

Supplementary Information

Competence-induced protein Ccs4 facilitates pneumococcal invasion into brain tissue and virulence in meningitis

Yujiro Hirose^a, Masaya Yamaguchi^{a, *}, Kana Goto^a, Tomoko Sumitomo^a, Masanobu Nakata^a, Shigetada Kawabata^a

^aDepartment of Oral and Molecular Microbiology, Osaka University Graduate School of Dentistry, Suita, Osaka 565-0871, Japan

*Address correspondence to: Masaya Yamaguchi, yamaguchi@dent.osaka-u.ac.jp

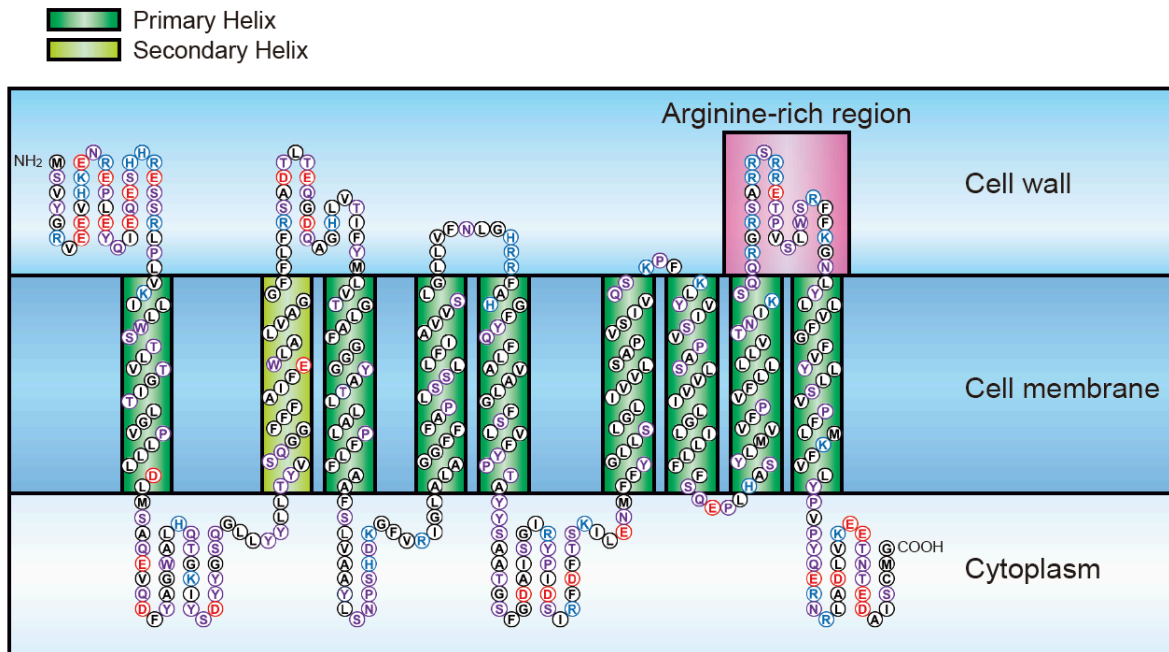


Figure S1. Deduced transmembrane topology of Ccs4. Transmembrane regions of Ccs4 were predicted using the SOSUI algorithm. Pink-shaded area indicates extracellular region with an arginine-rich region (-RFRRSARSRRS-).

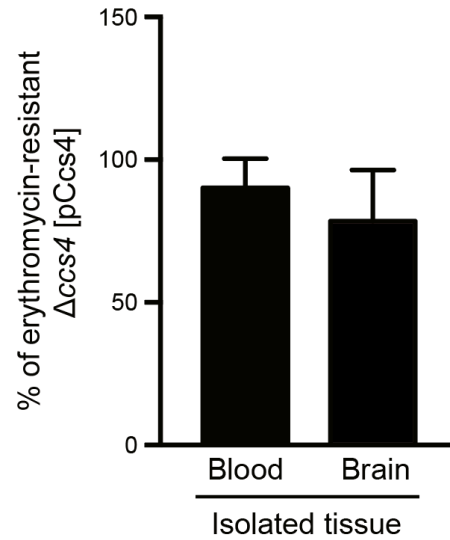


Figure S2. The percentage of $\Delta ccs4$ containing Ccs4-expressing vector in blood and brain homogenates. In the intravenous infection model, we counted CFU in blood and brain homogenates which isolated from complement strain-infected mice on THY agar with or without erythromycin (n = 18). The percentage of erythromycin-resistant $\Delta ccs4$ [pCcs4] = (CFU counts on THY ager with erythromycin / CFU counts on THY ager without erythromycin) \times 100.

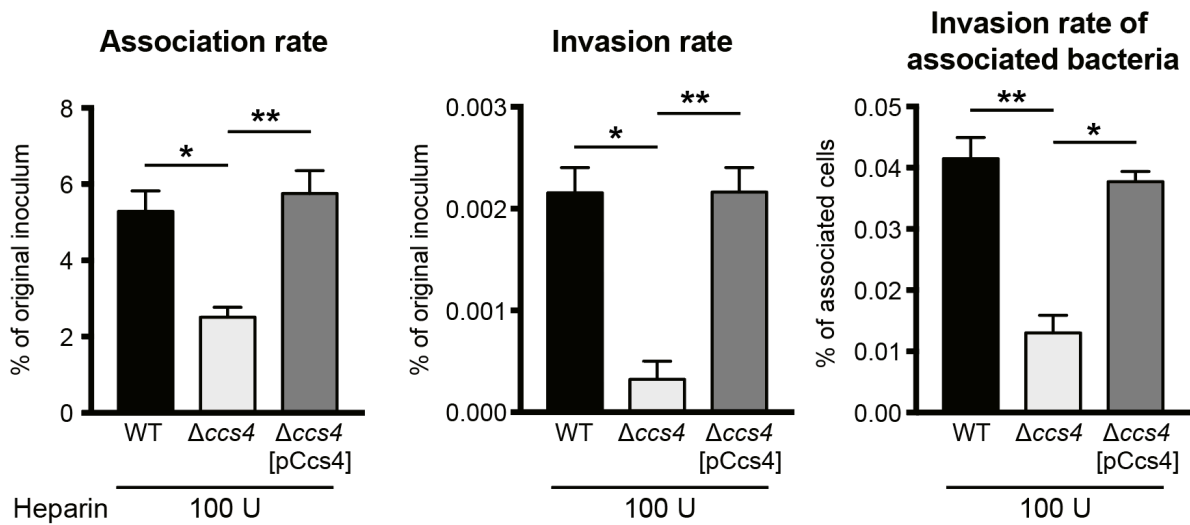


Figure S3. Effects of heparin pretreatment on association with and invasion into hBMECs by *S. pneumoniae*. Association rates were calculated by dividing the CFU value obtained at 1 hour after infection by the value for the original inoculum. Invasion rates were calculated by dividing the CFU value obtained at 1 hour after antibiotic addition by the value for the original inoculum. Values are presented as the mean of 6 wells from one of 3 independent experiments. Vertical lines represent the mean +S.E. Statistical differences between groups were analyzed using a Kruskal-Wallis test with Dunn's post hoc test. * $p < 0.05$ and ** $p < 0.01$.

Materials and Methods

Similarity search

We searched for proteins similar to Ccs4 of *S. pneumoniae* TIGR4 using the BLASTP program (NCBI BLAST). Proteins with an E-value $<2E-100$, coverage of the query $>99\%$, and identity $>40\%$ are listed. Evaluation criteria for capsule +/- were used as presented in literature or based on the existence of *cpsA-cpsD* encoding a putative polysaccharide capsule in *S. pneumoniae* TIGR4.

Supplementary Table 1. List of proteins similar to *S. pneumoniae* TIGR4 Csa4

Bacteria	Strain	Accession number	Protein ID	Product	Query cover	E-value	Identity	Capsule	Serotype		
<i>S. pneumoniae</i>	TIGR4	AE005672.3	AAK74380.1	Competence-induced protein Csa4	100%	0	100%	+	4		
	AP200	CP002121.1	ADM83843.1	Hypothetical protein	99%	0	100%	+	11A, ST62		
	INV200	FQ312029.1	CBW33819.1	Puative membrane protein	99%	0	100%	+	14		
	OXCI41	FQ312027.1	CBW31886.1	Puative membrane protein	99%	0	100%	+	3		
	INV104	FQ312030.1	CBW35848.1	Puative membrane protein	99%	0	100%	+	1		
	SPN034183	FQ312043.1	CCP31941.1	Puative membrane protein	99%	0	100%	+	3		
	SPN994038	FQ312041.2	CCP29955.1	Puative membrane protein	99%	0	100%	+	3		
	SPN994039	FQ312044.2	CCP33914.1	Competence-induced protein Csa4	99%	0	100%	+	3		
	A66	LN847353.1	CR160910.1	Competence-induced protein Csa4	99%	0	100%	+	3		
	NCTC7465	LN831051.1	COT02612.1	Competence-induced protein Csa4	99%	0	100%	+	1		
	D39	CP000410.1	ABJ53888.1	Competence-induced protein Csa4	99%	0	99%	+	2		
	R6	AE007317.1	AAK98986.1	Hypothetical protein	99%	0	99%	-	-		
<i>S. pneumoniae</i>	CGSP14	CP001033.1	ACB89463.1	Competence-induced protein Csa4	99%	0	99%	+	14		
	Hungary19A-6	CP000936.1	ACA36557.1	Competence-induced protein Csa4	99%	0	99%	+	19A		
	ST1556	CP003357.1	AFG93944.2	Damage-inducible protein CmaA	99%	0	99%	+	19F		
	Taiwan19F-14	CP000921.1	ACO24255.1	Competence-induced protein Csa4	99%	0	99%	+	19F		
	SPNA45	CP000936.1	CCM00114.1	Puative membrane protein	99%	0	99%	+	3		
	G54	CP001015.1	ACF56587.1	Hypothetical protein	99%	0	99%	+	19F		
	gamPN10373	CP001845.1	AFS42337.1	Competence-induced protein Csa4	99%	0	99%	+	1		
	TCH8431/19A	CP001993.1	ADJ68742.1	Competence-induced protein Csa4	99%	0	99%	+	19A		
	ATCC700669	FM211187.1	CAR68052.1	Puative membrane protein	99%	0	99%	+	23F		
	P1031	CP000920.1	AC020493.1	Competence-induced protein Csa4	99%	0	99%	+	1		
	70585	CP000918.1	ACO17022.1	Competence-induced protein Csa4	99%	0	99%	+	5		
	670-6B	CP002176.1	ADM90353.1	Competence-induced protein Csa4	99%	0	99%	+	6B		
<i>S. pneumoniae</i>	NT_110_58	CP007593.1	ADJ71143.1	Hypothetical protein	99%	0	99%	-	-		
	JJA	CP000919.1	ACO18425.1	Competence-induced protein Csa4	99%	0	99%	+	19F		
	SPN034156	FQ312045.1	CCP36950.1	Puative membrane protein	99%	0	99%	+	3		
	<i>S. pseudopneumoniae</i>	IST493	CP002925.1	Competence-induced protein Csa4	99%	0	96%	-	-		
	<i>S. mitis</i>	B6	FN568063.1	CBJ23149.1	Competence-induced protein Csa4	99%	0	91%	-	-	
		KCOM 1350	CP012646.1	ALD67418.1	Damage-inducible protein CmaA	99%	6.00E-133	54%	-	-	
		SVGS_061	CP014326.1	AMH89424.1	Damage-inducible protein CmaA	99%	4.00E-124	53%	+	-	
		Uo5	FR720602.1	CBZ201433.1	Competence-induced protein Csa4	99%	1.00E-108	48%	+	-	
		<i>S. pneumoniae</i>	S.MIT/ORA/LLIS-351	CP019562.1	AQA08377.1	Puative membrane protein	99%	1.00E-108	49%	+	-
			DORA_23_24	AZMK61000719.1	ETJ06019.1	Competence-induced protein Csa4	99%	1.00E-132	48%	-	-

(E-value < 2E-100, Coverage of the query > 99%, Identity > 48%)