

Supplemental material

Table S1 Bacterial strains used in the study

Strain	Description	Reference
<i>E. coli</i>		
S17-1	<i>hsdR17 recA1 RP4-2-tet::Mu-1kan::Tn7 Sm^R</i>	(1)
<i>P. aeruginosa</i>		
PAO1	Wild-type <i>P. aeruginosa</i>	(2)
<i>C. canimorsus</i>		
<i>C. canimorsus</i> 5 (Cc5)	Isolated from human fatal septicemia after dog bite, 1995, Libramont, Belgium	(3)
<i>Cc5ΔPUL1</i>	Substitution of <i>PUL1</i> by <i>ermF</i> ; Em ^R	(4)
<i>Cc5ΔPUL2</i>	Substitution of <i>PUL2</i> by <i>ermF</i> ; Em ^R	(4)
<i>Cc5ΔPUL3</i>	Substitution of <i>PUL3</i> by <i>ermF</i> ; Em ^R	(4)
<i>Cc5ΔPUL4</i>	Substitution of <i>PUL4</i> by <i>ermF</i> ; Em ^R	(4)
<i>Cc5ΔPUL5</i>	Substitution of <i>PUL5</i> by <i>ermF</i> ; Em ^R	(4)
<i>Cc5ΔPUL6</i>	Substitution of <i>PUL6</i> by <i>ermF</i> ; Em ^R	(4)
<i>Cc5ΔPUL7</i>	Substitution of <i>PUL7</i> by <i>ermF</i> ; Em ^R	(4)
<i>Cc5ΔPUL8</i>	Substitution of <i>PUL8</i> by <i>ermF</i> ; Em ^R	(4)
<i>Cc5ΔPUL9</i>	Substitution of <i>PUL9</i> by <i>ermF</i> ; Em ^R	(4)
<i>Cc5ΔPUL10</i>	Substitution of <i>PUL10</i> by <i>ermF</i> ; Em ^R	(4)
<i>Cc5ΔPUL11</i>	Substitution of <i>PUL11</i> by <i>ermF</i> ; Em ^R	(4)
<i>Cc5ΔPUL12</i>	Substitution of <i>PUL12</i> by <i>ermF</i> ; Em ^R	(4)
<i>Cc5ΔPUL13</i>	Substitution of <i>PUL13</i> by <i>ermF</i> ; Em ^R	(4)
<i>Cc5ΔCcan_03610</i>	Substitution of <i>Ccan_03610</i> by <i>ermF</i> using pFL1; Em ^R	This study
<i>Cc5ΔCcan_03620</i>	Substitution of <i>Ccan_03620</i> by <i>ermF</i> using pFL2; Em ^R	This study
<i>Cc5ΔCcan_03630</i>	Substitution of <i>Ccan_03630</i> by <i>ermF</i> using pFL3; Em ^R	This study
<i>Cc5ΔCcan_03640</i>	Substitution of <i>Ccan_03640</i> by <i>ermF</i> using pFL4; Em ^R	This study

<i>Cc5ΔCcan_03650</i>	Substitution of <i>Ccan_03650</i> by <i>ermF</i> using pFL5; Em ^R	This study
<i>Cc5ΔCcan_03660</i>	Substitution of <i>Ccan_03660</i> by <i>ermF</i> using pFL6; Em ^R	This study
<i>Cc5ΔCcan_03670</i>	Substitution of <i>Ccan_03670</i> by <i>ermF</i> using pFL7; Em ^R	This study
<i>Cc5ΔCcan_03680</i>	Substitution of <i>Ccan_03680</i> by <i>ermF</i> using pFL8; Em ^R	This study
<i>Cc5ΔCcan_03690</i>	Substitution of <i>Ccan_03690</i> by <i>ermF</i> using pFL9; Em ^R	This study
<i>Cc5ΔCcan_03700</i>	Substitution of <i>Ccan_03700</i> by <i>ermF</i> using pFL10; Em ^R	This study
<i>Cc5ΔCcan_03710</i>	Substitution of <i>Ccan_03710</i> by <i>ermF</i> using pFL11; Em ^R	This study
<i>Cc5ΔCcan_03720</i>	Substitution of <i>Ccan_03720</i> by <i>ermF</i> using pFL12; Em ^R	This study
<i>Cc5ΔCcan_03730</i>	Substitution of <i>Ccan_03730</i> by <i>ermF</i> using pFL13; Em ^R	This study
<i>Cc5ΔCcan_15860</i>	Substitution of <i>Ccan_15860</i> (<i>furA</i>) by <i>ermF</i> using pFL61; Em ^R	This study

Table S2 Primers used in the study

Lab.Ref.	Name	Sequence 5'-3' (restriction sites are underlined)	Restriction
Deletions			
6953	fwd_03640_1.1	cgctgcaggctacctatatgatggagcc	<i>Pst</i> I
6954	rev_03640_1.2	aaaaatttcaccttcgtagaaaaacttcttacgattttatttag	
7027	fwd_03640_2.1	gagtagataaaagcactgtttagggacaggacgtg	
7028	rev_03640_2.2	ggactagtatccgtctgtgccaataccc	<i>Spe</i> I
6957	fwd_03640_3.1	ctaaataaaaatcgtaagaagttttctacgaaggatgaaattttcagggacaac	
6958	rev_03640_3.2	ggacaggacacgtcctgtccctaaacagtgctttatctactccgatagcttc	
6959	fwd_03650_1.1	cgctgcagtttacgagcaggacatcc	<i>Pst</i> I
6960	rev_03650_1.2	aaaaatttcaccttcgtagaatgataatcttg	
7050	fwd_03650_2.1	gagtagataaaagcactgttcacttggttacaacgttcc	
7051	rev_03650_2.2	ggactagtatccgagtgtttctacc	<i>Spe</i> I
6963	fwd_03650_3.1	caaagattatcatttctacgaaggatgaaattttcagggacaac	
7052	rev_03650_3.2	ggaacgttgtaaccaagtgaaacagtgctttatctactccgatagcttc	
6965	fwd_03660_1.1	cgctgcagccaaaacagtttacattgacgg	<i>Pst</i> I
6966	rev_03660_1.2	aaaaatttcaccttcgtagtctctactatttcctatttttac	
6967	fwd_03660_2.1	gagtagataaaagcactgttaataacaatatataaaaaatagaatag	
6968	rev_03660_2.2	ggactagtacccaaatagcggaaagg	<i>Spe</i> I
6969	fwd_03660_3.1	gtaaaaaataggaaatagtagagactacgaaggatgaaattttcagggacaac	
6970	rev_03660_3.2	ctattctattttatataattgtattaacagtgctttatctactccgatagcttc	
6971	fwd_03670_1.1	cgctgcagaaatcagtggaagtaaccgc	<i>Pst</i> I
6972	rev_03670_1.2	aaaaatttcaccttcgtagtttatgttcttctgtgag	
6973	fwd_03670_2.1	gagtagataaaagcactgttttttagtattgccaacg	
6974	rev_03670_2.2	ggactagttttccgtccgtaagggtctgccc	<i>Spe</i> I

6975	fwd_03670_3.1	ctacaagaaagaacataaaaactacgaaggatgaaattttcagggacaac	
6976	rev_03670_3.2	cggtgggcaaataactaaaaaacagtgctttatctactccgatagcttc	
6977	fwd_03680_1.1	cgctgcagattgggggagagcctcgtgc	<i>PstI</i>
6978	rev_03680_1.2	aaaaattcatccttcgtagatcatctgatattttattattgattgatgc	
6979	fwd_03680_2.1	gagtagataaaagcactgttttgtaaggaaggacgtgtcc	
6980	rev_03680_2.2	ggactagtccttctcatcgaaattattgacatcg	<i>SpeI</i>
6981	fwd_03680_3.1	gcatcaaatcaataataaaaaatcagatgatctacgaaggatgaaattttcagggacaac	
6982	rev_03680_3.2	ggacacgtcccttcttacaacacagtgctttatctactccgatagcttc	
7527	fwd_03690_1.1	ggctgcaggattgtacgtaaccaatgtgctttcacc	<i>PstI</i>
7528	rev_03690_1.2	gttgcaaataccgatgagcgattttttattttaagcggaaaggacacg	
7529	fwd_03690_2.1	cctgaaaaattcatccttcgtagaatatgaaaaatcttattctgttggc	
7530	rev_03690_2.2	ccactagtgtattcacgagcgggtcaatagaattagtg	<i>SpeI</i>
7531	fwd_03690_3.1	cggtccttccgctaaaataaaaaataatcgctcatcggtatttgcaac	
7532	rev_03690_3.2	gccaacagaataagatatttttcatattctacgaaggatgaaattttcagg	
7533	fwd_03700_1.1	ggctgcagcgaagacttctgattgtacaagagaccg	<i>PstI</i>
7534	rev_03700_1.2	gttgcaaataccgatgagcattttattgatttacgtatgatttaagtcgc	
7535	fwd_03700_2.1	cctgaaaaattcatccttcgtagaaacaagctaaaaataatgatgac	
7536	rev_03700_2.2	ccactagtcctctttgaaacggctgagatacttgc	<i>SpeI</i>
7537	fwd_03700_3.1	gcgactaaatcatcgtaaatcaataaaatgctcatcggtatttgcaac	
7538	rev_03700_3.2	gtcatatttttttagctgtttctacgaaggatgaaattttcagg	
6995	fwd_03710_1.1	cgctgcagcagaaaataatgttcagaaagc	<i>PstI</i>
6996	rev_03710_1.2	aaaaattcatccttcgtagatttttttagctgtttctatttgc	
6997	fwd_03710_2.1	gagtagataaaagcactgttacgtgttggaaatgacagcgg	
6998	rev_03710_2.2	ggactagtttctgcaatcgcaacttgatac	<i>SpeI</i>
6999	fwd_03710_3.1	gacaaatagaacaagctaaaaataatctacgaaggatgaaattttcagggacaac	

7000	rev_03710_3.2	ccgctgtcattccaacacgtaacagtgctttatctactccgatagcttc	
7001	fwd_03720_1.1	cgctgcagtcattgataatcagcgagag	<i>PstI</i>
7002	rev_03720_1.2	aaaaattcatccttcgtagatTTTTctgttgaagaacaagaatcgcc	
7003	fwd_03720_2.1	gagtagataaaagcactgttagtaaaaaggattttcttttc	
7004	rev_03720_2.2	ggactagtctccttgaagagaggaagcc	<i>SpeI</i>
7005	fwd_03720_3.1	ggcgattctgttcttacaacagaaaaatctacgaaggatgaaattttcagggacaac	
7006	rev_03720_3.2	gaaaagaaaatccttttactaacagtgctttatctactccgatagcttc	
7059	fwd_03610_1.1	cgctgcagaatactctatttacacgg	<i>PstI</i>
7060	rev_03610_1.2	aaaaattcatccttcgtagtttataaatttggtg	
7061	fwd_03610_2.1	gagtagataaaagcactgtTTTTgaaactgtcattgg	
7062	rev_03610_2.2	ggactagtaagtgcccaatttctgc	<i>SpeI</i>
7063	fwd_03610_3.1	cacccaaaattataaaactacgaaggatgaaattttcagggacaac	
7064	rev_03610_3.2	ccaatgacagttcaaaaaaacagtgctttatctactccgatagcttc	
7065	fwd_03620_1.1	cgctgcagattagtagtggcattgg	<i>PstI</i>
7066	rev_03620_1.2	aaaaattcatccttcgtagaatTTTTctttaaagtagatc	
7067	fwd_03620_2.1	gagtagataaaagcactgttaattgTTTTtattacaatc	
7068	rev_03620_2.2	ggactagttgagacagagtaaaagc	<i>SpeI</i>
7069	fwd_03620_3.1	gatcactttaagaaaatattctacgaaggatgaaattttcagggacaac	
7070	rev_03620_3.2	gattgtaagataaaaaacaaattaacagtgctttatctactccgatagcttc	
7085	fwd_03730_1.1	cgctgcagatagggttatccctgctggggaagg	<i>PstI</i>
7086	rev_03730_1.2	aaaaattcatccttcgtagctTTTTctatttatctg	
7087	fwd_03730_2.1	gagtagataaaagcactgttaatctgtataaaaatgc	
7088	rev_03730_2.2	ggactagtcatcgagaggaagcaaaatataatcc	<i>SpeI</i>
7089	fwd_03730_3.1	cagatataaatagaaaagagctacgaaggatgaaattttcagggacaac	
7090	rev_03730_3.2	gcattttatacagattaacagtgctttatctactccgatagcttc	

7096	fwd_03630_1.1	cgctgcagacgctgataccagattgattgattttcaaacagg	<i>PstI</i>
7097	rev_03630_1.2	aaaaatttcaccttcgtagattcaatacttattcattgttttaatgc	
7098	fwd_03630_2.1	gagtagataaaagcactgttgcatcaatcagctacaaccaaaaatcc	
7099	rev_03630_2.2	ggactagttactccgagtattgggtggc	<i>SpeI</i>
7100	fwd_03630_3.1	gcattaaaaacaaatgataagtattgaaatctacgaaggatgaaattttcagggacaac	
7101	rev_03630_3.2	ggatttttgggtgtagctgattgatgcaacagtgctttatctactccgatagcttc	
7102	FR227-1.1furAKOPstI	cgctgcagggaaattggataaatacaataatg	<i>PstI</i>
7103	FR228-1.2-furAKO	gagtagataaaagcactgttctgcttgggtgtttcttttttag	
7104	FR229-2.1-furAKO	gaaaaatttcaccttcgtagccaagatggcagtagattattac	
7105	FR230-2.2-furAKOSpel	ggactagttattggcaaggttacgataacg	<i>SpeI</i>
7106	FR231-3.1-furAKO	ctaaaaagaaaacaccaagcagaacagtgctttatctactc	
7107	FR232-3.2-furAKO	gtaataaatctactgcatcttggtacgaaggatgaaattttc	

Trans-complementations and PCR screening

7036	fwd_Ccan_03640	cgtaccatggcgtgttaccaaagatagg	<i>NcoI</i>
7037	rev_Ccan_03640	tgactagttaaaacttcacattcactcc	<i>SpeI</i>
7038	fwd_Ccan_03650	cgtaccatggcgaatcaatcaatgataaagaaactactatatagcg	<i>NcoI</i>
7039	rev_Ccan_03650	tgactagttaaaacccaacatttacc	<i>SpeI</i>
7040	fwd_Ccan_03680	cgtaccatggcgccaacgaaagagcatcaaac	<i>NcoI</i>
7041	rev_Ccan_03680	tgactagttatcttgattgggtgctaaacc	<i>SpeI</i>
7042	fwd_Ccan_03690	cgtaccatggcgagaagaataacataatattaacattgg	<i>NcoI</i>
7043	rev_Ccan_03690	tgactagttattgatttacgtagtttaagtgcg	<i>SpeI</i>
7044	fwd_Ccan_03700	cgtaccatggcgaaaaaatatcttattctgttggc	<i>NcoI</i>
7045	rev_Ccan_03700	tgactagttattgtcaactatttcagc	<i>SpeI</i>
7046	fwd_Ccan_03710	cgtaccatggcgacaatgaatagaaaatatttttgataatattactgggg	<i>NcoI</i>

7047	rev_Ccan_03710	tgactagttatggcaaaataataactcgc	SpeI
7048	fwd_Ccan_03720	cgtaccatggcgtggaaatatacagttttaatagtgtccc	NcoI
7049	rev_Ccan_03720	tgactagttatcttttatatcattgattgaaattccg	SpeI
7077	fwd_Ccan_03660	cgtaccatggatagtcataattggatagaaaagtgg	NcoI
7078	rev_Ccan_03660	tgactagttattgcttccgtgctacaaaatcgg	SpeI
7079	fwd_Ccan_03670	cgtaccatggcgaagtcaaaaaaaaaatag	NcoI
7080	rev_Ccan_03670	tgactagttatcttctcaaaaataagc	SpeI
3451	fwd_16S	agagtttgatcctggctcag	
3454	rev_16S	gggttgcgctcgttg	

Sequencing			
3818	pMM25_fwd	gtttcccagtcacgac	
4730	pMM25_rev	ggcacgttccagttcttcag	
7125	ermF_seq1	atgctcaaattgtttgttctcc	
7126	ermF_seq2	gagcaaacatataaccgaggaacaaagtgc	

qPCR			
7335	03640-F-qPCR	caatgCGGctcgaatgactg	
7336	03640-R-qPCR	gggaatgggcgtagaaacca	
7337	03650-F-qPCR	tcggtgaggTggtgttacg	
7338	03650-R-qPCR	tacgCgtccttcgagcatac	
7339	03680-F-qPCR	caggcaaacaagcgttgga	
7340	03680-R-qPCR	gttccgtaaggTctgccca	
7341	08720-F-qPCR	ggacagTgttactgttatcaagtc	
7342	08720-R-qPCR	gctataatgtgacgagctaaatcac	
7343	16S -F-qPCR	tgagtggctaagcgaagtga	

7344	16S -R-qPCR	cttggaagggtcctcgcg	
pPM5 construction			
5470	PM177_Fj_Pompa_fw	cgatgctcgacttttttaacattgattttgtatttaaaaaattgggtgtactttgc	<i>Sall</i>
5471	PM178_Fj_Pompa_rv	cgatccatgggtaatttttaattacaattagttaattacaagcaaaagtaacacc	<i>NcoI</i>

Table S3 Plasmids used in the study

Plasmid	Description	Reference
Vectors		
pMM13	<i>oriColE1</i> , Em ^R .	(5)
pMM25	<i>oriColE1</i> , Km ^R , Cfx ^R .	(5)
pMM47.A	<i>oriColE1</i> , <i>ori pCC7</i> , Ap ^R , Cfx ^R .	(5)
pPM5	<i>oriColE1</i> , <i>ori pCC7</i> , Ap ^R , Cfx ^R ; promoter of <i>ompA</i> (<i>Fjoh_0697</i>) from <i>Flavobacterium johnsoniae</i> was amplified by PCR using primers 5470 and 5471, digested with <i>Sall</i> and <i>NcoI</i> , and inserted into the corresponding sites of pMM47.A, replacing the original <i>ermF</i> promoter.	This study
Mutator Plasmids		
pFL1	<i>ermF</i> framed by the 5' and 3' regions of <i>Ccan_03610</i> cloned into pMM25	This study
pFL2	<i>ermF</i> framed by the 5' and 3' regions of <i>Ccan_03620</i> cloned into pMM25	This study
pFL3	<i>ermF</i> framed by the 5' and 3' regions of <i>Ccan_03630</i> cloned into pMM25	This study
pFL4	<i>ermF</i> framed by the 5' and 3' regions of <i>Ccan_03640</i> cloned into pMM25	This study
pFL5	<i>ermF</i> framed by the 5' and 3' regions of <i>Ccan_03650</i> cloned into pMM25	This study
pFL6	<i>ermF</i> framed by the 5' and 3' regions of <i>Ccan_03660</i> cloned into pMM25	This study
pFL7	<i>ermF</i> framed by the 5' and 3' regions of <i>Ccan_03670</i> cloned into pMM25	This study

pFL8	<i>ermF</i> framed by the 5' and 3' regions of <i>Ccan_03680</i> cloned into pMM25	This study
pFL9	<i>ermF</i> framed by the 5' and 3' regions of <i>Ccan_03690</i> cloned into pMM25	This study
pFL10	<i>ermF</i> framed by the 5' and 3' regions of <i>Ccan_03700</i> cloned into pMM25	This study
pFL11	<i>ermF</i> framed by the 5' and 3' regions of <i>Ccan_03710</i> cloned into pMM25	This study
pFL12	<i>ermF</i> framed by the 5' and 3' regions of <i>Ccan_03720</i> cloned into pMM25	This study
pFL13	<i>ermF</i> framed by the 5' and 3' regions of <i>Ccan_03730</i> cloned into pMM25	This study
pFL61	<i>ermF</i> framed by the 5' and 3' regions of <i>Ccan_15860</i> cloned into pMM25	This study

<i>Trans-complementation Plasmids</i>		
pFL14	<i>Ccan_03640</i> amplified with 7036 & 7037 and cloned into pPM5	This study
pFL15	<i>Ccan_03650</i> amplified with 7038 & 7039 and cloned into pPM5	This study
pFL16	<i>Ccan_03660</i> amplified with 7077 & 7078 and cloned into pPM5	This study
pFL17	<i>Ccan_03670</i> amplified with 7079 & 7080 and cloned into pPM5	This study
pFL18	<i>Ccan_03680</i> amplified with 7040 & 7041 and cloned into pPM5	This study
pFL19	<i>Ccan_03690</i> amplified with 7042 & 7043 and cloned into pPM5	This study
pFL20	<i>Ccan_03700</i> amplified with 7044 & 7045 and cloned into pPM5	This study
pFL21	<i>Ccan_03710</i> amplified with 7046 & 7047 and cloned into pPM5	This study
pFL22	<i>Ccan_03720</i> amplified with 7048 & 7049 and cloned into pPM5	This study

Table S4 Protein and iron concentration of products used in the study

	[Iron] (μM)	[Protein] (g/l)
Serotransferrin	41.2 ± 3.0	5.4 ± 0.2
Apotransferrin	0.5 ± 0.2	4.4 ± 0.1
Lactoferrin	37.4 ± 7.2	3.5 ± 0.1
Bovine transferrin	38.1 ± 3.5	4.3 ± 0.2
Hemoglobin	257.4 ± 16.3	5.9 ± 0.6
Human serum, heat inactivated (Millipore)	18.1 ± 1.0	49.0 ± 1.1
Human serum, heat inactivated (University Hospital of Basel)	19.5 ± 1.7	57.2 ± 5.2
Protein depleted human serum, heat inactivated	0.9 ± 0.6	N/A

Fig.S1

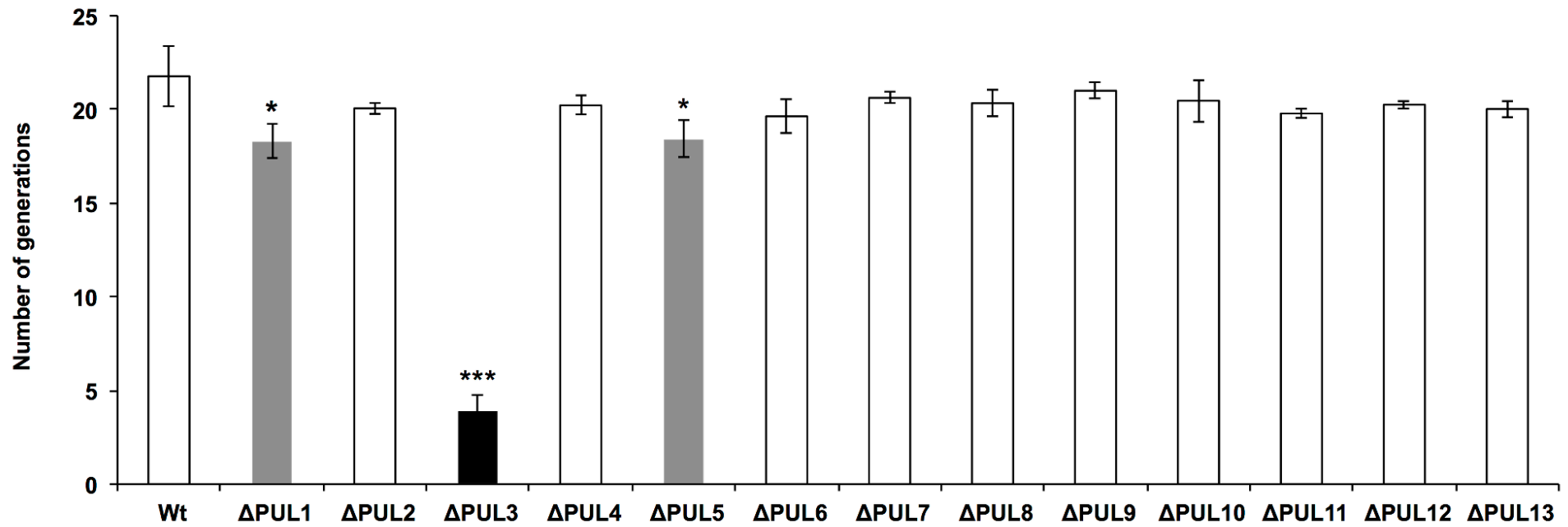


Figure S1.

Growth in HIHS of *C. canimorsus* 5 mutants deleted from individual PULs

Number of generations achieved by each of the individual *PUL* deletion mutant after 23 hours in HIHS. The black bar and to a lesser extent the dark grey bars indicate significantly reduced growth scores with respect to wt. Error bars indicate standard deviations (average of 3 experiments).

(*) and (***) apply to comparisons to wt values and stand for *t*-test based error probabilities of <0.05 and <0.001 respectively.

Fig.S2

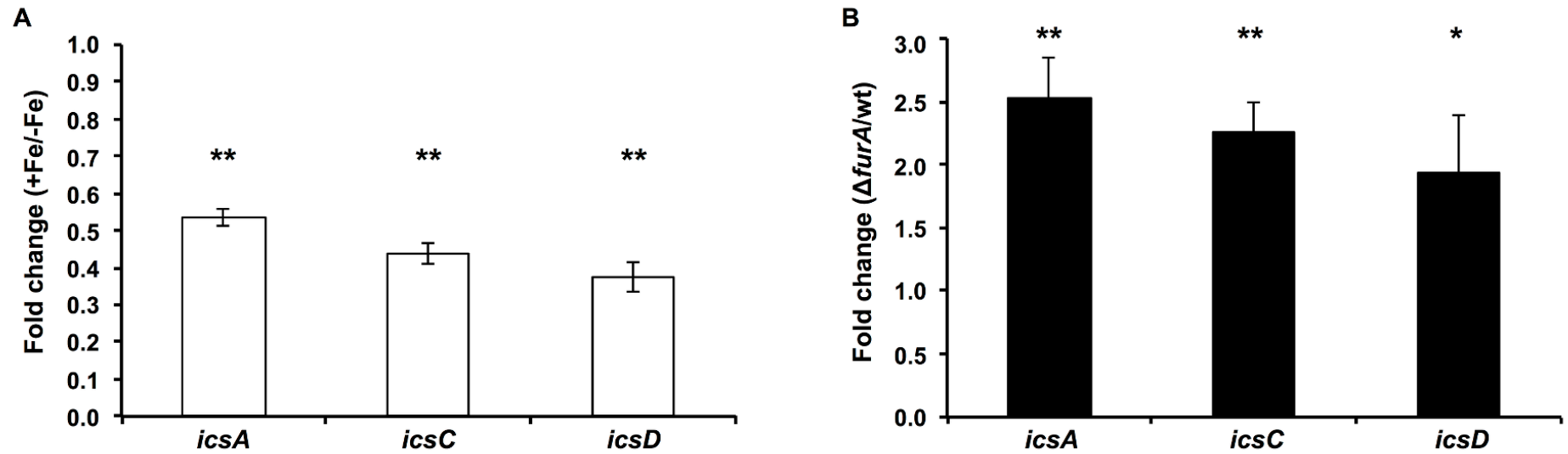


Figure S2.

Regulation of transcription of *PUL3* by free iron and the FurA transcriptional regulator

Fold change of mRNA levels of the two *susC* homologues *Ccan_03640* and *Ccan_03650* and the *susD* homologue *Ccan_03680*. (A) Relative mRNA levels from wt bacteria grown in HIHS plus iron (III) citrate (+Fe) vs. HIHS with no iron supplementation (-Fe). (B) Relative mRNA levels of $\Delta furA$ vs. wt Cc5 grown in HIHS. Error bars represent the standard deviation (average of 3 experiments). (*) and (**) apply to comparisons to wt values and stand for *t*-test based error probabilities of <0.05 and <0.01 respectively.

Fig.S3

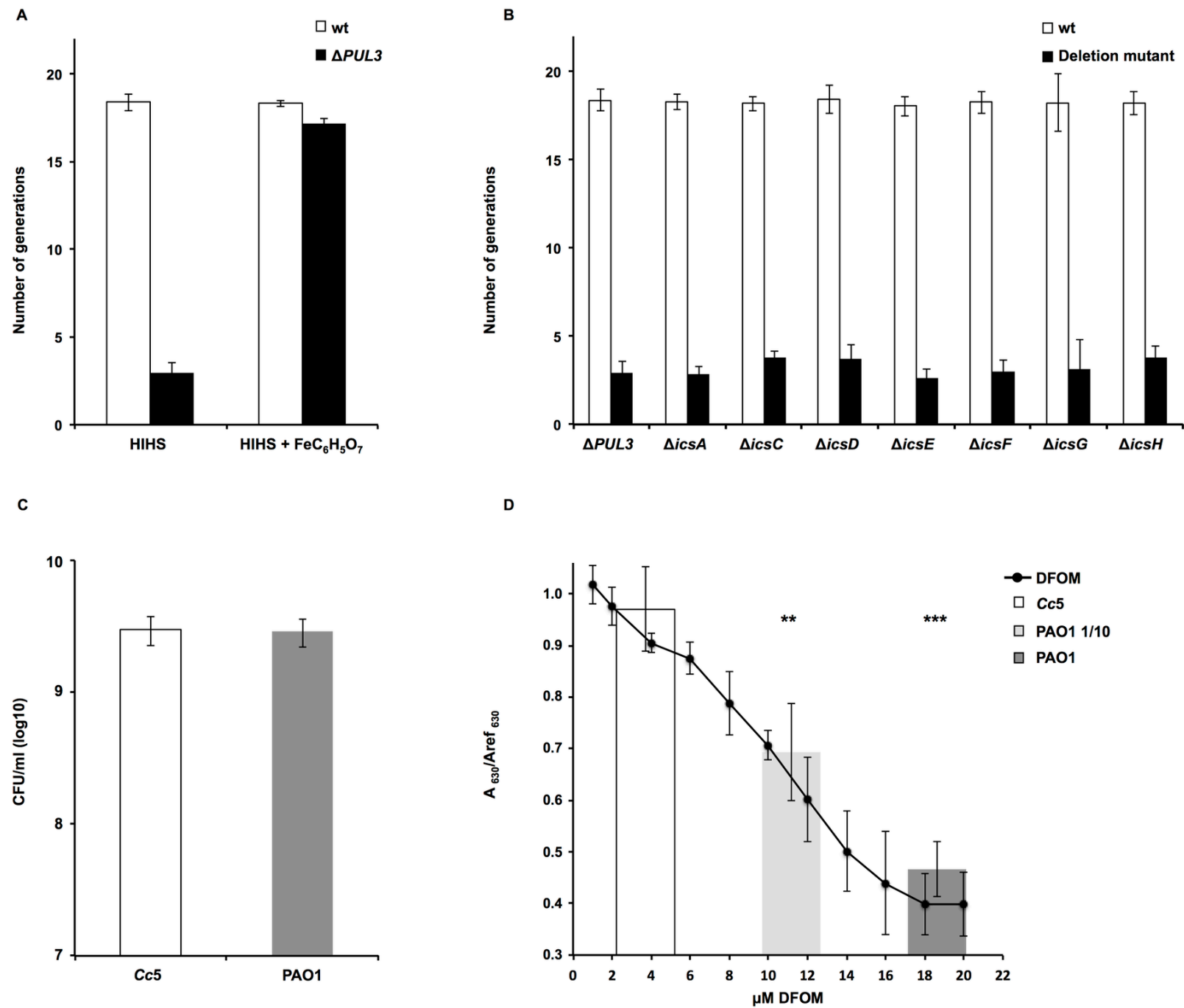


Figure S3.

Iron capture from transferrin does not involve soluble factors

(A) Number of generations achieved by wt (white bars) and $\Delta PUL3$ (black bars) *C. canimorsus* bacteria after 23 hours of co-culture in HIHS alone or supplemented with 0.25 mM iron (III) citrate ($\text{FeC}_6\text{H}_5\text{O}_7$). (B) Number of generations achieved by the wt (white bars) and each of the individual *ics* deletion mutants (black bars) after 23 hours of co-culture in HIHS. (C) Total growth in HIHS of *P. aeruginosa* PAO1 wt (dark grey bar) and *Cc5* wt (white bar). (D) Siderophore detection in *Cc5* wt (white bar) and *P. aeruginosa* PAO1 wt (light and dark grey bars) HIHS culture supernatants using the chrome azurol S assay. Decrease of the ratio A/A_{ref} at 630 nm indicates presence of siderophore. Dots: dilution series of the iron chelator desferrioxamine mesylate (DFOM) used as standard curve. PAO1 1/10: PAO1 supernatant diluted 1 to 10 in ddH₂O. Error bars in all panels represent standard deviation (average of 3 experiments). (**) and (***) apply to comparisons to *Cc5* wt values and stand for *t*-test based error probabilities of <0.01 and <0.001 respectively.

Fig.S4

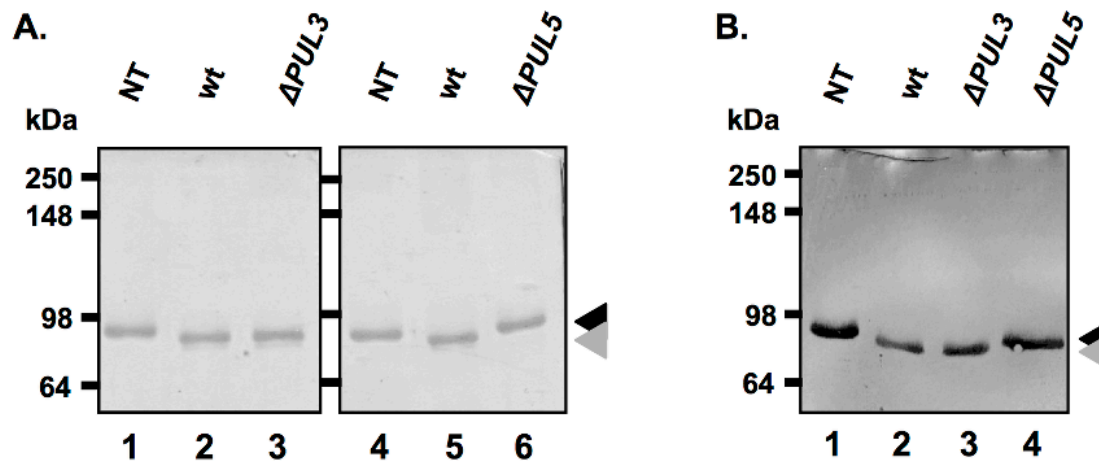


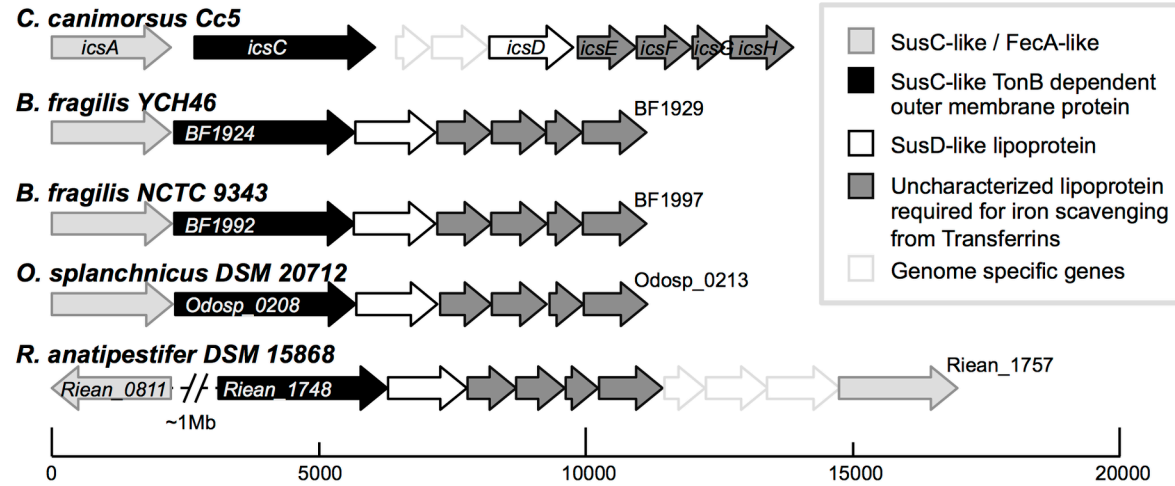
Figure S4.

Cleavage of human serotransferrin *N*-glycans by the Gpd complex

(A) Coomassie staining of SDS-PAGE loaded with STF incubated in absence (NT, lane 1 & 4) or presence of wt (lane 2 & 5), *PUL3* deleted (lane 3) or *PUL5* deleted (lane 6) *C. canimorsus*. (B) *Sambucus Nigra* Lectin (SNA) staining of human STF incubated in absence (NT, lane 1) or presence of wt (lane 2), *PUL3* deleted (lane 3) or *PUL5* deleted (lane 4) *C. canimorsus*. Numbers on the left indicate protein mass of the references in kDa. Grey and black arrows indicate a shift in electrophoretic mobility of STF.

Fig.S5

A.



B.

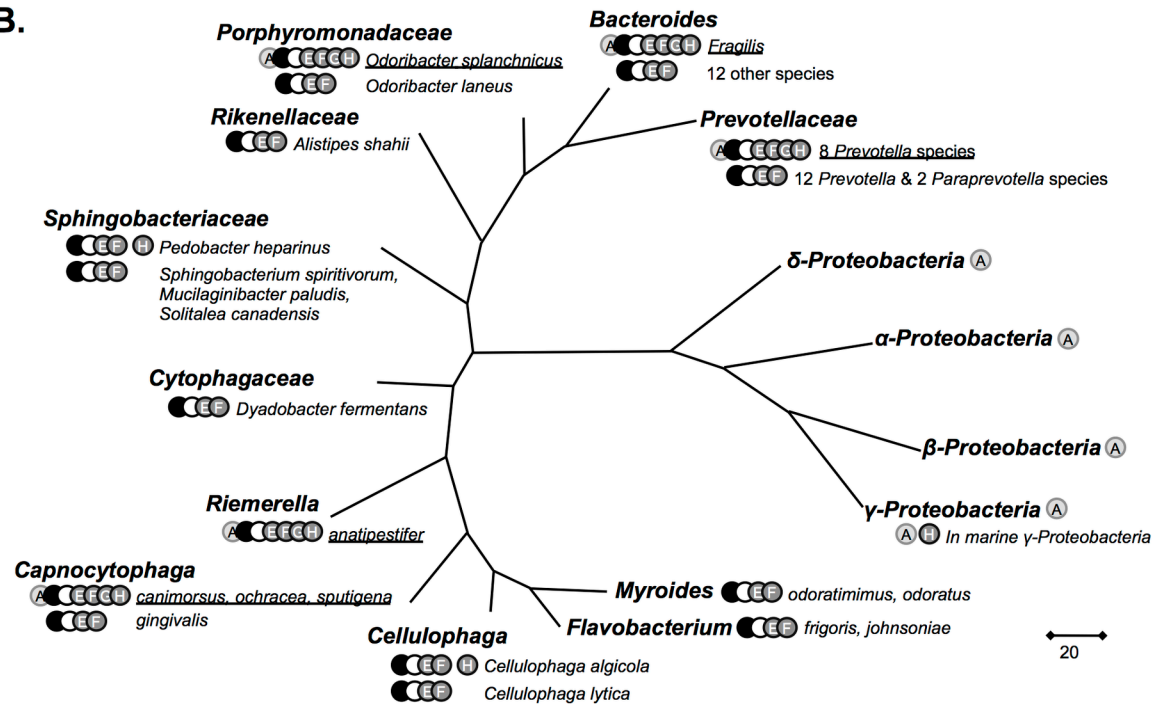


Figure S5.

The ICS is mostly found among pathogenic members from the *Bacteroidetes* phylum and has broad species specificity

(A) Orthologous *PULs* of *Cc5 PUL3* identified in the Complete Genomes database. The *PULs* display has been limited to the genes implicated in iron scavenging with a putative TonB-dependent outer membrane protein ortholog to either *icsA* or *icsC*. For the sake of readability, only the largest SusC like homologs and the last represented genes are tagged here. In the case of *R. anatipestifer* DSM 15868, an additional FecA-like protein that shares higher similarity with other orthologs than with its paralog is found at approximately 1 Mb from the SusC like protein. (B) Non-exhaustive occurrences of *PUL3* genes among other bacteria spotted on a representative 16S rRNA phylogenetic tree. The evolutionary history has been inferred using the Maximum Parsimony method and the consensual 16S rRNA sequences from the different taxa where genes from *PUL3* were found. Spots indicate major combinations of *PUL3* encoded genes found within each taxon. Letters in the round spots refer to the proteins encoded by *PUL3*, namely IcsA (A), IcsC (C), IcsD (D), IcsE (E), IcsF (F), IcsG (G) and IcsH (H). Underlined taxon names indicate the occurrence of strains exhibiting all seven *ics* genes in their genome.

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