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

3 Running title (54 characters): Cooperation between *S. aureus* and *P. aeruginosa*

4

5 Laura Camus¹, Paul Briaud¹, Sylvère Bastien¹, Sylvie Elsen², Anne Doléans-
6 Jordheim^{3,4}, François Vandenesch^{1,3,5} and Karen Moreau^{1#}.

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10 ¹ CIRI, Centre International de Recherche en Infectiologie, Université de Lyon, Inserm, U1111,
11 Université Claude Bernard Lyon 1, CNRS, UMR5308, ENS de Lyon, Lyon, France.

12 ² Université Grenoble Alpes, CNRS ERL5261, CEA-IRIG-BCI, INSERM UMR1036, Grenoble 38000,
13 France

14 ³ Institut des agents infectieux, Hospices Civils de Lyon, Lyon, France.

15 ⁴ Bactéries Pathogènes Opportunistes et Environnement, UMR CNRS 5557 Ecologie Microbienne,
16 Université Lyon 1 & VetAgro Sup, Villeurbanne, France.

17 ⁵ Centre National de Référence des Staphylocoques, Hospices Civils de Lyon, Lyon, France

18

19

20 # Address correspondence to Karen Moreau, karen.moreau@univ-lyon1.fr – Université Claude Bernard
21 Lyon1 – CIRI – Pathogénie des staphylocoques – 7 rue Guillaume Paradin – 69 008 Lyon - France –
22 tel. : +33 (0)4 78 77 86 57

23

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25

26 **Abstract**

27 In the context of infection, *Pseudomonas aeruginosa* and *Staphylococcus aureus* are frequently co-
28 isolated, particularly in cystic fibrosis (CF) patients. Within lungs, the two pathogens exhibit a range of
29 competitive and coexisting interactions. In the present study, we explored the impact of *S. aureus* on
30 *P. aeruginosa* physiology in the context of coexistence. Transcriptomic analyses showed that *S. aureus*
31 affects significantly and specifically the expression of numerous genes involved in *P. aeruginosa* carbon
32 and amino acid metabolism. In particular, 65% of the strains presented an important overexpression of
33 the genes involved in the acetoin catabolic (*aco*) pathway. We demonstrated that acetoin is (i) produced
34 by clinical *S. aureus* strains, (ii) detected in sputa from CF patients and (iii) involved in *P. aeruginosa*'s
35 *aco* system induction. Furthermore, acetoin is catabolized by *P. aeruginosa*, a metabolic way that
36 improves the survival of both pathogens by providing a new carbon source for *P. aeruginosa* and
37 avoiding toxic accumulation of acetoin on *S. aureus*. Due to its beneficial effects on both bacteria,
38 acetoin catabolism could testify to the establishment of trophic cooperation between *S. aureus* and
39 *P. aeruginosa* in the CF lung environment, promoting their persistence.

40 Introduction

41

42 Infectious sites constitute rich microbial ecosystems shared by a large diversity of
43 microorganisms, including the native microbiota and pathogens. Lungs of Cystic Fibrosis (CF) patients
44 are a well-known example of this microbial richness as they gather more than 60 genera of bacteria (1,
45 2). Such density of microorganisms promotes their interactions, themselves modeling their biological
46 activities and their environment (3, 4). However, microbial interactions are dynamic and range from
47 antagonism to cooperation according to species and environmental conditions (5). For instance, the
48 opportunistic pathogen *Pseudomonas aeruginosa* is well-known for its competitiveness in various
49 ecosystems thanks to many quorum-sensing-mediated factors such as phenazines, rhamnolipids or
50 type 6 secretion system (6). *P. aeruginosa* thus can alter growth, biofilm formation or respiration of
51 yeasts, fungi, Gram-negative and –positive bacteria (6). Among them, *Staphylococcus aureus* is
52 particularly sensitive to *P. aeruginosa* virulence factors and these can directly lyse staphylococci (7).
53 This competitive interaction between *P. aeruginosa* and *S. aureus* is observed for environmental but
54 also clinical strains as both pathogens are frequently co-isolated from wound and CF lung samples (7,
55 8). Until recently, the anti-staphylococcal behavior of *P. aeruginosa* was the only one observed between
56 the two species and was thus extensively described (7). In the context of CF lung infections, this
57 competitive interaction is highlighted by the decreased *S. aureus* prevalence as *P. aeruginosa* colonizes
58 lungs during adolescence (9). However, Baldan *et al.* first noted that a non-competitive state between
59 *P. aeruginosa* and *S. aureus* could establish during the development of CF chronic infections (10),
60 calling into question the antagonistic model between the two pathogens.

61

62 The establishment of this particular non-competitive state between *P. aeruginosa* and *S. aureus*
63 seems to be linked to *P. aeruginosa* adaptation to the pulmonary ecosystem. In fact, selective pressures
64 present in CF lung environment, such as host immune system or antibiotic treatments, drive
65 *P. aeruginosa* isolates towards a low-virulent but high-resistant state (11–14). Major virulence factors
66 involved in quorum-sensing or motility are often mutated in *P. aeruginosa* chronic infection isolates,
67 inducing a decrease of anti-staphylococcal factors production and then a non-competitive interaction
68 (15). In addition, a rewiring of metabolism networks and a decrease of its catabolic repertoire also
69 accompany *P. aeruginosa* adaptation to CF environment. This trophic specialization commonly leads to
70 a slowed growth of chronic isolates and thus a reduced competitive behavior towards shared resources

71 (16). Several independent studies thus observed this coexistence state between *S. aureus* and
72 *P. aeruginosa* isolated from chronic infections (10, 15, 17, 18). Briaud *et al.* recently demonstrated that
73 this interaction pattern appears to be more frequent than expected. Indeed, among the quarter of CF
74 patients co-infected by both pathogens, 65% were infected by a coexisting *S. aureus-P. aeruginosa* pair
75 (18, 19). Recent studies show that coexistence between *P. aeruginosa* and *S. aureus* could promote
76 their persistence throughout establishment of cooperative interaction. In these conditions, coexisting
77 bacteria demonstrated an increased tolerance to antibiotics: to tobramycin and tetracycline for *S. aureus*
78 and to gentamicin for *P. aeruginosa*; this appeared to be related to the induction of small colony variants
79 (15, 17, 18, 20). However, the effects of coexistence on bacterial general physiology, and not only
80 virulence-associated traits, have not been explored yet. Despite its significance in infectious ecosystem,
81 coexistence between *P. aeruginosa* and *S. aureus* remains thus poorly understood.

82

83 Using global and targeted transcriptomic approaches, we evaluated the impact of *S. aureus*
84 presence on *P. aeruginosa* gene expression on a set of clinical pairs of strains isolated from CF co-
85 infected patients. Coexistence with *S. aureus* induced the overexpression of many genes involved in
86 utilization of alternative carbon sources in *P. aeruginosa*, such as amino acids and acetoin. Acetoin was
87 shown to be produced by clinical *S. aureus* isolates *in vitro* and in CF sputum, and catabolized by
88 *P. aeruginosa*. The beneficial effects of acetoin catabolism on both bacteria during their interaction
89 highlight a trophic cooperation between *P. aeruginosa* and *S. aureus* in CF lung infections.

90

91 **Materials and Methods**

92

93 **Bacterial strains**

94 Bacterial strains and plasmids used in this study are listed in Table S1 and S2. CF clinical strains
95 were isolated by the Infectious Agents Institute (IAI) from sputa of patients monitored in the two CF
96 centers of Lyon hospitals (Hospices Civils de Lyon (HCL)). *S. aureus* and *P. aeruginosa* strains were
97 isolated from patients co-infected by both bacteria. Each strain pair indicated in Table S1 was recovered
98 from a single sample, obtained from different patients in most cases as indicated in Table S1. All the
99 methods were carried out in accordance with relevant French guidelines and regulations. This study

100 was submitted to the Ethics Committee of the HCL and registered under CNIL No 17-216. All patients
101 were informed of the study and consented to the use of their data.

102 As schematized in Figure S1A, interaction state was determined for each pair by growth
103 inhibition tests on Tryptic Soy Agar (TSA) and in liquid cultures (18). As previously described,
104 coexistence was characterized by: (i) an absence of inhibition halo on agar tests, and (ii) a similar growth
105 in mono and co-culture during an 8 hours (18).

106 The knock-out Δ *acoR* and Δ *aco* mutants were generated in *P. aeruginosa* PA2600 strain via
107 allelic exchange thanks to suicide plasmids pEXG2 Δ *acoR* and pEXG2 Δ *aco* constructed as described
108 previously (21, 22). Gentamicin (Euromedex) was used at final concentrations of 50 μ g/ml. Detailed
109 protocols are given in supplementary data.

110

111 **Cultures conditions**

112 Strains were grown in monoculture or co-culture in Brain-Heart Infusion (BHI) as described by
113 Briaud *et al.* (18). Briefly, *S. aureus*, *P. aeruginosa*, *Burkholderia cenocepacia*, *Stenotrophomonas*
114 *maltophilia* and *Bacillus subtilis* overnight precultures were diluted to OD₆₀₀ of 0.1 in BHI and grown 2h30
115 at 37°C and 200rpm. Cultures were then diluted to OD₆₀₀ of 2 in fresh medium and 10ml were mixed
116 with 10ml BHI for monocultures. Co-cultures were performed by mixing 10ml of *P. aeruginosa*
117 suspension with 10ml of *S. aureus*, *B. cenocepacia*, *S. maltophilia* or *B. subtilis* suspension. For
118 supernatant exposure, 10ml of *S. aureus* supernatant from a 4-hours culture were filtered on 0.22 μ M
119 filter and added to 10ml of *P. aeruginosa* suspension. Cultures were grown for 8 hours at 200rpm and
120 37°C for transcriptomic studies. Long-term survival assays were performed by extending incubation time
121 of *S. aureus* and *P. aeruginosa* mono- and co-cultures up to five days. Plating at day 0, 3 and 5 were
122 performed on mannitol salt agar (MSA, BBL™ Difco) and cefrimide (Difco™) for *S. aureus* or
123 *P. aeruginosa* counts, respectively. For growth monitoring in presence of acetoin, minimal medium M63
124 (76mM (NH₄)₂SO₄, 500mM KH₂PO₄, 9 μ M Fe.SO₄.7H₂O, 1mM MgSO₄.7H₂O) was inoculated with
125 *P. aeruginosa* to OD₆₀₀ of 0.1 and incubated for 25h at 37°C and 200rpm. Every two hours during 10
126 hours and at t=24h, acetoin was added to obtain a final concentration of 1.5mM in the culture. Plating
127 on TSA (Tryptic Soy Agar) was performed at t=0, one hour after each acetoin addition and at t=24h.

128

129 **Genome sequencing and annotation**

130 Genome sequencing and annotation of PA2596 and PA2600 strains were performed as
131 previously described (18). In order to compare CDS from PA2596 and PA2600, PAO1 strain
132 (NC_002745.2) was used as a reference. Protein sequences were compared and grouped using a
133 similarity threshold of 95% through Roary (V3.8.2) (23). Gene names and numbers were gathered from
134 PAO1 and used as ID tag for common genes. For non-common genes, CDS from PA2596 and PA2600
135 were tagged with a specific name (gene number and name recovered from UniprotKB database).
136 Functional classification was performed using KEGG database and by manual literature check of each
137 gene function. The complete genome sequences for the PA2596 and PA2600 strains have been
138 deposited in GenBank under the accession number GCA_009650455.1 and GCA_009650545.1.

139

140 **Transcriptomic analysis**

141 Figure S1 schematizes the global methodology used for transcriptomic analysis. RNAseq
142 analysis was performed on four pairs: the patient-specific pairs SA2597/PA2596 (competitive) and
143 SA2599/PA2600 (coexisting), and the crossed pairs SA2599/PA2596 (competitive) and
144 SA2597/PA2600 (coexisting). RNA extraction, cDNA libraries preparation and sequencing and reads
145 treatment were conducted as previously described (18). Gene expression was considered as
146 dysregulated when: (i) the fold change between co-culture and monoculture was at least 4-fold with an
147 adjusted P -value <0.05 , (ii) the dysregulation was observed in the two pairs of strains with the same
148 interaction state, (iii) the dysregulation was specific to either coexistence or competition state. RNAseq
149 data that support our findings are available in the SRAdatabase under the BioprojectID: PRJNA562449,
150 PRJNA562453, PRJNA554085, PRJNA552786, PRJNA554237, PRJNA554233.

151

152 Confirmation of gene expression was achieved by RT-qPCR as previously described on 14 or
153 21 coexisting pairs including the SA2599/PA2600 couple used in RNAseq experiment (**Fig. S1**) (18).
154 Housekeeping genes *gyrB* and *rpoD* were used as endogenous control. Table S3 lists primers used and
155 target genes.

156

157 **Acetoin and glucose dosages**

158 Acetoin was quantified using modified Voges-Proskauer test (24) optimized in 96-well
159 microplates. To 50 μ L of culture supernatant, 35 μ L of creatine (0.5% m/v, Sigma), 50 μ L of α -naphthol (5%

160 m/v, Sigma) and 50µL of KOH (40% m/v, Sigma) were sequentially added. The mix was incubated 15
161 minutes at room temperature and optical density at 560nm was read using spectrophotometry (Tecan
162 Infinite Pro2000, Tecan-Switzerland). Glucose dosages were performed with glucose (Trinder, GOPOD)
163 assay kit (LIBIOS) in microplates. 185µL of dosage reagent were added to 5µL of culture supernatant
164 and incubated at 37°C for 5 minutes. Optical density at 540nm was measured. Acetoin and glucose
165 standard (Sigma) were performed in water, BHI or M63 according to experiment.

166

167 **Statistical analysis**

168 Statistical analyses were performed using Prism GraphPad 8.0 software (San Diego, CA).
169 Differences in gene expression fold change and bacterial survival were studied using one-way ANOVA
170 with Dunnett's or Tukey's post-test comparisons, as specified in corresponding figures. Median acetoin
171 and glucose concentrations were compared through Mann-Whitney test or Kruskal-Wallis test with
172 Dunn's correction when appropriate. Differences were considered significant when *P*-values were lower
173 or equal to 0.05.

174

175 **Results**

176

177 ***P. aeruginosa* transcriptome is affected by *S. aureus* presence**

178 We studied the genetic expression of *P. aeruginosa* in absence or presence of *S. aureus* in two
179 contexts: when *P. aeruginosa* and *S. aureus* were in competition or when they were in coexistence. We
180 thus performed RNAseq analyses using a competitive strain pair (SA2597/PA2596) and a coexisting
181 one (SA2599/PA2600). Each pair was recovered from a single sample of a co-infected CF patient
182 **(Table S1)**. As nature of interaction is solely led by *P. aeruginosa* (18), the pairs were crossed to study
183 gene expression in additional competitive (SA2599/PA2596) and coexisting (SA2597/PA2600) pairs.
184 Transcriptomic effect was therefore evaluated during co-cultures of two competitive and two coexisting
185 strain pairs **(Fig. S1)**. *P. aeruginosa* gene expression was considered dysregulated when dysregulation
186 was common to both co-cultures in comparison to monoculture. Each dysregulated gene was then
187 associated to a functional class thanks to a KEGG analysis **(Fig. 1A, Tables S4 and S5)**.

188

189 Sixty-eight *P. aeruginosa* genes were dysregulated in co-culture in the context of competition, with
190 a majority of down-regulated genes (79.4%, **Fig. 1A**). Fifteen genes involved in nitrogen metabolism
191 and 19 genes involved in iron metabolism were down-regulated, making these two functional classes
192 the most affected in competition. Among these genes, the *nir* and *nos* systems involved in denitrification
193 but also genes implicated in iron uptake and transport (*isc* and *fec* genes) were down-regulated (**Table**
194 **S4**). Other functional classes were less affected, as only 4 genes linked to carbon and amino acids
195 metabolism (*bauA*, *ddaR*, *gntK*, *arcD*) and 3 genes encoding membrane and virulence factors (*rfaD*,
196 *PA2412*, *cdrA*) were dysregulated in presence of *S. aureus*.

197
198 More genes were affected during coexistence interaction, as a dysregulation of 105 genes was
199 observed in *P. aeruginosa* (**Fig. 1A**). In spite of a trend of up-regulation (56.4% of genes), we could
200 notice the down-regulation of 11 genes involved in nitrogen metabolism. Among these, 8 were also
201 down-regulated in the context of competition, especially genes from the *nir* system. We thus can
202 presume that the down-regulation of *nir* genes is not specific to coexistence or competition interaction
203 states. Conversely, the dysregulations of iron metabolism related genes appeared to be specific to
204 competition strains, as only one gene (*fhp*) of this functional class was down-regulated in coexistence.
205 However, coexistence specifically affected numerous genes belonging to functional classes of carbon
206 and amino acids metabolism (18 and 23 genes) and membrane and virulence factors (15 genes).
207 Concerning this last class, a trend towards lower expression was observed and probably due to the
208 down-regulation of membrane associated factors as the *flp-tad* system (*flp*, *tad* and *rcpC* genes
209 encoding Flp pilus) and the *PA1874-1876* operon (encoding an efflux pump) (**Table S5**).

210
211 The most affected classes in coexistence with *S. aureus* were related to *P. aeruginosa* energetic
212 metabolism (**Fig. S2**). We observed a down-regulation of genes coding for major pentose phosphate
213 pathway enzymes, as the gluconokinase GntK, its regulator GntR and the operon *zwf-edaA* (*PA3183-
214 PA3181*) encoding a glucose-6-phosphate 1-dehydrogenase, a 6-phosphogluconolactonase and a 2-
215 keto-3-deoxy-6-phosphogluconate aldolase (25). We also noticed the down-regulation of 5 other genes
216 clustered near to this operon and involved in the same pathway (*edd* and *gapA* genes) or glucose
217 transport (*gltB*, *gltF* and *gltK*) (25, 26).

218

219 In contrast, an up-regulation of numerous genes involved in the utilization of alternative carbon
220 sources as butanoate and amino acids was observed. The *aco* system, comprising the operon *PA4148-*
221 *PA4153* and the gene encoding its transcriptional regulator *acoR* (*PA4147*), was thus up-regulated in
222 *P. aeruginosa* coexisting with *S. aureus*. This system has been described in *P. aeruginosa* PAO1 to be
223 responsible for 2,3-butanediol and acetoin catabolism (27). According to KEGG analysis, the up-
224 regulated genes *acsA* (*PA0887*), *PA2555* (*acs* family) and *hdhA* (*PA4022*) are also involved in the
225 butanoate pathway and energy production from 2,3-butanediol and acetoin, as their products catalyse
226 the production of acetyl-coA from acetaldehyde and acetate (**Fig. S2**). Finally, 23 genes implicated in
227 amino acids metabolism were up-regulated in *P. aeruginosa* in presence of *S. aureus*. Most of them
228 were linked to the catabolism of several amino acids (**Fig. S2**). We can especially notice the *liu* operon
229 (*PA2015-PA2012*), the *mmsAB* operon (*PA3569-PA3570*) and the *hut* gene system (*PA5097-PA5100*),
230 involved in leucine, valine and histidine catabolism, respectively.

231
232 In order to confirm these transcriptomic effects, we co-cultivated a set of *P. aeruginosa-*
233 *S. aureus* coexistence CF pairs and performed RT-qPCR to evaluate *P. aeruginosa* gene expression in
234 presence of *S. aureus* (**Fig. 1B**). Each pair was isolated from a single sputum. Twenty-six genes were
235 tested, including 19 identified as dysregulated during RNAseq analysis and belonging to the most
236 impacted categories, *ie.* carbon and amino acids metabolism, and membrane and virulence factors.
237 Most of the genes were tested in a total of 14 *P. aeruginosa-S. aureus* pairs; expression of four of these
238 genes was assessed in seven additional pairs to confirm the observed dysregulations. The different
239 *P. aeruginosa* strains have shown very different transcriptomic patterns during co-cultivation with
240 *S. aureus*, especially for membrane-associated and virulence factor genes. We noticed an over-
241 expression of *rcpC*, *tadA*, *tadG* and *flp* from the *flp-tad* system from 52.4% to 35.7% of the *P. aeruginosa*
242 strains. We also tested 7 additional genes encoding virulence factors previously described as involved
243 in *P. aeruginosa* interaction with *S. aureus* as *las*, *rhl*, *pch* and *pvd* genes (7). Regarding these last
244 genes, no clear effect of *S. aureus* co-cultivation was observed.

245
246 However, clearer transcriptomic patterns were observed for genes linked to carbon and amino
247 acids metabolism, the two most impacted gene classes during RNAseq experiment (**Fig. 1B**). We
248 confirmed the up-regulation of *liuA* gene in 78.6% (11/14) of *P. aeruginosa* strains co-cultivated with

249 *S. aureus*. We also confirmed the down-regulation of glucose metabolism genes in a high proportion of
250 strains, ranging from 92.9% (13/14) for *zwf*, *glfF* and *edd* genes to 71.4% (10/14) for *gntK* gene. Finally,
251 the up-regulation of genes involved in butanoate metabolism was confirmed for *acoR* (57.2%, 12/21),
252 *PA4148* (66.7%, 14/21), *acoB* (57.1%, 8/14) and *PA4153* (64.3%, 9/14), suggesting an impact of co-
253 culture on the whole *aco* system in *P. aeruginosa*. In view of these results, we focused our study on four
254 genes: *liuA* and *zwf*, respectively involved in leucine and glucose catabolism, *PA4148*, first gene of the
255 *aco* operon and *acoR*, both responsible for acetoin catabolism.

256

257 ***P. aeruginosa* *aco* system is induced by *S. aureus* acetoin**

258 In order to determine if the transcriptomic dysregulations of *acoR*, *PA4148*, *liuA* and *zwf* in
259 *P. aeruginosa* PA2600 are specific to interaction with *S. aureus*, we tested the effect of three other
260 bacterial species: *B. cenocepacia* and *S. maltophilia* as they are sometimes associated with
261 *P. aeruginosa* in CF patients and *Bacillus subtilis* as it produces high amount of acetoin (28). Dosages
262 in *B. cenocepacia* and *S. maltophilia* monocultures confirmed that these bacteria do not produce acetoin
263 (**Fig. S3**) as previously described (29–31). We observed a down-regulation of *P. aeruginosa* *zwf* gene
264 expression in all co-cultures in comparison to monocultures (**Fig. 2A**). Regarding *aco* system genes
265 (*acoR* and *PA4148*) and *liuA* gene, only *B. subtilis* induced similar levels of overexpression than
266 *S. aureus* SA2599. We thus hypothesized that acetoin, produced by these two species during our
267 experiment (**Fig. S3**), may be the inductor signal for these genes during co-culture with *S. aureus*.

268

269 In order to test this hypothesis, we first explored whether an inductor signal was present in the
270 supernatant of *S. aureus* culture. We indeed observed an overexpression of *acoR* and *PA4148* when
271 *P. aeruginosa* PA2600 was cultivated in *S. aureus* SA2599 culture supernatant, as well as the down-
272 expression of *zwf* but to a lesser extent in comparison to co-culture (**Fig. S4**). On the contrary, we did
273 not observe overexpression of *liuA* gene, suggesting that this effect is not due to acetoin and requires
274 the presence of *S. aureus* cells. Secondly, we cultivated *P. aeruginosa* PA2600 in presence of *S. aureus*
275 UAMS-1 WT supernatant or its $\Delta alsSD$ derivative defective in acetoin synthesis (32). Supernatant of
276 wild-type UAMS-1 strain induced same transcriptomic patterns on *P. aeruginosa* as those obtained with
277 CF SA2599 strain (**Fig. S4**). In presence of UAMS-1 $\Delta alsSD$ supernatant, overexpression of *aco* genes
278 was almost totally abolished (**Fig. 2B**). However, acetoin addition to this supernatant restored this

279 overexpression in a dose-dependent manner but with threshold effects between 0.375mM and 1mM of
280 acetoin (**Fig. 2B**). This indicates that induction through acetoin is one of the mechanisms involved in
281 *aco* system overexpression in *P. aeruginosa*. According to this experiment, *zwf* gene down-regulation
282 did not seem to be mediated by acetoin.

283

284 **Coexisting isolates of *S. aureus* and *P. aeruginosa* efficiently metabolize acetoin**

285

286 As the *aco* system is involved in acetoin catabolism in *P. aeruginosa* (27) and *S. aureus*
287 produces this molecule (27, 28), we hypothesized that *P. aeruginosa* could catabolize *S. aureus* acetoin.
288 To explore this hypothesis, we monitored acetoin concentration during mono- and co-cultures of three
289 pairs of strains: SA2599/PA2600 (**Fig. 3A**), SA146/PA146 and SA153/PA153A (**Fig. S5A and B**).
290 *P. aeruginosa* strains did not produce acetoin, but an acetoin accumulation up to 1.3mM was observed
291 in *S. aureus* monocultures. Interestingly, a reduction of acetoin accumulation of at least 30% was
292 observed when *S. aureus* was co-cultivated with *P. aeruginosa*, in comparison to *S. aureus*
293 monoculture. The same trend could be observed in CF patient sputa, as a higher acetoin concentration
294 was detected in sputa from *S. aureus* mono-infected patients (n=9) in comparison to sputa from
295 *S. aureus-P. aeruginosa* co-infected patients (n=11) (**Fig. S6**). This effect could be due to a down-
296 regulation of *S. aureus* acetoin biosynthesis, or an acetoin catabolism in co-culture. Growth of
297 *P. aeruginosa* PA2600 in *S. aureus* SA2599 supernatant containing acetoin actually led to a reduction
298 of acetoin concentration, demonstrating the ability of *P. aeruginosa* to catabolize acetoin (**Fig. 3B**). This
299 ability was confirmed for the two other strains PA146 and PA153A when grown in supernatant of SA146
300 and SA153, respectively (**Fig. S5C and D**). We also noted that acetoin catabolism started after glucose
301 depletion in the supernatant and could be delayed by glucose addition (**Fig. 3B**). This suggests that
302 *P. aeruginosa* uses acetoin as an alternative carbon source in absence of easily available substrates
303 such as glucose. The early glucose depletion observed during co-culture supports this hypothesis
304 (**Fig. 3A**).

305

306 In order to test if this acetoin metabolism was specific to interaction state between *S. aureus*
307 and *P. aeruginosa*, we evaluated acetoin production and catabolism for 12 couples of competition and
308 12 couples of coexistence (**Table S1**). Cultivated in *P. aeruginosa* supernatant, *S. aureus* strains from

309 coexisting pairs were able to produce 4-times more acetoin (230 μ M) than strains from competitive pairs
310 (60 μ M) (**Fig. 4A**). Such distinction was not observed during culture in rich medium (**Fig. S7**). Regarding
311 *P. aeruginosa*, we cultivated the sets of competitive and coexisting *P. aeruginosa* strains in SA2599
312 supernatant and monitored acetoin catabolism (**Fig. 4B**). Both competitive and coexisting strains
313 catabolized acetoin since we observed a decrease in acetoin concentration for both groups. However,
314 coexisting strains showed an increased catabolism efficiency. Indeed, this group catabolized 98.6% of
315 acetoin after 4 hours of culture, while competitive strains catabolized only 47% of acetoin. This increased
316 efficiency of acetoin production and catabolism for coexisting strains could not be explained by a
317 difference in glucose utilization between competition and coexistence strains as both groups catabolized
318 glucose with the same efficiency (**Fig. S8**). Acetoin production by *S. aureus* and catabolism by
319 *P. aeruginosa* therefore seem to be more efficient in isolates from coexisting couples.

320

321

322 **Acetoin catabolism by *P. aeruginosa* increases survival rates of both pathogens in co-culture**

323 As *P. aeruginosa* catabolizes acetoin when medium is glucose-depleted, we tested the effect of
324 acetoin on PA2600 growth in minimal medium M63 (containing no glucose or amino acids)
325 supplemented with 1.5mM acetoin every 2 hours (**Fig. 5**). *P. aeruginosa* was able to grow up to 1.6 \times 10⁹
326 CFU/ml after 24h of culture with acetoin as sole carbon source while PA2600 Δ acoR and Δ aco mutants
327 grew significantly less, reaching a maximum cell concentration of 1.5 \times 10⁸ UFC/ml at 24h (**Fig. 5A**). In
328 parallel, we quantified acetoin concentration. We observed an accumulation of acetoin all along the
329 experiment in presence of PA2600 Δ acoR and Δ aco mutants. For WT strain, the accumulation of acetoin
330 reached its maximum at 5h of culture and afterwards slowly decreased to reach undetectable values at
331 10h of culture, demonstrating the consumption of acetoin in such conditions (**Fig. 5B**). A delay of 3h
332 between the start of acetoin catabolism and the growth augmentation of WT strain was noticed, and
333 might be due to metabolism adaptation. Acetoin catabolism nonetheless promoted a 10-fold increased
334 growth of *P. aeruginosa* during extended culture in glucose depleted medium.

335

336 In order to assess the impact of acetoin catabolism on survival of both pathogens, we co-
337 cultivated *P. aeruginosa* PA2600, Δ acoR and Δ aco mutants with *S. aureus* SA2599. As *S. aureus* was
338 not able to grow in M63 poor medium and acetoin affects its long term survival (33, 34), we performed

339 a long term culture (5 days) in BHI medium. We determined the survival rate of *S. aureus* co-cultivated
340 with *P. aeruginosa* in comparison to monoculture (**Fig. 6A**). Co-culture with the WT strain of
341 *P. aeruginosa* induced a *S. aureus* survival rate of 4.7×10^{-1} after 3 days of culture and of 5.1×10^{-3} after
342 5 days. *S. aureus* survival thus appears to be highly affected by long-term co-culture with *P. aeruginosa*,
343 even if the strains stably coexist during shorter spans of culture (18). Interaction state with *S. aureus*
344 thus seems to rely on nutritional conditions, a fact already observed elsewhere (35, 36). However, the
345 survival rate of *S. aureus* was even lower during co-culture with *P. aeruginosa* Δ *acoR* and Δ *aco*
346 mutants, reaching only 9.7×10^{-2} at 3 days of culture and 5.6×10^{-4} at 5 days. *S. aureus* survival was thus
347 4 to 10 times lower when *P. aeruginosa* was not able to catabolize acetoin, in comparison to co-culture
348 with a strain that efficiently used the molecule. These results suggest that accumulation of acetoin may
349 impact *S. aureus* survival during co-culture with *P. aeruginosa*. In parallel, we determined the survival
350 rate of *P. aeruginosa* (**Fig. 6B**). We noticed that in such conditions, *P. aeruginosa* WT strain presented
351 a growth advantage in co-culture, with a maximum of 6.6-fold increase of *P. aeruginosa* population after
352 5 days in presence of *S. aureus* in comparison to monoculture (**Fig. 6B**). The opposite effect was
353 observed for *aco* mutant strains, as their populations were reduced by 75% for Δ *acoR* and 32% for Δ *aco*
354 after 3 days of co-culture in comparison to monoculture, confirming the role of acetoin catabolism in
355 favouring *P. aeruginosa* growth and survival.

356
357 In order to figure out if acetoin accumulation was involved in the decrease of *S. aureus* survival
358 rate, we monitored acetoin concentration in *S. aureus* monoculture and co-cultures (**Fig. 6C**). As
359 expected, we did not detect acetoin in co-culture with PA2600 WT strain over the five days but we
360 observed an accumulation of acetoin with PA2600 Δ *acoR* and Δ *aco* mutants. The proportion of acetoin
361 in co-culture ($1300 \mu\text{M}$ to $1700 \mu\text{M}/10^6$ *S. aureus*) was more than 500 times higher than in monoculture
362 ($2.5 \mu\text{M}/10^6$ *S. aureus*) (**Fig. 6C**). We thus cultivated *S. aureus* in different acetoin/cells proportions and
363 observed that acetoin had an inhibitory dose-dependent effect on *S. aureus* growth from $20 \mu\text{M}/10^6$
364 *S. aureus* (**Fig. S9**). We concluded that acetoin accumulation may be responsible for the decreased
365 *S. aureus* survival during co-culture with PA2600 Δ *acoR* and Δ *aco* mutants. *P. aeruginosa* acetoin
366 catabolism thus allows a greater *S. aureus* survival during co-culture, in comparison to co-culture with
367 strains unable to catabolize acetoin.

368

369 Taken together, these results demonstrated that acetoin catabolism promotes *P. aeruginosa*
370 survival as a nutritional alternative carbon source, and improves *S. aureus* survival during co-culture
371 since a high concentration of acetoin appears to impair *S. aureus* growth.
372

373 **Discussion**

374

375 Coinfection with *S. aureus* and *P. aeruginosa* is a frequent situation especially in lungs of CF
376 patients, where coinfection accounts for 35% to 50% of cases (17, 18). In this context of coinfection, two
377 states of interaction between the two pathogens have been described: the well-known competitive
378 interaction where *P. aeruginosa* is able to inhibit the growth of *S. aureus* and the coexistence state
379 where growth of both species is not affected by each other. The first state has been extensively studied
380 and the leading bacterial determinants of *S. aureus* growth inhibition have been described (7). On the
381 contrary, little is known about the impact of coexistence state on bacterial physiology. In the present
382 study, we explored the impact of *S. aureus* on *P. aeruginosa* gene expression and physiology.

383 Comparing competitive and coexistence states, we observed that the down-regulation of genes
384 involved in iron metabolism was specific to competition. Most of these, such as *fec* genes or *PA4467-*
385 *PA4471* operon, are involved in ferrous iron uptake and down-regulated during iron-replete conditions
386 (37–39). These conditions are certainly generated by the lysis of *S. aureus* that provides an iron source
387 to *P. aeruginosa* during competitive interaction (7, 36), a situation not observed in coexistence. This
388 hypothesis is supported by the work of Tognon *et al.* (40) that also identified down-regulation of iron
389 metabolism genes during competitive interaction. Interestingly, they also noted typical responses of
390 amino acid starvation including the down-regulation of genes involved in branched-chain amino acid
391 degradation in competitive *P. aeruginosa* (40). While we did not identify such dysregulation in
392 competition, an overexpression of numerous genes involved in amino acid catabolism was noted during
393 coexistence (**Table S5**), emphasizing that these dysregulations depend on interaction state.

394

395 More interestingly, we observed that both carbon and amino acids metabolism was specifically
396 affected during coexisting interaction. Many genes involved in glucose catabolism were down-regulated
397 in coexisting isolates during co-culture with *S. aureus*, especially when the medium was glucose-
398 depleted (**Fig. 3**). It is worth noting that the *zwf* gene, down-regulated in almost all *P. aeruginosa* tested
399 strains, encodes a glucose-6-phosphate dehydrogenase that converts glucose- 6-phosphate to 6-
400 phosphogluconate; the first enzyme in the Entner-Doudoroff pathway, which is central to carbon
401 metabolism in *Pseudomonas sp.*

402 In such condition of glucose depletion, we demonstrated that *P. aeruginosa* was able to use an
403 alternative carbon source provided by *S. aureus*: acetoin. Acetoin is a four-carbon molecule produced
404 by the decarboxylation of α -acetolactate. Owing to its neutral nature, production and excretion of acetoin
405 during exponential growth prevents over acidification of the cytoplasm and the surrounding medium.
406 When other carbon sources are exhausted, it can constitute an external energy source for fermentive
407 bacteria (28).

408 Acetoin produced by *S. aureus* was shown to be an inductor of the *aco* operon and *acoR*
409 expression in *P. aeruginosa* (**Fig. 2B**) (27), allowing acetoin catabolism. This occurred in absence of
410 glucose and was potentially mediated by carbon catabolic repression (**Fig. 3**), a situation that was
411 already described in other bacteria such as *B. subtilis* (28). However, threshold effects in acetoin-
412 mediated induction and variability in *aco* system overexpression in *P. aeruginosa* strains (**Fig. 1B and**
413 **2B**) suggest that other regulatory mechanisms may be involved. Our study may also support the
414 relationship between acetoin and branched-chain amino acid pathways. Indeed, the biosynthesis
415 pathways of acetoin and leucine are co-regulated and share the same precursor α -acetolactate in
416 *S. aureus* (28). In response to co-culture with *S. aureus*, *P. aeruginosa* clinical strains showed
417 overexpression of acetoin and leucine catabolism genes (**Fig. 1B and 2**), suggesting the presence of
418 both compounds in our co-culture conditions. All of our analyses were performed *in vitro*. However,
419 using Voges-Proskauer dosage, we were able to confirm the presence of acetoin in CF patient sputa,
420 and in lower concentrations for *P. aeruginosa*-positive samples (**Fig. S6**). No direct correlation between
421 *P. aeruginosa* presence and acetoin quantity can be established as other microorganisms present in
422 sputa may also have an impact on acetoin concentration. Nevertheless, our data support the work of
423 Španěl *et al.* (41) and suggest that *P. aeruginosa* may catabolize and use *S. aureus* acetoin in the lung
424 environment.

425
426 More importantly, we observed that catabolism of acetoin by *P. aeruginosa* and acetoin
427 production by *S. aureus* were both more efficient for coexisting isolates, in comparison to competitive
428 ones (**Fig. 4**). This underlines the adapted metabolic regulation in coexisting isolates in comparison to
429 competitive ones. It is well known that the coexistence phenotype between *P. aeruginosa* and *S. aureus*
430 is a consequence of an adaptation process. Indeed, *P. aeruginosa* strains isolated from early infection
431 outcompete *S. aureus* while strains isolated from chronic infection are less antagonistic and can be co-

432 cultivated with *S. aureus* (7, 10, 17). It also has been widely described how both pathogens evolve
433 during colonization to evade the immune response and antibiotic treatment (42). Here, for the first time,
434 we suggest that evolution process leads to an adaptation of interspecies metabolic pathways between
435 *P. aeruginosa* and *S. aureus*.

436

437 Therefore, we suggest that acetoin produced by *S. aureus* could contribute to sputum nutritional
438 richness and be used by *P. aeruginosa* to survive in this nutritionally competitive environment during
439 chronic infection. This hypothesis is supported by the beneficial effect of acetoin catabolism on
440 *P. aeruginosa* growth and survival, especially during co-culture with *S. aureus* (**Fig. 5 and 6B**).
441 *S. aureus* survival in presence of *P. aeruginosa* was also shown to be highly affected by nutrient
442 availability induced by co-culture conditions (**Fig. 6A**). While coexistence is characterized by an absence
443 of *S. aureus* growth inhibition during 8-hour co-culture (18), it appears that nutritional competition can
444 still occur under unfavourable conditions and affect *S. aureus* survival. Therefore, coexistence between
445 the two pathogens is promoted in nutritionally rich environments, in line with previous observations (35,
446 36). Under adverse nutritional conditions induced by long-term culture, although its survival rate is
447 affected, acetoin catabolism benefits its producer, *S. aureus* (**Fig. 6A**). Although this effect seems to be
448 linked to acetoin accumulation in the medium as demonstrate by *P. aeruginosa* Δ *acoR* and Δ *aco*
449 mutants that do not catabolize acetoin anymore (**Fig. 5B and 6C**), the precise mechanism remains
450 unclear. In *S. aureus*, cell death in stationary phase may be induced by acetate production and ensuing
451 intracellular acidification. Thomas *et al.* showed that acetoin production counters cytoplasmic
452 acidification by consuming protons and promotes *S. aureus* survival in late-stationary phase (34). We
453 hypothesize that acetoin accumulation in the medium may induce a negative control of acetoin
454 synthesis, affecting *S. aureus* survival during co-culture conditions.

455

456 Previous studies demonstrated the potential benefits of *S. aureus* and *P. aeruginosa* during
457 coinfection. For example, *S. aureus* facilitates the survival of *P. aeruginosa* *lasR* mutants commonly
458 found in CF patients by detoxifying surrounding nitric oxide released by host immune cells (43). On the
459 other hand, 4-hydroxy-2-heptylquinoline-N-oxide (HQNO) produced by *P. aeruginosa* cells inhibits
460 respiration in *S. aureus* but also protects *S. aureus* cells from aminoglycosides (44). Additionally, we
461 recently demonstrated that *S. aureus* antibiotic resistance and internalization into epithelial cells were

462 increased in presence of coexisting *P. aeruginosa* (18). Here, we show that carbon metabolism is largely
463 affected and that *P. aeruginosa* uses the acetoin produced by *S. aureus* as an alternative carbon source.
464 This metabolic dialogue between the two pathogens is selected during bacterial adaptation in CF lungs
465 and promotes their survival. Thereby, we highlight for the first time a trophic cooperation between
466 *S. aureus* and *P. aeruginosa* during cooperative interaction.

467

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473

474 **Conflict of interest**

475 All authors declare no competing interests.

476

477 **Ethical statement**

478 All the strains used in this study were collected as part of the periodic monitoring of patients at the
479 Hospices Civils de Lyon. This study was submitted to the Ethics Committee of the Hospices Civils de
480 Lyon (HCL) and registered under CNIL No 17-216. All patients were informed of the study; however, as
481 the study was non-interventional no written informed consent were required under local regulations.

482

483

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614 **Figure legends**

615

616 **Figure 1: Alteration of *P. aeruginosa* transcriptome induced by co-culture with *S. aureus*.**

617 **A. Number of under-expressed (grey bars) and over-expressed (black bars) genes of**
618 ***P. aeruginosa* during co-culture with *S. aureus* for competitive (left) or coexisting (right) pairs.**

619 PA2596 competition and PA2600 coexistence strains were cultivated in absence or presence of SA2597
620 or SA2599 as described in Fig. S1. RNAs were extracted after 4 hours of culture and the RNAseq
621 analysis was performed as described in material and methods. A gene was considered as differentially
622 expressed when the Fold Change (FC) was $> |2\log_2|$ with an adjusted *P*-value <0.05 . Functional
623 classification was performed thanks to KEGG database and literature.

624 **B. Fold change of 26 *P. aeruginosa* gene expression induced by co-culture with *S. aureus* during**
625 **coexistence interaction.** Twenty-one *S. aureus-P. aeruginosa* coexisting pairs were isolated from

626 separate CF sputa recovered from 20 different patients. Each *P. aeruginosa* strain was cultivated in
627 absence or presence of its co-isolated *S. aureus* strain. RNAs were extracted after 4 hours of culture
628 and gene expression was assayed by RT-qPCR. A gene was considered as differentially expressed
629 when the Fold Change (FC) was $> |2|$, indicated by dotted lines. Black lines indicate the median. Genes
630 were tested in 14 (regular genes) or 21 (bold genes) strains. List of used strains is shown in Table S1.
631 Genes annotated with (*) were not identified as dysregulated during the RNAseq experiment.

632

633 **Figure 2: Fold changes of *P. aeruginosa* *acoR*, *PA4148*, *liuA* and *zwf* induced by culture**
634 **conditions.**

635 **A. Fold changes induced by co-culture with *S. aureus* (black bars), *B. subtilis* (grey bars), *B.***
636 ***ceenocepacia* (hatched white bars) or *S. maltophilia* (hatched black bars).** *P. aeruginosa* PA2600

637 strain was cultivated in absence or presence of *S. aureus* SA2599, *B. subtilis*, *B. ceenocepacia* or *S.*
638 *maltophilia*. RNAs were extracted after 4 hours of culture and gene expression was assayed by RT-
639 qPCR. Bars represent the mean fold change + SEM from three independent experiments. Dotted lines
640 indicate a fold change = $|2|$. **P*_{adj} <0.05 , ***P*_{adj} <0.01 , ****P*_{adj} <0.001 ANOVA with Dunnett's correction
641 (*S. aureus* vs. condition).

642 **B. Fold changes induced by culture in supernatant of *S. aureus* UAMS1 wild type (WT, black**
643 **bars), $\Delta alsSD$ mutant (white bars), $\Delta alsSD$ mutant complemented with increasing acetoin**
644 **concentrations (grey bars).** *P. aeruginosa* PA2600 strain was cultivated in absence or presence of
645 filtered supernatant from *S. aureus* UAMS-1 WT, $\Delta alsSD$ or $\Delta alsSD$ complemented with acetoin
646 concentrations ranging from 0.375mM to 2mM. RNAs were extracted after 4 hours of culture and gene
647 expression was assayed by RT-qPCR. Bars represent the mean fold change + SEM from three
648 independent experiments. Dotted line indicates a fold change = |2|. * P_{adj} <0.05, ** P_{adj} <0.01 ANOVA with
649 Tukey's correction.

650

651 **Figure 3: Monitoring of acetoin (black lines) and glucose (grey lines) concentrations in *S. aureus***
652 **and *P. aeruginosa* monocultures or co-culture (A) or in *S. aureus* supernatant inoculated with**
653 ***P. aeruginosa* (B), during coexisting interaction.**

654 **A.** *S. aureus* SA2599 and *P. aeruginosa* PA2600 were cultivated in monoculture or co-culture. Acetoin
655 and glucose were quantified from supernatant each hour. Points represent the mean acetoin or glucose
656 concentration \pm SEM from three independent experiments. Similar results of acetoin accumulation are
657 shown in Figure S5 for couples 146 and 153A.

658 **B.** A 4-hours filtered supernatant of *S. aureus* SA2599 was inoculated with *P. aeruginosa* PA2600
659 culture or sterile medium for controls. Supernatant was used unaltered (dotted lines) or complemented
660 with glucose (solid lines). Acetoin and glucose were quantified from supernatant each hour. Points
661 represent the mean acetoin or glucose concentration \pm SEM from three independent experiments.
662 Similar results of acetoin catabolism are shown in Figure S5 for couples 146 and 153A.

663

664 **Figure 4: Ability of acetoin production by *S. aureus* (A) and catabolism by *P. aeruginosa* (B) for**
665 **competitive (grey bars) or coexisting (black bars) strains.**

666 **A. Acetoin concentration in *P. aeruginosa* supernatant inoculated with *S. aureus* strains from**
667 **competition and coexistence couples.** Each *S. aureus* strain from competition (n=12) and
668 coexistence (n=12) couples was cultivated in *P. aeruginosa* PA2600 filtered supernatant and acetoin
669 was quantified from supernatant after 6 hours of culture. Bars represent the median acetoin
670 concentration \pm 95% CI. **** P <0.0001 Mann-Whitney test.

671 **B. Acetoin concentration in *S. aureus* supernatant inoculated with *P. aeruginosa* strains from**
672 **competition and coexistence couples.** Each *P. aeruginosa* strain from competition (n=12) and
673 coexistence (n=12) couples was cultivated in *S. aureus* SA2599 filtered supernatant and acetoin was
674 quantified from supernatant after 4 hours of culture. Sterile supernatant was used as control for acetoin
675 degradation. Bars represent the median acetoin concentration \pm 95% CI. Dotted line indicate the initial
676 acetoin concentration. * $P_{adj}<0.05$, ** $P_{adj}<0.01$ Kruskal-Wallis test with Dunn's correction.

677
678 **Figure 5: Monitoring of *P. aeruginosa* growth (A) and acetoin concentration (B) in minimal**
679 **medium supplemented in acetoin.** *P. aeruginosa* PA2600 WT, Δ *acoR* and Δ *aco* strains were grown
680 in M63 medium and 1.5mM acetoin was added every 2 hours, indicated by black arrows.

681 **A.** Cultures were plated on TSA each 2 hours to count bacteria. Points represent the mean bacterial
682 concentration \pm SEM from three independent experiments.

683 **B.** Acetoin was quantified from supernatant each hour. Points represent the mean acetoin concentration
684 \pm SEM from three independent experiments.

685
686

687 **Figure 6: Monitoring of *S. aureus* survival (A), *P. aeruginosa* survival (B) and acetoin**
688 **concentration (C) during long-term co-culture.** *S. aureus* SA2599 was cultivated in presence of
689 *P. aeruginosa* PA2600 WT, Δ *acoR* and Δ *aco* during 5 days. Cultures were plated at J0, J3 and J5 on
690 MSA and cetrimide to count *S. aureus* and *P. aeruginosa* respectively and acetoin was quantified from
691 supernatant.

692 **A, B.** Survival rate was estimated by dividing the bacterial concentration in co-culture by bacterial
693 concentration in monoculture for each bacterium. Bars represent the mean survival rate + SEM from
694 five independent experiments. * $P_{adj}<0.05$, **** $P_{adj}<0.0001$ one-way ANOVA with Dunnett correction (WT
695 vs. condition).

696 **C.** Acetoin concentration was normalized to *S. aureus* counts. Bars represent the median acetoin
697 concentration per 10^6 *S. aureus* \pm 95% CI from five independent experiments. * $P_{adj}<0.05$, ** $P_{adj}<0.01$,
698 Kruskal-Wallis with Dunn's correction.

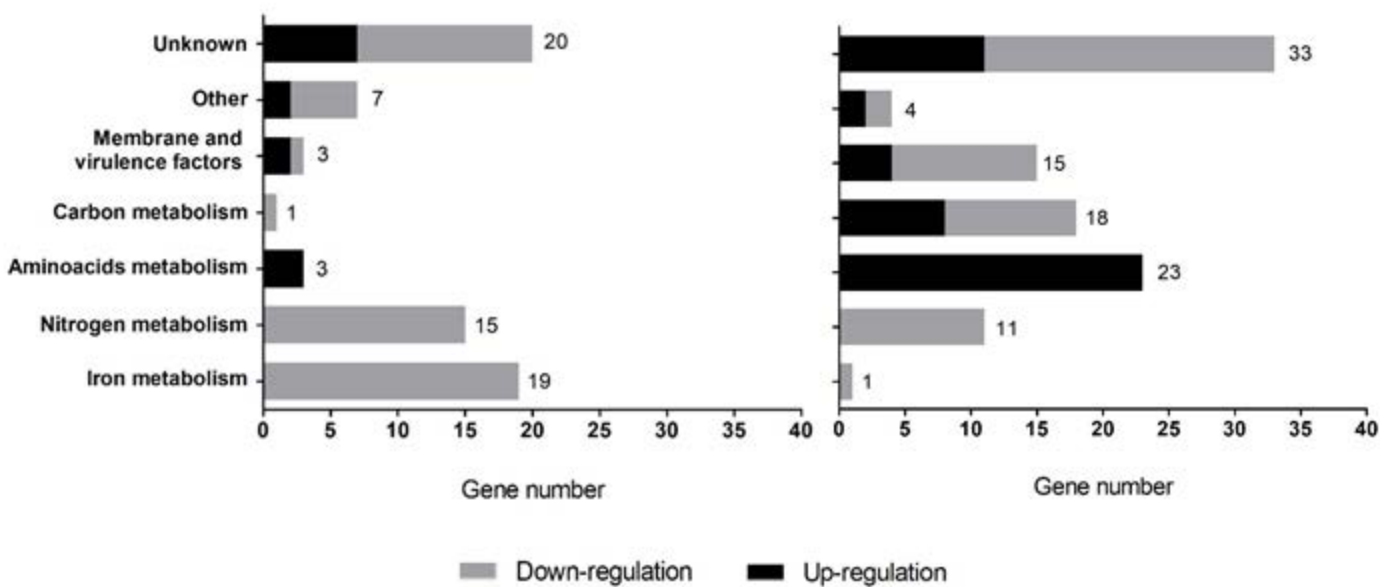
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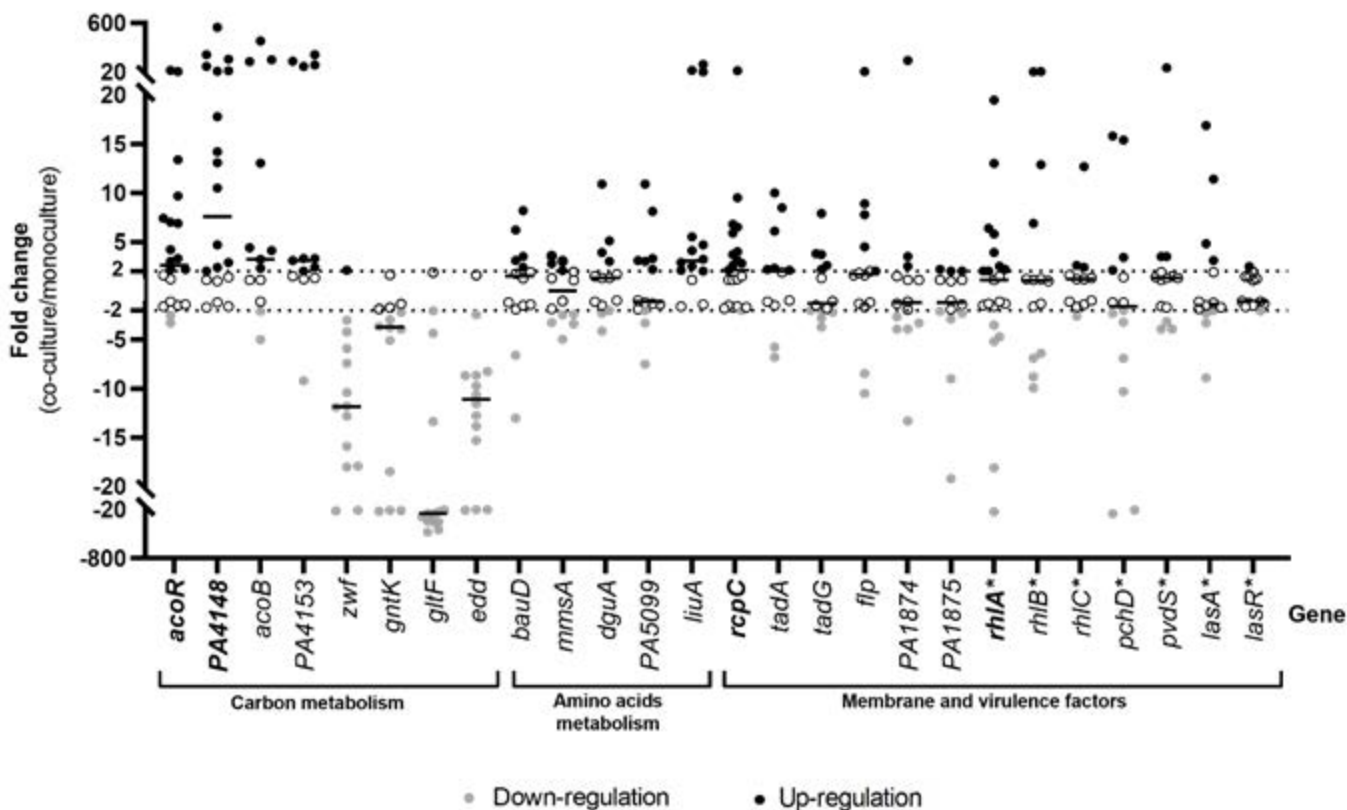
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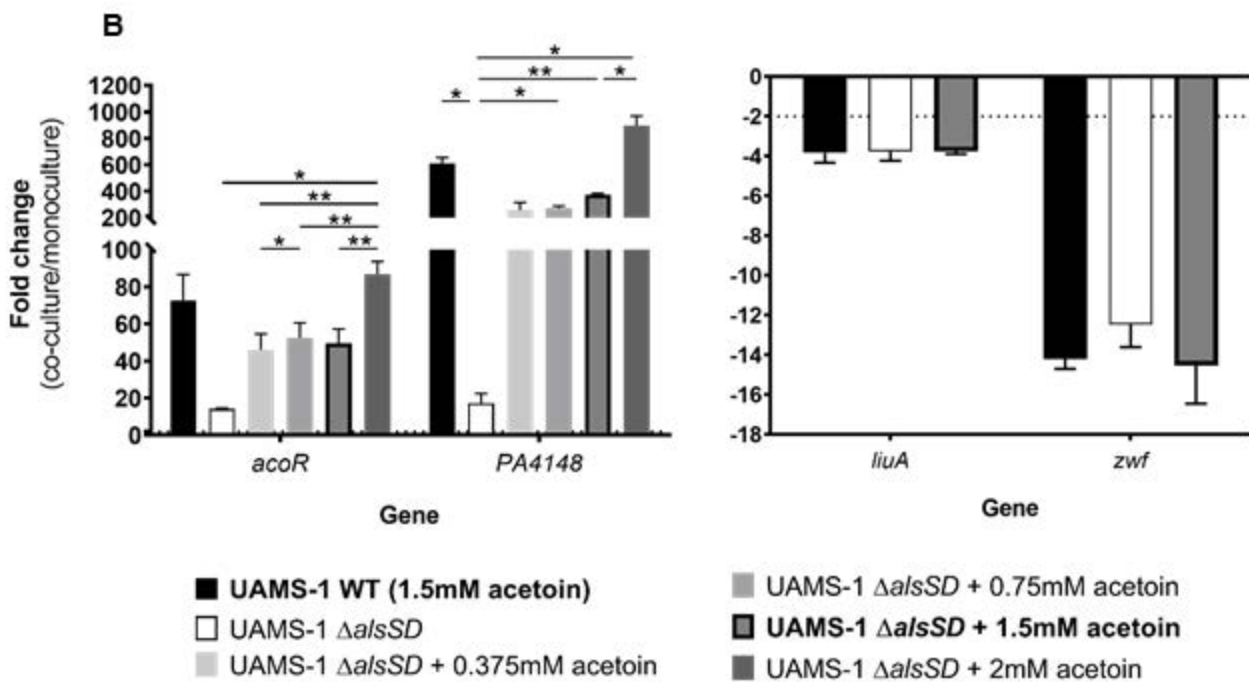
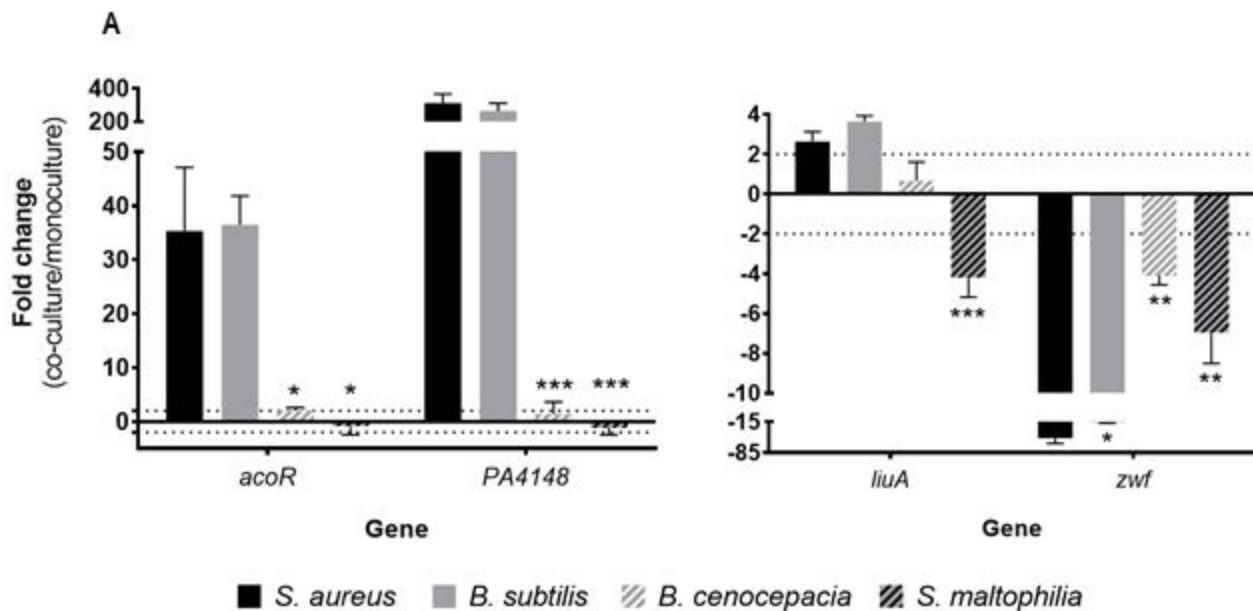
A Competition

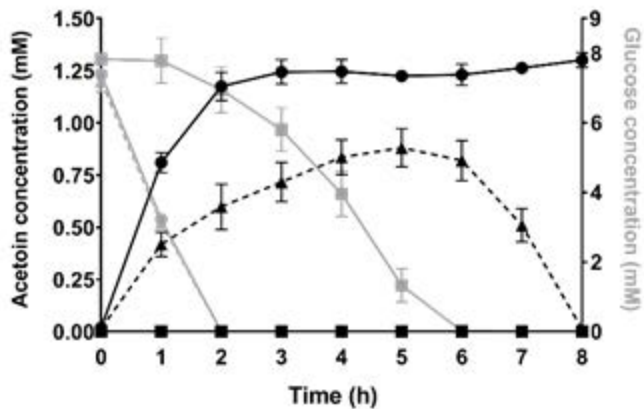
Coexistence



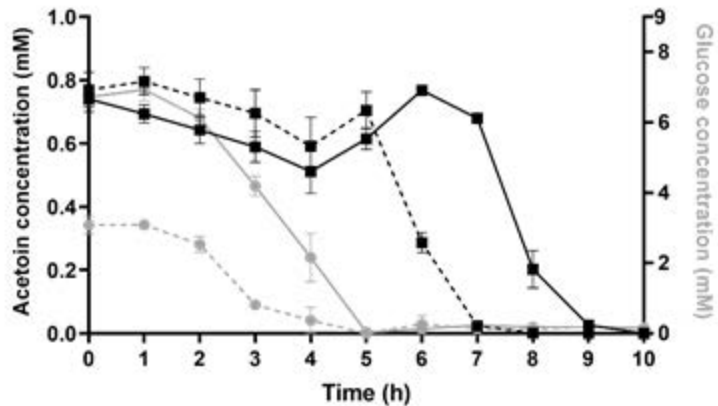
B



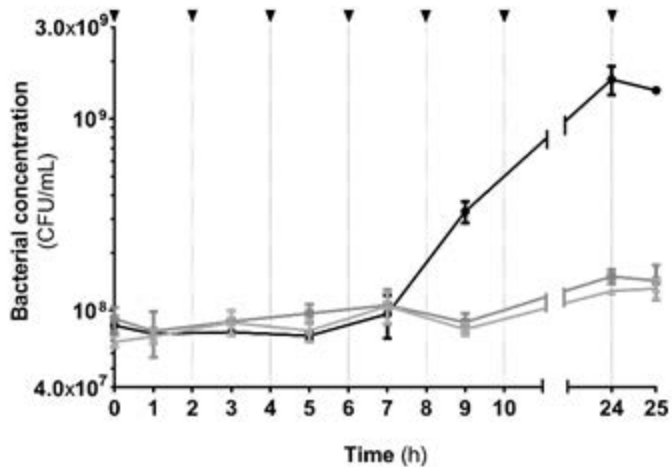


A

- ◆ ● *S. aureus* monoculture
- ■ *P. aeruginosa* monoculture
- ★ ★ Co-culture

B

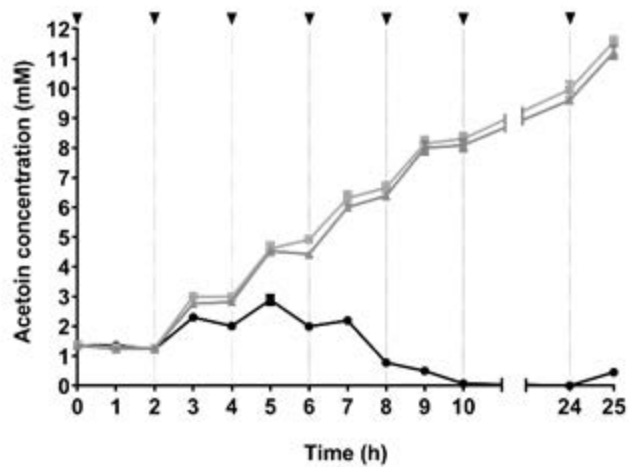
- ● *S. aureus* supernatant
- ● *S. aureus* supernatant + glucose

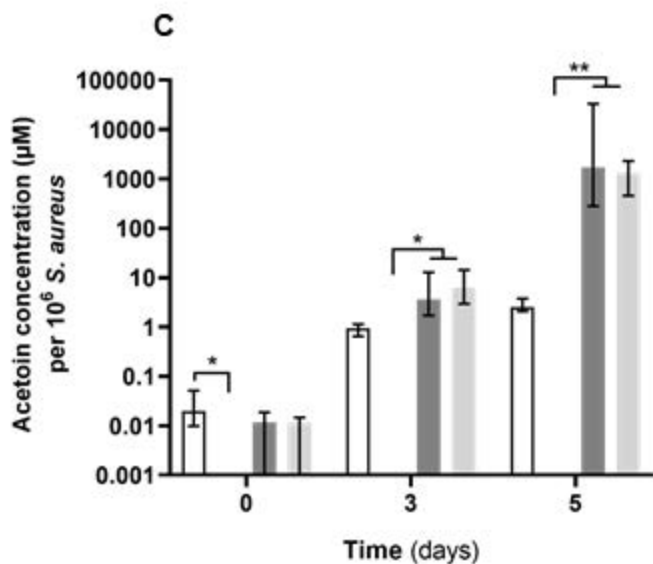
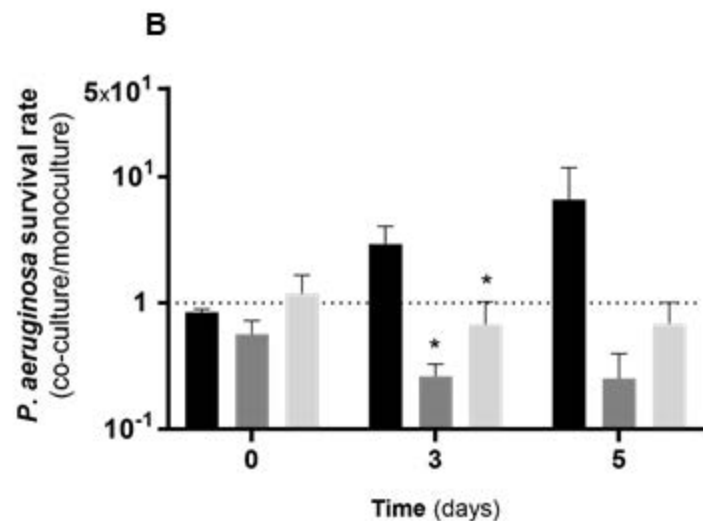
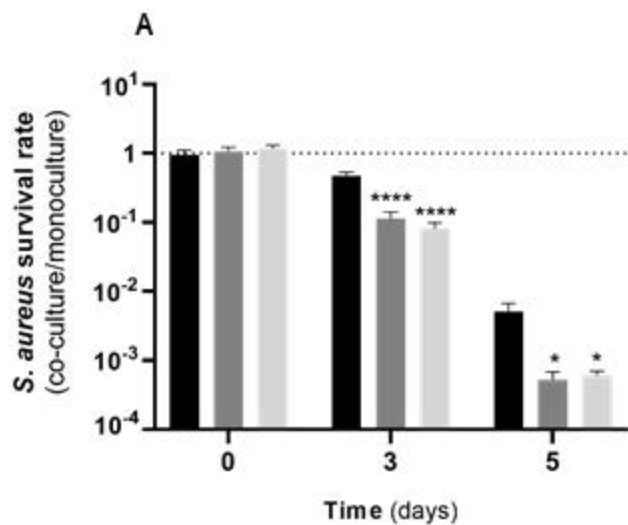
A

● *P. aeruginosa* WT

■ *P. aeruginosa* Δ acoR

▲ *P. aeruginosa* Δ aco

B



□ *S. aureus* monoculture

Co-cultures

■ *P. aeruginosa* WT

■ *P. aeruginosa* Δ *acoR*

■ *P. aeruginosa* Δ *aco*

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

Laura Camus, Paul Briaud, Sylvère Bastien, Sylvie Elsen, Anne Doléans-Jordheim, François Vandenesch and Karen Moreau.

Supplementary data

Materials and methods: Generation of PA2600 knock-out Δ *acoR* and Δ *aco* mutants.

Table S1: Clinical CF strains used in this study.

Table S2: Non-CF strains and plasmids used in this study.

Table S3: Primers used in this study.

Table S4: List of *P. aeruginosa* genes differentially expressed in presence of *S. aureus* in the context of a competitive interaction.

Table S5: List of *P. aeruginosa* genes differentially expressed in presence of *S. aureus* in the context of coexistence.

Figure S1: Schematic representation of the employed methodology.

Figure S2: *P. aeruginosa* metabolic pathways and associated genes up-regulated or down-regulated in coexistence with *S. aureus*.

Figure S3: Acetoin concentration in supernatant of *S. aureus*, *B. subtilis*, *B. cenocepacia* and *S. maltophilia* monocultures or co-cultures with *P. aeruginosa*.

Figure S4: Fold change of *P. aeruginosa* *acoR*, *PA4148*, *liuA* and *zwf* gene expression induced by culture with *S. aureus* or its supernatant.

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

Figure S6: Acetoin concentration in CF sputa from patients.

Figure S7: Acetoin concentration in cultures of *S. aureus* strains from competition and coexistence couples.

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

Figure S9: Growth kinetic of *S. aureus* cultivated in absence or presence of acetoin.

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	CGACGGTATCCAGGTCGATG	<i>PA1874</i>	70
	OLC22-R	GAACCTGACCGTGACCACCT		
	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 AGGGCGATGCCCGGCCGATG	<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	Flavo-hemoprotein	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
fecl_3	PA4896	PA4896		Q9HUR7	Putative RNA polymerase sigma factor Fecl3	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 Q9I4I9	Putative protein YybH	Unknown	2.16	1.03E-03	2.73	1.94E-05		
ilvA_1	PA1326	ilvA	j Q9I4I8	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 1 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 PuuA	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	Psi 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	Flavoheporein	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, hydrazone 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	Q9HWM6	Dihydrolipoyllysine-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		Q9HTI1	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 > |2log₂| 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). Functional classification was performed thanks to KEGG database and literature.

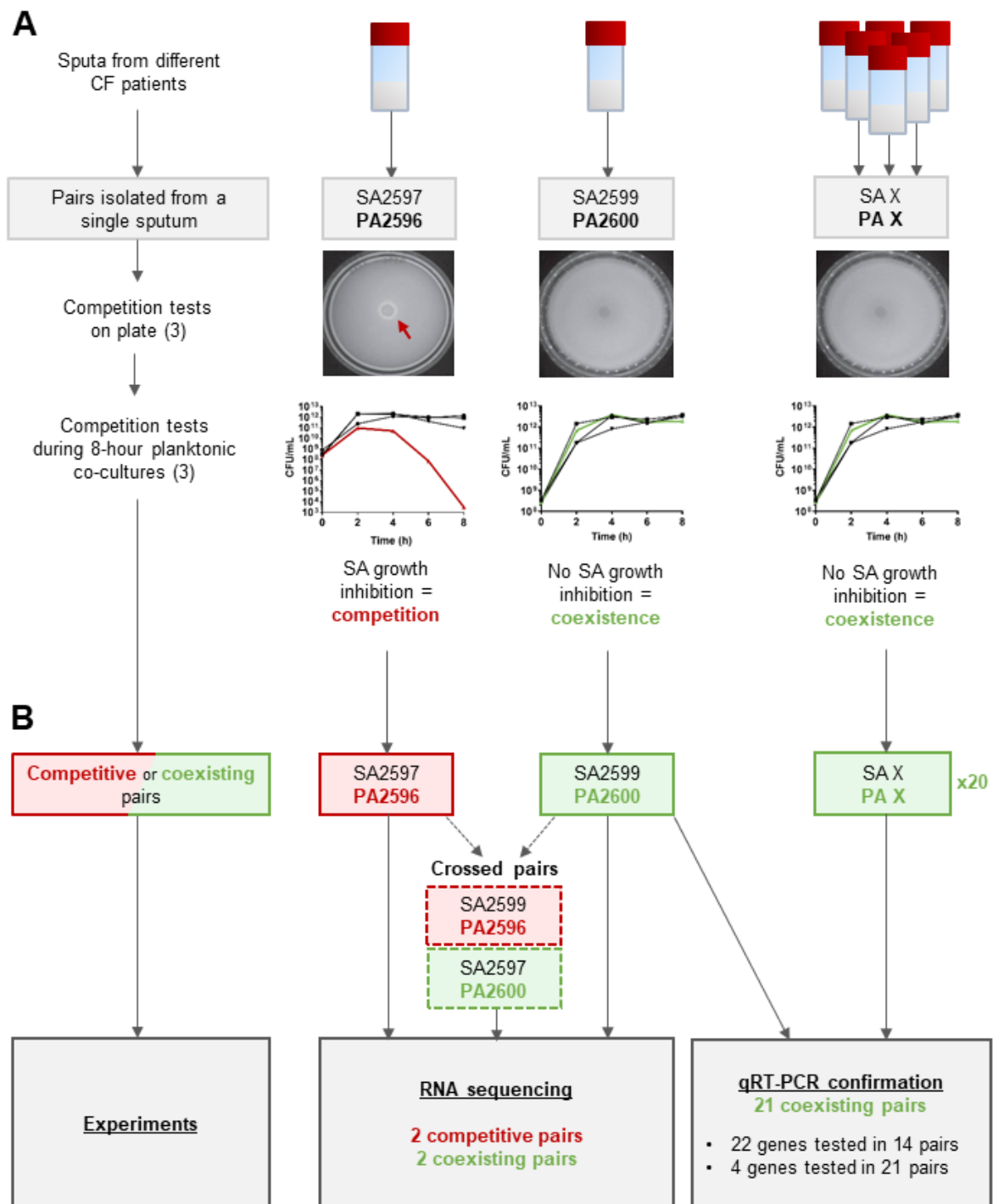


Figure S1: Schematic representation of the employed methodology.

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).

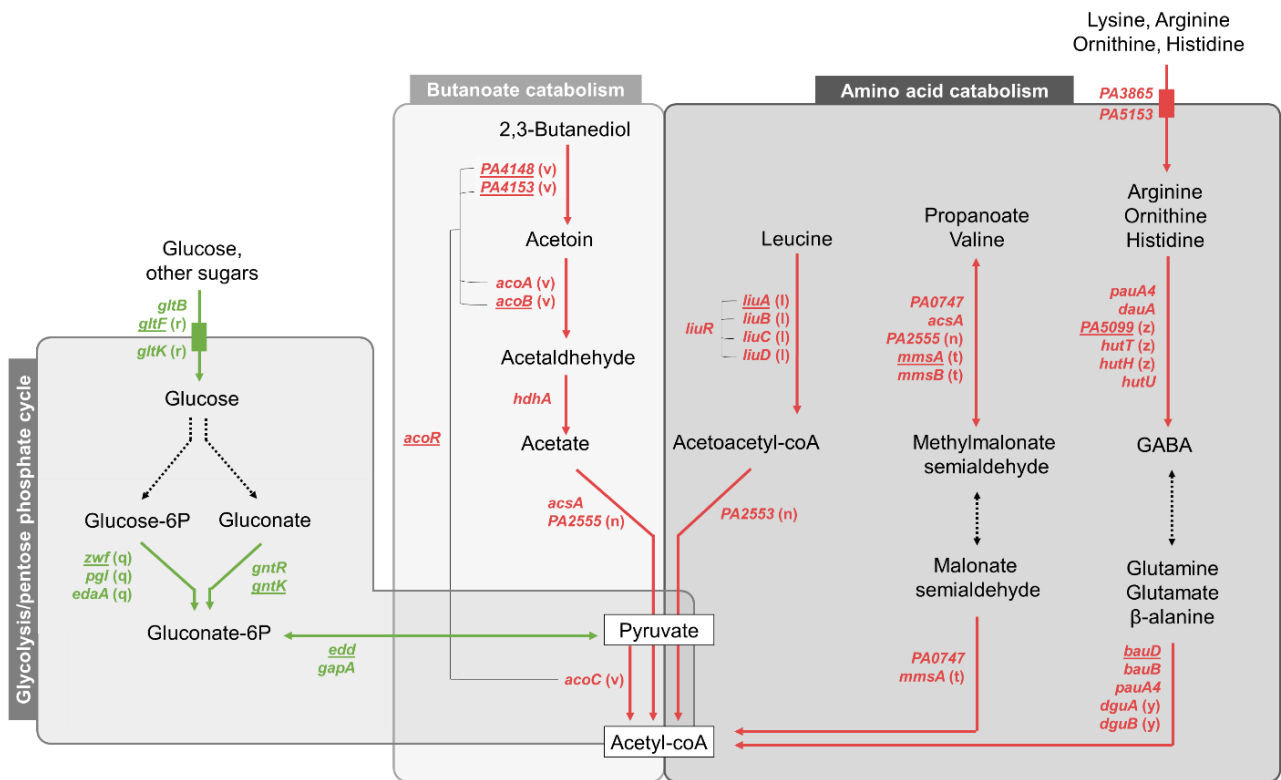


Figure S2: *P. aeruginosa* metabolic pathways and associated genes up-regulated (red) or down-regulated (green) in coexistence with *S. aureus*. PA2600 coexistence strain was cultivated in absence or presence of SA2599 or SA2597. RNAs were extracted after 4 hours of culture and RNAseq analysis was performed. 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.

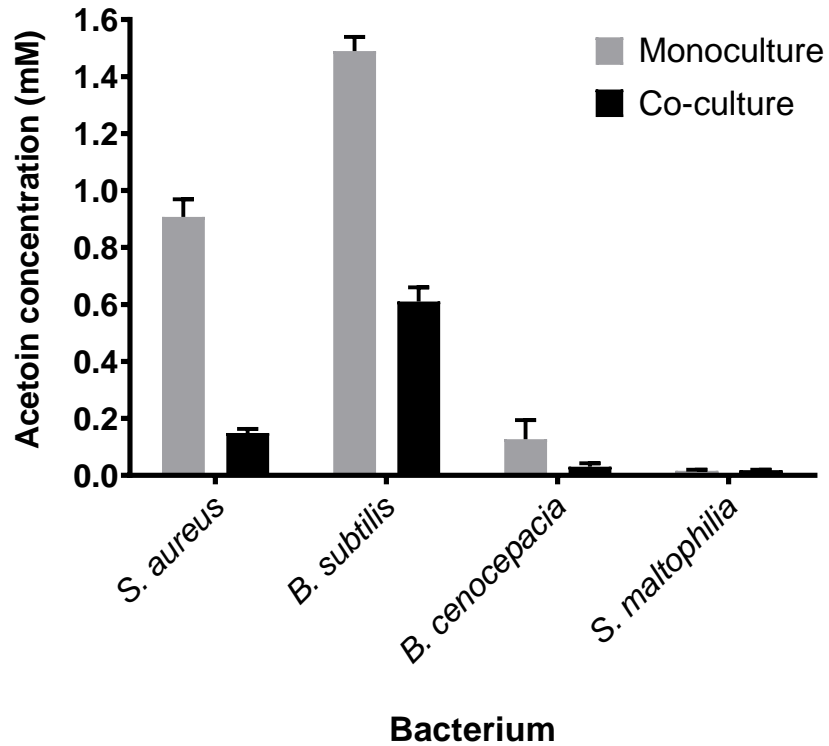


Figure S3: Acetoin concentration in supernatant of *S. aureus* SA2599, *B. subtilis*, *B. cenocepacia* and *S. maltophilia* monocultures (grey bars) or co-cultures with *P. aeruginosa* PA2600 (black bars). Acetoin was quantified from supernatant after 4h of culture. Bars represent the mean acetoin concentration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

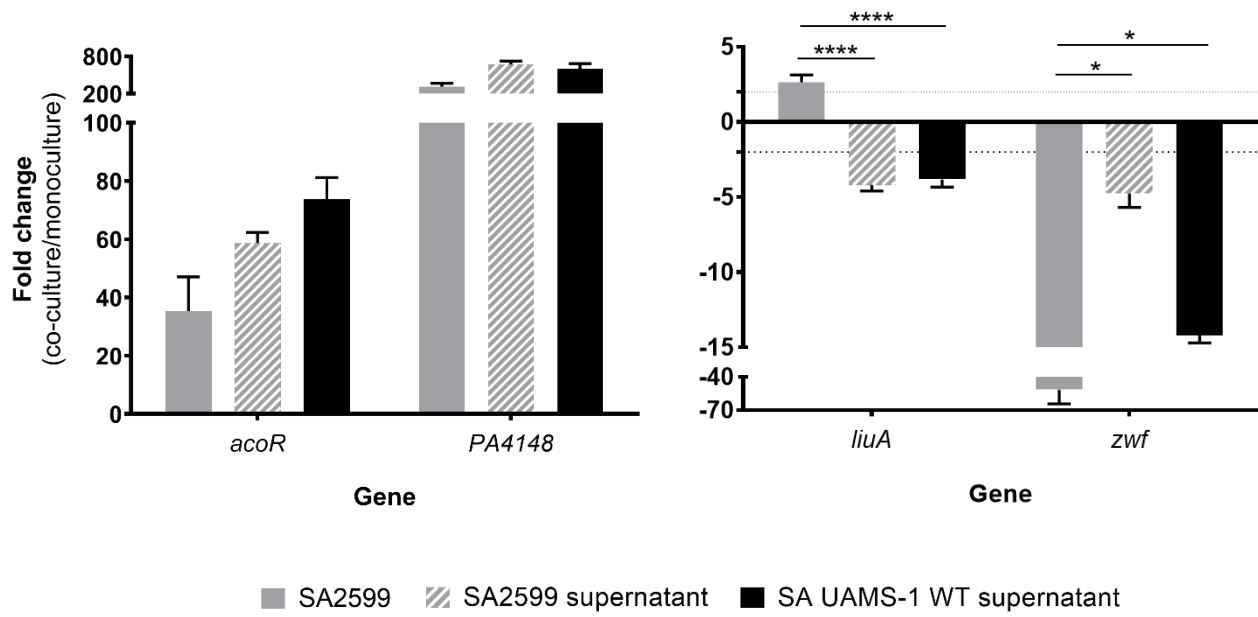


Figure S4: Fold change of *P. aeruginosa acoR*, *PA4148*, *liuA* and *zwf* gene expression induced by culture with *S. aureus* (grey bars) or its supernatant (hatched and black bars). *P. aeruginosa* PA2600 strain was cultivated in the absence or presence of *S. aureus* SA2599 or filtered supernatant 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.**

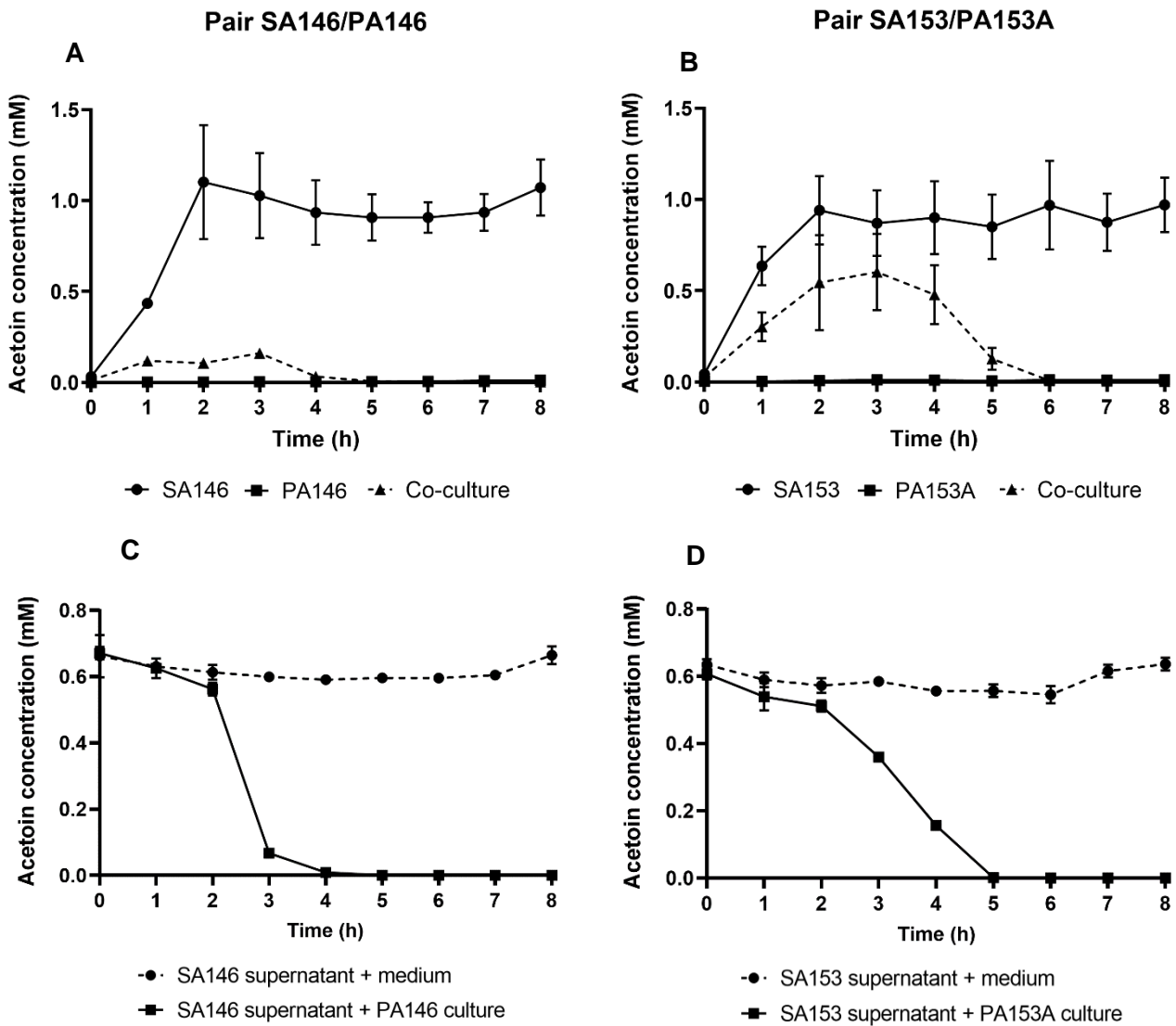


Figure S5: Monitoring of acetoin concentration in *S. aureus* and *P. aeruginosa* monocultures or co-culture (A,B) or in *S. aureus* supernatant inoculated with *P. aeruginosa* (B,C), for the pairs SA146/PA146 (A,C) and SA153/PA153A (B,D).

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.

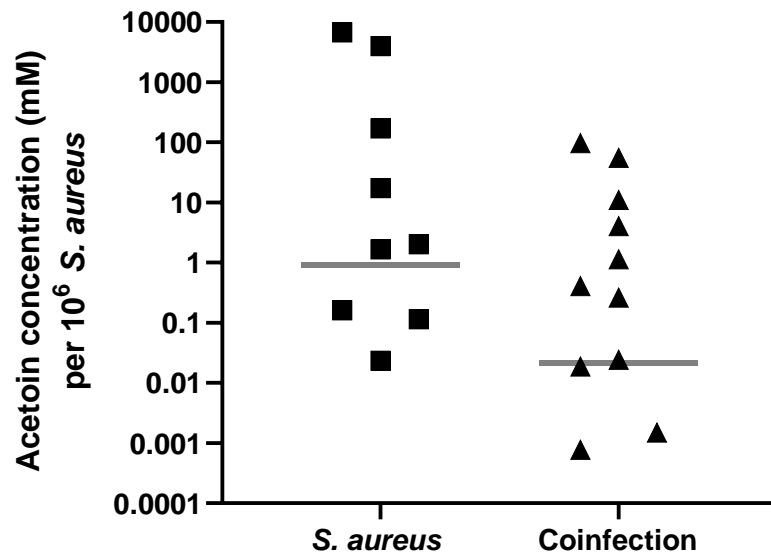


Figure S6: Acetoin concentration in CF sputa from patients. Sputa from *S. aureus* mono-infected patients (n=9) or *S. aureus* and *P. aeruginosa* co-infected patients (n=11) were gathered and acetoin concentration was quantified. Bars represent the median acetoin concentration normalized on *S. aureus*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

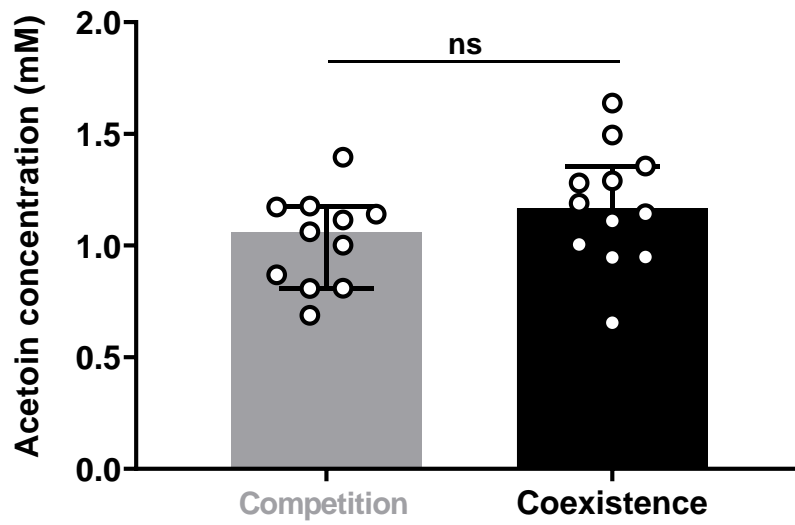


Figure S7: Acetoin concentration in cultures of *S. aureus* strains from competition and coexistence couples. Each *S. aureus* strain from competition (n=11) and coexistence (n=12) couples was cultivated for 6 hours in BHI and acetoin was dosed from supernatant. Bars represent the median acetoin concentration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

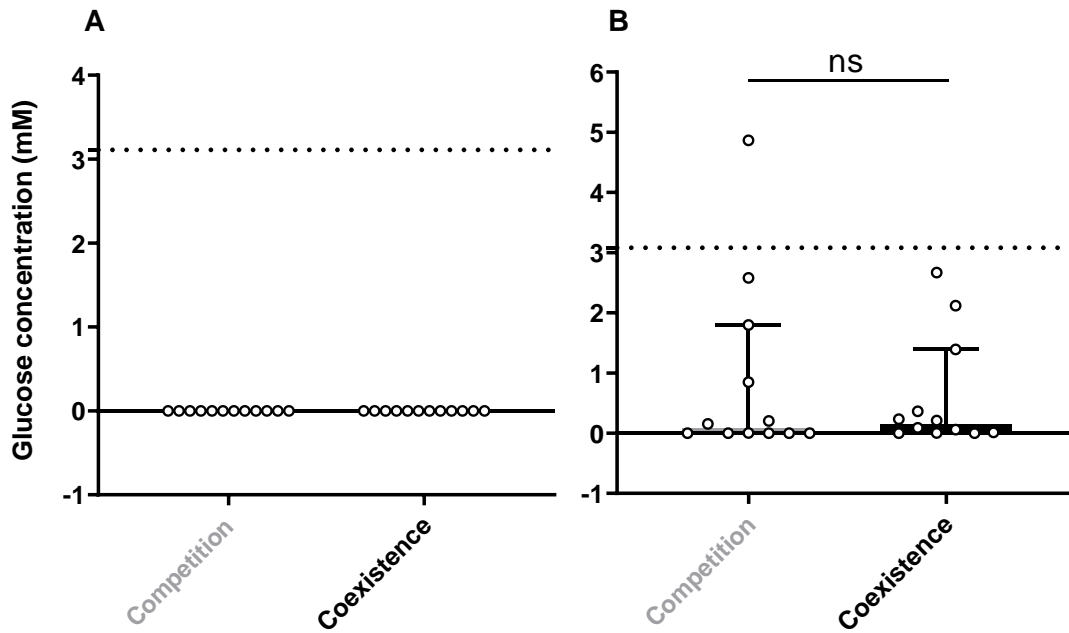


Figure S8: Glucose concentrations in cultures of *S. aureus* (A) and *P. aeruginosa* (B) strains from competition and coexistence pairs.

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.

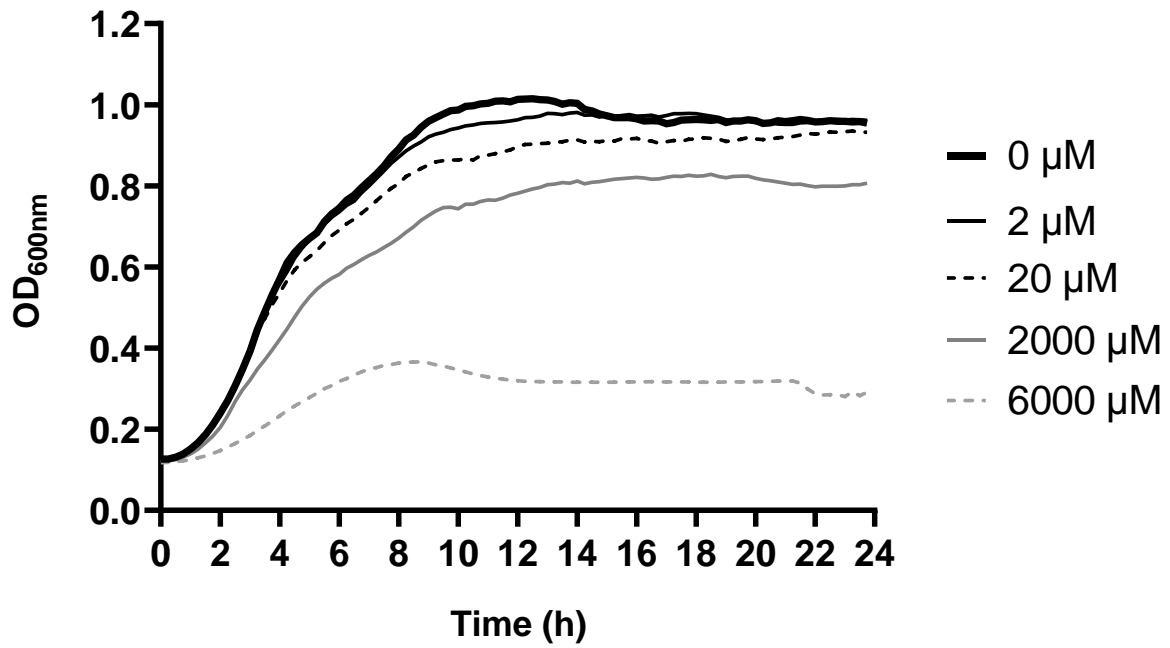


Figure S9: Growth kinetic of *S. aureus* cultivated in absence or presence of acetoin. SA2599 was cultivated during 24h in the absence of acetoin or in the presence of acetoin in different proportions ranging from 0.2μM to 6000μM per 10⁶ *S. aureus*. Lines represent the mean optical density of three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

Supplementary data references

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

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