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

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Abstract

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

Introduction

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

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

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

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

Materials and Methods

Bacterial strains

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

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

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

The knock-out Δ*acoR* and Δ*aco* mutants were generated in *P. aeruginosa* PA2600 strain via allelic exchange thanks to suicide plasmids pEXG2Δ*acoR* and pEXG2Δ*aco* constructed as described previously (21, 22). Gentamicin (Euromedex) was used at final concentrations of 50µg/ml. Detailed protocols are given in supplementary data.

Cultures conditions

Strains were grown in monoculture or co-culture in Brain-Heart Infusion (BHI) as described by Briaud *et al*. (18). Briefly, *S. aureus*, *P. aeruginosa*, *Burkholderia cenocepacia*, *Stenotrophomonas maltophilia* and *Bacillus subtilis* overnight precultures were diluted to OD_{600} of 0.1 in BHI and grown 2h30 at 37°C and 200rpm. Cultures were then diluted to OD_{600} of 2 in fresh medium and 10ml were mixed with 10ml BHI for monocultures. Co-cultures were performed by mixing 10ml of *P. aeruginosa* suspension with 10ml of *S. aureus*, *B. cenocepacia*, *S. maltophilia* or *B. subtilis* suspension. For supernatant exposure, 10ml of *S. aureus* supernatant from a 4-hours culture were filtered on 0.22µM filter and added to 10ml of *P. aeruginosa* suspension. Cultures were grown for 8 hours at 200rpm and 37°C for transcriptomic studies. Long-term survival assays were performed by extending incubation time of *S. aureus* and *P. aeruginosa* mono- and co-cultures up to five days. Plating at day 0, 3 and 5 were performed on mannitol salt agar (MSA, BBL™ Difco) and cetrimide (Difco™) for *S. aureus* or *P. aeruginosa* counts, respectively. For growth monitoring in presence of acetoin, minimal medium M63 (76mM $(NH_4)_2SO_4$, 500mM KH_2PO_4, 9µM $Fe.SO_4.7H_2O$, 1mM $MgSO_4.7H_2O$) was inoculated with *P. aeruginosa* to OD_{600} of 0.1 and incubated for 25h at 37°C and 200rpm. Every two hours during 10 hours and at t=24h, acetoin was added to obtain a final concentration of 1.5mM in the culture. Plating on TSA (Tryptic Soy Agar) was performed at t=0, one hour after each acetoin addition and at t=24h.

Genome sequencing and annotation

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

Transcriptomic analysis

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

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

Acetoin and glucose dosages

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

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

Statistical analysis

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

Results

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

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

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

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

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

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

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

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

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

P. aeruginosa aco system is induced by _S. aureus_ acetoin

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

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

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

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

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

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

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

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

As *P. aeruginosa* catabolizes acetoin when medium is glucose-depleted, we tested the effect of acetoin on PA2600 growth in minimal medium M63 (containing no glucose or amino acids) supplemented with 1.5mM acetoin every 2 hours **(Fig. 5)**. *P. aeruginosa* was able to grow up to 1.6×10^9 CFU/ml after 24h of culture with acetoin as sole carbon source while PA2600 Δ*acoR* and Δ*aco* mutants grew significantly less, reaching a maximum cell concentration of 1.5×10^8 UFC/ml at 24h **(Fig. 5A)**. In parallel, we quantified acetoin concentration. We observed an accumulation of acetoin all along the experiment in presence of PA2600 Δ*acoR* and Δ*aco* mutants. For WT strain, the accumulation of acetoin reached its maximum at 5h of culture and afterwards slowly decreased to reach undetectable values at 10h of culture, demonstrating the consumption of acetoin in such conditions **(Fig. 5B)**. A delay of 3h between the start of acetoin catabolism and the growth augmentation of WT strain was noticed, and might be due to metabolism adaptation. Acetoin catabolism nonetheless promoted a 10-fold increased growth of *P. aeruginosa* during extended culture in glucose depleted medium.

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

a long term culture (5 days) in BHI medium. We determined the survival rate of *S. aureus* co-cultivated with *P. aeruginosa* in comparison to monoculture **(Fig. 6A)**. Co-culture with the WT strain of *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 5 days. *S. aureus* survival thus appears to be highly affected by long-term co-culture with *P. aeruginosa*, even if the strains stably coexist during shorter spans of culture (18). Interaction state with *S. aureus* thus seems to rely on nutritional conditions, a fact already observed elsewhere (35, 36). However, the survival rate of *S. aureus* was even lower during co-culture with *P. aeruginosa* Δ*acoR* and Δ*aco* 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 4 to 10 times lower when *P. aeruginosa* was not able to catabolize acetoin, in comparison to co-culture with a strain that efficiently used the molecule. These results suggest that accumulation of acetoin may impact *S. aureus* survival during co-culture with *P. aeruginosa*. In parallel, we determined the survival rate of *P. aeruginosa* **(Fig. 6B)**. We noticed that in such conditions, *P. aeruginosa* WT strain presented a growth advantage in co-culture, with a maximum of 6.6-fold increase of *P. aeruginosa* population after 5 days in presence of *S. aureus* in comparison to monoculture **(Fig. 6B)**. The opposite effect was observed for *aco* mutant strains, as their populations were reduced by 75% for Δ*acoR* and 32% for Δ*aco* after 3 days of co-culture in comparison to monoculture, confirming the role of acetoin catabolism in favouring *P. aeruginosa* growth and survival.

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

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

Discussion

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

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

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

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

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

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

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

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

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

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

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Conflict of interest

All authors declare no competing interests.

Ethical statement

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

References

1. Sibley CD, Rabin H, Surette MG. 2006. Cystic fibrosis: a polymicrobial infectious disease. Future Microbiol 1:53–61.

2. Guss AM, Roeselers G, Newton ILG, Young CR, Klepac-Ceraj V, Lory S, Cavanaugh CM. 2011. Phylogenetic and metabolic diversity of bacteria associated with cystic fibrosis. ISME J 5:20–29.

3. Peters BM, Jabra-Rizk MA, O'May GA, Costerton JW, Shirtliff ME. 2012. Polymicrobial interactions: impact on pathogenesis and human disease. Clin Microbiol Rev 25:193–213.

4. Murray JL, Connell JL, Stacy A, Turner KH, Whiteley M. 2014. Mechanisms of synergy in polymicrobial infections. J Microbiol Seoul Korea 52:188–199.

5. Abisado RG, Benomar S, Klaus JR, Dandekar AA, Chandler JR. 2018. Bacterial Quorum Sensing and Microbial Community Interactions. mBio 9.

6. Tashiro Y, Yawata Y, Toyofuku M, Uchiyama H, Nomura N. 2013. Interspecies interaction between Pseudomonas aeruginosa and other microorganisms. Microbes Environ 28:13–24.

7. Hotterbeekx A, Kumar-Singh S, Goossens H, Malhotra-Kumar S. 2017. In vivo and In vitro Interactions between Pseudomonas aeruginosa and Staphylococcus spp. Front Cell Infect Microbiol 7.

8. Serra R, Grande R, Butrico L, Rossi A, Settimio UF, Caroleo B, Amato B, Gallelli L, de Franciscis S. 2015. Chronic wound infections: the role of Pseudomonas aeruginosa and Staphylococcus aureus. Expert Rev Anti Infect Ther 13:605–613.

9. O'Brien TJ, Welch M. 2019. Recapitulation of polymicrobial communities associated with cystic fibrosis airway infections: a perspective. Future Microbiol 14:1437–1450.

10. Baldan R, Cigana C, Testa F, Bianconi I, De Simone M, Pellin D, Di Serio C, Bragonzi A, Cirillo DM. 2014. Adaptation of Pseudomonas aeruginosa in Cystic Fibrosis airways influences virulence of Staphylococcus aureus in vitro and murine models of co-infection. PloS One 9:e89614.

11. Smith EE, Buckley DG, Wu Z, Saenphimmachak C, Hoffman LR, D'Argenio DA, Miller SI, Ramsey BW, Speert DP, Moskowitz SM, Burns JL, Kaul R, Olson MV. 2006. Genetic adaptation by Pseudomonas aeruginosa to the airways of cystic fibrosis patients. Proc Natl Acad Sci U S A 103:8487–8492.

12. Hoffman LR, Kulasekara HD, Emerson J, Houston LS, Burns JL, Ramsey BW, Miller SI. 2009. Pseudomonas aeruginosa lasR mutants are associated with cystic fibrosis lung disease progression. J Cyst Fibros Off J Eur Cyst Fibros Soc 8:66–70.

13. Folkesson A, Jelsbak L, Yang L, Johansen HK, Ciofu O, Høiby N, Molin S. 2012. Adaptation of Pseudomonas aeruginosa to the cystic fibrosis airway: an evolutionary perspective. Nat Rev Microbiol 10:841–851.

14. Marvig RL, Sommer LM, Molin S, Johansen HK. 2015. Convergent evolution and adaptation of Pseudomonas aeruginosa within patients with cystic fibrosis. Nat Genet 47:57–64.

15. Limoli DH, Whitfield GB, Kitao T, Ivey ML, Davis MR, Grahl N, Hogan DA, Rahme LG, Howell PL, O'Toole GA, Goldberg JB. 2017. Pseudomonas aeruginosa Alginate Overproduction Promotes Coexistence with Staphylococcus aureus in a Model of Cystic Fibrosis Respiratory Infection. mBio 8.

16. La Rosa R, Johansen HK, Molin S. 2019. Adapting to the Airways: Metabolic Requirements of Pseudomonas aeruginosa during the Infection of Cystic Fibrosis Patients. Metabolites 9.

17. Michelsen CF, Christensen A-MJ, Bojer MS, Høiby N, Ingmer H, Jelsbak L. 2014. Staphylococcus aureus alters growth activity, autolysis, and antibiotic tolerance in a human host-adapted Pseudomonas aeruginosa lineage. J Bacteriol 196:3903–3911.

18. Briaud P, Camus L, Bastien S, Doléans-Jordheim A, Vandenesch F, Moreau K. 2019. Coexistence with Pseudomonas aeruginosa alters Staphylococcus aureus transcriptome, antibiotic resistance and internalization into epithelial cells. Sci Rep 9.

19. Briaud P, Bastien S, Camus L, Boyadijian M, Reix P, Mainguy C, Vandenesch F, Doléans-Jordheim A, Moreau K. 2020. Impact of coexistence phenotype between Staphylococcus aureus

and Pseudomonas aeruginosa isolates on clinical outcomes among Cystic Fibrosis patients. Front Cell Infect Microbiol 10.

20. Frydenlund Michelsen C, Hossein Khademi SM, Krogh Johansen H, Ingmer H, Dorrestein PC, Jelsbak L. 2016. Evolution of metabolic divergence in Pseudomonas aeruginosa during long-term infection facilitates a proto-cooperative interspecies interaction. ISME J 10:1323–1336.

21. Carriel D, Simon Garcia P, Castelli F, Lamourette P, Fenaille F, Brochier-Armanet C, Elsen S, Gutsche I. 2018. A Novel Subfamily of Bacterial AAT-Fold Basic Amino Acid Decarboxylases and Functional Characterization of Its First Representative: Pseudomonas aeruginosa LdcA. Genome Biol Evol 10:3058–3075.

22. Rietsch A, Vallet-Gely I, Dove SL, Mekalanos JJ. 2005. ExsE, a secreted regulator of type III secretion genes in Pseudomonas aeruginosa. Proc Natl Acad Sci U S A 102:8006–8011.

23. Page AJ, Cummins CA, Hunt M, Wong VK, Reuter S, Holden MTG, Fookes M, Falush D, Keane JA, Parkhill J. 2015. Roary: rapid large-scale prokaryote pan genome analysis. Bioinforma Oxf Engl 31:3691–3693.

24. Nicholson WL. 2008. The Bacillus subtilis ydjL (bdhA) Gene Encodes Acetoin Reductase/2,3-Butanediol Dehydrogenase. Appl Environ Microbiol 74:6832–6838.

25. Lessie TG, Phibbs PV. 1984. Alternative pathways of carbohydrate utilization in pseudomonads. Annu Rev Microbiol 38:359–388.

26. Chevalier S, Bouffartigues E, Bodilis J, Maillot O, Lesouhaitier O, Feuilloley MGJ, Orange N, Dufour A, Cornelis P. 2017. Structure, function and regulation of Pseudomonas aeruginosa porins. FEMS Microbiol Rev 41:698–722.

27. Liu Q, Liu Y, Kang Z, Xiao D, Gao C, Xu P, Ma C. 2018. 2,3-Butanediol catabolism in *Pseudomonas aeruginosa* PAO1: 2,3-Butanediol catabolism in *Pseudomonas aeruginosa*. Environ Microbiol 20:3927–3940.

28. Xiao Z, Xu P. 2007. Acetoin Metabolism in Bacteria. Crit Rev Microbiol 33:127–140.

29. Gade N, Negi SS, Jindal A, Gaikwad U, Das P, Bhargava A. 2016. Dual Lower Respiratory Tract Infection by Burkholderia cepacia and Acinetobacter baumannii in A Neonate: A Case Report. J Clin Diagn Res JCDR 10:DD01–DD03.

30. Amoli RI, Nowroozi J, Sabokbar A, Rajabniya R. 2017. Isolation of Stenotrophomonas maltophilia from clinical samples: An investigation of patterns motility and production of melanin pigment. Asian Pac J Trop Biomed 7:826–830.

31. Dryahina K, Sovová K, Nemec A, Španěl P. 2016. Differentiation of pulmonary bacterial pathogens in cystic fibrosis by volatile metabolites emitted by their in vitro cultures: Pseudomonas aeruginosa, Staphylococcus aureus, Stenotrophomonas maltophilia and the Burkholderia cepacia complex. J Breath Res 10:037102.

32. Tsang LH, Cassat JE, Shaw LN, Beenken KE, Smeltzer MS. 2008. Factors Contributing to the Biofilm-Deficient Phenotype of Staphylococcus aureus sarA Mutants. PLoS ONE 3:e3361.

33. Chaudhari SS, Thomas VC, Sadykov MR, Bose JL, Ahn DJ, Zimmerman MC, Bayles KW. 2016. The LysR-type transcriptional regulator, CidR, regulates stationary phase cell death in *Staphylococcus aureus*: Metabolic control of cell death in *S. aureus*. Mol Microbiol 101:942–953.

34. Thomas VC, Sadykov MR, Chaudhari SS, Jones J, Endres JL, Widhelm TJ, Ahn J-S, Jawa RS, Zimmerman MC, Bayles KW. 2014. A Central Role for Carbon-Overflow Pathways in the Modulation of Bacterial Cell Death. PLoS Pathog 10:e1004205.

35. Miller CL, Van Laar TA, Chen T, Karna SLR, Chen P, You T, Leung KP. 2017. Global transcriptome responses including small RNAs during mixed-species interactions with methicillin-resistant Staphylococcus aureus and Pseudomonas aeruginosa. MicrobiologyOpen 6.

36. Mashburn LM, Jett AM, Akins DR, Whiteley M. 2005. Staphylococcus aureus serves as an iron source for Pseudomonas aeruginosa during in vivo coculture. J Bacteriol 187:554–566.

37. Visca P, Imperi F. 2018. An essential transcriptional regulator: the case of Pseudomonas aeruginosa Fur. Future Microbiol 13:853–856.

38. Cornelis P, Matthijs S, Van Oeffelen L. 2009. Iron uptake regulation in Pseudomonas aeruginosa. Biometals Int J Role Met Ions Biol Biochem Med 22:15–22.

39. Ochsner UA, Wilderman PJ, Vasil AI, Vasil ML. 2002. GeneChip expression analysis of the iron starvation response in Pseudomonas aeruginosa: identification of novel pyoverdine biosynthesis genes. Mol Microbiol 45:1277–1287.

40. Tognon M, Köhler T, Luscher A, van Delden C. 2019. Transcriptional profiling of Pseudomonas aeruginosa and Staphylococcus aureus during in vitro co-culture. BMC Genomics 20:30.

41. Španěl P, Sovová K, Dryahina K, Doušová T, Dřevínek P, Smith D. 2016. Do linear logistic model analyses of volatile biomarkers in exhaled breath of cystic fibrosis patients reliably indicate *Pseudomonas aeruginosa* infection? J Breath Res 10:036013.

42. Baishya J, Wakeman CA. 2019. Selective pressures during chronic infection drive microbial competition and cooperation. NPJ Biofilms Microbiomes 5:16.

43. Hoffman LR, Richardson AR, Houston LS, Kulasekara HD, Martens-Habbena W, Klausen M, Burns JL, Stahl DA, Hassett DJ, Fang FC, Miller SI. 2010. Nutrient availability as a mechanism for selection of antibiotic tolerant Pseudomonas aeruginosa within the CF airway. PLoS Pathog 6:e1000712.

44. Hoffman LR, Déziel E, D'Argenio DA, Lépine F, Emerson J, McNamara S, Gibson RL, Ramsey BW, Miller SI. 2006. Selection for Staphylococcus aureus small-colony variants due to growth in the presence of Pseudomonas aeruginosa. Proc Natl Acad Sci U S A 103:19890–19895.

Figure legends

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

A. Number of under-expressed (grey bars) and over-expressed (black bars) genes of *P. aeruginosa* during co-culture with *S. aureus* for competitive (left) or coexisting (right) pairs. PA2596 competition and PA2600 coexistence strains were cultivated in absence or presence of SA2597 or SA2599 as described in Fig. S1. RNAs were extracted after 4 hours of culture and the 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. Functional classification was performed thanks to KEGG database and literature.

B. Fold change of 26 *P. aeruginosa* gene expression induced by co-culture with *S. aureus* during coexistence interaction. Twenty-one *S. aureus-P. aeruginosa* coexisting pairs were isolated from separate CF sputa recovered from 20 different patients. Each *P. aeruginosa* strain was cultivated in absence or presence of its co-isolated *S. aureus* strain. RNAs were extracted after 4 hours of culture and gene expression was assayed by RT-qPCR. A gene was considered as differentially expressed when the Fold Change (FC) was > |2|, indicated by dotted lines. Black lines indicate the median. Genes were tested in 14 (regular genes) or 21 (bold genes) strains. List of used strains is shown in Table S1. Genes annotated with (*) were not identified as dysregulated during the RNAseq experiment.

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

A. Fold changes induced by co-culture with *S. aureus* (black bars), *B. subtilis* (grey bars), *B. cenocepacia* (hatched white bars) or *S. maltophilia* (hatched black bars). *P. aeruginosa* PA2600 strain was cultivated in absence or presence of *S. aureus* SA2599, *B. subtilis, B. cenocepacia* or *S. maltophilia*. 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. Dotted lines indicate a fold change = |2|. $*P_{adj}<0.05$, $**P_{adj}<0.01$, $***P_{adj}<0.001$ ANOVA with Dunnett's correction (*S. aureus vs*. condition).

B. Fold changes induced by culture in supernatant of *S. aureus* UAMS1 wild type (WT, black bars), Δ*alsSD* mutant (white bars), Δ*alsSD* mutant complemented with increasing acetoin concentrations (grey bars). *P. aeruginosa* PA2600 strain was cultivated in absence or presence of filtered supernatant from *S. aureus* UAMS-1 WT, Δ*alsSD* or Δ*alsSD* complemented with acetoin concentrations ranging from 0.375mM to 2mM. 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. Dotted line indicates a fold change = |2|. *P_{adj}<0.05, **P_{adj}<0.01 ANOVA with Tukey's correction.

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

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

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

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

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

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

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

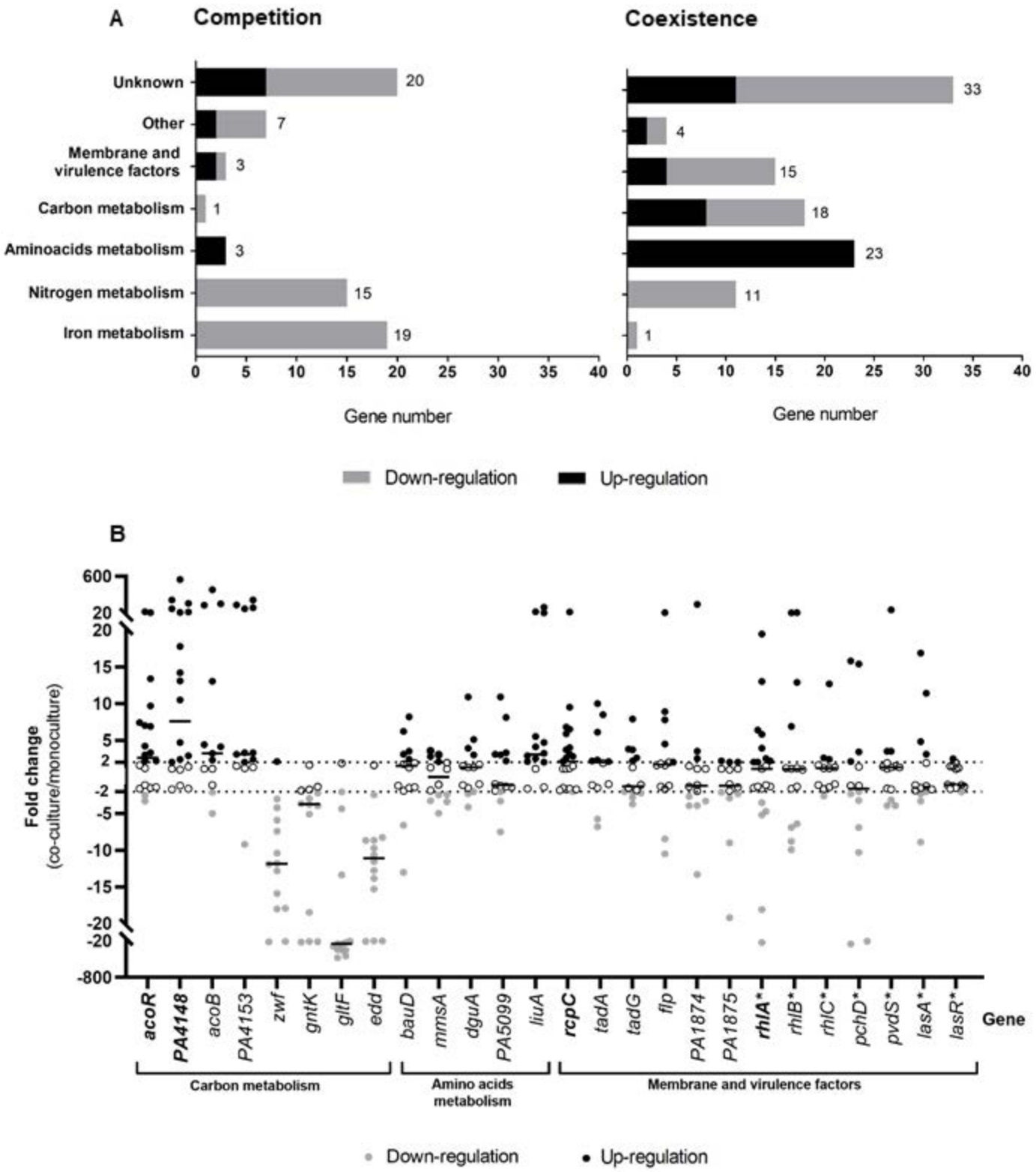
A. Cultures were plated on TSA each 2 hours to count bacteria. Points represent the mean bacterial concentration ± SEM from three independent experiments.

B. Acetoin was quantified from supernatant each hour. Points represent the mean acetoin concentration ± SEM from three independent experiments.

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

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

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


A
Competition
Coexistence
Unknown
Other
Membrane and virulence factors
Carbon metabolism
Aminoacids metabolism
Nitrogen metabolism
Iron metabolism
20
7
3
1
3
15
19
33
4
15
18
23
11
1
0 5 10 15 20 25 30 35 40
Gene number
Down-regulation
Up-regulation
B
Fold change
(co-culture/monoculture)
600
20
20
15
10
5
2
-2
-5
-10
-15
-20
-20
-800
acoR
PA4148
acoB
PA4153
zwf
gntK
gltF
edd
bauD
mmsA
dguA
PA5099
liuA
rcpC
tadA
tadG
flp
PA1874
PA1875
rhlA*
rhlB*
rhlC*
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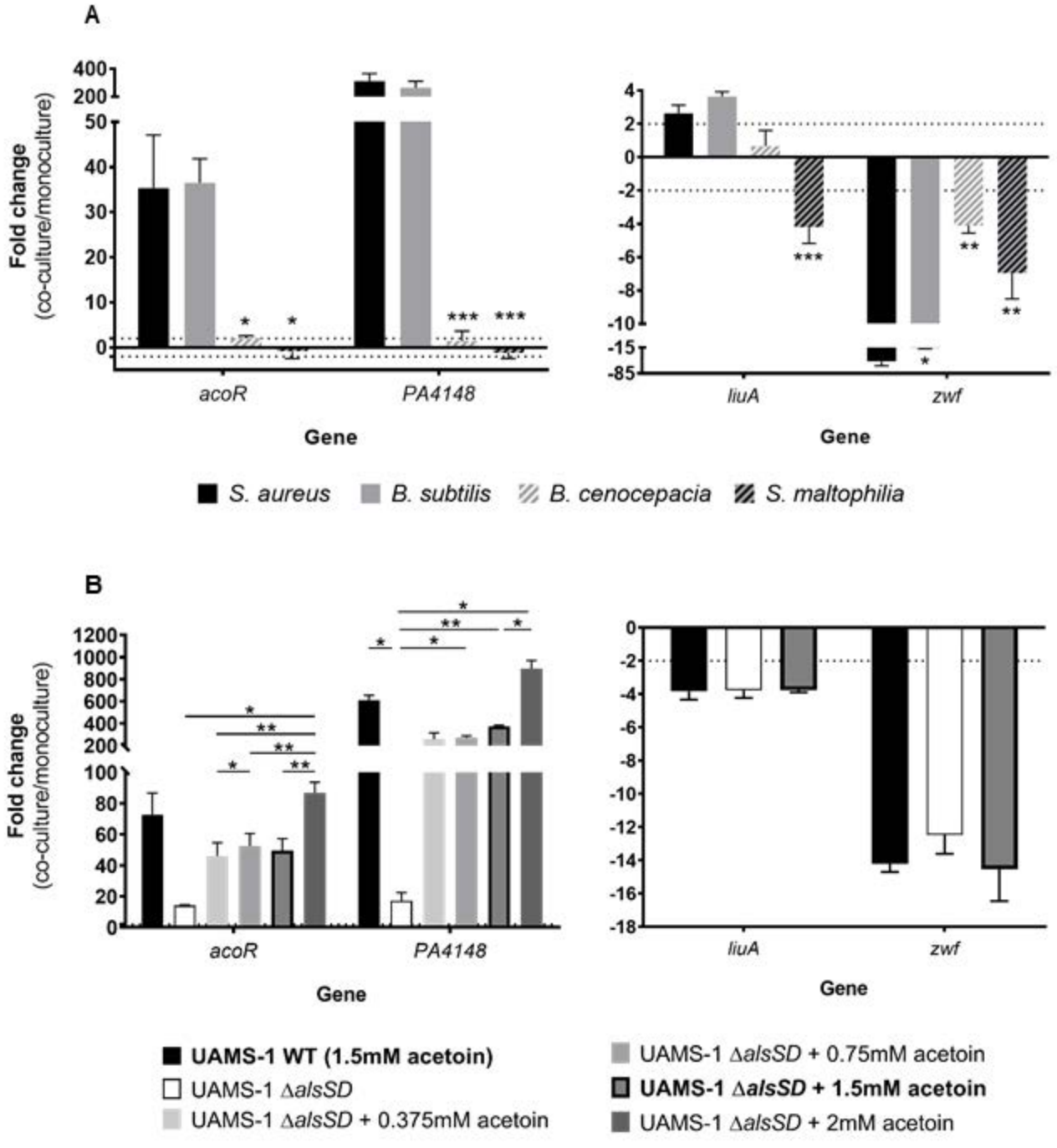
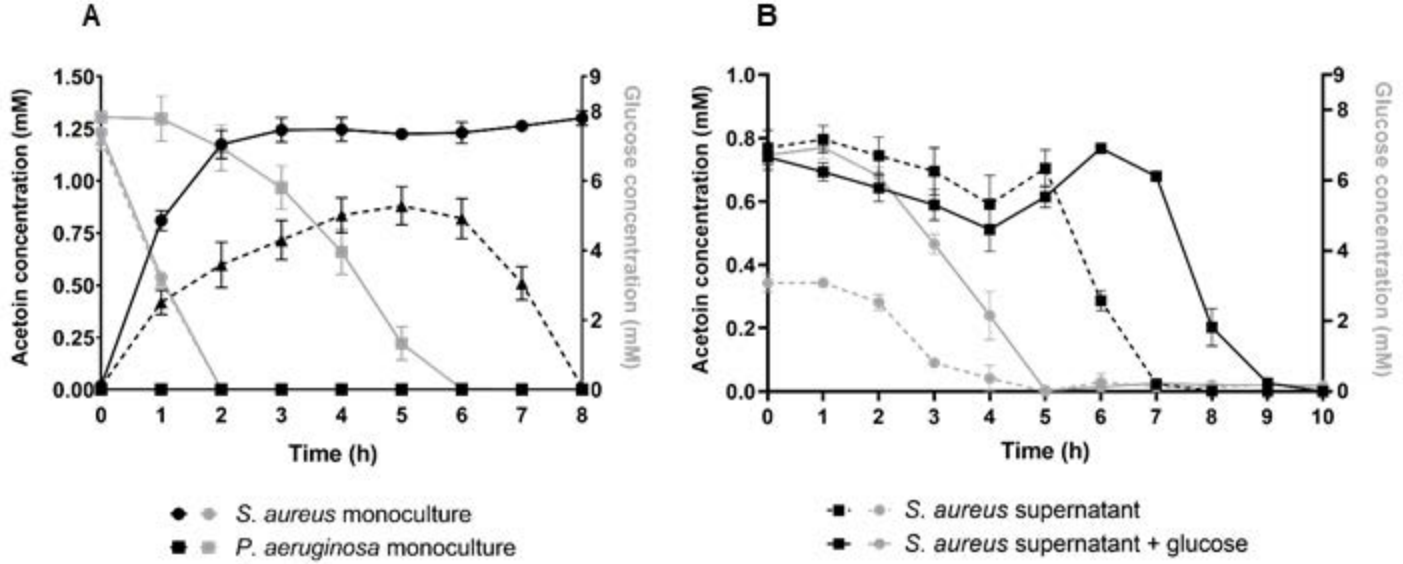
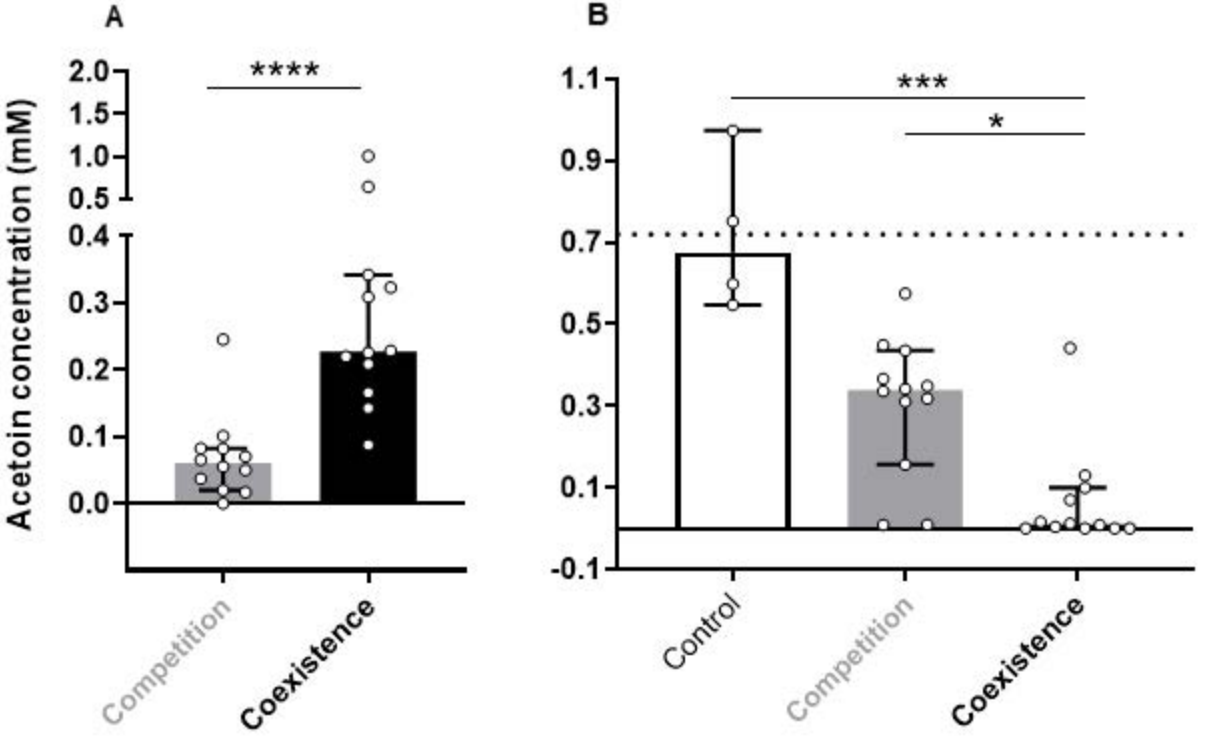
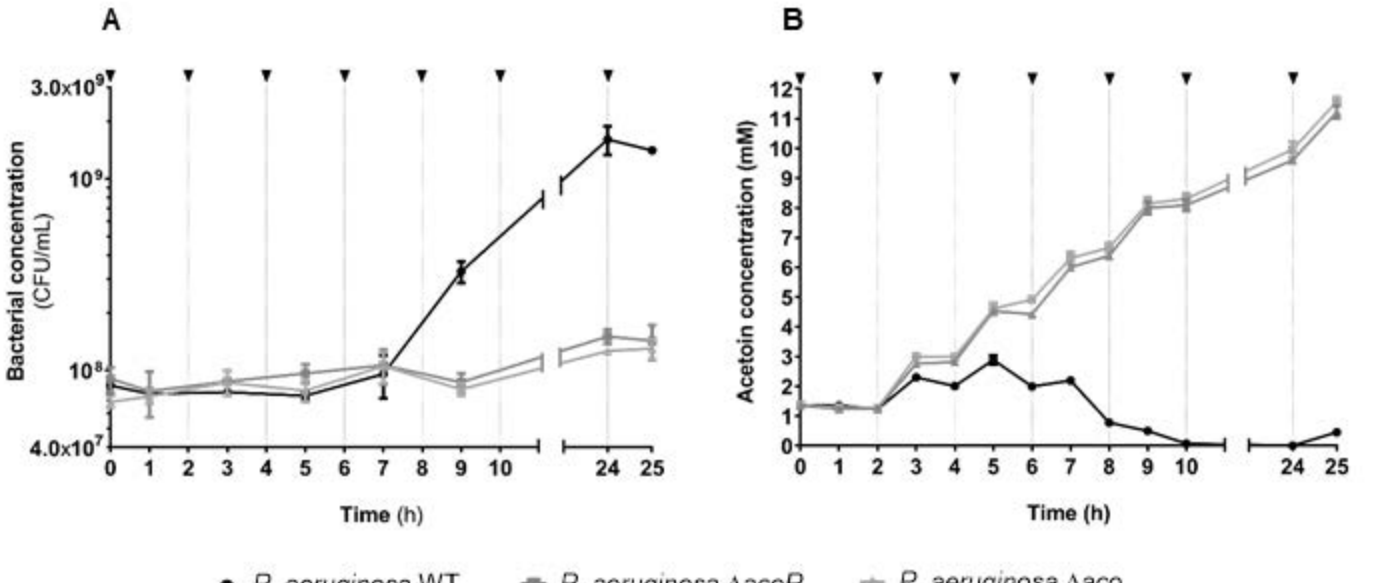
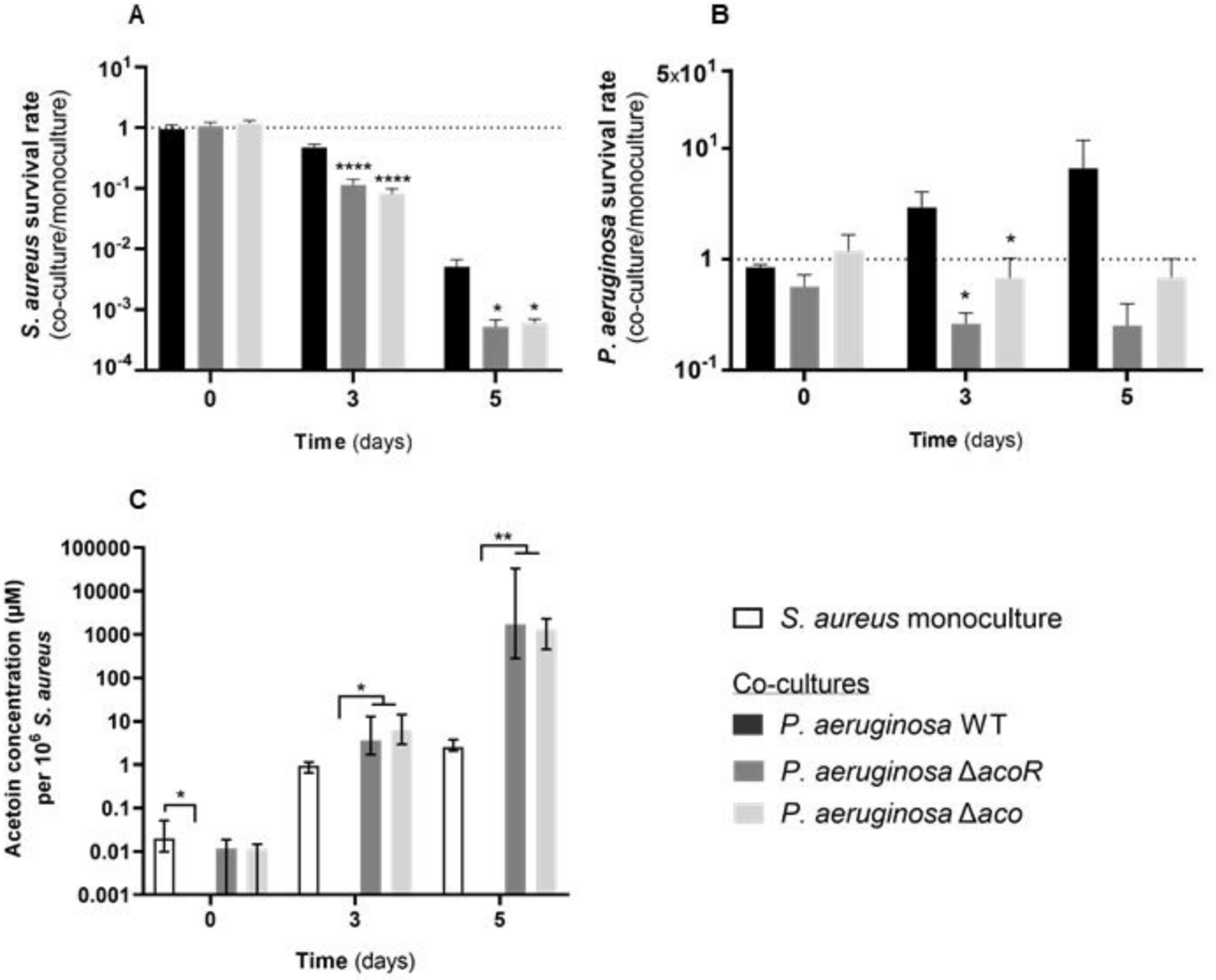
lasR*
Gene
Carbon metabolism
Amino acids metabolism
Membrane and virulence factors
Down-regulation
Up-regulation


A
Fold change
(co-culture/monoculture)
400
200
50
40
30
20
10
0
*
*
***
***
acoR
PA4148
Gene
4
2
0
-2
-4
-6
-8
-10
-15
-85
***
**
*
**
liuA
zwf
Gene
S. aureus
B. subtilis
B. cenocepacia
S. maltophilia
B
Fold change
(co-culture/monoculture)
1200
1000
800
600
400
200
100
80
60
40
20
0
acoR
PA4148
Gene
0
-2
-4
-6
-8
-10
-12
-14
-16
-18
liuA
zwf
Gene
UAMS-1 WT (1.5mM acetoin)
UAMS-1 ΔalsSD
UAMS-1 ΔalsSD + 0.375mM acetoin
UAMS-1 ΔalsSD + 0.75mM acetoin
UAMS-1 ΔalsSD + 1.5mM acetoin
UAMS-1 ΔalsSD + 2mM acetoin


A
Acetoin concentration (mM)
Glucose concentration (mM)
Time (h)
S. aureus monoculture
P. aeruginosa monoculture
Co-culture
B
Acetoin concentration (mM)
Glucose concentration (mM)
Time (h)
S. aureus supernatant
S. aureus supernatant + glucose


A
Acetoin concentration (mM)
2.0
1.5
1.0
0.5
0.4
0.3
0.2
0.1
0.0
****
Competition
Coexistence
B
1.1
0.9
0.7
0.5
0.3
0.1
-0.1
***
*
Control
Competition
Coexistence


A
B
3.0x10^9
10^9
10^8
4.0x10^7
Bacterial concentration (CFU/mL)
0 1 2 3 4 5 6 7 8 9 10 24 25
Time (h)
12 11 10 9 8 7 6 5 4 3 2 1 0
Acetoin concentration (mM)
0 1 2 3 4 5 6 7 8 9 10 24 25
Time (h)
P. aeruginosa WT
P. aeruginosa ΔacoR
P. aeruginosa Δaco


A
S. aureus survival rate
(co-culture/monoculture)
10^1
1
10^-1
10^-2
10^-3
10^-4
****
****
*
*
0
3
5
Time (days)
B
P. aeruginosa survival rate
(co-culture/monoculture)
5x10^1
10^1
1
10^-1
*
*
0
3
5
Time (days)
C
Acetoin concentration (µM)
per 10^6 S. aureus
100000
10000
1000
100
10
1
0.1
0.01
0.001
*
*
**
0
3
5
Time (days)
S. aureus monoculture
Co-cultures
P. aeruginosa WT
P. aeruginosa ΔacoR
P. aeruginosa Δaco

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

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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.
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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 30µL of pre-culture of each strain on LB plates. After two hours at 37°C, 30µL 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
P. aeruginosa (PA) and *S. aureus* (SA)	1	PA2596	Competition (SA2599, SA2597)	RNAseq	3
		SA2597		RNAseq	3
	2 (■)	PA2600	Coexistence (SA2599, SA2597)	RNAseq; qRT-PCR screening; *aco* 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; *aco* 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	Coexistence	qRT-PCR screening	This study
		SA7		qRT-PCR screening	This study
	4	PA13	Coexistence	qRT-PCR screening	This study
		SA13		qRT-PCR screening	This study
	5 (●)	PA27	Coexistence	qRT-PCR screening	3
		SA27		qRT-PCR screening	3
	6	PA30	Coexistence	qRT-PCR screening	3
		SA30		qRT-PCR screening	3
	7	PA31	Coexistence	qRT-PCR screening	3
		SA31		qRT-PCR screening	3
	8	PA37	Coexistence	qRT-PCR screening	This study
		SA37		qRT-PCR screening	This study
	9	PA42	Coexistence	qRT-PCR screening	3
		SA42		qRT-PCR screening	3
	10	PA48	Coexistence	qRT-PCR screening	This study
		SA48		qRT-PCR screening	This study
	11	PA53	Coexistence	qRT-PCR screening	This study
		SA53		qRT-PCR screening	This study
	12	PA54	Coexistence	qRT-PCR screening	This study
		SA54		qRT-PCR screening	This study
	13	PA69	Coexistence	qRT-PCR screening	3
		SA69		qRT-PCR screening	3
	14 (▲)	PA80	Coexistence	qRT-PCR screening	3
		SA80		qRT-PCR screening	3
	15	PA82	Coexistence	qRT-PCR screening	3
		SA82		qRT-PCR screening	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	Coexistence	qRT-PCR screening; Acetoin catabolism screening	3
		SA148		qRT-PCR screening; Acetoin production screening	3
	17	PA152	Coexistence	qRT-PCR screening; Acetoin catabolism screening	3
		SA152		qRT-PCR screening; Acetoin production screening	3
	18	PA153A	Coexistence	qRT-PCR screening; Acetoin catabolism screening; Acetoin monitoring in co-culture; Acetoin catabolism in SA supernatant	3
		PA153B		Transcriptomic (qRT-PCR)	3
		SA153		qRT-PCR screening; Acetoin production screening; Acetoin monitoring in co-culture; Production of SA supernatant	3

	19	PA154A	Coexistence	qRT-PCR screening; Acetoin catabolism screening	3
		PA154B		qRT-PCR screening; Acetoin catabolism screening	3
		SA154		qRT-PCR screening; Acetoin production screening	3
	20	PA156	Coexistence	qRT-PCR screening; Acetoin catabolism screening	3
		SA156		qRT-PCR screening; Acetoin production screening	3
	21	PA166A	Coexistence	qRT-PCR screening; Acetoin catabolism screening	3
		SA166		qRT-PCR screening; Acetoin production screening	3
	22	PA167	Competition	Acetoin catabolism screening	3
		SA167		Acetoin production screening	3
	2 (■)	PA171A	Coexistence	Acetoin catabolism screening	3
		SA171		Acetoin production screening	3
	23	PA172	Competition	Acetoin catabolism screening	This study
		SA172		Acetoin production screening	This study
	24	PA178	Coexistence	Acetoin catabolism screening	3
		SA178		Acetoin production screening	3
	25	PA179	Competition	Acetoin catabolism screening	3
		SA179		Acetoin production screening	3
	26	PA181	Competition	Acetoin catabolism screening	3
		SA181		Acetoin production screening	3
	27 (♦)	PA186	Competition	Acetoin catabolism screening	3
		SA186		Acetoin production screening	3
	28	PA187	Competition	Acetoin catabolism screening	This study
	29	PA188A	Competition	Acetoin catabolism screening	This study
		SA188		Acetoin production screening	This study
	30	PA193A	Competition	Acetoin catabolism screening	This study
		SA193		Acetoin production screening	This study
	5 (●)	PA194A	Coexistence	Acetoin catabolism screening	This study
		PA194B		Acetoin catabolism screening	This study
		SA194		Acetoin production screening	This study
	31	PA197	Competition	Acetoin catabolism screening	This study
		SA197		Acetoin production screening	This study
	32	SA198	Competition	Acetoin production screening	This study
	33	PA199A	Competition	Acetoin catabolism screening	This study
		PA199C		Acetoin catabolism screening	This study
	34	PA200	Competition	Acetoin catabolism screening	This study
		SA200		Acetoin production screening	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
B. cenocepacia	37	LUG2886	Coexistence (PA2600)	qRT-PCR (*aco* induction)	This study
S. maltophilia	38	LUG2884	Coexistence (PA2600)	qRT-PCR (*aco* 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
P. aeruginosa	PA2600 Δ*acoR*	*acoR* deletion mutant	Coexistence (SA2599)	Culture in minimal medium; 5-day co-cultures	This study
	PA2600 Δ*aco*	*aco* operon deletion mutant	Coexistence (SA2599)	Culture in minimal medium; 5-day co-cultures	This study
S. aureus	SA UAMS-1	WT strain	Coexistence (PA2600)	Production of SA supernatant (*aco* induction)	4
	SA UAMS-1 Δ*alsSD*	UAMS-1489, *alsSD* deletion mutant	Coexistence (PA2600)	Production of SA supernatant (*aco* induction)	4
B. subtilis	LUG2953	WT strain	Coexistence (PA2600)	qRT-PCR (*aco* induction)	This study
Plasmids	pEXG2	Gm^R ; mobilizable, non-replicative vector in *P. aeruginosa*		*acoR* and *aco* deletions	2
	pEXG2-ΔacoR	pEXG2 carrying upstream and downstream sequences of *acoR* for gene deletion		*acoR* deletion	This study
	pEXG2-Δaco	pEXG2 carrying upstream and downstream sequences of *aco* for operon deletion		*aco* deletion	This study
	pRK2013	Km^R, helper plasmid with conjugative properties		*acoR* and *aco* 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	*rpoD*	177
	PArpoD-R	ATCCGGGGCTGTCTCGAATA		
	PAgyrB-F	ATCTCGGTGAAGGTACCGGA	*gyrB*	160
	PAgyrB-R	TGCCTTCGTTGGGATTCTCC		
	OLC8-F	GCGAGGATCTCTACTTCCGC	*acoR*	140
	OLC8-R	CTCACCGAGTTCGATGCGTA		
	OLC9-F	GCGGATCGTCAACCTGTCAT	*PA4148*	86
	OLC9-R	CGATCACGGCAAACTTCGAG		
	OLC14-F	GTTCTTCAGGCTCCAGTCGG	*pvdS*	81
	OLC14-R	TTGCGGACGATCTGGAACAG		
	OLC16-F	CGCGACAAGAGCGAATACCT	*lasA*	71
	OLC16-R	AGGGTCAGCAACACTTTCGG		
	OLC17-F	CTGGACTGAACCAGGCGATG	*rhlA*	113
	OLC17-R	CAGGTATTTGCCGACGGTCT		
	OLC22-F	CGACGGTATCCAGGTCGATG	*PA1874*	70
	OLC22-R	GAACTTGACCGTGACCACCT		
	OLC23-F	CGGTGTTGCTCGGATACCTC	*rcpC*	120
	OLC23-R	GCTTGCGTTCGAGCTTTTCC		
	OLC25-F	ATCGCCTGCTTCCAGTTGTC	*pchD*	166
	OLC25-R	AGAGAGTGAAGTTGTGCGCC		
	OLC29-F	GAGCGACGAACTGACCTACC	*rhlB*	200
	OLC29-R	TACTTCTCGTGAGCGATGCG		
	OLC31-F	TGCTCACTTCGCTATGGACC	*acoB*	117
	OLC31-R	AGAAGATCACCGGGTCGTTG		
	OLC33-F	CCGACGTGATCGCCTTCATA	*PA4153*	83
	OLC33-R	GACGATTTCTTCCAGGCCGA		
	OLC35-F	GCCCATCGAGTTCCGTATGT	*bauD*	151
	OLC35-R	GAGGCAGACGGTGAAGATCG		
	OLC39-F	GACGAAGACGGCATGAACCT	*tadA*	119
	OLC39-R	TCCAGTTCGTAGCGGGAGAT		
	OLC41-F	CCACCACGAAACTGCAACTG	*tadG*	107
	OLC41-R	GCTTCTCCAACCGAAGTCCA		
	OLC54-F	TTGCCGTATTGAGTCCCACG	*flp*	77
	OLC54-R	GACTTTTTCGCCGACTCCGT		
	OLC56-F	GGACTGACGCTCAGGCAAT	*rhlC*	74
	OLC56-R	CCGGAGGAGATCAGGAACGA		
	OLC71-F	CAGCCGGACGAAGGTATCTC	*zwf*	113
	OLC71-R	GCGTGGTAGGTCTCGGAAAA		
	OLC72-F	GCTATACCCGCTCAAGGGC	*dguA*	92
	OLC72-R	TCTTGCGGTCGTAATCGGTG		
	OLC73-F	CTTCGCCTACCTGTTCAGCC	*PA5099*	163
	OLC73-R	CGGCAGGTAGCGTGAATAGT		
	OLC74-F	GCAGTTCGGAGCACATCAAC	*PA1874*	130
	OLC74-R	TCGATGCTGACGAAATCGGT		
	OLC75-F	TGGTATCCGGCGAACACATC	*liuA*	127
	OLC75-R	CACATCTTGCTGCCGTTGAG		
	OLC92-F	CAGCCAGGACTACGAGAACG	*lasR*	153

	OLC92-R	TGGTAGATGGACGGTTCCCA		
	OLC93-F	GGCATTCCCCTCACCGAC	*gntK*	181
	OLC93-R	GGGTCAGTTCCAGGTAGACGA		
	OLC94-F	TGGAAGTGGCTGCTCAATCC	*gltF*	81
	OLC94-R	CCAGTCGAAGCGGAAACCTT		
	OLC95-F	CACCTGCACCTTCTATGGCA	*edd*	96
	OLC95-R	GTGTTCGGGTTGACGAAGGA		
	OLC96-F	CAACGGCACCTCGATCTTCA	*mmsA*	94
	OLC96-R	AATCGGGATGTTGATGCCCA		
Cloning	OLC58-F	GGTCGACTCTAGAGGATCCCC AGGGCGATGCCCCGGCCGATG	*acoR* downstream	474
	OLC58-R	CACATGGTCCTTCGAGTGTGC		
	OLC59-F	GCACACTCGAAGGACCATGTG CGCCGGCATGGCATCCGCATG	*acoR* upstream	486
	OLC59-R	ACCGAATTCGAGCTCGAGCCC CTCGATCGCGCGGACGAACCA		
	OLC64-F	GGTCGACTCTAGAGGATCCCC TAGTCGGCATCGCGCACCCGT	*aco* operon downstream	654
	OLC64-R	GGAGAAATCGTCGGACGACGT		
	OLC65-F	ACGTCGTCCGACGATTTCTCCA GTTGGTGAACAACAAGGAGC	*aco* 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***		Q9I700	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	Q9I609	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***		Q9I4J8	Phthiocerol/phenolphthiocerol synthesis polyketide synthase	Unknown	**-4.31**	1.74E-21	**-3.55**	1.62E-19		
rocR	***PA1196***	***ddaR***		Q9I4E2	Arginine utilization regulatory protein RocR	Aminoacids (arginine) metabolism	**2.51**	3.23E-05	**3.22**	5.81E-21		10
fecI_5	***PA1300***	***PA1300***	c	Q9I444	Putative RNA polymerase sigma factor FecI	Iron metabolism	**-3.17**	3.51E-11	**-2.50**	2.25E-11		6
fecR_1	***PA1301***	***PA1301***	c	Q9I443	Protein FecR	Iron metabolism	**-3.02**	1.71E-08	**-2.53**	1.01E-13		6
ctpF	***PA1429***	***PA1429***		Q9I3R5	Putative cation-transporting ATPase F	Unknown	**2.89**	8.45E-09	**3.63**	7.15E-35		
group_3703	***PA1673***	***PA1673***		Q9I352	Bacteriohemerythrin	Unknown	**2.16**	1.57E-04	**2.57**	1.30E-14		
group_5526	***PA1746***	***PA1746***		Q9I2Z1	Hypothetical protein	Unknown	**2.42**	1.59E-06	**2.42**	1.38E-09		
group_2540	***PA1747***	***PA1747***		Q9I2Z0	Hypothetical protein	Unknown	**2.57**	8.64E-09	**2.46**	1.07E-08		
group_269	***PA2033***	***PA2033***		Q9I282	Hypothetical protein	Unknown	**-2.24**	1.65E-03	**-2.84**	7.11E-23		
ybdM	***PA2126***	***cgrC***		Q9I1Y9	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***		Q9I195	Ferric uptake regulation protein	Iron metabolism	**-2.44**	1.09E-04	**-2.56**	8.25E-10		6
mbtH	***PA2412***	***PA2412***		Q9I169	Hypothetical protein	Monobactam biosynthesis	**-2.12**	1.66E-03	**-2.27**	6.19E-08		
group_3129	***PA2468***	***foxI***		Q9I114	Putative RNA polymerase sigma factor FecI	Iron metabolism	**-2.32**	1.63E-07	**-2.03**	1.07E-08		6
hmp	***PA2664***	***fhp***		Q9I0H4	Flavohemoprotein	Iron metabolism, NO detoxification	**-2.41**	1.85E-05	**-2.38**	1.86E-03		11
group_3982	***PA2691***	***PA2691***		Q9I0F1	NADH dehydrogenase-like protein	Oxidative phosphorylation	**-4.38**	1.03E-18	**-3.45**	3.32E-07		
hldD	***PA3337***	***rfaD***		Q9HYQ8	ADP-L-glycero-D-manno-heptose-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***	***nosL***	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***	***bfd***		Q9HY80	Bacterioferritin-associated ferredoxin	Unknown	**-2.24**	3.28E-10	**-2.04**	1.81E-10		
ykgO	***PA3600***	***rpl36***	e	Q9HY26	50S ribosomal protein L36 2	Ribosome structure	**-2.99**	6.77E-10	**-2.87**	3.60E-08		
rpmE2	***PA3601***	***ykgM***	e	Q9HY25	50S ribosomal protein L31 type B	Ribosome structure	**-2.92**	2.37E-10	**-3.21**	2.69E-26		
fdx_1	***PA3809***	***fdx2***	f	Q51383	2Fe-2S ferredoxin	Iron-sulfur protein	**-2.72**	4.17E-12	**-3.02**	7.98E-16		
hscA	***PA3810***	***hscA***	f	Q51382	Chaperone protein HscA	Protein stabilization	**-2.56**	4.41E-11	**-2.60**	6.55E-16		12
hscB	***PA3811***	***hscB***	f	Q9HXJ1	Co-chaperone protein HscB	Protein stabilization	**-2.04**	1.40E-06	**-2.28**	1.35E-14		12
iscA	***PA3812***	***iscA***	f	Q9HXJ0	Iron-binding protein IscA	[Fe-S] cluster biogenesis	**-2.51**	2.80E-10	**-3.37**	7.39E-26		13
iscU	***PA3813***	***iscU***	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***	***iscS***	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***	***iscR***	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***	***yvgX***		Q9HX93	Copper-exporting P-type ATPase	Unknown	**-2.28**	3.75E-07	**-2.63**	4.15E-14		
ntaA	***PA4155***	***PA4155***		Q9HWM6	Nitrilotriacetate monooxygenase component A	Unknown	**-3.09**	5.86E-10	**-2.23**	6.24E-06		
fyuA_1	***PA4156***	***fvbA***		Q9HWM5	Pesticin receptor	Iron metabolism	**-2.70**	8.32E-12	**-2.48**	2.30E-09		14
fepC_1	***PA4158***	***fepC***		Q9HWM3	Ferric enterobactin transport ATP-binding protein FepC	ABC transporters (iron complex)	**-2.30**	6.59E-05	**-2.21**	3.50E-04		
fepB	***PA4159***	***fepB***		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***	***sodM***	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***	***fumC1***	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***	***fagA***	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***	***ccpR***		P14532	Cytochrome c551 peroxidase	Oxydative stress response	**3.16**	3.49E-11	**3.27**	6.90E-16	Repression	7,16
group_1579	***PA4625***	***cdrA***		Q9HVG6	Cyclic diguanylate-regulated TPS partner A, CdrA	Adhesion and biofilm matrix structure	**2.53**	8.83E-05	**2.06**	6.78E-10		17
fecI_3	***PA4896***	***PA4896***		Q9HUR7	Putative RNA polymerase sigma factor FecI	Iron metabolism	**-3.04**	6.78E-10	**-2.23**	1.49E-08		6
group_4196	***PA5027***	***PA5027***		Q9HUE2	Hypothetical protein	Unknown	**2.35**	7.70E-05	**2.82**	2.70E-20	Activation	9
arcD	***PA5170***	***arcD***		P18275	Arginine/ornithine antiporter	Aminoacids (arginine) metabolism	**2.36**	4.52E-04	**2.49**	5.13E-04		10
NA	***PA5369.3***	***PA5369.3***		NA	tRNA-Ala	Aminoacyl-tRNA biosynthesis	**2.12**	7.09E-03	**4.50**	3.69E-21		
pka	***PA5475***	***PA5475***		Q9HT95	Protein lysine acetyltransferase Pka	Unknown	**2.04**	4.85E-04	**2.30**	1.81E-10		

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

Gene (clinical strains)	Gene (PAO1)			UniProt	Product	Function	SA2599/PA2600		SA2597/PA2600		LasR regulation	Ref.
							Log_2 fold-change	Adjusted p-value	Log_2 fold-change	Adjusted p-value		
group_955	**NA**	**NA**		NA	Hypothetical protein	Unknown	**-2.20**	3.84E-09	**-2.56**	1.54E-05		
eamB_1	**NA**	**NA**		P38101	Cysteine/O-acetylserine efflux protein	Aminoacids transport	**2.04**	5.37E-04	**2.31**	2.69E-03		
group_511	**NA**	**NA**		NA	Hypothetical protein	Unknown	**-2.04**	2.34E-03	**-2.77**	5.28E-03		
bauD	***PA0129***	***bauD***		Q9I703	Putative GABA permease	Aminoacids (β-alanine) catabolism	**2.90**	1.53E-15	**3.07**	2.83E-14		18
bauB	***PA0131***	***bauB***		Q9I701	Beta-alanine degradation protein BauB	Aminoacids (β-alanine) catabolism	**2.27**	2.30E-05	**2.61**	2.31E-04		18
cysG_2	***PA0510***	***nirE***	a	G3XD80	Siroheme synthase NirE	Nitrogen, porphyrin and chlorophyll metabolism	**-2.42**	6.73E-05	**-2.72**	1.36E-03	Repression	8,9
group_3026	***PA0513***	***nirG***	a	P95414	NirG	Nitrogen metabolism	**-2.69**	3.29E-05	**-2.97**	2.87E-03		8
group_3339	***PA0515***	***nirD***	a	P95412	Probable transcriptional regulator	Nitrogen metabolism	**-2.62**	1.99E-03	**-3.37**	3.73E-04		8
group_5595	***PA0516***	***nirF***	a	Q51480	Heme d1 biosynthesis protein NirF	Nitrogen metabolism	**-3.53**	1.60E-08	**-3.88**	1.44E-06		8
nirM	***PA0518***	***nirM***	a	P00099	Cytochrome c-551	Nitrogen metabolism	**-2.62**	4.27E-03	**-3.27**	3.88E-03		8
nirS_2	***PA0519***	***nirS***	a	P24474	Nitrite reductase	Nitrogen metabolism	**-3.27**	7.25E-06	**-4.43**	2.95E-08		8
nirQ	***PA0520***	***nirQ***		Q51481	Denitrification regulatory protein NirQ	Nitrogen metabolism	**-3.25**	7.06E-05	**-4.12**	2.10E-06		8
norC	***PA0523***	***norC***	h	Q59646	Nitric oxide reductase subunit C	Nitrogen metabolism	**-3.59**	2.99E-05	**-6.23**	4.50E-14		8
norB	***PA0524***	***norB***	h	Q59647	Nitric oxide reductase subunit B	Nitrogen metabolism	**-5.82**	3.20E-18	**-5.77**	2.52E-12		8
group_4191	***PA0525***	***norD***	h	Q51484	Probable dinitrification protein NorD	Nitrogen metabolism	**-3.86**	1.68E-06	**-4.82**	1.23E-08		8
group_364	***PA0526***	***PA0526***		G3XDA0	Hypothetical protein	Unknown	**-3.38**	6.53E-05	**-4.60**	3.45E-06		
NA	***PA0668.3***	**NA**		NA	tRNA-Ala(tgc)	Aminoacyl-tRNA biosynthesis	**3.45**	5.66E-05	**7.57**	1.86E-22		
acrC_2	***PA0746***	***PA0746***	i	Q9I5I3	Acryloyl-CoA reductase (NADH)	Unknown	**2.80**	1.01E-12	**3.12**	2.02E-15		
mmsA_2	***PA0747***	***PA0747***	i	Q9I5I2	Methylmalonate-semialdehyde dehydrogenase	Aminoacids (isoleucine, leucine, valine) catabolism, propanoate and carbon metabolism	**2.08**	1.04E-05	**2.31**	1.44E-06		
acnM	***PA0794***	***PA0794***		Q9I5E4	Aconitate hydratase A	Propanoate metabolism	**2.18**	5.28E-10	**2.90**	1.80E-07		
aroP_2	***PA0866***	***aroP2***		Q9I575	Aromatic amino acid transport protein AroP	Unknown	**2.30**	8.73E-06	**2.74**	1.02E-03		
acs_1	***PA0887***	***acsA***		Q9I558	Acetyl-coenzyme A synthetase	Propanoate, carbon and pyruvate metabolism	**3.08**	5.58E-10	**3.27**	2.24E-07	Repression	7
yybH	***PA1325***	***yybH***	j	Q9I419	Putative protein YybH	Unknown	**2.16**	1.03E-03	**2.73**	1.94E-05		
ilvA_1	***PA1326***	***ilvA***	j	Q9I418	L-threonine dehydratase biosynthetic IlvA	Carbon metabolism, aminoacids (isoleucine, leucine, valine) biosynthesis	**2.19**	4.61E-05	**2.46**	6.93E-04		
group_1804	***PA1874***	***PA1874***	k	Q9I2M3	Hypothetical protein	Antibiotic resistance, quorum sensing	**-2.54**	5.92E-05	**-2.68**	2.83E-04	Activation	9,19
group_4895	***PA1875***	***PA1875***	k	Q9I2M2	Hypothetical protein	Antibiotic resistance	**-2.94**	1.72E-09	**-2.80**	1.14E-05	Activation	9,19
prsE_3	***PA1877***	***PA1877***	k	Q9I2M0	Type I secretion system membrane fusion protein PrsE	Antibiotic resistance	**-2.29**	8.13E-03	**-3.04**	1.51E-07		19
group_6609	***PA1878***	***PA1878***		Q9I2L9	Hypothetical protein	Unknown	**-2.03**	2.47E-05	**-2.38**	1.01E-03		
group_2034	***PA1914***	***hvn***		Q9I2J0	Hypothetical protein	Unknown	**-3.87**	9.10E-16	**-4.61**	3.05E-20	Activation	7
degU_4	***PA1978***	***erbR***		P29369	Transcriptional regulatory protein DegU	Ethanol stress response	**2.28**	2.43E-05	**3.06**	1.45E-07	Repression	9,20
luxQ	***PA1992***	***ercS***		Q9I2B7	Autoinducer 2 sensor kinase/phosphatase LuxQ	Ethanol stress response	**2.13**	9.50E-08	**2.11**	2.65E-04		20
accA1_2	***PA2012***	***liuD***	l	Q9I299	Acetyl-/propionyl-coenzyme A carboxylase alpha chain	Aminoacids (leucine) and monoterpenes catabolism	**2.30**	7.81E-07	**2.69**	2.15E-06		21
menB	***PA2013***	***liuC***	l	Q9I298	1%2C4-dihydroxy-2-naphthoyl-CoA synthase	Aminoacids (leucine) and monoterpenes catabolism	**3.02**	1.20E-11	**3.29**	7.09E-12		21
group_2911	***PA2014***	***liuB***	l	Q9I297	Methylmalonyl-CoA carboxyltransferase 12S subunit	Aminoacids (leucine) and monoterpenes catabolism	**2.61**	1.20E-08	**2.85**	4.87E-10	Activation	9,21
mmgC_7	***PA2015***	***liuA***	l	Q9I296	Acyl-CoA dehydrogenase	Aminoacids (leucine) and monoterpenes catabolism	**2.62**	3.07E-08	**2.76**	5.26E-09		21
cueR_2	***PA2016***	***liuR***		Q9I295	HTH-type transcriptional regulator CueR	Aminoacids (leucine) and monoterpenes catabolism	**2.61**	4.02E-08	**3.17**	4.01E-06		21

group_13	***PA2040***	***pauA4***		Q9I275	Gamma-glutamylputrescine synthetase 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
thlA_1	***PA2553***	***PA2553***	n	Q9I0T1	Acetyl-CoA acetyltransferase	Carbon and fatty acids metabolism	**2.46**	7.14E-11	**2.72**	1.52E-08	Activation	9
group_5789	***PA2554***	***PA2554***	n	Q9I0T0	Putative oxidoreductase	Unknown	**2.78**	1.75E-13	**3.05**	1.43E-09	Activation	9
acsA_1	***PA2555***	***PA2555***	n	Q9I0S9	Acetyl-coenzyme A synthetase	Propanoate, carbon and pyruvate metabolism	**2.83**	1.51E-10	**2.93**	8.32E-07	Activation	9
fadD3	***PA2557***	***PA2557***		Q9I0S7	3-[(3aS%2C4S%2C7aS)-7a-methyl-1%2C5-dioxo-octahydro-1H-inden	Unknown	**3.04**	2.30E-14	**3.16**	2.62E-11		
lecA	***PA2570***	***lecA***		Q05097	PA-I galactophilic lectin LecA	Adhesion, biofilm formation	**-3.40**	1.59E-12	**-2.61**	1.13E-03	Activation	7,9,24
group_5847	***PA2662***	***PA2662***	o	Q9I0H6	Hypothetical protein	Unknown	**-3.18**	1.66E-07	**-3.57**	3.71E-05		
group_3164	***PA2663***	***ppyR***	o	Q9I0H5	Psl and pyoverdine operon regulator, PpyR	Iron metabolism, biofilm formation and virulence	**-2.83**	7.91E-04	**-4.04**	9.15E-07		25
hmp	***PA2664***	***fhp***		Q9I0H4	Flavohemoprotein	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	Q9HWM9	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
pucI_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 > $|2\log_2|$ with an adjusted *P*-value<0.05 in presence of both SA strains. Genes from the same operon are annotated with an identical letter. Grey cells indicate genes that were also dysregulated in competition couples (Table S4). Functional classification was performed thanks to KEGG database and literature.

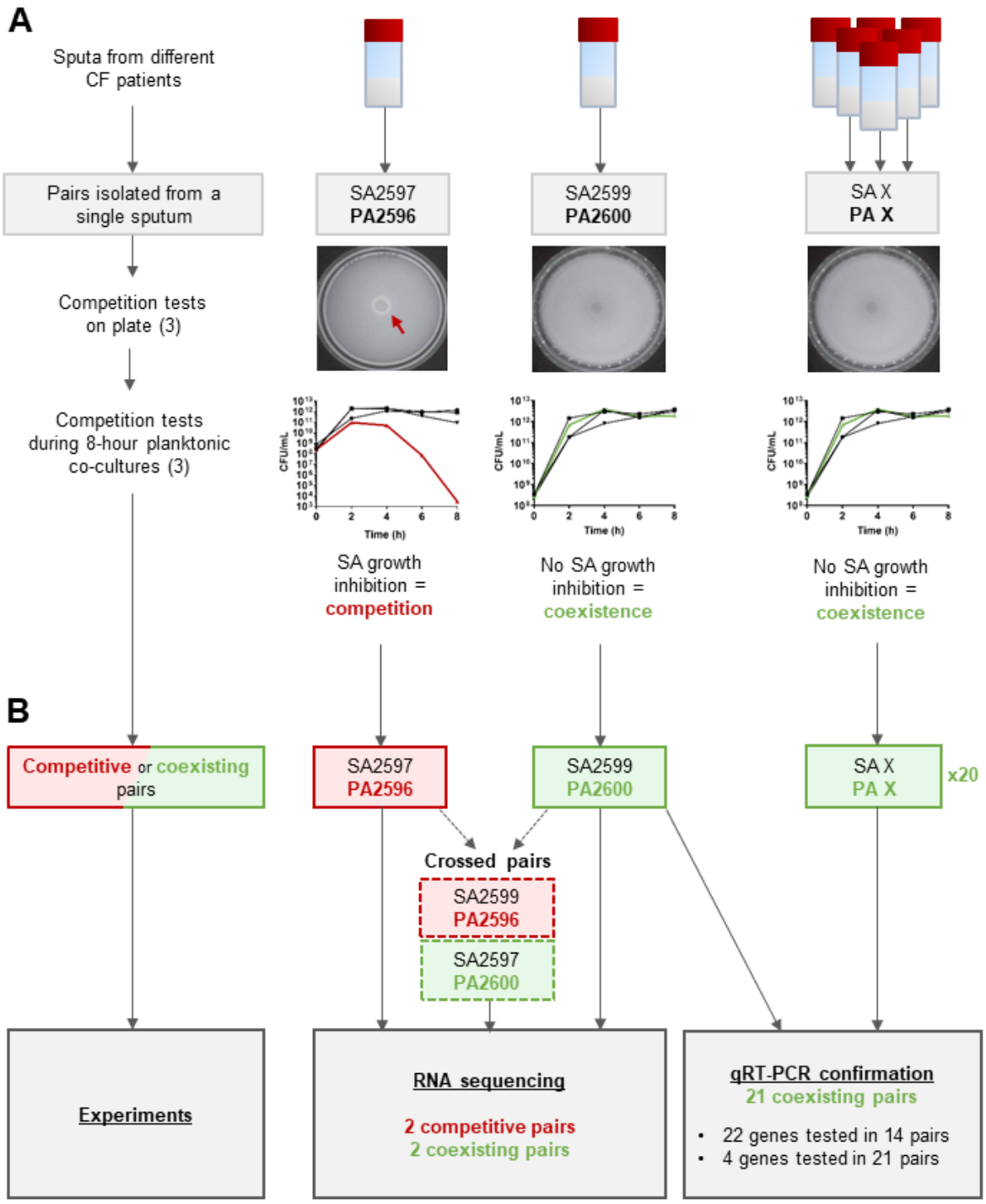

Figure S1: Schematic representation of the employed methodology.

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).

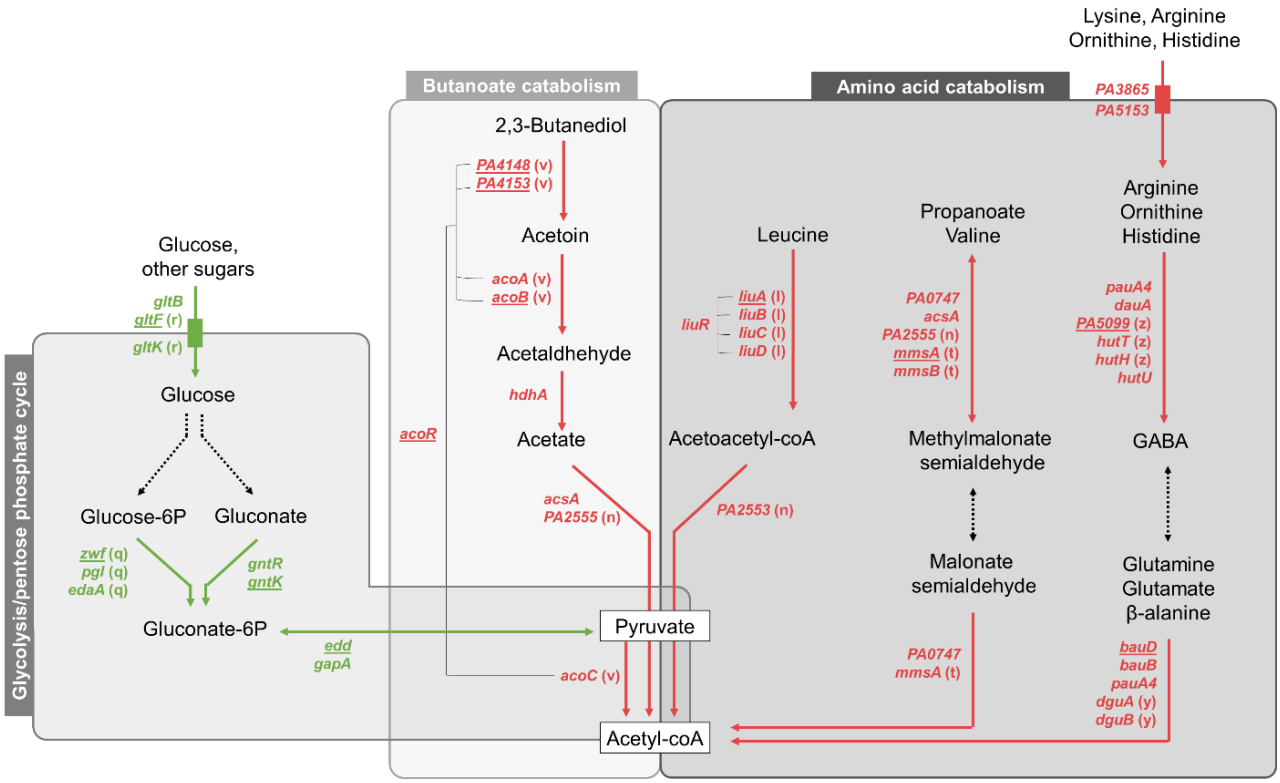

Figure S2: *P. aeruginosa* metabolic pathways and associated genes up-regulated (red) or down-regulated (green) in coexistence with *S. aureus*. PA2600 coexistence strain was cultivated in absence or presence of SA2599 or SA2597. RNAs were extracted after 4 hours of culture and RNAseq analysis was performed.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

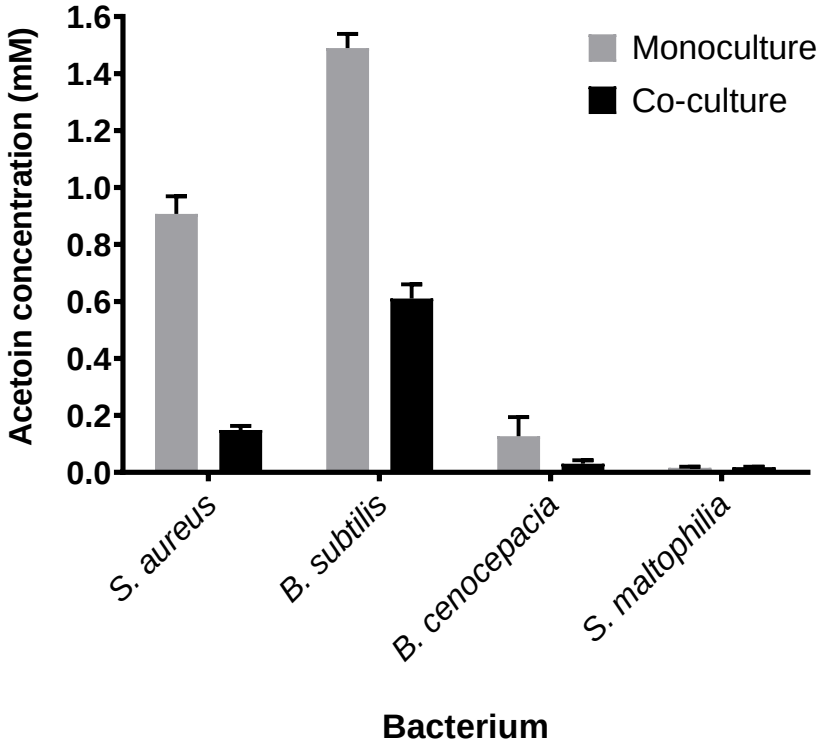

Figure S3: Acetoin concentration in supernatant of *S. aureus* SA2599, *B. subtilis*, *B. cenocepacia* and *S. maltophilia* monocultures (grey bars) or co-cultures with *P. aeruginosa* PA2600 (black bars). Acetoin was quantified from supernatant after 4h of culture. Bars represent the mean acetoin concentration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

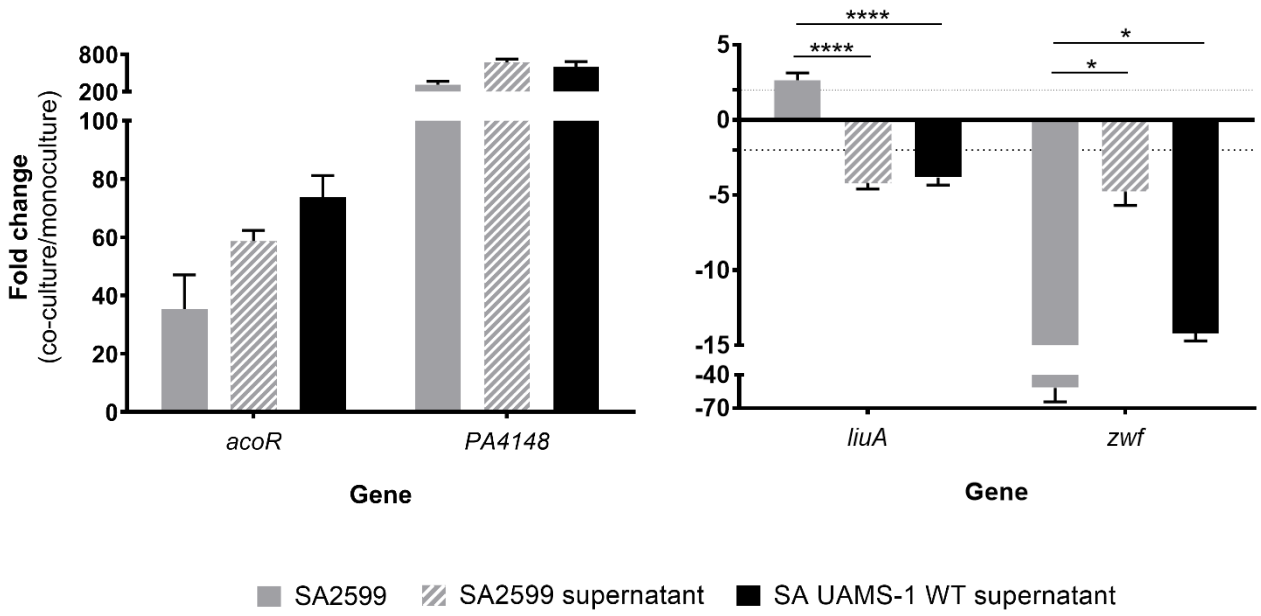

Figure S4: Fold change of *P. aeruginosa acoR*, *PA4148*, *liuA* and *zwf* gene expression induced by culture with *S. aureus* (grey bars) or its supernatant (hatched and black bars). *P. aeruginosa* PA2600 strain was cultivated in the absence or presence of *S. aureus* SA2599 or filtered supernatant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

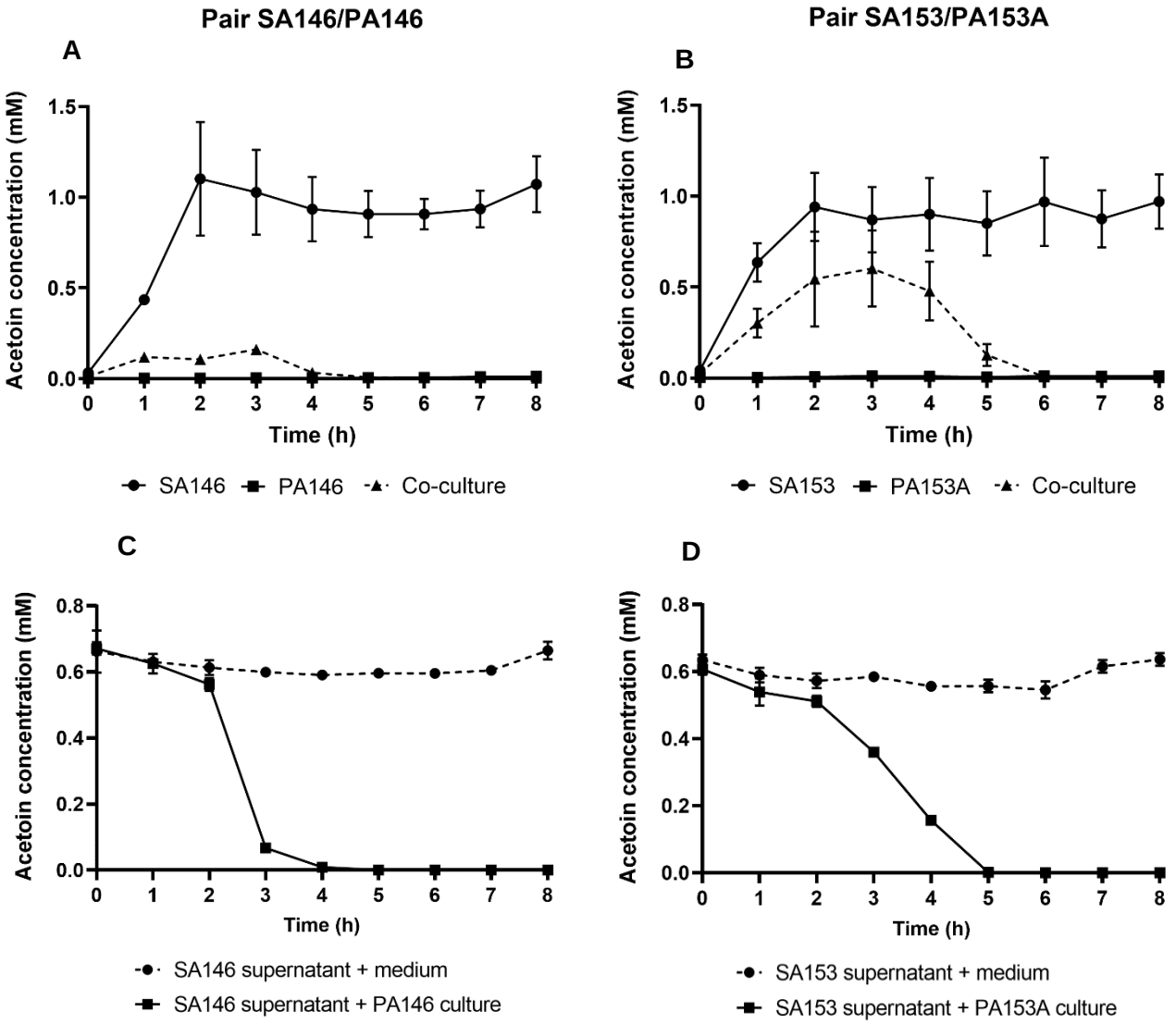

Figure S5: Monitoring of acetoin concentration in *S. aureus* and *P. aeruginosa* monocultures or co-culture (A,B) or in *S. aureus* supernatant inoculated with *P. aeruginosa* (B,C), for the pairs SA146/PA146 (A,C) and SA153/PA153A (B,D).

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 ± 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 ± SEM from three independent experiments per pair.

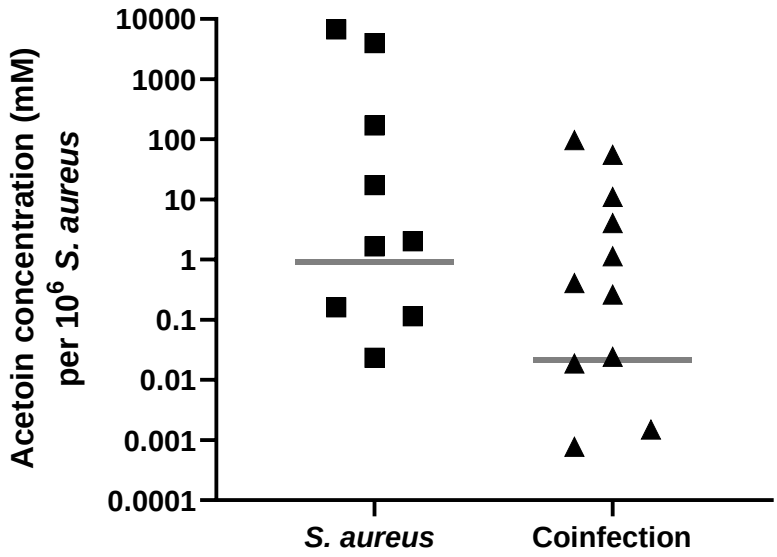

Figure S6: Acetoin concentration in CF sputa from patients. Sputa from *S. aureus* mono-infected patients (n=9) or *S. aureus* and *P. aeruginosa* co-infected patients (n=11) were gathered and acetoin concentration was quantified. Bars represent the median acetoin concentration normalized on *S. aureus*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

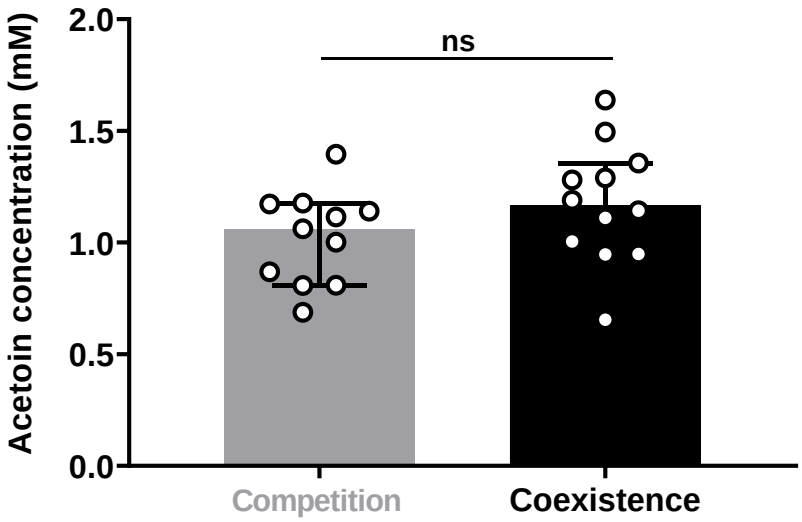

Figure S7: Acetoin concentration in cultures of *S. aureus* strains from competition and coexistence couples. Each *S. aureus* strain from competition (n=11) and coexistence (n=12) couples was cultivated for 6 hours in BHI and acetoin was dosed from supernatant. Bars represent the median acetoin concentration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

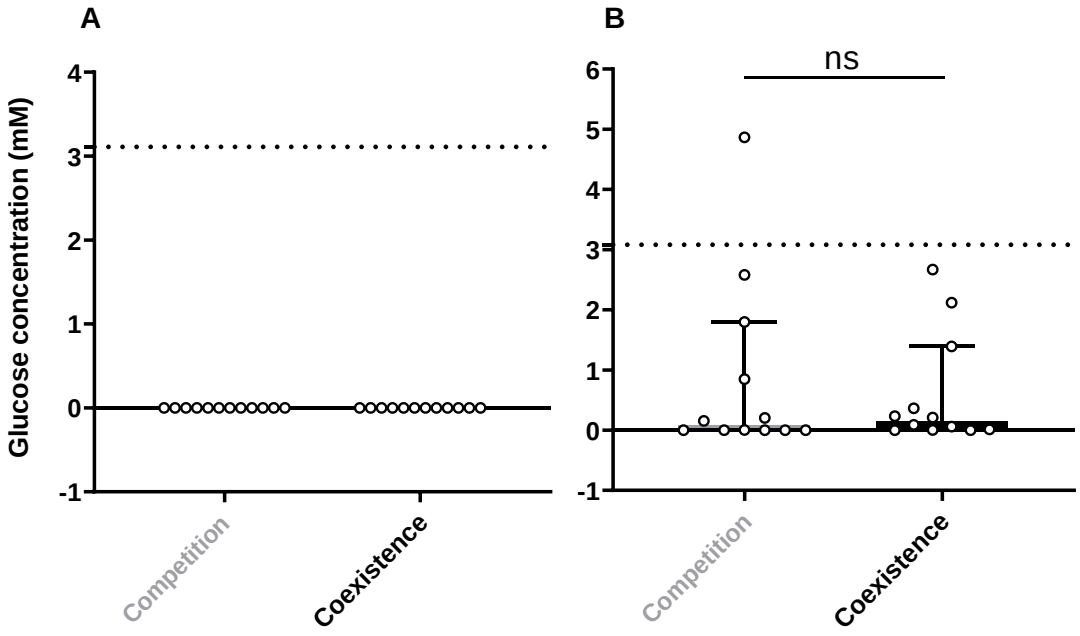

Figure S8: Glucose concentrations in cultures of *S. aureus* (A) and *P. aeruginosa* (B) strains from competition and coexistence pairs.

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 ± 95% CI. Dotted line indicates the initial glucose concentration in *S. aureus* supernatant. ns $P>0.05$ Mann-Whitney test.

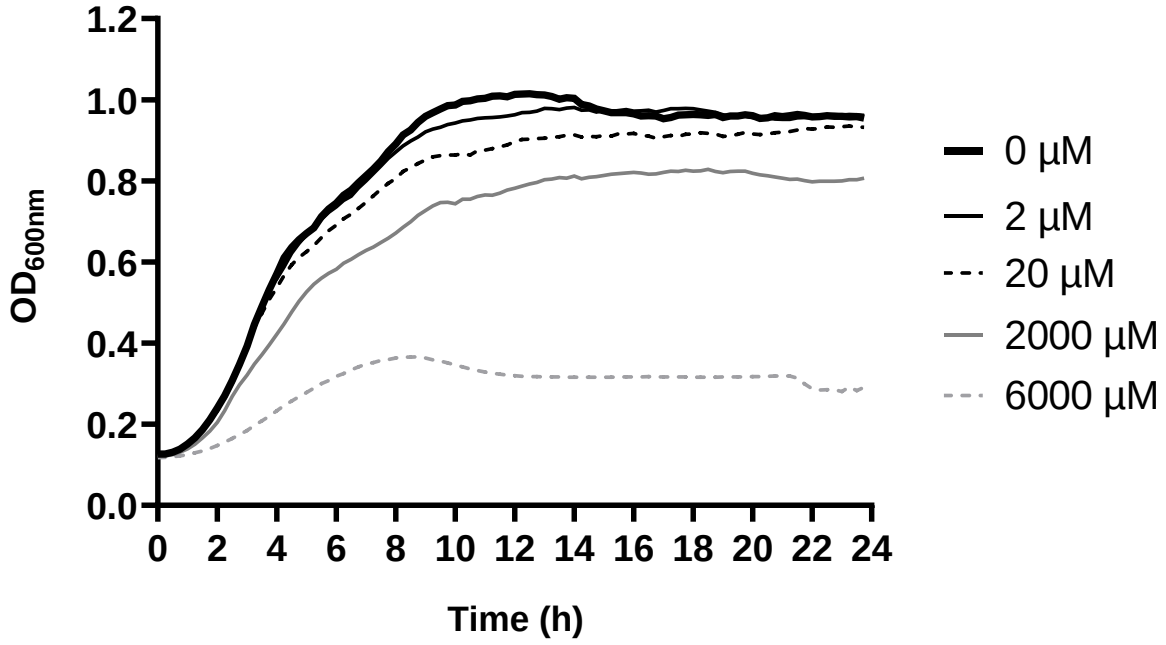

Figure S9: Growth kinetic of *S. aureus* cultivated in absence or presence of acetoin. SA2599 was cultivated during 24h in the absence of acetoin or in the presence of acetoin in different proportions ranging from 0.2µM to 6000µM per 10^6 *S. aureus*. Lines represent the mean optical density of three technical replicates.

Supplementary data references

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

1. Carriel D, Simon Garcia P, Castelli F, Lamourette P, Fenaille F, Brochier-Armanet C, Elsen S, Gutsche I. 2018. A Novel Subfamily of Bacterial AAT-Fold Basic Amino Acid Decarboxylases and Functional Characterization of Its First Representative: Pseudomonas aeruginosa LdcA. Genome Biol Evol 10:3058–3075.

2. Rietsch A, Vallet-Gely I, Dove SL, Mekalanos JJ. 2005. ExsE, a secreted regulator of type III secretion genes in Pseudomonas aeruginosa. Proc Natl Acad Sci U S A 102:8006–8011.

3. Briaud P, Camus L, Bastien S, Doléans-Jordheim A, Vandenesch F, Moreau K. 2019. Coexistence with Pseudomonas aeruginosa alters Staphylococcus aureus transcriptome, antibiotic resistance and internalization into epithelial cells. Sci Rep 9.

4. Tsang LH, Cassat JE, Shaw LN, Beenken KE, Smeltzer MS. 2008. Factors Contributing to the Biofilm-Deficient Phenotype of Staphylococcus aureus sarA Mutants. PLoS ONE 3:e3361.

5. Figurski DH, Helinski DR. 1979. Replication of an origin-containing derivative of plasmid RK2 dependent on a plasmid function provided in trans. Proc Natl Acad Sci USA 76:1648–1652.

6. Chevalier S, Bouffartigues E, Bodilis J, Maillot O, Lesouhaitier O, Feuilloley MGJ, Orange N, Dufour A, Cornelis P. 2017. Structure, function and regulation of Pseudomonas aeruginosa porins. FEMS Microbiol Rev 41:698–722.

7. Schuster M, Lostroh CP, Ogi T, Greenberg EP. 2003. Identification, timing, and signal specificity of Pseudomonas aeruginosa quorum-controlled genes: a transcriptome analysis. J Bacteriol 185:2066–2079.

8. Borrero-de Acuña JM, Rohde M, Wissing J, Jänsch L, Schobert M, Molinari G, Timmis KN, Jahn M, Jahn D. 2016. Protein Network of the Pseudomonas aeruginosa Denitrification Apparatus. J Bacteriol 198:1401–1413.

9. Wagner VE, Bushnell D, Passador L, Brooks AI, Iglewski BH. 2003. Microarray analysis of Pseudomonas aeruginosa quorum-sensing regulons: effects of growth phase and environment. J Bacteriol 185:2080–2095.

10. Lundgren BR, Sarwar Z, Pinto A, Ganley JG, Nomura CT. 2016. Ethanolamine Catabolism in Pseudomonas aeruginosa PAO1 Is Regulated by the Enhancer-Binding Protein EatR (PA4021) and the Alternative Sigma Factor RpoN. J Bacteriol 198:2318–2329.

11. Arai H, Hayashi M, Kuroi A, Ishii M, Igarashi Y. 2005. Transcriptional regulation of the flavohemoglobin gene for aerobic nitric oxide detoxification by the second nitric oxide-responsive regulator of Pseudomonas aeruginosa. J Bacteriol 187:3960–3968.

12. Campos-García J, G Ordóñez L, Soberón-Chávez G. 2000. The Pseudomonas aeruginosa hscA gene encodes Hsc66, a DnaK homologue. Microbiology (Reading, Engl) 146 (Pt 6):1429–1435.

13. Romsang A, Duang-Nkern J, Leesukon P, Saninjuk K, Vattanaviboon P, Mongkolsuk S. 2014. The iron-sulphur cluster biosynthesis regulator IscR contributes to iron homeostasis and resistance to oxidants in Pseudomonas aeruginosa. PLoS ONE 9:e86763.

14. Elias S, Degtyar E, Banin E. 2011. FvbA is required for vibriobactin utilization in Pseudomonas aeruginosa. Microbiology (Reading, Engl) 157:2172–2180.

15. Hassett DJ, Howell ML, Ochsner UA, Vasil ML, Johnson Z, Dean GE. 1997. An operon containing fumC and sodA encoding fumarase C and manganese superoxide dismutase is controlled by the ferric uptake regulator in Pseudomonas aeruginosa: fur mutants produce elevated alginate levels. J Bacteriol 179:1452–1459.

16. Eichner A, Günther N, Arnold M, Schobert M, Heesemann J, Hogardt M. 2014. Marker genes for the metabolic adaptation of Pseudomonas aeruginosa to the hypoxic cystic fibrosis lung environment. Int J Med Microbiol 304:1050–1061.

17. Borlee BR, Goldman AD, Murakami K, Samudrala R, Wozniak DJ, Parsek MR. 2010. Pseudomonas aeruginosa uses a cyclic-di-GMP-regulated adhesin to reinforce the biofilm extracellular matrix. Mol Microbiol 75:827–842.

18. Yao X, He W, Lu C-D. 2011. Functional characterization of seven γ-Glutamylpolyamine synthetase genes and the bauRABCD locus for polyamine and β-Alanine utilization in Pseudomonas aeruginosa PAO1. J Bacteriol 193:3923–3930.

19. Zhang L, Mah T-F. 2008. Involvement of a novel efflux system in biofilm-specific resistance to antibiotics. J Bacteriol 190:4447–4452.

20. Mern DS, Ha S-W, Khodaverdi V, Gliese N, Görisch H. 2010. A complex regulatory network controls aerobic ethanol oxidation in Pseudomonas aeruginosa: indication of four levels of sensor kinases and response regulators. Microbiology (Reading, Engl) 156:1505–1516.

21. Aguilar JA, Zavala AN, Díaz-Pérez C, Cervantes C, Díaz-Pérez AL, Campos-García J. 2006. The atu and liu clusters are involved in the catabolic pathways for acyclic monoterpenes and leucine in Pseudomonas aeruginosa. Appl Environ Microbiol 72:2070–2079.

22. Chou HT, Li J-Y, Peng Y-C, Lu C-D. 2013. Molecular characterization of PauR and its role in control of putrescine and cadaverine catabolism through the γ-glutamylation pathway in Pseudomonas aeruginosa PAO1. J Bacteriol 195:3906–3913.

23. Daddaoua A, Corral-Lugo A, Ramos J-L, Krell T. 2017. Identification of GntR as regulator of the glucose metabolism in Pseudomonas aeruginosa. Environ Microbiol 19:3721–3733.

24. Worstell NC, Singla A, Saenkham P, Galbadage T, Sule P, Lee D, Mohr A, Kwon JS-I, Cirillo JD, Wu H-J. 2018. Hetero-Multivalency of Pseudomonas aeruginosa Lectin LecA Binding to Model Membranes. Sci Rep 8:8419.

25. Attila C, Ueda A, Wood TK. 2008. PA2663 (PpyR) increases biofilm formation in Pseudomonas aeruginosa PAO1 through the psl operon and stimulates virulence and quorum-sensing phenotypes. Appl Microbiol Biotechnol 78:293–307.

26. Fowler RC, Hanson ND. 2015. The OpdQ porin of Pseudomonas aeruginosa is regulated by environmental signals associated with cystic fibrosis including nitrate-induced regulation involving the NarXL two-component system. Microbiologyopen 4:967–982.

27. Taniyama K, Itoh H, Takuwa A, Sasaki Y, Yajima S, Toyofuku M, Nomura N, Takaya N. 2012. Group X aldehyde dehydrogenases of Pseudomonas aeruginosa PAO1 degrade hydrazones. J Bacteriol 194:1447–1456.

28. Wang Y, Ha U, Zeng L, Jin S. 2003. Regulation of membrane permeability by a two-component regulatory system in Pseudomonas aeruginosa. Antimicrob Agents Chemother 47:95–101.

29. Bernard CS, Bordi C, Termine E, Filloux A, de Bentzmann S. 2009. Organization and PprB-dependent control of the Pseudomonas aeruginosa tad Locus, involved in Flp pilus biology. J Bacteriol 191:1961–1973.

30. He W, Li G, Yang C-K, Lu C-D. 2014. Functional characterization of the dguRABC locus for D-Glu and d-Gln utilization in Pseudomonas aeruginosa PAO1. Microbiology (Reading, Engl) 160:2331–2340.

31. Gerth ML, Ferla MP, Rainey PB. 2012. The origin and ecological significance of multiple branches for histidine utilization in Pseudomonas aeruginosa PAO1. Environ Microbiol 14:1929–1940.