

Title: Peptide signaling in the Staphylococci

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Table of Contents

1. Introduction

2. Overview of *agr*

- 2.1. Molecular arrangement of the *agr* locus
- 2.2. *agr* regulon
- 2.3. AIP signal
- 2.4. *agr* interference
- 2.5. AIP structure-activity relationships

3. AIP production

- 3.1. AgrD
- 3.2. AgrB
- 3.3. Mechanism of processing
- 3.4. Type-specific processing

4. AgrC

- 4.1. Structural homology of AgrC family members
- 4.2. Structural features of HPK₁₀ cytoplasmic domains
- 4.3. Atypical topology of HPK₁₀ sensory domains
- 4.4. Ligand specificity determinants
- 4.5. Constitutive mutations
- 4.6. Symmetric transmembrane signal transduction

5. AgrA

- 5.1. P2/P3 and PSM promoter binding
- 5.2. Structural studies

6. RNAIII

- 6.1. Structure
- 6.2. Delta-toxin
- 6.3. Mechanism of action
- 6.4. Positive regulation of alpha-toxin
- 6.5. Negative regulation of Protein A
- 6.6. Negative regulation of Rot
- 6.7. Regulation of other targets

7. Regulation of *agr*

- 7.1. Environmental cues
- 7.2. Positive regulation
- 7.3. Negative regulation
- 7.4. Other regulators that affect *agr* function

- 8. Interconnection of *agr* and biofilms**
 - 8.1. Effect of *agr* mutations on biofilms
 - 8.2. Biofilm dispersal with *agr* activation
- 9. Role of *agr* in *S. aureus* pathogenesis**
 - 9.1. Community-associated MRSA
 - 9.2. Neutrophils and macrophage interactions
 - 9.3. Animal models of infection
 - 9.4. Association with diseases and colonization
 - 9.5. *agr* variation across strains
- 10. Mechanisms of *agr* inhibition**
 - 10.1. Host factors that inhibit *agr*
 - 10.2. AgrC receptor antagonists
 - 10.3. Other strategies of *agr* inhibition
- 11. *Staphylococcus epidermidis***
 - 11.1. *S. epidermidis* *agr* system
 - 11.2. AIP structure
 - 11.3. *agr* interference
 - 11.4. *agr* regulation of biofilms
- 12. Other Staphylococci**
- 13. Conclusions and future perspectives**

Panel A.

		TRANSMEMBRANE SENSOR DOMAIN																				
residue		1234567890					1234567890					1234567890					1234567890					count
		TM1					OUTSIDE					TM2					INSIDE					
AgrC1	1	---VELLNSYNFVLFVLTQMILMFT---	IPAIISGI	--KYS	KL	YDFYFIIVISTLSL	--FLFK	MFDSASLIILTSFII	-I	68	<i>S. aureus</i>											
AgrC4	1	---VESLNSYNFVLFVLTQIIIMFT---	VPSIISGI	--KYS	KSDYLYITGITALS	--ILFN	FIDSVTLIILTIFII	-I	68	<i>S. aureus</i>												
AgrC3	1	---MEALNDYNYVLFVIVQVSLMFF---	ISAFISGI	--RYK	KSDYIYIIGIVLSSV	--YFFD	KIGSISLVVITIFII	-I	68	<i>S. aureus</i>												
AgrC2	1	---VAYGNKQ-LSYGTFLQVILFT---	VAKFISFV	--KF	NLRDYFIIVGIIPTM	--FLYY	FYGSRAVLIPTFSSI	-I	67	<i>S. aureus</i>												
AgrC	1	-----MDDINLFPFAGLQIFLMIW---	VTKVIINM	--KF	NFRDYIIVFTIVIPSA	--IMYY	FWQSKALIVLVIIIT	-I	65	<i>S. epidermidis</i>												
VirS	1	MLMNQMFWDFMENASMIIEVVSFYLI-	ISQFSPKK	-RKN	KQEVYSFVIIIFSILLKVLNV	--YPNER	IVICFFIGL	-I	74	<i>C. perfringens</i>												
AgrC	1	-----MFSILMAIIQITGIFI---	AIQILTNK	--VF	SIKEGLVTIAIAMLAF	--PLFT	LVQYWSMIFVIVFV	-S	62	<i>L. monocytogenes</i>												
FsrC	1	-----MILSLLATNVLLVSSFIVFV---	LRVTLIKI	--E	KIPLLSLLIVINLCSFAALMLG	--YSW	LIYALTVVIFTG		68	<i>E. faecalis</i>												
PltK	1	-----MLVF---	LLQILINREL	RQ	KRVYATVAVMTVLLTV	--IYM	LYDDYSDIITFIVNMGL		52	<i>L. plantarum</i>												
hpk9	1	---MIGFREYVTVRIAIIWLSLVIITMSL	GEVASNLW	P	VP	PLAYFWIIPIVCVTTF	--MSS	RLLYTQWILMLPVAIFL	--	73	<i>L. plantarum</i>											
hpk10	1	-----MKIIFVQTLFLFL---	FVKVLLRDWR	VLS	IWDISYAVVSSLVSG	--WIFS	VIGQYALPLLGLVV	-L	61	<i>L. plantarum</i>												
LamC	1	-----MLIIFLQEFFLV---	ITKAILKK	-RS	IQLFDISFVAVSLGVTY	--AFYS	LLQDRVSFLVLLCI	-I	60	<i>L. plantarum</i>												
PlsK	1	-----MVDFLNIIVVQVTSLLL---	IYHRISSI	--K	LTMREIALVFLGTFSS	--ILL	STFFFVAVVIMMLTAIGI	-K	63	<i>L. plantarum</i>												
PlnB	1	---MVEISIFDSVIQSFFIYLGIIIL---	VYNFIFSN	-T	NIKRIIYSLILLVLSLVGAIL	D---	DTTSLILV---	-L	63	<i>L. plantarum</i>												
SppK	1	---MLYTDVSVSMLQNFVAILLIFL---	LYRYIQRK	-I	TFKRIILDILIAIFS	----	ILYL	FISDASLLVMVLMRL	--	66	<i>L. sakei</i>											
ComD1	1	-----MDLFGFGTVIVHFLIISH---	SYHFICKG	--Q	INRKELFVFGAYTLTE	--IV	---	FDFPLYILYLDGLGI	-E	62	<i>S. pneumoniae R6</i>											
ComD2	1		L		RL	R	Y		LE	SF	L	KI	62	<i>S. pneumoniae A66</i>								

Panel B.

		TRANSMEMBRANE SENSOR DOMAIN																				
residue		1234567890					1234567890					1234567890					1234567890					count
		INSIDE					TM3					OUTSIDE					TM4					
AgrC1	69	MYFVKIKWYSILLIMTSQI	-----	ILYCAN	MYIVI	-YAYIT	---	KI	SDSIFVIFP	--SFF	VVYVTISILFSYIIN		133	<i>S. aureus</i>								
AgrC4	69	LYFSKIKWYSILLIMTSQI	-----	ILYCAN	MYIVI	-FTYIV	---	KI	VDSIFVIFP	--IFF	VVYVTISILFSYIIN		133	<i>S. aureus</i>								
AgrC3	69	FLYFKIRLYSVFLVMVTQI	-----	ILYCAN	FVYIII	-FSYII	---	T	ISHSVFIVLP	--IFL	VVYSISYALAYILN		133	<i>S. aureus</i>								
AgrC2	68	FLFFKLKYAIVTILVTMI	-----	IMYLS	NFATVGL	-FLTLR	---	KY	TTDPAILLP	--LY	ILSFSSVSLLATYLV	R	132	<i>S. aureus</i>								
AgrC	66	FFYTKIKLYSILVVLFTTM	-----	ILYIT	NFITVYI	-HLTIK	---	DY	IPFKFALQL	--IH	FTSFVITLIIAYLTQ		130	<i>S. epidermidis</i>								
VirS	75	YKFNFRVNNIKCIMISLIF	-----	WLFML	TVEALSISFIVKINSL	--VNV	SELLGKN	-IYR	METMILSKVILISLI				143	<i>C. perfringens</i>								
AgrC	63	ALYWKKNVVSASITLVV	-----	IILL	TISDSIVG	-FILV	PGLNF	KY	DEIFNELLPTLIYCAGMLANLLVFSFLLR				133	<i>L. monocytogenes</i>								
FsrC	69	FLLIHKKRFSIFKAIFLSVF	-----	TLLM	SFINYTEQTILSVFFQ	-IY	QNKLLWIASN	---	VLLLL	INIWIWALKIPN			138	<i>E. faecalis</i>								
PltK	53	LAFVRRKKYFIVTSLTATLA	-----	YIL	SFFGN	YATESIFHLFNLG	--LND	WSGWLFEA	--IG	ETIVTACMLAVAASF			122	<i>L. plantarum</i>								
hpk9	74	-NALKRLVGAIMGVIKAVA	-QSASP	MLLRQA	IGLHSTA	-----	DIN	FLASI	----	MML	PVILGMLAH		133	<i>L. plantarum</i>								
hpk10	62	FTWWHTHSFSESLFVSLQV	-----	IIS	IVVDHISS	-LLTYM	---	LK	DD	-GY	LLIFMGLQLVGLITLN		120	<i>L. plantarum</i>								
LamC	61	YAYWYEHNISKSVIIAMEI	-----	TML	SMVADHLAS	-LVMLF	---	FDS	MR	-LY	LLLFSIFQSLIIMVY		119	<i>L. plantarum</i>								
PlsK	64	D-----GLHQ-----	KKY	---	LAFYS	VYSVTF	FFSLVGN	-LS	LLGTF	-VD	GENI	----	YI	PIFF	-TAL	PVVINE	118	<i>L. plantarum</i>				
PlnB	64	GAIIKEKCPKINYHNLNVLMLISS	Q	IV	ILALASYLSRGLYIYLD	D	KISGL	-----	SE	-Y	GDLFIGIEIIVMYIIG		136	<i>L. plantarum</i>								
SppK	67	GWHFHQQKENKIKTTDTANLIL	IIVI	Q	LLLAVAGTII	ISQ	-FTIS	I	IKS	D	FSQNILNNSA	T	D	--IT	LLGI	FFAVLFDGLFF	143	<i>L. sakei</i>				
ComD1	63	RFLFPLGLYSYFRWMKQYERDR	GFLS	LLL	SLLYESTHN	-FLSV	TFSS	-IT	GD	NFV	LQY	---	H	P	FFFV	TVLTYFVTL	136	<i>S. pneumoniae R6</i>				

Panel E.

		CYTOPLASMIC HK DOMAIN																					
residue		1234567890	1234567890	1234567890	1234567890	1234567890	1234567890	1234567890	1234567890	1234567890	1234567890	1234567890	1234567890	1234567890	1234567890	1234567890	1234567890	1234567890	1234567890	1234567890	count		
		N-BOX																					
		h h N																					
AgrC1	276	QMNAIKLNGIENLKVREIKGLITAKILRAQEMNIPISIEIPDE-VSSINLNMIDLSRSIGIILLDNAIEASTEIDDPIIRV	354	<i>S. aureus</i>																			
AgrC4	276	QMNAIKLNGIENLKVREIKGLITAKILRAQEMSIPIISIEIPDE-VTHINLNMIDLSRSIGIILLDNAIEASTEIDDPIIRV	354	<i>S. aureus</i>																			
AgrC3	276	QMNAIKLNGIENLKVREIKGLITAKILRAQEMNIPISIEIPDE-VTRINLNMIDLSRSIGIILLDNAIEASTEIDDPIIRV	354	<i>S. aureus</i>																			
AgrC2	275	QMNAIKLNGIENLKVREIKGLITAKILRAQEMNIPISIEIPDE-VSSINLNMIDLSRSIGIILLDNAIEASTEIDDPIIRV	353	<i>S. aureus</i>																			
AgrC	273	KTRSIKMNGIEKLVREIKGLITTKIIQAQEKRIPIISIEVPDE-IDRIDMNTVELSRIIGIIVDNAIEASENLEEPLINI	351	<i>S. epidermidis</i>																			
VirS	282	NSEIDKLNLFNTGNEVLVDVILNNKREKCMENNISLKVIFIDFS-KVN-FIEYFDICTIFSNCIDNAIEACKKIKDNNRYI	359	<i>C. perfringens</i>																			
AgrC	279	ESNNYKISLLQNIHVIELKGLLAVKLIRAQELKIDAILEVVEP-IDKISMSIDLCKVVGILLDNAIEAALTTCENPVIRI	357	<i>L. monocytogenes</i>																			
FsrC	282	DNEEFELMKNLRLKKNMELKALISMKINTAKQAKLKVIVDVPEVFILDTSIDLVVVIRLLAILLDNAIEENSAKSELKMF	361	<i>E. faecalis</i>																			
PltK	267	NRDFGHFDNLEQIKDKPLKAI IQSKFSVARQAGIQVRLEANDP-IAAIAIDSVTLRSVSGILLDNAIEAVKQSGGQIAV	345	<i>L. plantarum</i>																			
hpk9	271	NRQS-GLSDLSRLDMPTVSGLIYAKYEAANGQNVHLVVTILEK-VTLADIPQIKVVRILGNLLDNAIEDAAARANH-QVAL	347	<i>L. plantarum</i>																			
hpk10	262	SDEQYNLEDLSRVQVKSILFVSKLSYAQSQGVKQVFDLQV-LNGVTTNELDLAIALGILLDNAIEATAGHYHGELMS	340	<i>L. plantarum</i>																			
LamC	261	LKEYNLEDLSRVKVKSIKLSILFVSKLSYAQSQIEVHFDLKEP-LIDITVNELDLAIALGIMLDNAIEASVGHADGEIMS	339	<i>L. plantarum</i>																			
PlsK	271	QSSKYSLINLSKLVPAIKSILSSKIIYAQQNGIEVNIIEISSD-VTTTYFDLLDYIRVIAIFLDNAIEASLSTDLPEMSV	349	<i>L. plantarum</i>																			
PlnB	288	SLNDGSIASVQHLKNEILRGLVQKFFYAKQCGVKLTIEIANT-DFILSHGVTVAVRIIGNLLDNAIEQAQKMTDKIVTV	366	<i>L. plantarum</i>																			
SppK	294	EIQGAVIAQLDYLKNDPIRGLVIQKFLAAKQAGVTLKFEMTEP-IELATANLLTVIRIIGILLDNAIEQAVQETDQLVSC	372	<i>L. sakei</i>																			
ComD1	285	RSDKYTYFDLNNIEDSALRSLVAQSIYARNNGVEFTLEVKDT-ITKLPIELLDLVRIMSVLLNNAVEGSADS	363	<i>S. pneumoniae R6</i>																			
		DHp Subdomain					CA Subdomain																

panel F.

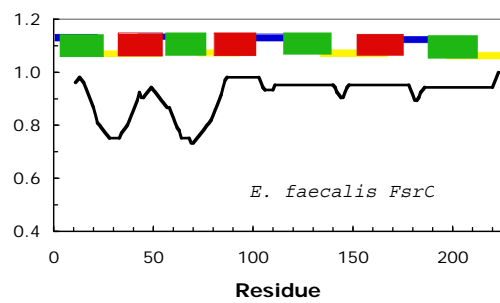
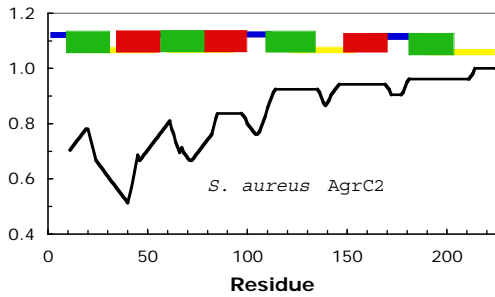
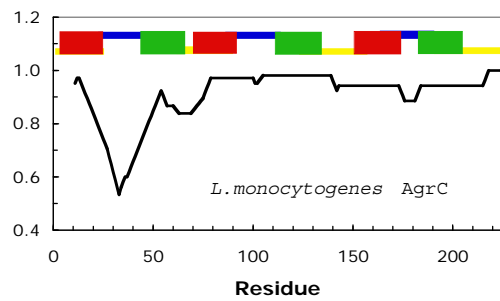
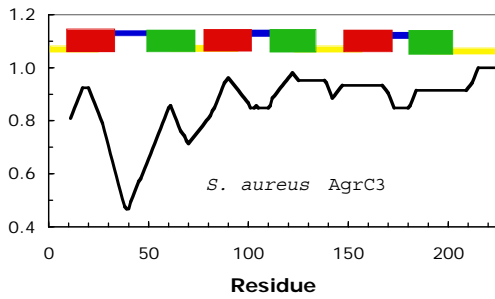
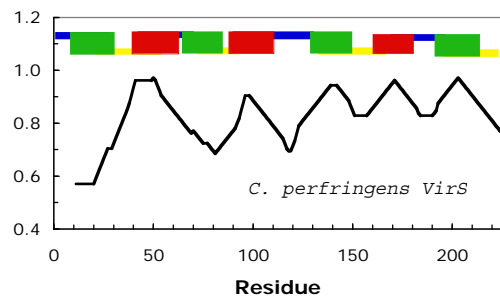
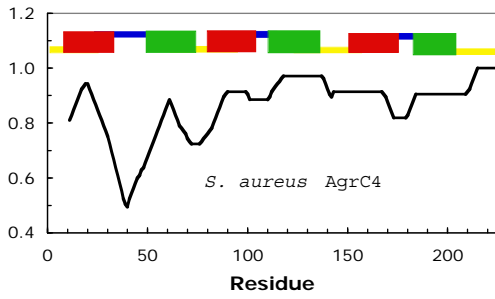
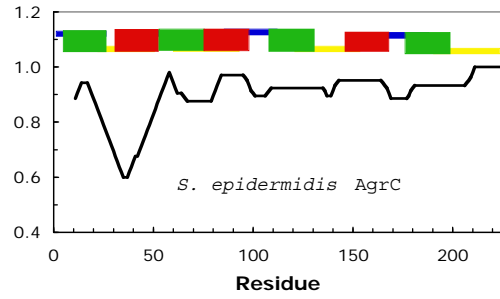
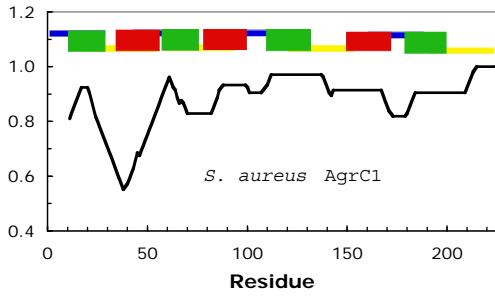
		CYTOPLASMIC HK DOMAIN																					
residue		1234567890	1234567890	1234567890	1234567890	1234567890	1234567890	1234567890	1234567890	1234567890	1234567890	1234567890	1234567890	1234567890	1234567890	1234567890	1234567890	1234567890	1234567890	1234567890	count		
		h G-BOX GhG h h h																					
AgrC1	355	AFIESE-NSVTFIVMNCAD-DIPRIHELFFQESFSTKGE--GRGLGLSTLKEIADNADNVLLDITIENG	429	<i>S. aureus</i>																			
AgrC4	355	AFIESE-NSVTFIVMNCAD-DIPRIHELFFQESFSTKGE--GRGLGLSTLKEIADNADNVLLDITIENG	429	<i>S. aureus</i>																			
AgrC3	355	AFIESE-NSVTFIVMNCAD-DIPRIHELFFQESFSTKGE--GRGLGLSTLKEIADNADNVLLDITIENG	429	<i>S. aureus</i>																			
AgrC2	354	AFIESE-NSVTFIVMNCAD-DIPRIHELFFQESFSTKGE--GRGLGLSTLKEIADNADNVLLDITIENG	428	<i>S. aureus</i>																			
AgrC	352	AFIDNE-ESVTFIVMNCASN-DIPKIHelfEQGFSTKGD--NRGLGLSTLKEITDSNENVLLDITVIENGY-FVQKVEINN	426	<i>S. epidermidis</i>																			
VirS	360	SLKGNVCVNNFYVIKIENSKT-N--KIKKLNDFLTDKDKFLHGIIGLKNIRLALAEKYNGEIIIEPLDDKFIKMLIPINQ	436	<i>C. perfringens</i>																			
AgrC	358	AFVKKG-DSIIIVFANSLPV-NMPPYKIFEEGFSTKGE--GRGLGLASLREIMKKYSHVALDTKVTVDRE-VIQELEIM-	431	<i>L. monocytogenes</i>																			
FsrC	362	SIFNKN-ETQEFVITNSVQA--EFDFKVMKTKFSSKSNPEEHGWGLLYVKEIVDFSDQFDLQTSFNEGA-VTQHLLIEK	437	<i>E. faecalis</i>																			
PltK	346	ALVKYP-RMVELIFANSLNH-PIERLDELVMAGHTSKGA--GHGQGLATVRELLDPLANVTYEVSSHQRF---EFIISI	417	<i>L. plantarum</i>																			
hpk9	348	TVKNRDAHTTVFSITNQIPTHEAVDLSVIKKSRTTKPG--HLGYGLSSIEQLT--TDQLVVNYCIEEGN-FIAELIIKH	422	<i>L. plantarum</i>																			
hpk10	341	AIFVTA-HSTVFLVQNNVFD-SLPLWLQKEAGYSTKGG--ERGLGLSLSAIVNRNENMILETRLLASA-FVQRLTVKR	415	<i>L. plantarum</i>																			
LamC	340	AIFIEK-NSTVFLIQNNVFE-QLPLWKLKEAGYSTKGG--NRGIGLNSLSKIVNRNENMILETRVLGAV-FLQRLTVKR	414	<i>L. plantarum</i>																			
PlsK	350	AFIEED-DSQTVIIIVNAPD-SYIDKRRIFESGFSTKGGH--NRGIGLATVKDILNKYPNASLETETFKNSL-FTQKLAIKK	424	<i>L. plantarum</i>																			
PlnB	367	AFNEID-NTAEIAISNPI-D-SDFNQHQIFETGYSTKGS--NRGLGLTNVRDLVEQQKGFYMDIETKKNY-VTMTLIVTE	440	<i>L. plantarum</i>																			
SppK	373	AFLQSD-GLIEITIENTA-S-QVKNLQAFSELGYSTKGA--GRGTGLANVQDLIAKQTNLFLETQIENRK-LRQTLMITE	446	<i>L. sakei</i>																			
ComD1	364	AVIKME-TETVIVIQNSCKM-TMTPSGDLFALGFSTKGR--NRGVGLNNVKELLDKYNIIILETEMEGST-FRQIIRFKR	438	<i>S. pneumoniae R6</i>																			
		CA Subdomain																					

Panel G.

		HK DOMAIN											
residue		1	2	3	4	5	6	7	8	9	10	count	
AgrC1	430	N	-	-	-	-	-	-	-	-	-	430	<i>S. aureus</i>
AgrC4	430	N	-	-	-	-	-	-	-	-	-	430	<i>S. aureus</i>
AgrC3	430	N	-	-	-	-	-	-	-	-	-	430	<i>S. aureus</i>
AgrC2	429	N	-	-	-	-	-	-	-	-	-	429	<i>S. aureus</i>
AgrC	427	K	E	S	-	-	-	-	-	-	-	429	<i>S. epidermidis</i>
VirS	437	E	K	E	A	-	-	-	-	-	-	440	<i>C. perfringens</i>
AgrC		-	-	-	-	-	-	-	-	-	-		<i>L. monocytogenes</i>
FsrC	438	N	H	N	S	K	K	V	V	N	E	447	<i>E. faecalis</i>
PltK	418	E	S	E	-	-	-	-	-	-	-	420	<i>L. plantarum</i>
hpk9		-	-	-	-	-	-	-	-	-	-		<i>L. plantarum</i>
hpk10	416	E	-	-	-	-	-	-	-	-	-	416	<i>L. plantarum</i>
LamC	415	V	S	Q	N	D	-	-	-	-	-	419	<i>L. plantarum</i>
PlsK	425	D	V	-	-	-	-	-	-	-	-	426	<i>L. plantarum</i>
PlnB	441	D	K	-	-	-	-	-	-	-	-	442	<i>L. plantarum</i>
SppK	447	E	T	-	-	-	-	-	-	-	-	448	<i>L. sakei</i>
ComD1	439	E	F	E	-	-	-	-	-	-	-	441	<i>S. pneumoniae R6</i>

CA Subdomain

Figure S1. COBALT generated alignment of AgrC and representative AgrC homologs (HPK₁₀'s). Highlighted residue are as follows: gray, residues predicted to be part of trans-membrane helices; red, highly conserved residues; green, residues where mutations lead to constitutive activity; magenta, residues where mutations result in broader ligand specificity; blue, residues predicted to have high propensity for coiled-coil formation. Residues in magenta test have been shown to influence ligand binding specificity. Residues enclosed in boxes have been shown to be located on the outside (black boxes) or inside (red boxes) of the membrane by β -galactosidase (*lacZ*) and/or alkaline phosphatase (*phoA*) fusion analyses. TM5 residues that are part of a four-element leucine zipper motif are shown as bold, underlined red or black text. Boundaries of the transmembrane sensor domain and cytoplasmic HK domain are indicated by the bars at the top of each panel, and boundaries of the DHp and CA subdomains are indicated by the bars at the bottom of the panels. Protein and bacteria names as well as protein specific residue numbering are indicated in the margins, and residue count line is provided at the top of each panel to aid in counting residues.



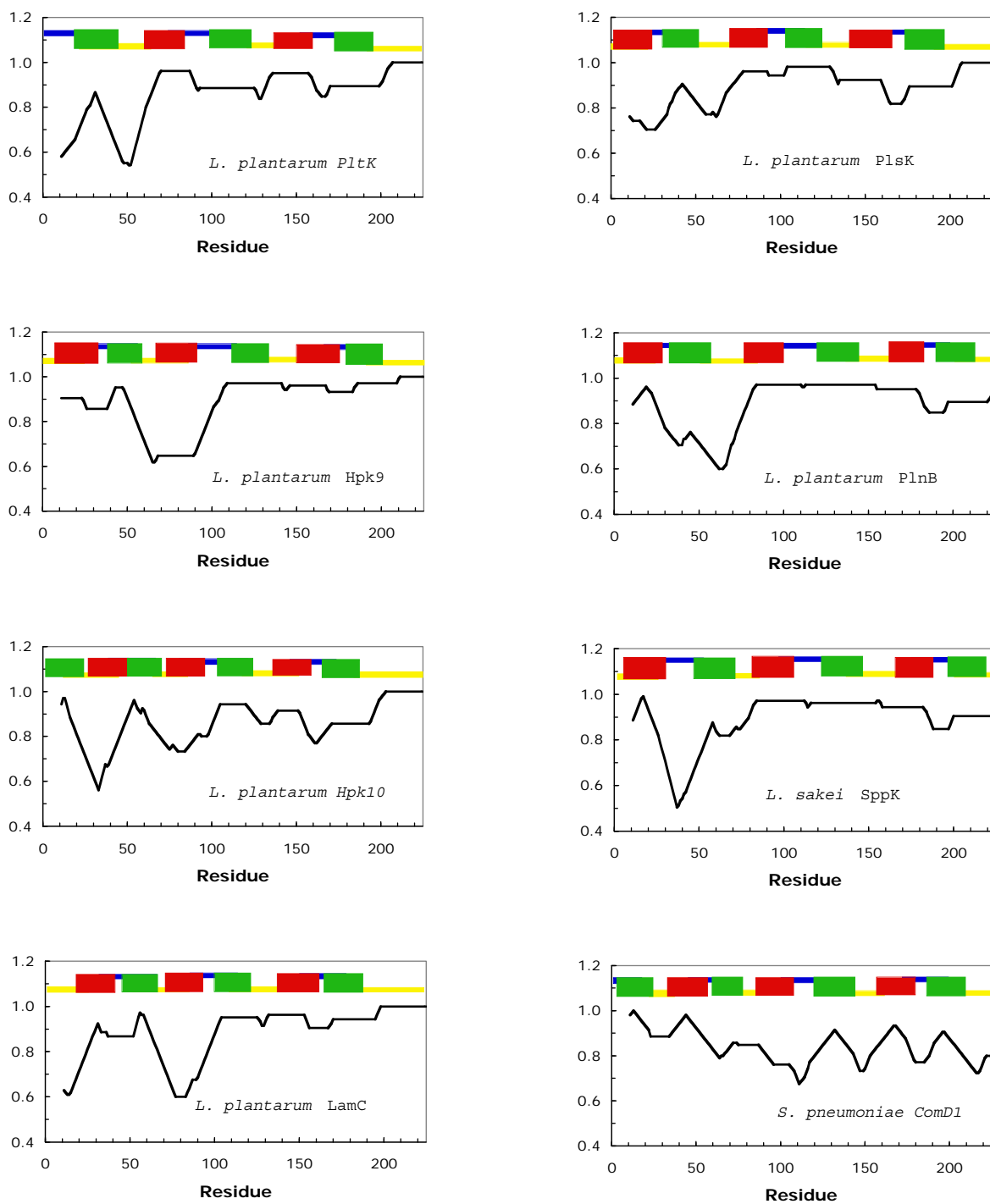


Figure S2. TOPCONS consensus topology predictions (outside, thin blue bars; TM_{OUT-to-IN}, wide green bars; inside, thin yellow bars; TM_{IN-to-OUT}, thick red bars) and reliability plots for the 16 HPK₁₀ sequences in Figure S1.