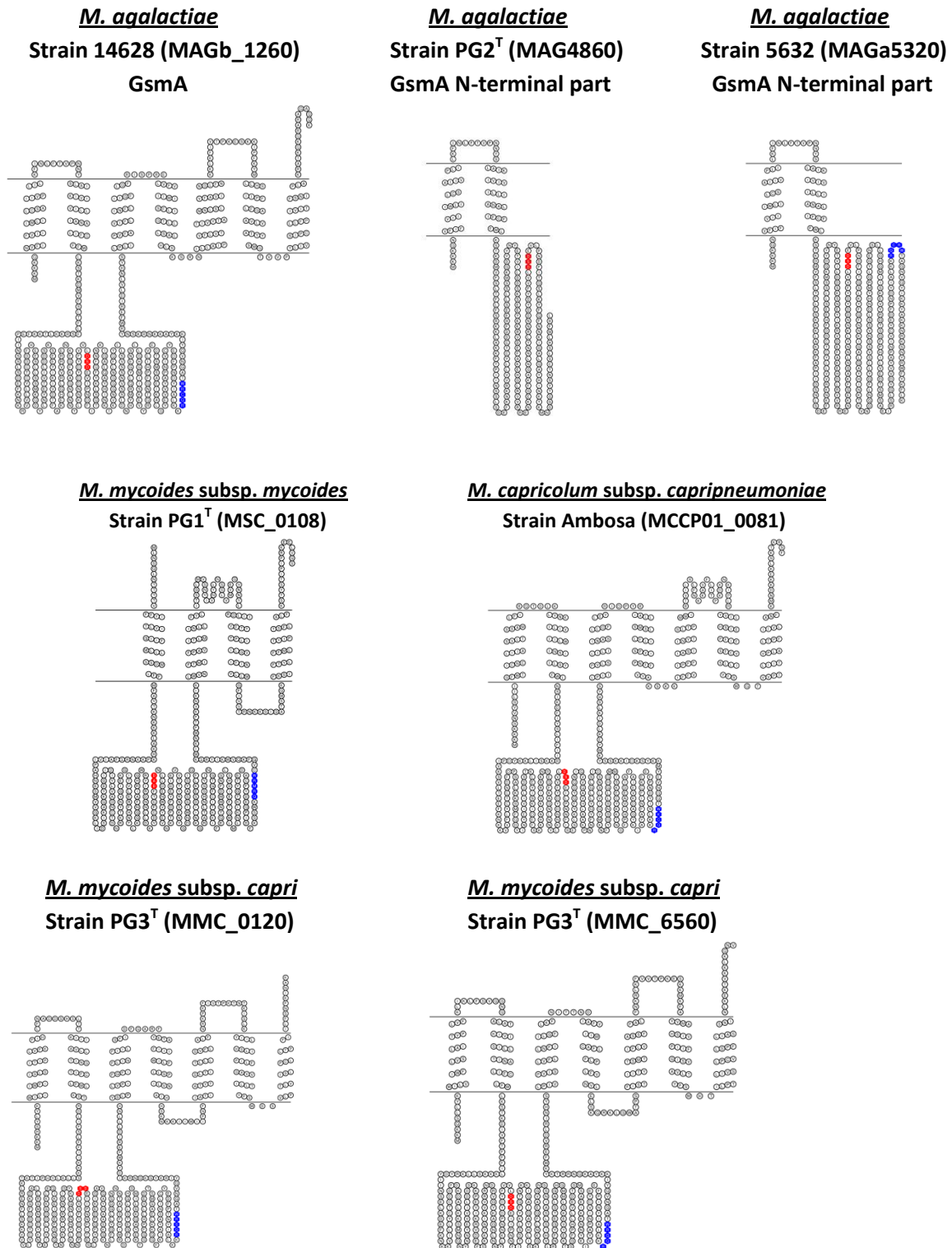


Figure S2. Colony immunostaining of *M. agalactiae* strains and transformants with mmc4 serum. Mmc4-positive colonies are dark-blue and mmc4-negative colonies are pink. Examples of negative or sectored colonies are marked with a red arrow. Numbers of nucleotides in the polyG tract of the wild-type *gsmA* gene are indicated in brackets. ¹ *gsmA* is disrupted by an IS or by a single base deletion upstream of the polyG, in strain 5632 and 4867, respectively.

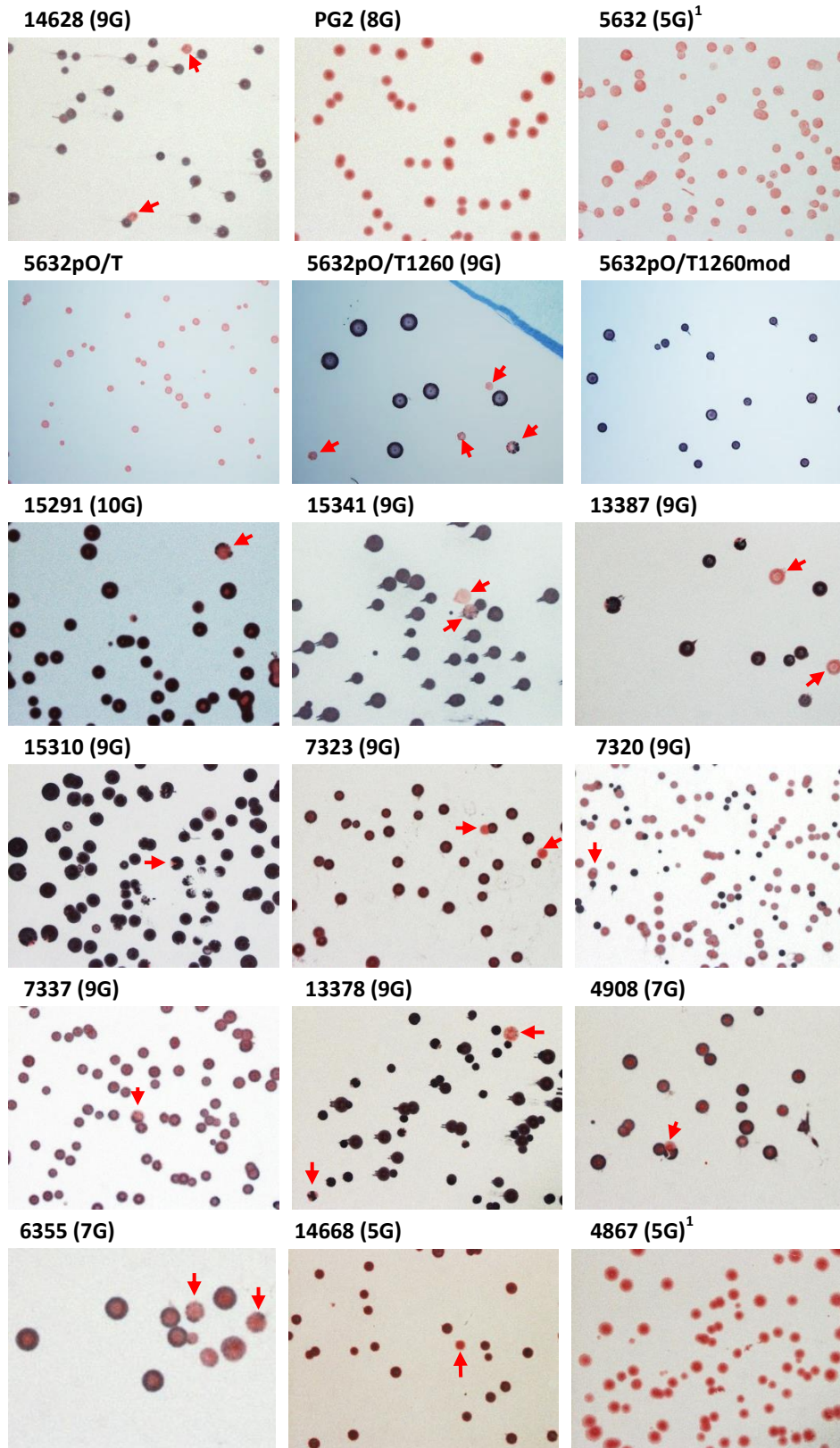


Table S1. Non-exhaustive list of mycoplasma species harboring genes coding for synthases and enzymes involved in sugar activation.

Phylogenetic group	Mycoplasma				UDP-glucose synthesis	UDP-galactopyranose synthesis			UDP-galactofuranose synthesis	UDP-sugar synthesis prediction			Synthase ¹				Secreted polysaccharide
	species	strains	genome accession number	host	UTP glucose-1P uridylyltransferase GalU	UDP-glucose 4 epimerase GalE	galactokinase	UTP galactose-1P uridylyltransferase	UDP-galactopyranose mutase Gif	UDP-glc	UDP-galp	UDP-galf	mnemonic or accession number	TMD	motifs		
Spiroplasma	<i>M. mycoides</i> subsp. <i>mycoides</i>	PG1 ^T	BX293980.2	cattle	MSC_0110	MSC_0971 MSC_0978	ND	ND	MSC_0984 MSC_0970	yes	yes	yes	MSC_0108	4	DXD	QXXRW	β-(1→6)-galactofuranose
	<i>M. mycoides</i> subsp. <i>capri</i> serovar LC	95010	FQ377874.1	goat	MLC_1070 MLC_8170	MLC_8200	ND	ND	MLC_8210	yes	yes	yes	MLC_1040 MLC_8190	4 4	DXD DXD	QXXRW QXXRW	β-(1→6)-galactofuranose
	<i>M. mycoides</i> subsp. <i>capri</i> serovar capri	PG3 ^T	JFAE00000000.1	goat	MMC_6550	ND	ND	ND	ND	yes	no	no	MMC_0120 MMC_06560	7 7	DXD DXD	RXXRW RXXRW	β-(1→6)-glucan
	<i>M. leachii</i>	PG50 ^T	CP002108.1	cattle	MSB_A0105	ND	ND	ND	ND	yes	no	no	MSB_A0104	7	DXD	RXXKW	β-(1→2)-glucan
	<i>M. capricolum</i> subsp. <i>capricolum</i>	ATCC 27343	CP000123.1	goat	MCA0064	ND	ND	ND	ND	yes	no	no	MCA0063	7	DXD	RXXKW	β-(1→2)-glucan
	<i>M. capricolum</i> subsp. <i>capripneumoniae</i>	Ambosa	LM995445.1	goat	MCCP01_0082	ND	ND	ND	ND	yes	no	no	MCCP01_0081	7	DXD	RXXKW	β-(1→2)-glucan
Hominis	<i>M. bovigenitalium</i>	CL-51080	AORH00000000.1	cattle	Mbvg_s01_3210	Mbvg_s01_2500	ND	ND	Mbvg_s01_2490	yes	yes	yes	Mbvg_s01_6580 Mbvg_S01_6980	4 7	DXD DXD	QXXRW RXXKF	Not done
	<i>M. agalactiae</i>	14628	AJPR00000000.1	goat	MAGb_0980	ND	ND	ND	ND	yes	no	no	MAGb_1260	7	DXD	RXXRW	β-(1→6)-glucan
	<i>M. bovis</i>	PG45 ^T	CP002188.1	cattle	MBOVPG45_0358	ND	ND	ND	ND	yes	no	no	MBOVPG45_0329	7	DXD	RXXRW	Not done
	<i>M. gallinarum</i>	DSM 19816	JHZE00000000.1	galliform	WP_027333022.1	ND	ND	ND	ND	yes	no	no	WP_027332911.1	4	DXD	QXXRW	Not done
	<i>M. alligatoris</i>	A21JP2	ADNC00000000.1	alligator	WP_005683645.1	WP_005683604.1	ND	ND	WP_005683646.1	yes	yes	yes	EFF41433.1	4	DXD	QXXRW	Not done
	<i>M. fermentans</i>	JER	CP001995.1	human	MFE_05250	MFE_00490	ND	ND	ND	yes	yes	no	MFE_00520	4	DXD	QXXRW	Not done
Pneumoniae	<i>M. penetrans</i>	HF-2	BA000026.2	human	MYPE160	ND	ND	ND	ND	yes	no	no	MYPE6340	7	DXD	RXXRW	Not done
	<i>M. iowae</i>	695	AGFP00000000.1	turkey	GUU_02277 GUU_04379	GUU_04409	GUU_04404	GUU_04399	ND	yes	yes	no	GUU_02272 GUU_00732	7 7	DXD DXD	RXXRW RXXRI	Not done

¹Specific identification of synthases is based on the secondary structure predicted by TMHMM 2.0 and similarity with other known synthases using Phyre. Glc, glucose; galp, galactopyranose; galf, galactofuranose; TMDs, transmembrane domains; UDP-glc, UDP-glucose; UDP-galp, UDP-galactopyranose; UDP-galf, UDP-galactofuranose. UDP-sugar synthesis prediction is based on the detection of the corresponding enzymes by BLASTP. ND, homologues were not detected by BLASTP searches.

Figure S3. Phylogenetic analysis of Gsm (Glycan synthase of mollicutes) proteins.

The tree was generated using the maximum likelihood method (bootstrap values are indicated in red at nodes). Synthases with 4 transmembrane domains are in the grey box and those with 7 transmembrane domains are in the blue box.

