SUPPLEMENTAL MATERIALS FOR:

A type I signal peptidase is required for pilus assembly in the Gram-positive biofilm-forming bacterium *Actinomyces oris*

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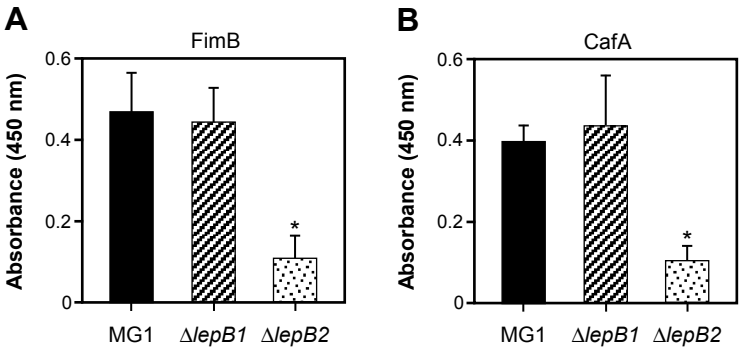

Figure S1: Requirement of LepB2 for surface expression of tip pilins
The presence of FimB (**A**) and CafA (**B**) on the bacterial cell surface was analyzed by ELISA with specific antibodies to FimB and CafA, respectively. The absorbance values, as compared to those of corresponding mutants as background, were determined from at least two independent experiments performed in triplicate. Error bars represent standard deviations. Asterisks (*) indicate P values of 0.02 (A) and 0.03 (B); all determined using the paired, two-tailed t-test with Prism GraphPad.

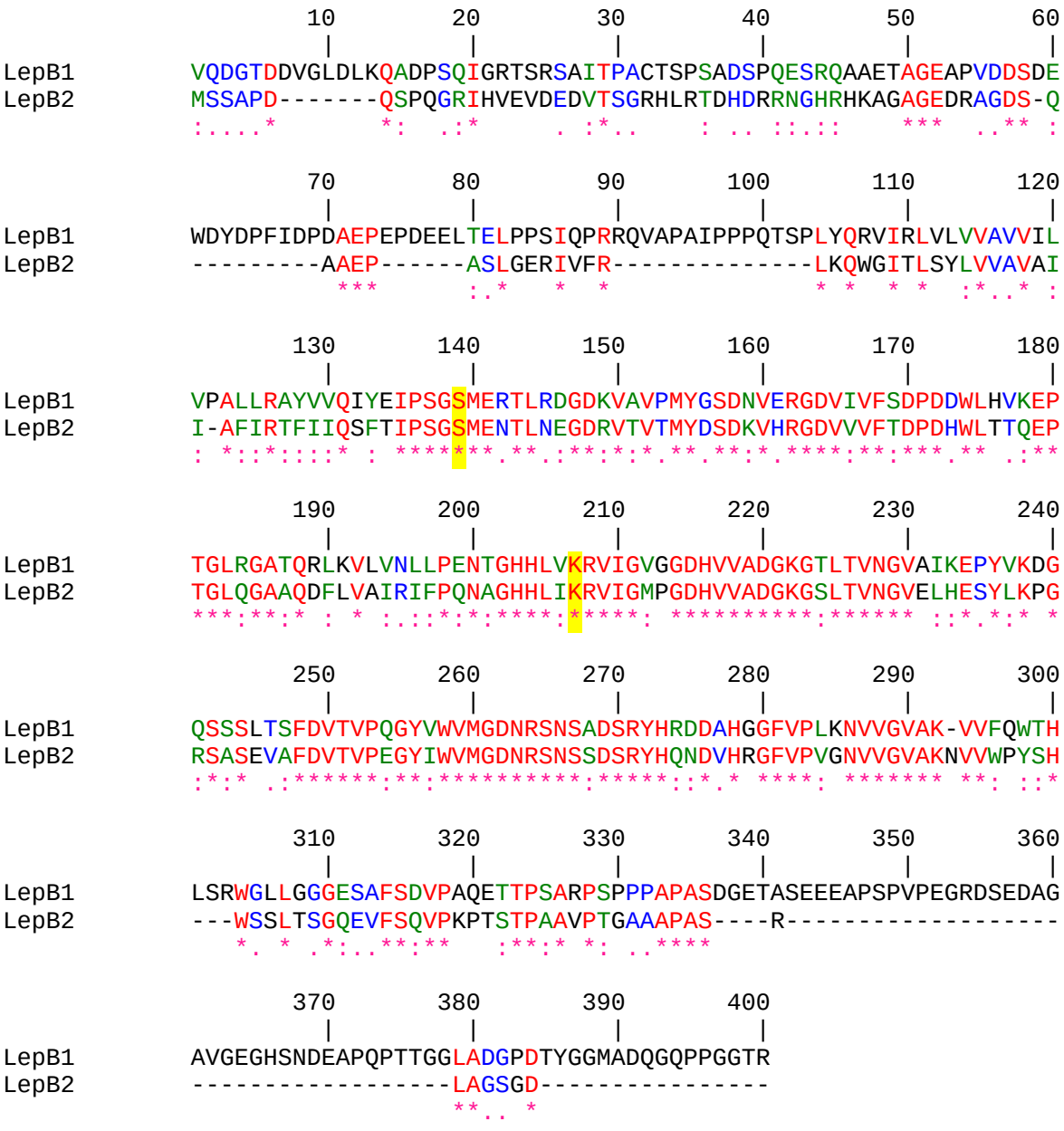

Figure S2: Sequence alignment of *A. oris* LepB1 and LepB2

Sequence alignment of *A. oris* LepB1 and LepB2 was performed using CLUSTALW (1). Highlighted in yellow are the conserved catalytic Ser and Lys residues.

Table S1: Bacterial strains and plasmids used in this study

Strain & Plasmid	Description	Reference
Strain		
A. oris MG1	Parental strain	(2)
A. oris CW1	Δ*galK*; an isogenic derivative of MG-1	(3)
A. oris AR4	Δ*fimA*; an isogenic derivative of CW1	(3)
A.oris WU36	Conditional *srtA* deletion mutant (Δ*srtA*), containing pTetR-Ω-SrtA	(4)
A.oris WU51	Deletion of *1291* (Δ*gspA*); an isogenic derivative of CW1	(4)
A.oris WU73	Deletion of *gspA* and *srtA*; Δ*GspA*/Δ*srtA*	(4)
A.oris WU42	Δ*lepB2*; an isogenic derivative of MG1	This study
A.oris WU50	Δ*lepB1*; an isogenic derivative of MG1	This study
A.oris WU80	Δ*lepB1/2*; an isogenic derivative of MG1	This study
A.oris WU81	Deletion of *lepB2* and *srtA*; Δ*lepB2*/Δ*srtA*	This study
A.oris WU42c1	WU42 containing pLepB2	This study
A.oris WU42c2	WU42 containing pLepB2(S101A)	This study
A.oris WU42c3	WU42 containing pLepB2(K169A)	This study
A.oris WU51c1	WU51 containing pGspA$_{\Delta cwss}$	This study
S. oralis So34	RPS positive	(5)
S. oralis OC1	a RPS-negative isogenic mutant of So34	(5)
Plasmid		
pJRD215	*Actinomyces*/*E. coli* shuttle vector, Kan^R, Str^R	(6)
pGspA$_{\Delta cwss}$	pCWU10 expressing GspA lacking the cell wall sorting signal (CWS)	This study
pCWU2	Derivative of pHTT177, expressing GalK under the control of the *rpsJ* promoter	(3)
pCWU2-Δ*lepB2*	An allelic replacement vector of *Ana_1190 (lepB2)* using pCWU2	This study
pCWU2-Δ*lepB1*	An allelic replacement vector of *Ana_1180 (lepB1)* using pCWU2	This study
pLepB2	pJRD215 derivative expressing wild-type LepB2 from MG-1	This study
pLepB2(S101A)	pJRD215 derivative expressing LepB2 with S101A mutation	This study

pLepB2(K169A)	pJRD215 derivative expressing LepB2 with K169A mutation	This study
pCVD047	Broad host range vector	(7)
pCWU10	pCVD047 derivative with kanamycin resistance gene from pJRD215 replacing with its original ampicillin resistance gene	This study
$pFimA_{\Delta cwss}$	pCWU10 expressing FimA lacking the cell wall sorting signal (CWSS)	This study

Table S2: Primers used in this study

Primer	Sequence[(a)]	Application
1190upF	GGCGTCTAGATCCGGACAAACCGTTCCATG CCCCGA	pCWU2-Δ*lepB2*
1190upR	GGCGAAGCTTTGCGCTGCTCATAGGCCTTC TCCTG	pCWU2-Δ*lepB2*
1190dnF	GGCGAAGCTTGACTGATCGCCCCGAAAGCG TGCTG	pCWU2-Δ*lepB2*
1190dnR	GGCGGAATTCACCGACCTCGTCCAGGCCGC CGACGT	pCWU2-Δ*lepB2*
Pro-1192F	GGCGGAATTCCCCTCGGCCGAGTCATCGGCC GCTCG	pLepB2
pro-1192R	GGCGGGTACCCTCCTGGGATCGGGGCATGGAACGG	pLepB2
com-1190F	GGCGGGTACCGTGATGAGCAGCGCACCCGACCA GAGC	pLepB2
com-1190R	GGCGAAGCTTTCAGTGGTGGTGGTGGTGGT GGTCCCCG GAGCCCGCCA GCCTG	pLepB2
1188upF	GGCGGAATTCACTGATCGCCCCGAAAGCGT GCTGG	pCWU2-Δ*lepB1*
1188upR	GGCGTCTAGAACGGGGAAGTGCAGGCCGGT GTG	pCWU2-Δ*lepB1*
1188dnF	GGCGTCTAGAGGCCCGGACACTTACGGCGG CATGG	pCWU2-Δ*lepB1*
1188dnR	GGCGGGTACCCTAGCCCCATGACGCATCCA CCG	pCWU2-Δ*lepB1*
1190S102A-F	GCGATGGAGAACACCCTCAACGAGGGCG	pLepB2(S101A)
1190S102A-R	CCCCGAGATGGTGAAGCTGCTCTGGATG	pLepB2(S101A)
1190K169A-F	GCGCGGGTCATCGGAATGCCCGGTGACCACG	pLepB2(K169A)
1190K169A-R	GATGAGGTGGTGACCGGCGTTCTGGGG	pLepB2(K169A)
pCVD047-noAmp-F	GGCGGAGTCATGATTTAGAAAAATAAACAAATAGGG G	$pCVD047_{\Delta Amp}$
pCVD047-noAmp-R	GGCGAAGCTTCTGTCAGACCAAGTTACTCATATATA	$pCVD047_{\Delta Amp}$
$215Kan_{MCS}$-F	GGCGGAGCTCTCAGAAGAACTCGTCAAGAA GGCGA	pCWU10
$215Kan_{MCS}$-R	GGCGAAGCTTATCG ATGATAAGCTGTCAA	pCWU10
GspAΔcws-His6-R	GGCGGAATTCTCAGTGGTGGTGGTGGTGGTGGGGC TTGCCGGAGGTGGAGGCCGCC	$pGspA_{\Delta cwss}$
prFimB-BamHI-F	AAAAAGGATCCGACGTCACCGGTGTCATCACCCTCC	$pFimA_{\Delta cwss}$
prFimB-R	GGGACCGCCTTCTCTTAGGCGTCG	$pFimA_{\Delta cwss}$
FimA-F	GTGACGCCGTCGGACAAGACGGAG	$pFimA_{\Delta cwss}$
FimAΔcws-His6-EcoRI-R	AAAAAGAATTCTCAGTGGTGGTGGTGGTGGTGAAC CGA CTG CTT GGT GTT CTC AAC GG	$pFimA_{\Delta cwss}$
RT-16s-R	GGTGTTGCCGACTTTCATG	RT- PCR (*16s rRNA*)

RT-16s-F	GTCGCTAGTAATCGCAGATCAG	RT- PCR (*16s rRNA*)
RT-1291F	GACGGCACCTACAAGATCAC	RT-PCR (*gspA*)
RT-1291R	AGGAGTCGGTCTTGCTGA	RT-PCR (*gspA*)
P1	CCTCCAGGTCC CGATCACAC	RT and qRT-PCR
P2	GCCTGCGGGGTTGGATAGAGG	RT and qRT-PCR
P3	CCCGGCCGGTCAGCCTCCGAGGTCG	RT and qRT-PCR
P4	GCTGGACCAGTGGGAGTATGGCCAC	RT and qRT-PCR
P5	GTGTGATCGCGGACCTGGAGG	RT- PCR
P6	GGCCATACTCCCACTGGTCCAG	RT- PCR

[a] Underlined are the restriction sites in the primers.

Table S3: Putative signal peptides of non-fimbrial and fimbrial surface proteins in *Actinomyces oris*

Surface Protein	Signal Peptide[a]
AcaA	MSIIPIRLRRLSAPTSALLAALALAAAGP**AVA**APASNT
AcaB	MKRRIVAQMAILALSLSAPVLAVTHAPQ**AAA**ADGNII
AcaC/GspA	MRLGRPLAAASVMAAAIGASLAASP**ALA**GDSLAF
AcaD	MRLGRPLAAASAVAAMTGASLIASP**VLA**NSSNSN
AcaE	MNTMSTGAASRPALTRGAVRGAAAILTLFLVAILSQFAPQ**ASA**EQNKSV
AcaF	MLRRFFVRSHRRELTSSRQPSRQSRRRLR SGAAISTFALIAGALGTATLPAPP**AEA**VHGNPA
AcaG	MRFSPLIPVNIELKFATASIECRTSPERFAVSKIPAPRALRAPVR ATGALLLLVVMVAAMLTVLPMQ**ARA**AINPKI
AcaH	MFLSSSSPGSPSLRLIRNDMRLRPTRAVR VSAVLCALTLTALSPFPLSPP**AGA**DEPAAP
AcaI	MTSHSPFSRRRLPALLGSLPLAATGLIAAAPP**ALA**VPTSDG
AcaJ	MRRRAPRLVGLAGAVVLSSSLSIPMMAP**VSA**EPTSGD
AcaK	MNIKRVIAAAALTVVPLTASSA**ALA**APVAPE
AcaL	MNRPSQHDKPNVAHYMKQLTFIILVTLLLFNQELPLPTTLPT**ASA**AITTNV
AcaM	MSRTARALSALTAFCLLAAASTLTSVP**AAA**SGQARP MLVWEGPAALRTAGAQVGADQWCDKVDPVVVSYHTG
AcaN	KNRSSSGLPQEMTRQLDGQFTALSLMSAKEILAGM**ATF**DERKRS
FimP	MHSLNTRRGLGLAAAMTLAAGALVAPT**GAA**APADPN
FimQ	MSQSSRRPQRGREGLLRRFCAVVGSLAIVLVTMVSLPWTAPP**ADA**STSDLS
FimA	MKHNASTLGRRAAAAAGVLTLAFLGLAPS**AVA**TETPNY
FimB	MFPLLSRRAIKPRLRKLVAGAAALACVAAALVGTEQPS**AGA**APSGLP

[a]The n-regions are followed by h-regions, which are highlighted by grey color, and the AXA motif is underlined and bolded.

Table S4: Edman Degradation of GspA proteins purified from four *A. oris* strains

Sequencing Cycle	Residue (pmol)			
	MG1	**Δ*lepB1***	**Δ*lepB2***	**Δ*lepB1*/Δ*lepB2*** [a]
1	G (4.761)	G (13.250)	G (7.833)	L (3.150); A (3.665)
2	D (4.438)	D (13.830)	D (8.280)	A (3.887)
3	S (2.529)	S (5.692)	S (4.623)	G (2.938); P (1.390)
4	L (3.972)	L (10.650)	L (6.026)	D (2.962); A (2.836)
5	A (4.082)	A (11.050)	A (6.184)	S (1.774)
6	F (2.913)	F (8.309)	F (5.394)	L (3.162)
7	K (3.882)	K (10.280)	K (5.761)	A (4.124); G (2.501)
8	I (2.661)	I (8.125)	I (4.958)	F (2.520); D (2.494)
9	A (3.427)	A (9.922)	A (5.492)	K (2.520); S (1.729)
10	D (2.794)	D (7.996)	D (5.265)	I (1.875); L (2.144)

[a] Two major residues were detected in the majority of the first 10 sequencing cycles.

Table S5: Edman Degradation of FimA proteins purified from three *A. oris* strains

Sequencing Cycle	Residue (pmol)		
	MG1	**Δ*lepB1***	**Δ*lepB2*** [a]
1	T (8.363)	T (2.341); G (1.394)	A (10.082); T (8.999); G (70.840);
2	E (6.034)	E (1.391)	V (6.512); E (6.271); L (6.009)
3	T (7.885)	T (2.137)	A (10.563); T (9.934)
4	P (6.338)	P (1.786)	T (10.321); P (8.828)
5	N (4.784)	N (1.467)	E (5.881); N (4.545)
6	Y (5.789)	Y (1.512)	T (11.027); A (9.149); Y (5.218)
7	G (5.246)	G (2.061)	P (7.555); G (11.852); V (6.863)
8	N (4.597)	N (1.412)	N (5.679); A (9.687)
9	I (4.777)	I (1.771)	I (6.770); Y (4.509)
10	K (5.922)	K (1.133)	K (6.659); G (12.054); E (5.618)

[a] Multiple residues were detected in each of the first 10 sequencing cycles.

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