

Trophic cooperation promotes bacterial survival of *Staphylococcus aureus* and *Pseudomonas aeruginosa*

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Materials and methods

Generation of PA2600 knock-out Δ *acoR* and Δ *aco* mutants: upstream and downstream flanking regions of *acoR* and *aco* operon (474 bp and 486 bp fragments for *acoR* ; 654 bp and 708 bp for *aco*) were PCR amplified (GoTaq polymerase, Promega) and cloned into pEXG2 by Sequence Ligation and Independent Cloning (SLIC) method (1,2). Resulting plasmids pEXG2-*acoR* and pEXG2-*aco* were then transferred into PA2600 by triparental mating. A first conjugation was performed between the two *E. coli* strains carrying either the pRK2013 helper plasmid or the constructed pEXG2 plasmid by spotting 30 μ L of pre-culture of each strain on LB plates. After two hours at 37°C, 30 μ L of PA2600 pre-culture were added on the dried spot and the plate was incubated five hours at 37°C. The spot was then re-suspended in LB medium and plated on Cetrimide plates supplemented with gentamycin. Resulting clones were then plated on LB containing 10% sucrose to select for plasmid excision by crossing-over. The resulting strains were checked for gentamicin sensitivity and gene deletion by PCR.

CF clinical strains	CF patient	Strain name	Interaction state	Experiments	Reference
<i>P. aeruginosa</i> (PA) and <i>S. aureus</i> (SA)	1	PA2596	Competition (SA2599, SA2597)	RNAseq	3
		SA2597		RNAseq	3
	2 (■)	PA2600	Coexistence (SA2599, SA2597)	RNAseq; qRT-PCR screening; <i>aco</i> induction qRT-PCR; Acetoin monitoring in co-culture; Acetoin catabolism in SA supernatant; Culture in minimal medium; 5-day co-cultures	3
		SA2599		RNA seq; qRT-PCR screening; <i>aco</i> induction qRT-PCR; Acetoin production screening; Acetoin monitoring in co-culture; Production of SA supernatant; 5 days co-cultures; Growth in presence of acetoin	3
	3	PA7A SA7	Coexistence	qRT-PCR screening qRT-PCR screening	This study This study
	4	PA13 SA13	Coexistence	qRT-PCR screening qRT-PCR screening	This study This study
	5 (●)	PA27 SA27	Coexistence	qRT-PCR screening qRT-PCR screening	3 3
	6	PA30 SA30	Coexistence	qRT-PCR screening qRT-PCR screening	3 3
	7	PA31 SA31	Coexistence	qRT-PCR screening qRT-PCR screening	3 3
	8	PA37 SA37	Coexistence	qRT-PCR screening qRT-PCR screening	This study This study
	9	PA42 SA42	Coexistence	qRT-PCR screening qRT-PCR screening	3 3
	10	PA48 SA48	Coexistence	qRT-PCR screening qRT-PCR screening	This study This study
	11	PA53 SA53	Coexistence	qRT-PCR screening qRT-PCR screening	This study This study
	12	PA54 SA54	Coexistence	qRT-PCR screening qRT-PCR screening	This study This study
	13	PA69 SA69	Coexistence	qRT-PCR screening qRT-PCR screening	3 3
	14 (▲)	PA80 SA80	Coexistence	qRT-PCR screening qRT-PCR screening	3 3
	15	PA82 SA82	Coexistence	qRT-PCR screening qRT-PCR screening	3 3
	16	PA146	Coexistence	qRT-PCR screening; Acetoin catabolism screening; Acetoin monitoring in co-culture; Acetoin catabolism in SA supernatant	3
		SA146		qRT-PCR screening; Acetoin production screening; Acetoin monitoring in co-culture; Production of SA supernatant	3
	14 (▲)	PA148B SA148	Coexistence	qRT-PCR screening; Acetoin catabolism screening qRT-PCR screening; Acetoin production screening	3 3
	17	PA152 SA152	Coexistence	qRT-PCR screening; Acetoin catabolism screening qRT-PCR screening; Acetoin production screening	3 3
	18	PA153A	Coexistence	qRT-PCR screening; Acetoin catabolism screening; Acetoin monitoring in co-culture; Acetoin catabolism in SA supernatant	3
		PA153B SA153		Transcriptomic (qRT-PCR) qRT-PCR screening; Acetoin production screening; Acetoin monitoring in co-culture; Production of SA supernatant	3 3

	19	PA154A PA154B SA154	Coexistence	qRT-PCR screening; Acetoin catabolism screening qRT-PCR screening; Acetoin catabolism screening qRT-PCR screening; Acetoin production screening	3 3 3
	20	PA156 SA156	Coexistence	qRT-PCR screening; Acetoin catabolism screening qRT-PCR screening; Acetoin production screening	3 3
	21	PA166A SA166	Coexistence	qRT-PCR screening; Acetoin catabolism screening qRT-PCR screening; Acetoin production screening	3 3
	22	PA167 SA167	Competition	Acetoin catabolism screening Acetoin production screening	3 3
	2 (■)	PA171A SA171	Coexistence	Acetoin catabolism screening Acetoin production screening	3 3
	23	PA172 SA172	Competition	Acetoin catabolism screening Acetoin production screening	This study This study
	24	PA178 SA178	Coexistence	Acetoin catabolism screening Acetoin production screening	3 3
	25	PA179 SA179	Competition	Acetoin catabolism screening Acetoin production screening	3 3
	26	PA181 SA181	Competition	Acetoin catabolism screening Acetoin production screening	3 3
	27 (♦)	PA186 SA186	Competition	Acetoin catabolism screening Acetoin production screening	3 3
	28	PA187	Competition	Acetoin catabolism screening	This study
	29	PA188A SA188	Competition	Acetoin catabolism screening Acetoin production screening	This study This study
	30	PA193A SA193	Competition	Acetoin catabolism screening Acetoin production screening	This study This study
	5 (●)	PA194A PA194B SA194	Coexistence	Acetoin catabolism screening Acetoin catabolism screening Acetoin production screening	This study This study This study
	31	PA197 SA197	Competition	Acetoin catabolism screening Acetoin production screening	This study This study
	32	SA198	Competition	Acetoin production screening	This study
	33	PA199A PA199C	Competition	Acetoin catabolism screening Acetoin catabolism screening	This study This study
	34	PA200 SA200	Competition	Acetoin catabolism screening Acetoin production screening	This study This study
	35	SA205	Competition	Acetoin production screening	This study
	36	SA207	Coexistence	Acetoin production screening	This study
	27 (♦)	SA213	Competition	Acetoin production screening	This study
<i>B. cenocepacia</i>	37	LUG2886	Coexistence (PA2600)	qRT-PCR (<i>aco</i> induction)	This study
<i>S. maltophilia</i>	38	LUG2884	Coexistence (PA2600)	qRT-PCR (<i>aco</i> induction)	This study

Table S1: Clinical CF strains used in this study. Unless indicated, interaction state was tested between strains from the same clinical pair (*ie.* isolated from a single patient). Four strain pairs were recovered from a same patient but at different time points and are annotated with identical patient number and symbol (■, ●, ▲ or ♦).

Strains / plasmids	Name	Characteristics	Interaction state	Experiments	Reference
<i>P. aeruginosa</i>	PA2600 Δ <i>acoR</i>	<i>acoR</i> deletion mutant	Coexistence (SA2599)	Culture in minimal medium; 5-day co-cultures	This study
	PA2600 Δ <i>aco</i>	<i>aco</i> operon deletion mutant	Coexistence (SA2599)	Culture in minimal medium; 5-day co-cultures	This study
<i>S. aureus</i>	SA UAMS-1	WT strain	Coexistence (PA2600)	Production of SA supernatant (<i>aco</i> induction)	4
	SA UAMS-1 Δ <i>alsSD</i>	UAMS-1489, <i>alsSD</i> deletion mutant	Coexistence (PA2600)	Production of SA supernatant (<i>aco</i> induction)	4
<i>B. subtilis</i>	LUG2953	WT strain	Coexistence (PA2600)	qRT-PCR (<i>aco</i> induction)	This study
Plasmids	pEXG2	Gm ^R ; mobilizable, non-replicative vector in <i>P. aeruginosa</i>		<i>acoR</i> and <i>aco</i> deletions	2
	pEXG2- Δ <i>acoR</i>	pEXG2 carrying upstream and downstream sequences of <i>acoR</i> for gene deletion		<i>acoR</i> deletion	This study
	pEXG2- Δ <i>aco</i>	pEXG2 carrying upstream and downstream sequences of <i>aco</i> for operon deletion		<i>aco</i> deletion	This study
	pRK2013	Km ^R , helper plasmid with conjugative properties		<i>acoR</i> and <i>aco</i> deletions	5

Table S2: Non-CF strains and plasmids used in this study. *P. aeruginosa* Δ *acoR* and Δ *aco* mutants were constructed from the clinical CF isolate PA2600 (Table S1). Interaction state was tested with the strain indicated in brackets.

Use	Name	Sequence	Target	Amplicon Size
qPCR	PArpoD-F	GCGCAACAGCAATCTCGTCT	<i>rpoD</i>	177
	PArpoD-R	ATCCGGGGCTGTCTCGAATA		
	PAgyrB-F	ATCTCGGTGAAGGTACCGGA	<i>gyrB</i>	160
	PAgyrB-R	TGCCTTCGTTGGGATTCTCC		
	OLC8-F	GCGAGGATCTCTACTTCCGC	<i>acoR</i>	140
	OLC8-R	CTCACCGAGTTCGATGCGTA		
	OLC9-F	GCGGATCGTCAACCTGTCAT	<i>PA4148</i>	86
	OLC9-R	CGATCACGGCAAACCTTCGAG		
	OLC14-F	GTTCTTCAGGCTCCAGTCGG	<i>pvdS</i>	81
	OLC14-R	TTGCGGACGATCTGGAACAG		
	OLC16-F	CGCGACAAGAGCGAATACCT	<i>lasA</i>	71
	OLC16-R	AGGGTCAGCAACACTTTCGG		
	OLC17-F	CTGGACTGAACCAGGCGATG	<i>rhlA</i>	113
	OLC17-R	CAGGTATTTGCCGACGGTCT		
	OLC22-F	CGACGGTATCCAGGTGATG	<i>PA1874</i>	70
	OLC22-R	GAACTTGACCGTGACCACCT		
	OLC23-F	CGGTGTTGCTCGGATACCTC	<i>rcpC</i>	120
	OLC23-R	GCTTGCGTTCGAGCTTTTCC		
	OLC25-F	ATCGCCTGCTTCCAGTTGTC	<i>pchD</i>	166
	OLC25-R	AGAGAGTGAAGTTGTGCGCC		
	OLC29-F	GAGCGACGAACTGACCTACC	<i>rhlB</i>	200
	OLC29-R	TACTTCTCGTGAGCGATGCG		
	OLC31-F	TGCTCACTTCGCTATGGACC	<i>acoB</i>	117
	OLC31-R	AGAAGATCACCGGGTCGTTG		
	OLC33-F	CCGACGTGATCGCCTTCATA	<i>PA4153</i>	83
	OLC33-R	GACGATTTCTTCCAGGCCGA		
	OLC35-F	GCCCATCGAGTTCGGTATGT	<i>bauD</i>	151
	OLC35-R	GAGGCAGACGGTGAAGATCG		
	OLC39-F	GACGAAGACGGCATGAACCT	<i>tadA</i>	119
	OLC39-R	TCCAGTTCGTAGCGGGAGAT		
	OLC41-F	CCACCACGAACTGCAACTG	<i>tadG</i>	107
	OLC41-R	GCTTCTCCAACCGAAGTCCA		
	OLC54-F	TTGCCGTATTGAGTCCCACG	<i>flp</i>	77
	OLC54-R	GACTTTTTCGCCGACTCCGT		
	OLC56-F	GACTGACGCTCAGGCAAT	<i>rhlC</i>	74
	OLC56-R	CCGGAGGAGATCAGGAACGA		
	OLC71-F	CAGCCGGACGAAGGTATCTC	<i>zwf</i>	113
	OLC71-R	GCGTGGTAGGTCTCGGAAAA		
	OLC72-F	GCTATACCCGCTCAAGGGC	<i>dguA</i>	92
	OLC72-R	TCTTGCGGTCGTAATCGGTG		
	OLC73-F	CTTCGCCTACCTGTTTCAGCC	<i>PA5099</i>	163
	OLC73-R	CGGCAGGTAGCGTGAATAGT		
OLC74-F	GCAGTTCGGAGCACATCAAC	<i>PA1874</i>	130	
OLC74-R	TCGATGCTGACGAAATCGGT			
OLC75-F	TGGTATCCGGCGAACACATC	<i>liuA</i>	127	
OLC75-R	CACATCTTGCTGCCGTTGAG			
OLC92-F	CAGCCAGGACTACGAGAACG	<i>lasR</i>	153	

	OLC92-R	TGGTAGATGGACGGTTCCCA		
	OLC93-F	GGCATTCCCCTCACCGAC	<i>gntK</i>	181
	OLC93-R	GGGTCAGTTCCAGGTAGACGA		
	OLC94-F	TGGAAGTGGCTGCTCAATCC	<i>gltF</i>	81
	OLC94-R	CCAGTCGAAGCGGAAACCTT		
	OLC95-F	CACCTGCACCTTCTATGGCA	<i>edd</i>	96
	OLC95-R	GTGTTCTGGGTTGACGAAGGA		
	OLC96-F	CAACGGCACCTCGATCTTCA	<i>mmsA</i>	94
	OLC96-R	AATCGGGATGTTGATGCCCA		
Cloning	OLC58-F	GGTCGACTCTAGAGGATCCCC AGGGCGATGCCCCGGCCGATG	<i>acoR</i> downstream	474
	OLC58-R	CACATGGTCCTTCGAGTGTGC		
	OLC59-F	GCACACTCGAAGGACCATGTG CGCCGGCATGGCATCCGCATG	<i>acoR</i> upstream	486
	OLC59-R	ACCGAATTCGAGCTCGAGCCC CTCGATCGCGCGGACGAACCA		
	OLC64-F	GGTCGACTCTAGAGGATCCCC TAGTCGGCATCGCGCACCCGT	<i>aco</i> operon downstream	654
	OLC64-R	GGAGAAATCGTCGGACGACGT		
	OLC65-F	ACGTCGTCCGACGATTTCTCCA GTTGGTGAACAACAAGGAGC	<i>aco</i> operon upstream	708
	OLC65-R	ACCGAATTCGAGCTCGAGCCC ACAGCCTGACCACTTTCGTGC		

Table S3: Primers used in this study.

Gene (clinical strains)	Gene (PAO1)	UniProt	Product	Function	SA2599/PA2596		SA2597/PA2596		LasR regulation	Ref.	
					Log ₂ fold- change	Adjusted p- value	Log ₂ fold- change	Adjusted p- value			
fecI_2	NA	NA	P23484	Putative RNA polymerase sigma factor FecI	Iron metabolism	-2.67	4.02E-06	-2.51	1.85E-04		6
group_1318	NA	NA	P40883	Regulatory protein PchR	Iron metabolism	-3.57	3.88E-23	-3.13	6.04E-25		6
group_1539	NA	NA	NA	Hypothetical protein	Unknown	-2.96	3.51E-11	-3.58	3.20E-13		
bauA_1	PA0132	bauA	Q91700	Beta-alanine-pyruvate aminotransferase	Aminoacids (ILV) catabolism, propanoate metabolism	3.15	1.00E-06	3.44	6.25E-39	Activation	7
nirS_1	PA0509	nirN	a Q91609	Nitrite reductase	Nitrogen metabolism	-2.92	6.77E-10	-2.93	3.30E-09	Repression	8,9
cysG_2	PA0510	nirE	a G3XD80	Siroheme synthase NirE	Nitrogen, porphyrin and chlorophyll metabolism	-3.00	8.64E-09	-2.56	5.61E-06	Repression	8,9
group_5012	PA0512	nirH	a P95415	NirH	Nitrogen metabolism	-3.27	8.64E-09	-2.23	4.94E-04	Repression	8,9
group_3026	PA0513	nirG	a P95414	NirG	Nitrogen metabolism	-2.79	5.29E-06	-2.35	9.60E-04		8
group_4848	PA0514	nirL	a P95413	Heme d1 biosynthesis protein NirL	Nitrogen metabolism	-3.09	1.71E-08	-3.47	2.61E-08		8
group_3339	PA0515	nirD	a P95412	Probable transcriptional regulator	Nitrogen metabolism	-2.99	2.04E-07	-2.03	1.30E-03		8
group_5595	PA0516	nirF	a Q51480	Heme d1 biosynthesis protein NirF	Nitrogen metabolism	-3.34	1.03E-10	-3.47	6.65E-16		8
nirS_2	PA0519	nirS	a P24474	Nitrite reductase	Nitrogen metabolism	-3.29	3.53E-07	-5.39	6.48E-51		8
nirQ	PA0520	nirQ	b Q51481	Denitrification regulatory protein NirQ	Nitrogen metabolism	-4.27	3.41E-28	-4.51	3.08E-33		8
qoxC	PA0521	nirO	b G3XD44	Quinol oxidase subunit 3	Unknown	-4.65	2.60E-22	-4.60	4.38E-15		
group_6044	PA0522	nirP	b Q51483	Hypothetical protein	Unknown	-2.90	1.59E-05	-2.99	7.07E-05		
norB	PA0524	norB	Q59647	Nitric oxide reductase subunit B	Nitrogen metabolism	-5.53	1.99E-23	-8.08	1.91E-79		8
group_3360	PA0672	hemO	G3XCZ8	Heme oxygenase	Porphyrin and chlorophyll metabolism	-2.38	9.28E-05	-2.53	1.57E-17		
ppsC	PA1137	PA1137	Q914J8	Phthiocerol/phenolphthiocerol synthesis polyketide synthase	Unknown	-4.31	1.74E-21	-3.55	1.62E-19		
rocR	PA1196	ddaR	Q914E2	Arginine utilization regulatory protein RocR	Aminoacids (arginine) metabolism	2.51	3.23E-05	3.22	5.81E-21		10
fecI_5	PA1300	PA1300	c Q91444	Putative RNA polymerase sigma factor FecI	Iron metabolism	-3.17	3.51E-11	-2.50	2.25E-11		6
fecR_1	PA1301	PA1301	c Q91443	Protein FecR	Iron metabolism	-3.02	1.71E-08	-2.53	1.01E-13		6
ctpF	PA1429	PA1429	Q913R5	Putative cation-transporting ATPase F	Unknown	2.89	8.45E-09	3.63	7.15E-35		
group_3703	PA1673	PA1673	Q91352	Bacteriohemerythrin	Unknown	2.16	1.57E-04	2.57	1.30E-14		
group_5526	PA1746	PA1746	Q912Z1	Hypothetical protein	Unknown	2.42	1.59E-06	2.42	1.38E-09		
group_2540	PA1747	PA1747	Q912Z0	Hypothetical protein	Unknown	2.57	8.64E-09	2.46	1.07E-08		
group_269	PA2033	PA2033	Q91282	Hypothetical protein	Unknown	-2.24	1.65E-03	-2.84	7.11E-23		
ybdM	PA2126	cgrC	Q911Y9	CupA gene regulator C, CgrC	Unknown	2.60	3.18E-07	2.46	1.05E-06		
group_6509	PA2321	gntK	G3XD53	Gluconokinase	Carbon metabolism (pentose phosphate)	-2.73	4.03E-06	-2.41	2.68E-05	Repression	9
group_3203	PA2384	PA2384	Q91195	Ferric uptake regulation protein	Iron metabolism	-2.44	1.09E-04	-2.56	8.25E-10		6
mbtH	PA2412	PA2412	Q91169	Hypothetical protein	Monobactam biosynthesis	-2.12	1.66E-03	-2.27	6.19E-08		
group_3129	PA2468	foxI	Q91114	Putative RNA polymerase sigma factor FecI	Iron metabolism	-2.32	1.63E-07	-2.03	1.07E-08		6
hmp	PA2664	fhp	Q910H4	Flavohepomeprotein	Iron metabolism, NO detoxification	-2.41	1.85E-05	-2.38	1.86E-03		11
group_3982	PA2691	PA2691	Q910F1	NADH dehydrogenase-like protein	Oxidative phosphorylation	-4.38	1.03E-18	-3.45	3.32E-07		
hldD	PA3337	rfaD	Q9HYQ8	ADP-L-glycero-D-mannoheptose-6-epimerase	Lipopolysaccharide biosynthesis	3.40	1.45E-12	4.26	1.62E-48		
yccM	PA3391	nosR	d Q9HYL3	Regulatory protein NosR	Nitrogen metabolism	-3.57	7.09E-14	-4.08	1.98E-30	Repression	8,9
nosZ	PA3392	nosZ	d Q9HYL2	Nitrous-oxide reductase	Nitrogen metabolism	-3.62	1.57E-10	-5.50	6.62E-51	Repression	8,9
nosD	PA3393	nosD	d Q9HYL1	Putative ABC transporter binding protein NosD	Nitrogen metabolism	-3.13	1.38E-10	-3.97	3.44E-20	Repression	8,9
nosY	PA3395	nosY	d Q9HYK9	Putative ABC transporter permease protein NosY	ABC transporters (nitrogen metabolism)	-3.14	1.69E-07	-2.91	1.48E-06	Repression	8,9

nosL	PA3396	<i>nosL</i>	d	Q9HYK8	Copper-binding lipoprotein NosL	Nitrogen metabolism	-2.60	3.86E-05	-2.46	5.00E-05	Repression	8,9
group_3537	PA3411	PA3411		Q9HYJ4	Hypothetical protein	Unknown	-2.59	3.63E-06	-2.05	1.41E-03		
bfd	PA3530	<i>bfd</i>		Q9HY80	Bacterioferritin-associated ferredoxin	Unknown	-2.24	3.28E-10	-2.04	1.81E-10		
ykgO	PA3600	<i>rpl36</i>	e	Q9HY26	50S ribosomal protein L36 2	Ribosome structure	-2.99	6.77E-10	-2.87	3.60E-08		
rpmE2	PA3601	<i>ykgM</i>	e	Q9HY25	50S ribosomal protein L31 type B	Ribosome structure	-2.92	2.37E-10	-3.21	2.69E-26		
fdx_1	PA3809	<i>fdx2</i>	f	Q51383	2Fe-2S ferredoxin	Iron-sulfur protein	-2.72	4.17E-12	-3.02	7.98E-16		
hscA	PA3810	<i>hscA</i>	f	Q51382	Chaperone protein HscA	Protein stabilization	-2.56	4.41E-11	-2.60	6.55E-16		12
hscB	PA3811	<i>hscB</i>	f	Q9HXJ1	Co-chaperone protein HscB	Protein stabilization	-2.04	1.40E-06	-2.28	1.35E-14		12
iscA	PA3812	<i>iscA</i>	f	Q9HXJ0	Iron-binding protein IscA	[Fe-S] cluster biogenesis	-2.51	2.80E-10	-3.37	7.39E-26		13
iscU	PA3813	<i>iscU</i>	f	Q9HXI9	Iron-sulfur cluster assembly scaffold protein IscU	[Fe-S] cluster biogenesis	-2.40	3.46E-06	-3.34	1.95E-27		13
iscS_1	PA3814	<i>iscS</i>	f	Q9HXI8	Cysteine desulfurase IscS	Sulfur relay system, thiamine metabolism, [Fe-S] cluster biogenesis	-2.24	5.79E-05	-3.44	3.85E-29		13
iscR	PA3815	<i>iscR</i>	f	Q9HXI7	HTH-type transcriptional regulator IscR	[Fe-S] cluster biogenesis regulation	-2.42	4.44E-06	-3.65	4.38E-31		13
copA_4	PA3920	<i>yvgX</i>		Q9HX93	Copper-exporting P-type ATPase	Unknown	-2.28	3.75E-07	-2.63	4.15E-14		
ntaA	PA4155	PA4155		Q9HWM6	Nitritotriacetate monooxygenase component A	Unknown	-3.09	5.86E-10	-2.23	6.24E-06		
fyuA_1	PA4156	<i>fvbA</i>		Q9HWM5	Pesticin receptor	Iron metabolism	-2.70	8.32E-12	-2.48	2.30E-09		14
fepC_1	PA4158	<i>fepC</i>		Q9HWM3	Ferric enterobactin transport ATP-binding protein FepC	ABC transporters (iron complex)	-2.30	6.59E-05	-2.21	3.50E-04		
fepB	PA4159	<i>fepB</i>		Q9HWM2	Ferrienterobactin-binding periplasmic protein	ABC transporters (iron complex)	-3.21	1.20E-10	-2.48	7.35E-06		
zupT	PA4467	PA4467	g	Q9HVV1	Zinc transporter ZupT	Unknown	-3.12	1.92E-08	-2.97	1.40E-15		
sodB_2	PA4468	<i>sodM</i>	g	P53652	Superoxide dismutase [Mn/Fe]	Oxydative and iron stress response	-3.17	1.19E-09	-3.09	4.37E-19		15
group_2514	PA4469	PA4469	g	Q9HVV0	Hypothetical protein	Unknown	-3.32	8.79E-09	-3.28	2.91E-23		
fumC_1	PA4470	<i>fumC1</i>	g	Q51404	Fumarate hydratase class II	Carbon metabolism (cytrate circle), iron stress response	-3.14	6.08E-08	-3.16	2.75E-18		15
group_4225	PA4471	<i>fagA</i>	g	G3XD99	Hypothetical protein	Unknown	-3.52	3.14E-17	-3.23	1.64E-25		
group_3398	PA4570	PA4570		Q9HVL4	Hypothetical protein	Unknown	-4.12	3.14E-17	-4.32	4.87E-31		
ccpA	PA4587	<i>ccpR</i>		P14532	Cytochrome c551 peroxidase	Oxydative stress response	3.16	3.49E-11	3.27	6.90E-16	Repression	7,16
group_1579	PA4625	<i>cdrA</i>		Q9HVG6	Cyclic diguanylate-regulated TPS partner A, CdrA	Adhesion and biofilm matrix structure	2.53	8.83E-05	2.06	6.78E-10		17
fecI_3	PA4896	PA4896		Q9HUR7	Putative RNA polymerase sigma factor FecI	Iron metabolism	-3.04	6.78E-10	-2.23	1.49E-08		6
group_4196	PA5027	PA5027		Q9HUE2	Hypothetical protein	Unknown	2.35	7.70E-05	2.82	2.70E-20	Activation	9
arcD	PA5170	<i>arcD</i>		P18275	Arginine/ornithine antiporter	Aminoacids (arginine) metabolism	2.36	4.52E-04	2.49	5.13E-04		10
NA	PA5369.3	PA5369.3	NA		tRNA-Ala	Aminoacyl-tRNA biosynthesis	2.12	7.09E-03	4.50	3.69E-21		
pka	PA5475	PA5475		Q9HT95	Protein lysine acetyltransferase Pka	Unknown	2.04	4.85E-04	2.30	1.81E-10		

Table S4: List of *P. aeruginosa* genes differentially expressed in presence of *S. aureus* in the context of a competitive interaction. PA2596 competition strain was cultivated in absence or presence of SA2599 or SA2597. RNAs were extracted after 4 hours of culture and RNAseq analysis was performed as described in material and methods. A gene was considered as differentially expressed when the Fold Change (FC) was $> |2\log_2|$ with an adjusted P -value < 0.05 in presence of both SA strains. Genes from the same operon are annotated with an identical letter. Grey cells indicate genes that were also dysregulated in the context of coexistence (Table S5). Functional classification was performed thanks to KEGG database and literature.

Gene (clinical strains)	Gene (PAO1)		UniProt	Product	Function	SA2599/PA2600		SA2597/PA2600		LasR regulation	Ref.
						Log ₂ fold-change	Adjusted p-value	Log ₂ fold-change	Adjusted p-value		
group_955	NA	NA	NA	Hypothetical protein	Unknown	-2,20	3,84E-09	-2,56	1,54E-05		
eamB_1	NA	NA	P38101	Cysteine/O-acetylserine efflux protein	Aminoacids transport	2,04	5,37E-04	2,31	2,69E-03		
group_511	NA	NA	NA	Hypothetical protein	Unknown	-2,04	2,34E-03	-2,77	5,28E-03		
▶ bauD	PA0129	bauD	Q9I703	Putative GABA permease	Aminoacids (β-alanine) catabolism	2,90	1,53E-15	3,07	2,83E-14		18
bauB	PA0131	bauB	Q9I701	Beta-alanine degradation protein BauB	Aminoacids (β-alanine) catabolism	2,27	2,30E-05	2,61	2,31E-04		18
cysG_2	PA0510	nirE	a G3XD80	Siroheme synthase NirE	Nitrogen, porphyrin and chlorophyll metabolism	-2,42	6,73E-05	-2,72	1,36E-03	Repression	8,9
group_3026	PA0513	nirG	a P95414	NirG	Nitrogen metabolism	-2,69	3,29E-05	-2,97	2,87E-03		8
group_3339	PA0515	nirD	a P95412	Probable transcriptional regulator	Nitrogen metabolism	-2,62	1,99E-03	-3,37	3,73E-04		8
group_5595	PA0516	nirF	a Q51480	Heme d1 biosynthesis protein NirF	Nitrogen metabolism	-3,53	1,60E-08	-3,88	1,44E-06		8
nirM	PA0518	nirM	a P00099	Cytochrome c-551	Nitrogen metabolism	-2,62	4,27E-03	-3,27	3,88E-03		8
nirS_2	PA0519	nirS	a P24474	Nitrite reductase	Nitrogen metabolism	-3,27	7,25E-06	-4,43	2,95E-08		8
nirQ	PA0520	nirQ	Q51481	Denitrification regulatory protein NirQ	Nitrogen metabolism	-3,25	7,06E-05	-4,12	2,10E-06		8
norC	PA0523	norC	h Q59646	Nitric oxide reductase subunit C	Nitrogen metabolism	-3,59	2,99E-05	-6,23	4,50E-14		8
norB	PA0524	norB	h Q59647	Nitric oxide reductase subunit B	Nitrogen metabolism	-5,82	3,20E-18	-5,77	2,52E-12		8
group_4191	PA0525	norD	h Q51484	Probable dinitrification protein NorD	Nitrogen metabolism	-3,86	1,68E-06	-4,82	1,23E-08		8
group_364	PA0526	PA0526	G3XDA0	Hypothetical protein	Unknown	-3,38	6,53E-05	-4,60	3,45E-06		
NA	PA0668.3	NA	NA	tRNA-Ala(tgc)	Aminoacyl-tRNA biosynthesis	3,45	5,66E-05	7,57	1,86E-22		
acrC_2	PA0746	PA0746	i Q9I5I3	Acryloyl-CoA reductase (NADH)	Unknown	2,80	1,01E-12	3,12	2,02E-15		
mmsA_2	PA0747	PA0747	i Q9I5I2	Methylmalonate-semialdehyde dehydrogenase	Aminoacids (isoleucine, leucine, valine) catabolism, propanoate and carbon metabolism	2,08	1,04E-05	2,31	1,44E-06		
acnM	PA0794	PA0794	Q9I5E4	Aconitate hydratase A	Propanoate metabolism	2,18	5,28E-10	2,90	1,80E-07		
aroP_2	PA0866	aroP2	Q9I575	Aromatic amino acid transport protein AroP	Unknown	2,30	8,73E-06	2,74	1,02E-03		
acs_1	PA0887	acsA	Q9I558	Acetyl-coenzyme A synthetase	Propanoate, carbon and pyruvate metabolism	3,08	5,58E-10	3,27	2,24E-07	Repression	7
yybH	PA1325	yybH	j Q9I419	Putative protein YybH	Unknown	2,16	1,03E-03	2,73	1,94E-05		
ilvA_1	PA1326	ilvA	j Q9I418	L-threonine dehydratase biosynthetic IlvA	Carbon metabolism, aminoacids (isoleucine, leucine, valine) biosynthesis	2,19	4,61E-05	2,46	6,93E-04		
▶ group_1804	PA1874	PA1874	k Q9I2M3	Hypothetical protein	Antibiotic resistance, quorum sensing	-2,54	5,92E-05	-2,68	2,83E-04	Activation	9,19
▶ group_4895	PA1875	PA1875	k Q9I2M2	Hypothetical protein	Antibiotic resistance	-2,94	1,72E-09	-2,80	1,14E-05	Activation	9,19
prsE_3	PA1877	PA1877	k Q9I2M0	Type I secretion system membrane fusion protein PrsE	Antibiotic resistance	-2,29	8,13E-03	-3,04	1,51E-07		19
group_6609	PA1878	PA1878	Q9I2L9	Hypothetical protein	Unknown	-2,03	2,47E-05	-2,38	1,01E-03		
group_2034	PA1914	hvn	Q9I2J0	Hypothetical protein	Unknown	-3,87	9,10E-16	-4,61	3,05E-20	Activation	7
degU_4	PA1978	erbR	P29369	Transcriptional regulatory protein DegU	Ethanol stress response	2,28	2,43E-05	3,06	1,45E-07	Repression	9,20
luxQ	PA1992	ercS	Q9I2B7	Autoinducer 2 sensor kinase/phosphatase LuxQ	Ethanol stress response	2,13	9,50E-08	2,11	2,65E-04		20
accA1_2	PA2012	liuD	l Q9I299	Acetyl-/propionyl-coenzyme A carboxylase alpha chain	Aminoacids (leucine) and monoterpenes catabolism	2,30	7,81E-07	2,69	2,15E-06		21
menB	PA2013	liuC	l Q9I298	1%2C4-dihydroxy-2-naphthoyl-CoA synthase	Aminoacids (leucine) and monoterpenes catabolism	3,02	1,20E-11	3,29	7,09E-12		21
group_2911	PA2014	liuB	l Q9I297	Methylmalonyl-CoA carboxyltransferase 12S subunit	Aminoacids (leucine) and monoterpenes catabolism	2,61	1,20E-08	2,85	4,87E-10	Activation	9,21
▶ mmgC_7	PA2015	liuA	l Q9I296	Acyl-CoA dehydrogenase	Aminoacids (leucine) and monoterpenes catabolism	2,62	3,07E-08	2,76	5,26E-09		21
cueR_2	PA2016	liuR	Q9I295	HTH-type transcriptional regulator CueR	Aminoacids (leucine) and monoterpenes catabolism	2,61	4,02E-08	3,17	4,01E-06		21

group_13	PA2040	pauA4	Q9I275	Gamma-glutamylputrescine synthetase PuaA	Polyamines catabolism, aminoacids (glutamine) biosynthesis	2,36	2,02E-08	2,77	8,62E-09		22
kynU	PA2080	kynU	Q9I235	Kynureninase KynU	Aminoacids (tryptophan) catabolism	2,63	9,29E-10	2,67	7,28E-07	Activation	7
group_1851	PA2166	PA2166	Q9I1U9	Hypothetical protein	Unknown	-2,23	5,77E-05	-2,82	1,56E-04	Activation	7
gntR_3	PA2320	gntR	Q9I1F6	HTH-type transcriptional regulator GntR	Carbon metabolism (pentose phosphate)	-2,63	9,55E-11	-2,71	1,51E-05		
▶ group_6509	PA2321	gntK	G3XD53	Gluconokinase GntK	Carbon metabolism (pentose phosphate)	-3,67	1,18E-14	-3,42	5,37E-06	Repression	9,23
group_1686	PA2462	PA2462	Q9I120	Hypothetical protein	Unknown	2,36	1,33E-10	2,34	7,08E-11		
tsdA	PA2481	PA2481	m Q9I101	Thiosulfate dehydrogenase	Unknown	2,68	1,47E-07	2,09	1,59E-04		
group_3818	PA2482	PA2482	m Q9I100	Cytochrome c4	Unknown	2,73	8,24E-08	2,39	6,15E-04		
mmgC_5	PA2552	acdB	n Q9I0T2	Acyl-CoA dehydrogenase	Unknown	2,49	6,18E-12	2,82	2,35E-10	Activation	9
thiA_1	PA2553	PA2553	n Q9I0T1	Acetyl-CoA acetyltransferase	Carbon and fatty acids metabolism	2,46	7,14E-11	2,72	1,52E-08	Activation	9
group_5789	PA2554	PA2554	n Q9I0T0	Putative oxidoreductase	Unknown	2,78	1,75E-13	3,05	1,43E-09	Activation	9
acsA_1	PA2555	PA2555	n Q9I0S9	Acetyl-coenzyme A synthetase	Propanoate, carbon and pyruvate metabolism	2,83	1,51E-10	2,93	8,32E-07	Activation	9
fadD3	PA2557	PA2557	Q9I0S7	3-[(3aS%2C4S%2C7aS)-7a-methyl-1%2C5-dioxo-octahydro-1H-inden	Unknown	3,04	2,30E-14	3,16	2,62E-11		
lecA	PA2570	lecA	Q05097	PA-I galactophilic lectin LecA	Adhesion, biofilm formation	-3,40	1,59E-12	-2,61	1,13E-03	Activation	7,9,24
group_5847	PA2662	PA2662	o Q9I0H6	Hypothetical protein	Unknown	-3,18	1,66E-07	-3,57	3,71E-05		
group_3164	PA2663	ppyR	o Q9I0H5	Psl and pyoverdine operon regulator, PpyR	Iron metabolism, biofilm formation and virulence	-2,83	7,91E-04	-4,04	9,15E-07		25
hmp	PA2664	fhp	Q9I0H4	Flavoheмоprotein	Iron metabolism, NO detoxification	-5,40	1,82E-18	-5,66	3,69E-14		11
lip_2	PA2862	lipA	P26876	Triacylglycerol lipase	Glycerolipid metabolism, virulence (lipase activity)	2,31	6,63E-05	2,47	4,68E-04		
group_2901	PA3038	opdQ	Q9HZH0	Porin-like protein NicP	Membrane transports (in response to stress)	2,75	8,42E-07	2,98	2,71E-06	Repression	7,26
ydfJ	PA3079	PA3079	p Q9HZC9	Membrane protein YdfJ	Unknown	2,43	2,32E-07	2,29	2,55E-06		
group_6150	PA3080	PA3080	p Q9HZC8	Ycf48-like protein	Unknown	2,33	5,77E-09	2,24	1,34E-04		
eda_2	PA3181	edaA	q O68283	2-dehydro-3-deoxy-phosphogluconate aldolase	Carbon metabolism (pentose phosphate)	-2,85	2,30E-14	-3,17	6,61E-11	Activation	7
pgl_1	PA3182	pgl	q Q9X2N2	6-phosphogluconolactonase	Carbon metabolism (pentose phosphate)	-3,10	1,45E-13	-3,13	1,21E-08	Activation	7
▶ zwf_2	PA3183	zwf	q O68282	Glucose-6-phosphate 1-dehydrogenase	Carbon metabolism (pentose phosphate)	-2,77	4,39E-08	-3,05	4,13E-11	Activation	7
ugpC	PA3187	gltK	r Q9HZ51	Sn-glycerol-3-phosphate import ATP-binding protein UgpC	ABC transporter (oligosaccharides, polyol, lipids and monosaccharides)	-3,88	1,55E-18	-3,03	4,95E-08		
▶ ugpa	PA3189	gltF	r Q9HZ49	Sn-glycerol-3-phosphate transport system permease	ABC transporter (glucose/mannose)	-2,66	7,46E-08	-2,25	4,99E-03	Activation	7
group_5842	PA3190	gltB	Q9HZ48	Putative sugar-binding periplasmic protein	Carbon metabolism (pentose phosphate)	-5,31	1,43E-59	-4,96	2,08E-23	Activation	7
▶ edd	PA3194	edd	P31961	Phosphogluconate dehydratase	Carbon metabolism (pentose phosphate)	-2,16	1,26E-04	-2,55	2,31E-10	Activation	7
epd_1	PA3195	gapA	P27726	D-erythrose-4-phosphate dehydrogenase	Carbon metabolism (pentose phosphate)	-2,27	1,15E-05	-2,42	1,87E-07	Activation	7
group_5870	PA3233	PA3233	Q9HZ07	Hypothetical protein	Unknown	2,04	1,84E-08	2,35	1,90E-05	Repression	7
actP_1	PA3234	yjcG	s Q9HZ06	Cation/acetate symporter ActP	Unknown	2,86	3,07E-07	2,89	5,65E-05	Repression	7
yjcH_1	PA3235	yjcH	s Q9HZ05	Inner membrane protein YjcH	Unknown	2,09	4,22E-04	2,31	6,97E-05	Repression	7
nosL	PA3396	nosL	Q9HYK8	Copper-binding lipoprotein NosL	Nitrogen metabolism	-2,63	2,89E-04	-3,00	7,77E-03	Repression	9
mmsB	PA3569	mmsB	t P28811	3-hydroxyisobutyrate dehydrogenase	Aminoacids (ILV) catabolism	2,30	2,60E-10	3,27	1,43E-09		
▶ mmsA_1	PA3570	mmsA	t P28810	Methylmalonate-semialdehyde dehydrogenase	Aminoacids (ILV) catabolism, propanoate and carbon metabolism	2,32	8,63E-09	3,32	3,75E-08		
iscR	PA3815	iscR	Q9HXI7	HTH-type transcriptional regulator IscR	isc operon regulation	-2,41	2,64E-06	-2,10	3,56E-03		13
dauA	PA3863	dauA	Q9HXE3	FAD-dependent catabolic D-arginine dehydrogenase DauA	Aminoacids (arginine, ornithine) catabolism	2,12	6,53E-05	2,10	3,23E-03		
argT_1	PA3865	PA3865	Q9HXE1	Lysine/arginine/ornithine-binding periplasmic protein	ABC transporter (arginine, ornithine)	2,47	2,45E-05	2,54	2,08E-04		

group_3022	PA3922	PA3922	u	Q9HX91	Hypothetical protein	Unknown	2,18	3,16E-07	2,13	8,05E-11		
group_4203	PA3923	PA3923	u	Q9HX90	Hypothetical protein	Unknown	2,39	2,29E-07	2,38	6,61E-11	Activation	9
group_2095	PA4022	hdhA		Q9HX05	Hypothetical protein	Pyruvate and carbon metabolism, hydrazine utilization	2,35	8,00E-12	2,50	4,71E-07		27
yhdG_3	PA4023	eutP		Q9HX04	Putative amino acid permease YhdG	Unknown	2,79	7,48E-07	3,33	4,81E-08		
▶ acoR_1	PA4147	acoR		Q9HWN4	Acetoin catabolism regulatory protein	Unknown	2,02	1,07E-05	2,26	1,51E-05		
▶ fabG_10	PA4148	PA4148	v	Q9HWN3	3-oxoacyl-[acyl-carrier-protein] reductase FabG	Butanoate metabolism	2,52	7,65E-03	4,20	2,95E-08		
acoA	PA4150	acoA	v	Q9HWN1	Acetoin:2%2C6-dichlorophenolindophenol oxidoreductase subunit a	Unknown	2,42	6,84E-03	3,89	5,15E-16		
▶ acoB	PA4151	acoB	v	Q9HWN0	Acetoin:2%2C6-dichlorophenolindophenol oxidoreductase subunit b	Unknown	2,95	8,68E-07	3,51	3,12E-15		
acoC	PA4152	acoC	v	Q9HWM9	Dihydrolypoyllysine-residue acetyltransferase component of acetoin cleaving system	Carbon and pyruvate metabolism	3,00	4,18E-04	4,89	3,22E-19		
▶ ydjJ_2	PA4153	PA4153	v	Q9HWM8	2,3-butanediol dehydrogenase	Butanoate metabolism	2,33	9,28E-03	3,94	1,07E-13		
pchA_2	PA4231	pchA		Q51508	Salicylate biosynthesis isochorismate synthase	Ubiquinone and non-ribosomal siderophore peptides synthesis	-2,72	3,55E-05	-2,18	5,58E-03		
cckA	PA4293	pprA	w	Q9HWA7	Sensor kinase CckA	Membrane permeability	-2,54	2,98E-06	-3,28	1,55E-08	Activation	9,28
group_719	PA4294	PA4294	w	Q9HWA6	Hypothetical protein	Unknown	-3,35	9,82E-14	-5,10	7,29E-14	Activation	9
group_2484	PA4300	tadC	x	Q9HWA0	TadC	Flp pilus assembly	-2,45	8,38E-06	-2,73	4,22E-05	Activation	7,9
group_6095	PA4301	tadB	x	Q9HW99	TadB	Flp pilus assembly	-2,47	1,84E-06	-2,73	1,06E-04		31
▶ group_4498	PA4302	tadA	x	Q9HW98	TadA ATPase	Flp pilus assembly	-2,07	3,10E-05	-2,99	4,73E-06	Activation	7,9,29
outD	PA4304	rcpA	x	Q9HW96	RcpA	Flp pilus assembly	-2,48	2,53E-07	-2,92	1,79E-08	Activation	7,9,29
▶ group_95	PA4306	flp		Q9HW94	Type IVb pilin, Flp	Flp pilus assembly	-2,94	1,73E-05	-2,67	1,65E-03	Activation	7,9,29
group_1582	PA4638	PA4638		Q9HVF3	Hypothetical protein	Unknown	-2,33	3,36E-08	-2,42	1,55E-04		
yabJ_1	PA5083	dguB	y	Q9HUA0	2-iminobutanoate/2-iminopropanoate deaminase	Aminoacids (glutamine) metabolism	2,70	1,04E-04	3,77	1,29E-09		30
▶ dadA1_1	PA5084	dguA	y	Q9HU99	D-amino acid dehydrogenase 1	Aminoacids (glutamine, phenylalanine) metabolism	2,70	7,78E-05	4,01	2,78E-08		30
group_5220	PA5096	PA5096		Q9HU87	Glycine betaine-binding periplasmic protein OusX	ABC transporter (glycine, betaine, proline)	2,51	2,24E-10	2,54	7,31E-06		
proY_1	PA5097	hutT	z	Q9HU86	Proline-specific permease ProY	Aminoacids (histidine) catabolism	2,46	1,71E-09	2,37	3,88E-07		31
hutH_1	PA5098	hutH	z	Q9HU85	Histidine ammonia-lyase	Aminoacids (histidine) catabolism	3,16	3,35E-16	2,95	2,39E-12		31
▶ pucL_1	PA5099	PA5099	z	Q9HU84	Putative allantoin permease	Aminoacids (histidine) catabolism	2,84	6,41E-08	2,07	1,95E-05		31
hutU	PA5100	hutU		Q9HU83	Urocanate hydratase	Aminoacids (histidine) catabolism	2,65	4,00E-06	2,19	2,65E-08		31
artJ	PA5153	PA5153		Q9HU31	ABC transporter arginine-binding protein 1	ABC transporter (arginine)	2,06	3,96E-05	2,23	8,34E-13		
NA	PA5160.1	PA5160.1	NA		tRNA-Thr(tgt)	Aminoacyl-tRNA biosynthesis	3,26	5,20E-08	6,82	3,05E-20		
group_4368	PA5383	yeiH		Q9HT11	Hypothetical protein	Unknown	-2,93	3,32E-06	-3,25	1,16E-03		
group_3039	PA5460	PA5460		Q9HTB0	Hypothetical protein	Unknown	-2,24	7,66E-09	-2,23	4,96E-03		
group_5371	PA5469	PA5469		Q9HTA1	Hypothetical protein	Unknown	2,26	1,56E-03	3,02	4,99E-04		

Table S5: List of *P. aeruginosa* genes differentially expressed in presence of *S. aureus* in the context of coexistence. PA2600 coexistence strain was cultivated in the absence or presence of SA2599 or SA2597. RNAs were extracted after 4 hours of culture and a RNAseq analysis was performed as described in material and methods. A gene was considered as differentially expressed when the Fold Change (FC) was $> |2\log_2|$ with an adjusted P -value < 0.05 in presence of both SA strains. Genes from the same operon are annotated with an identical letter. Grey cells indicate genes that were also dysregulated in competition couples (Table S4). Symbol ▶ indicates genes tested in RT-qPCR (Fig. 1B). Functional classification was performed thanks to KEGG database and literature.

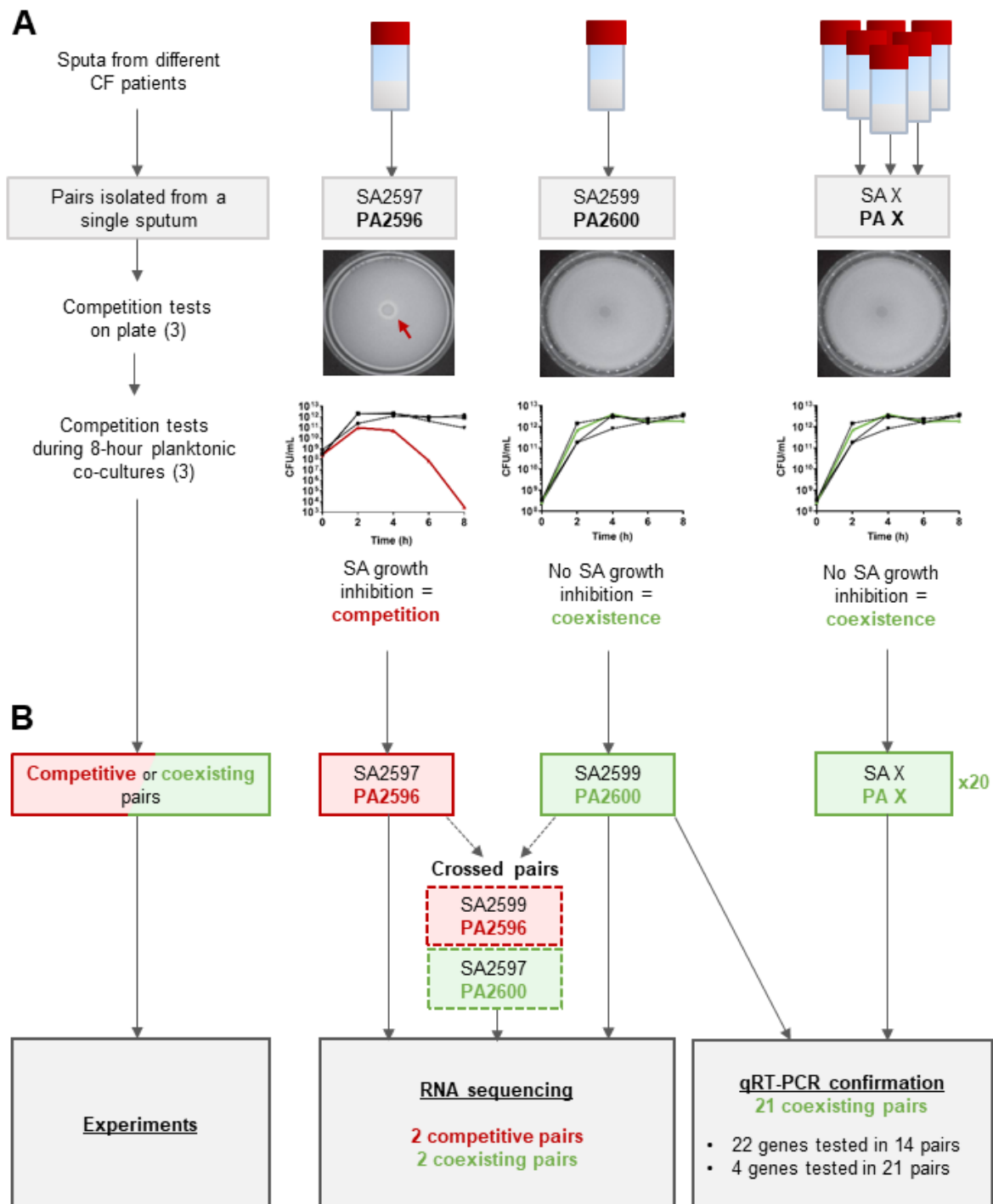


Figure S1: Schematic representation of the employed methodology.

A. Determination of interaction state within *S. aureus*-*P. aeruginosa* co-isolated pairs. Pairs of strains are co-isolated from a single sputum sample. Interaction state is tested during plate and liquid tests, as described in materials and methods and by Briaud *et al.* (3). Results of competition tests (pictures and kinetics) were obtained for a previous study (3).

B. Strain pairs used in transcriptomic analyses. Pairs SA2597/PA2596 and SA2599/PA2600 were isolated from two different patients. Interaction state of crossed pairs was determined as above and confirmed that it is solely led by *P. aeruginosa* (3). The 21 strain pairs used for qRT-PCR confirmation were both isolated from different patients, except in one case (Table S1).

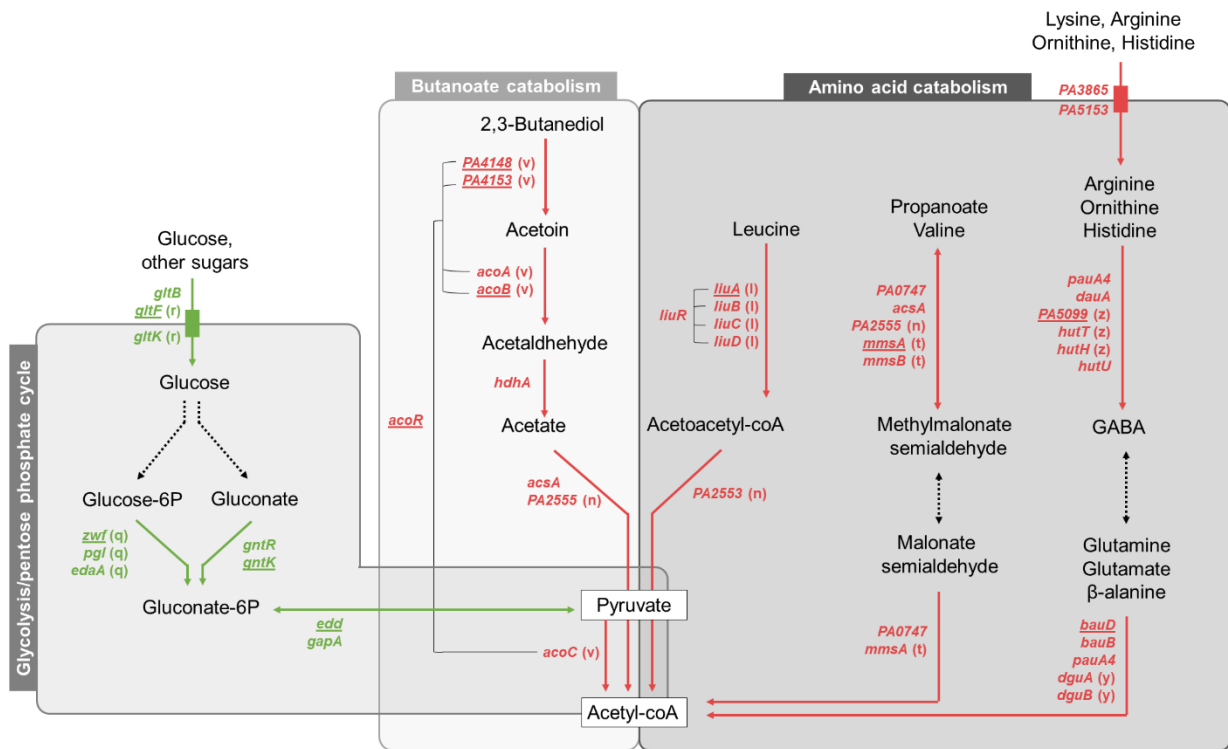


Figure S2: *P. aeruginosa* metabolic pathways and associated genes up-regulated (red) or down-regulated (green) in coexistence with *S. aureus*. PA2600 coexistence strain was cultivated in absence or presence of SA2599 or SA2597. RNAs were extracted after 4 hours of culture and RNAseq analysis was performed. A gene was considered as differentially expressed when the Fold Change (FC) was $> |2\log_2|$ with an adjusted P -value < 0.05 . Genes from the same operon are annotated with an identical letter. Genes tested in RT-qPCR and confirmed for PA2600 are underlined. Functional classification and pathway constructions were performed thanks to KEGG database and literature.

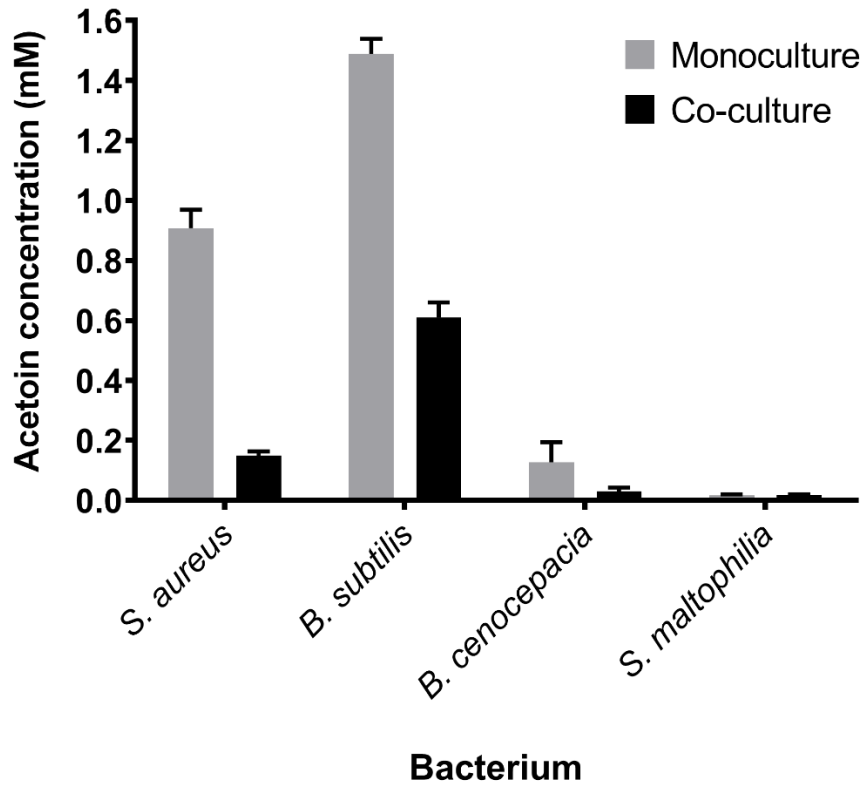


Figure S3: Acetoin concentration in supernatant of *S. aureus* SA2599, *B. subtilis*, *B. cenocepacia* and *S. maltophilia* monocultures (grey bars) or co-cultures with *P. aeruginosa* PA2600 (black bars). Acetoin was quantified from supernatant after 4h of culture. Bars represent the mean acetoin concentration + SEM from three independent experiments.

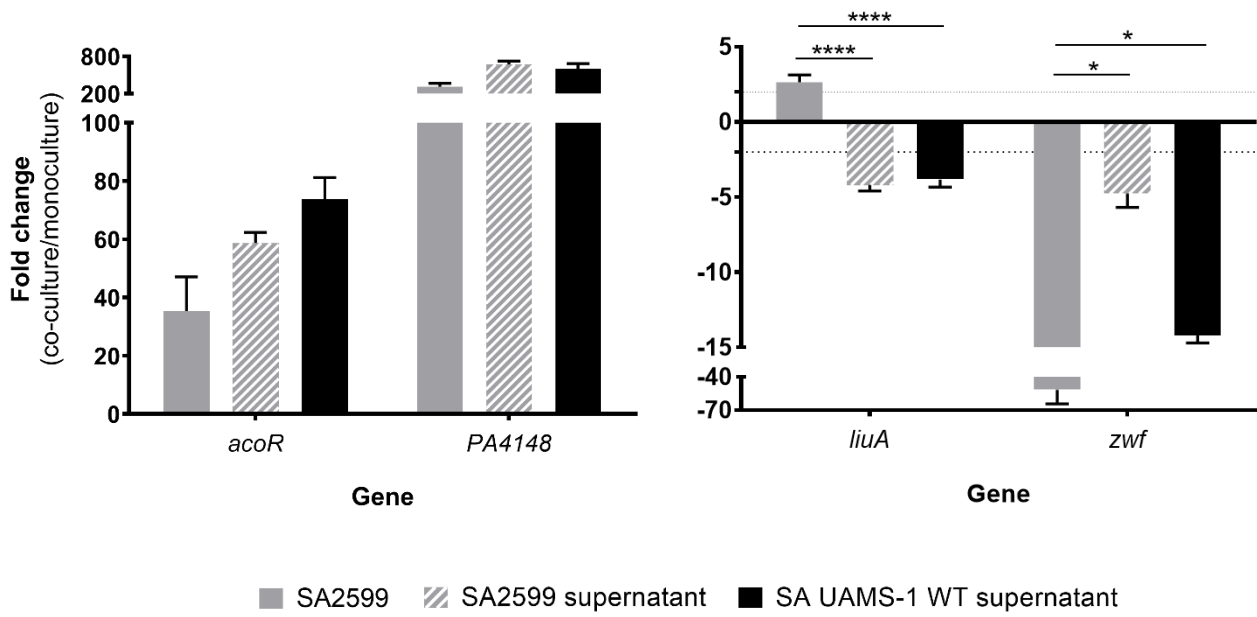


Figure S4: Fold change of *P. aeruginosa acoR*, *PA4148*, *liuA* and *zwf* gene expression induced by culture with *S. aureus* (grey bars) or its supernatant (hatched and black bars). *P. aeruginosa* PA2600 strain was cultivated in the absence or presence of *S. aureus* SA2599 or filtered supernatant of *S. aureus* SA2599 and UAMS-1 WT. RNAs were extracted after 4 hours of culture and gene expression was assayed by RT-qPCR. Bars represent the mean fold change + SEM from three independent experiments. Dot lines indicate a fold change = |2|. * $P_{adj} < 0.05$, ** $P_{adj} < 0.0001$ ANOVA with Tukey's correction.**

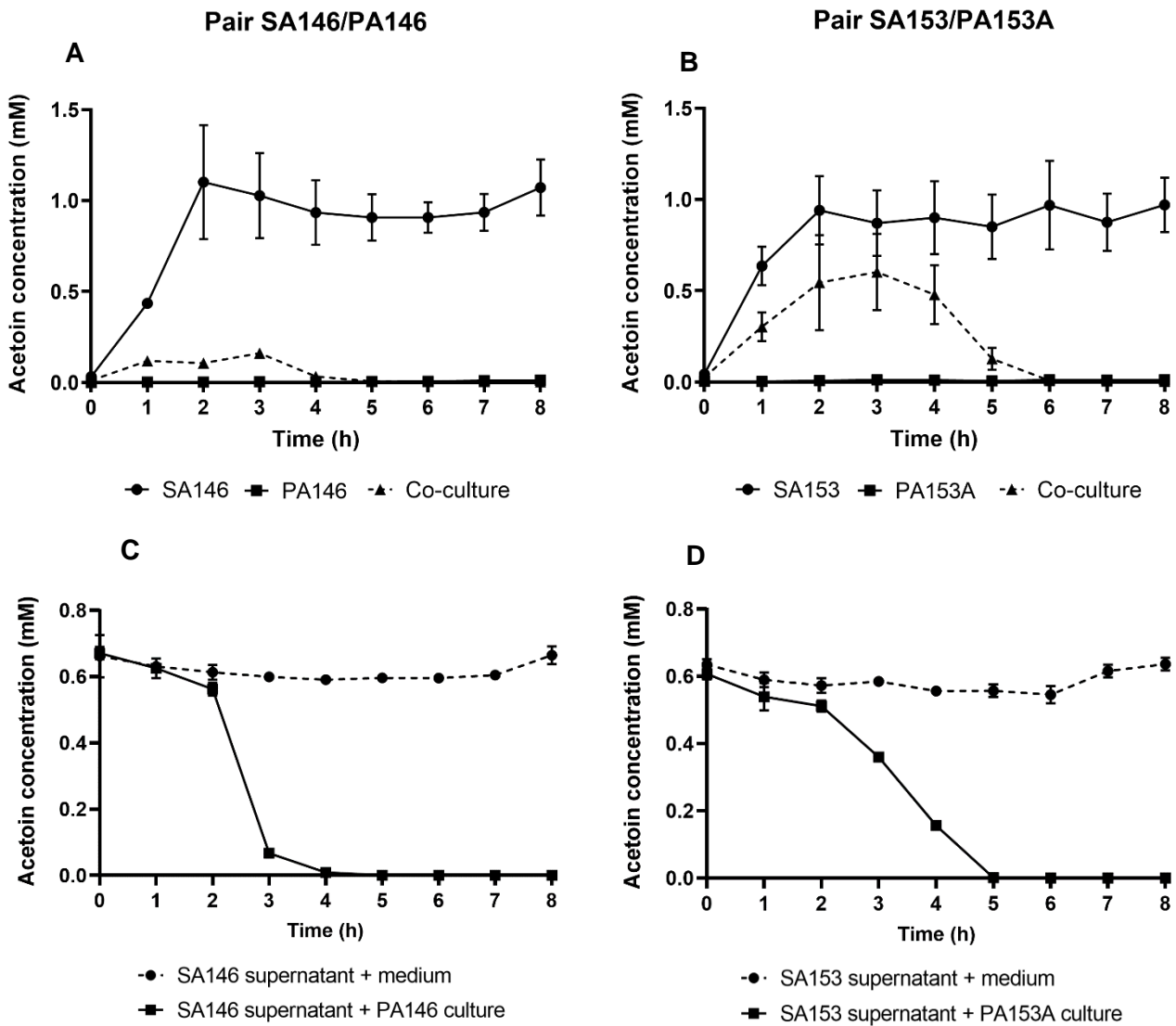


Figure S5: Monitoring of acetoin concentration in *S. aureus* and *P. aeruginosa* monocultures or co-culture (A,B) or in *S. aureus* supernatant inoculated with *P. aeruginosa* (B,C), for the pairs SA146/PA146 (A,C) and SA153/PA153A (B,D).

A, B. *S. aureus* and *P. aeruginosa* were cultivated in monoculture or co-culture. Acetoin was quantified from supernatant each hour. Points represent the mean acetoin concentration \pm SEM from two independent experiments per pair.

C, D. A 4-hour filtered supernatant of *S. aureus* was inoculated with *P. aeruginosa* culture or sterile medium for controls. Acetoin was quantified from supernatant each hour. Points represent the mean acetoin \pm SEM from three independent experiments per pair.

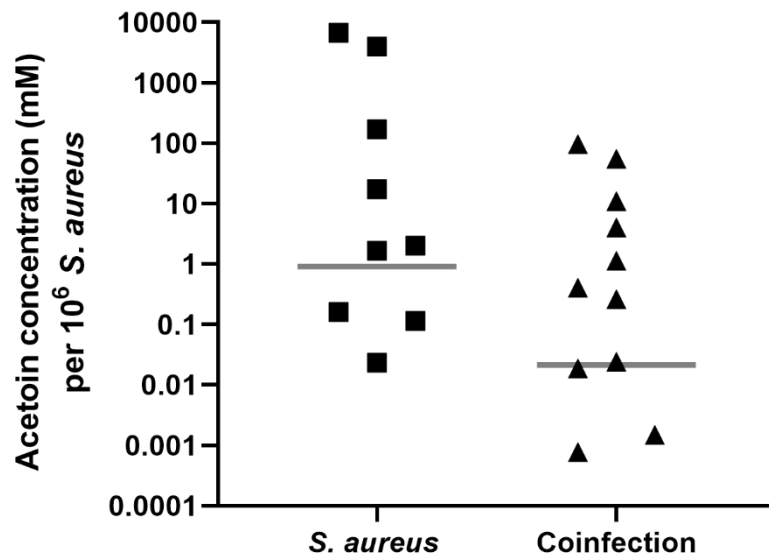


Figure S6: Acetoin concentration in CF sputa from patients. Sputa from *S. aureus* mono-infected patients (n=9) or *S. aureus* and *P. aeruginosa* co-infected patients (n=11) were gathered and acetoin concentration was quantified. Bars represent the median acetoin concentration normalized on *S. aureus* concentration in each sputum.

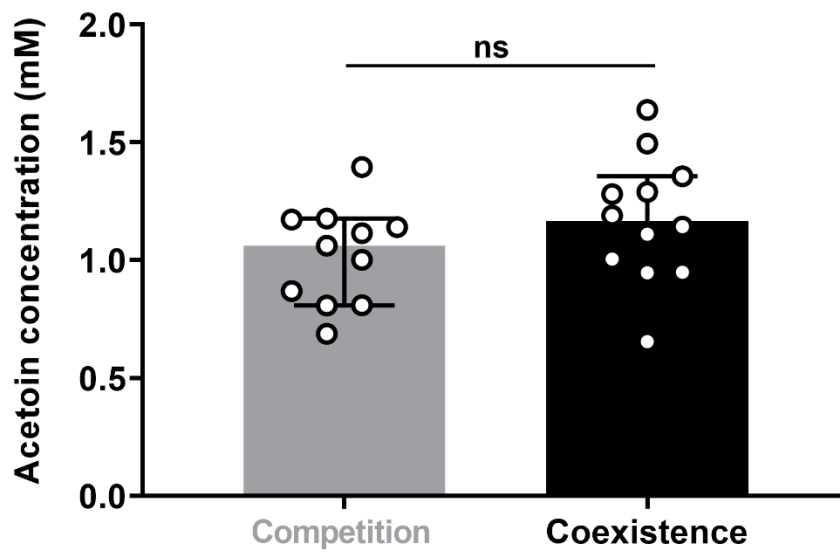


Figure S7: Acetoin concentration in cultures of *S. aureus* strains from competition and coexistence couples. Each *S. aureus* strain from competition (n=11) and coexistence (n=12) couples was cultivated for 6 hours in BHI and acetoin was dosed from supernatant. Bars represent the median acetoin concentration \pm 95% CI. ns $P > 0.05$ Mann-Whitney test.

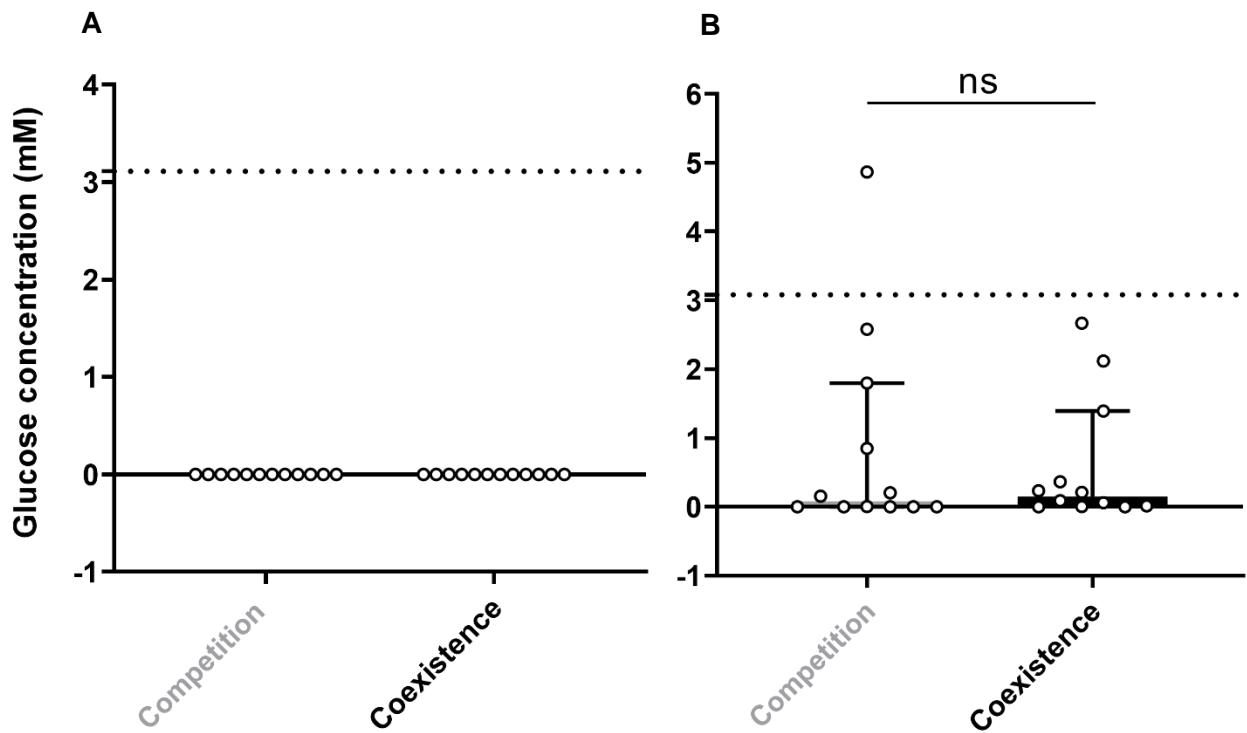


Figure S8: Glucose concentrations in cultures of *S. aureus* (A) and *P. aeruginosa* (B) strains from competition and coexistence pairs.

A. Each *S. aureus* strain from competition (n=12) and coexistence (n=12) couples was cultivated in *P. aeruginosa* PA2600 filtered supernatant for 6 hours and glucose was quantified from supernatant. No glucose was detected. Dotted line indicates the initial glucose concentration in *P. aeruginosa* supernatant.

B. Each *P. aeruginosa* strain from competition (n=12) and coexistence (n=12) couples was cultivated in *S. aureus* SA2599 filtered supernatant for 4 hours and glucose was quantified from supernatant. Bars represent the median glucose concentration \pm 95% CI. Dotted line indicates the initial glucose concentration in *S. aureus* supernatant. ns $P > 0.05$ Mann-Whitney test.

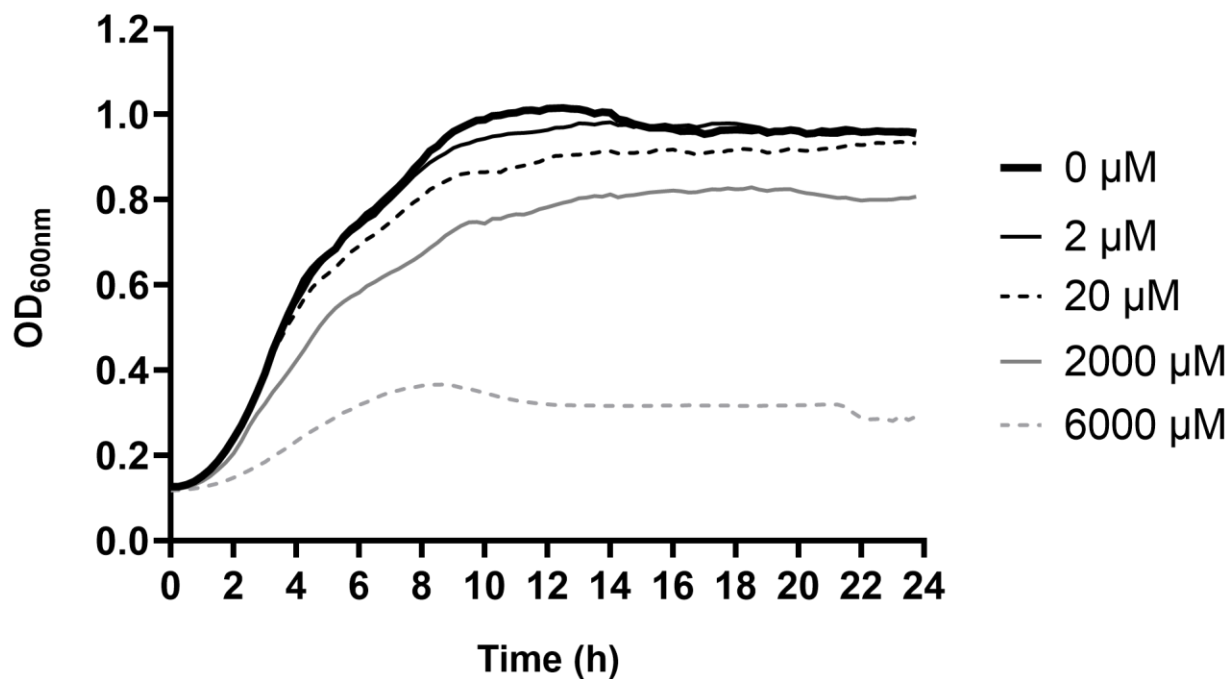


Figure S9: Growth kinetic of *S. aureus* cultivated in absence or presence of acetoin. SA2599 was cultivated during 24h in the absence of acetoin or in the presence of acetoin in different proportions ranging from 0.2μM to 6000μM per 10⁶ *S. aureus*. Lines represent the mean optical density of three technical replicates.

Supplementary data references

(1–5)(6–10)(11–15)(16–20)(21–25)(26–31)

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