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Indole Production Promotes *Escherichia coli* Mixed-Culture Growth with *Pseudomonas aeruginosa* by Inhibiting Quorum Signaling

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Indole production by *Escherichia coli*, discovered in the early 20th century, has been used as a diagnostic marker for distinguishing *E. coli* from other enteric bacteria. By using transcriptional profiling and competition studies with defined mutants, we show that cyclic AMP (cAMP)-regulated indole formation is a major factor that enables *E. coli* growth in mixed biofilm and planktonic populations with *Pseudomonas aeruginosa*. Mutants deficient in cAMP production (*cyaA*) or the cAMP receptor gene (*crp*), as well as indole production (*tnaA*), were not competitive in coculture with *P. aeruginosa* but could be restored to wild-type competitiveness by supplementation with a physiologically relevant indole concentration. *E. coli sdiA* mutants, which lacked the receptor for both indole and *N*-acyl-homoserine lactones (AHLs), showed no change in competitive fitness, suggesting that indole acted directly on *P. aeruginosa*. An *E. coli tnaA* mutant strain regained wild-type competitiveness if grown with *P. aeruginosa* AHL synthase (*rhlI* and *rhlI lasI*) mutants. In contrast to the wild type, *P. aeruginosa* AHL synthase mutants were unable to degrade indole. Indole produced during mixed-culture growth inhibited pyocyanin production and other AHL-regulated virulence factors in *P. aeruginosa*. Mixed-culture growth with *P. aeruginosa* stimulated indole formation in *E. coli cpdA*, which is unable to regulate cAMP levels, suggesting the potential for mixed-culture gene activation via cAMP. These findings illustrate how indole, an early described feature of *E. coli* central metabolism, can play a significant role in mixed-culture survival by inhibiting quorum-regulated competition factors in *P. aeruginosa*.

In nature, bacteria normally occur in polymicrobial communities. Interactions between community members typically involve several mechanisms, including responses to antimicrobial compounds, nutritional interactions, and signaling (9, 12, 42). Chemical signaling is widespread in bacteria, and in Gram-negative bacteria it involves several compounds, including *N*-acyl derivatives of homoserine lactone (AHLs), furans, small peptides, quinolones, and indole (37). In *Pseudomonas aeruginosa*, many genes involved with virulence and competition are regulated by AHL- and quinolone-based quorum signaling (35, 50). Quorum signal disruption (quenching) has been shown to alter bacterial competition and reduce virulence (18). In one animal model study of *P. aeruginosa* lung infections, administration of a ginseng extract caused a reduction in AHL levels and AHL-regulated elastase without affecting bacterial growth (44). Microbial nutrition is also important in species' interactions. Central metabolism, long regarded as reflecting a number of routine housekeeping functions, is now being reexamined for its role in mixed-culture interactions within biofilm and planktonic populations (9).

One component of central metabolism in *Escherichia coli* involves purine and pyrimidine nucleic acid synthesis (reviewed in reference 36). Pathways for *de novo* synthesis and salvage pathways exist for both nucleotides. During purine synthesis, ribose-5-phosphate, an intermediate in the pentose phosphate cycle, is converted through a series of intermediates to the end products AMP and GMP. Aside from being nucleic acid components, purines serve other important functions in bacteria. These functions include energy transfer (ATP and GTP) and cell signaling [cyclic AMP (cAMP), bis-(3'-5')-cyclic di-GMP (c-di-GMP), and guanosine tetraphosphate (ppGpp)] (11, 17). The secondary sig-

nal molecule, cAMP, is synthesized from AMP by adenylate cyclase, which is encoded by *cyaA* (21) and is broken down to 5'-AMP by cAMP phosphodiesterase, encoded by *cpdA* (22). The receptor for cAMP is the cAMP receptor protein, encoded by *crp* (21). The second messenger cAMP has been linked to a number of cellular functions in *E. coli*, most notably regulation of carbon catabolism (8) and more recently a number of stress responses (14).

One *E. coli* metabolite, indole, is produced from the amino acid tryptophan by tryptophanase (encoded by *tnaA*), which is regulated by cAMP (23). Since the discovery of its formation from tryptophan in the early 20th century (19), indole production has been employed as a biochemical test for distinguishing *E. coli* from other members of the *Enterobacteriaceae* (7). Here we show that cAMP-regulated indole production, traditionally associated with central metabolism, quenches the production of AHL-regulated pyocyanin production in *P. aeruginosa* (29) and facilitates *E. coli* growth in mixed culture.

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TABLE 1 Bacterial strains used

Species and strain	Description	Source and/or reference(s) ^a
<i>E. coli</i> BW25113	wt	3
<i>E. coli</i> BW25113 <i>cyaA</i> ::kan	Lacks adenylate cyclase gene	3, 48
<i>E. coli</i> BW25113 <i>cpd</i> ::kan	Lacks cAMP-phosphodiesterase gene	3, 22
<i>E. coli</i> BW25113 <i>crp</i> ::kan	Lacks cAMP receptor protein	3, 14
<i>E. coli</i> BW25113 <i>sdiA</i> ::kan	Lacks <i>luxR</i> homologue (receptor for AHLs and indole)	3, 30, 43
<i>E. coli</i> BW25113 <i>tnaA</i> ::kan	Lacks tryptophanase	3, 23
<i>E. coli</i> MG1655	wt	D. A. Siegele (6)
<i>E. coli</i> MG1655 <i>purH</i>	Lacks IMP cyclohydrolase (purine synthesis)	F. R. Blattner (25)
<i>E. coli</i> MG1655 <i>purD</i>	Lacks phosphoribosyltransferase (purine synthesis)	F. R. Blattner (25)
<i>E. coli</i> ZK126	wt	D. A. Siegele (1)
<i>Pseudomonas aeruginosa</i> PAO1	wt	V. Deretic (45)
<i>P. aeruginosa</i> PDO100 Δ <i>rhII</i> ::Tn501	Lacks AHL synthase for C4-HSL	E. P. Greenberg (50)
<i>P. aeruginosa</i> PAO-MW1 Δ <i>rhII</i> Δ <i>lasI</i>	Lacks AHL synthases for C4-HSL and 3-o-C12-HSL	E. P. Greenberg (50)

^a *E. coli* BW25113 strains were obtained from the Genome Analysis Project in Japan.

MATERIALS AND METHODS

Bacterial strains and media. The strains used in this study are listed in Table 1 and were maintained in Luria-Bertani (LB) medium supplemented with kanamycin (50 μ g/ml) for *E. coli* mutants. Prior to experimentation, cultures were revived from frozen stocks and cultured overnight in LB agar. The cultures were then subcultured in LB broth and incubated overnight at 37°C with aeration (100 rpm). Unless stated otherwise, during experimentation the turbidity of the overnight cultures was adjusted to an optical density at 600 nm (OD₆₀₀) of 0.1 ($[1.08 \pm 0.15] \times 10^5$ CFU/ml [mean \pm standard error of the mean]) using sterile LB broth. During this study, we used several strains of *E. coli*, including ZK126 and MG1655 (for transcriptional profiling) and BW25113 (for the majority of the gene investigations, due to the availability of a mutant collection for it [3]). All three strains are *E. coli* K-12 derivatives (3, 6). During preliminary experiments, we tested several media to differentiate *E. coli* and *P. aeruginosa* during dilution plating. Strain ZK126 showed the most promise in exhibiting different colony morphologies from *P. aeruginosa* on chromogenic agar (Hi-Chrome ECC agar; Fluka) and so was used initially. However, due to concern about colony appearance being affected by mixed growth, we developed selective media with LB plus ampicillin (100 μ g/ml) to select for *P. aeruginosa* and LB plus cefsulodin (20 μ g/ml) to select for *E. coli* (27). In preliminary experiments all three *E. coli* parent strains showed similar competition profiles in mixed culture. Although spontaneous *E. coli* ampicillin-resistant and *P. aeruginosa* cefsulodin-resistant mutants did occur, the estimated frequency was 10^{-6} , so we do not consider spontaneous mutations to be a source of error.

Microarray. *P. aeruginosa* PAO1 and *E. coli* ZK126 were grown in LB at 37°C with a shaking speed of 200 rpm to an OD₆₀₀ of 0.3. The cultures were mixed 1:1 and subsequently incubated for an additional 45 min at 37°C. All cultures were mixed 1:1 with RNAlater (Ambion) and stored at 4°C for a maximum of 2 weeks until processed. RNA extraction, purification, processing for cDNA, and microarray analysis using *E. coli* and *P. aeruginosa* Gene Chip arrays (Affymetrix) were performed as described previously (34). During RNA purification, we monitored genomic DNA contamination by multiplex PCR using *lacZ* as an indicator of *E. coli* (primers Ec-LacZ-F, 5'-ACT ATC CCG ACC GCC TTA CT-3'; Ec-LacZ-R, 5'-TAG CGG CTG ATG TTG AAC TG-3') and *rplU* as an indicator of *P. aeruginosa* genomic contamination (primers and PCR conditions were as described in reference 20).

Competition experiments. For mixed-culture experiments, cells were inoculated into 50 ml LB as described above. The 125-ml culture flasks contained eight sterile silicon discs (7-mm diameter) (49) as biofilm colonization substrata. Competition experiments were performed for up to 7 days. Samples were removed periodically for dilution plating (27) and chemical analysis. Pure culture experiments for each strain were also conducted as controls. Each experiment was replicated a minimum of three times.

Exogenous addition of cAMP and indole. Stock solutions of cAMP and indole were prepared in distilled and dimethyl formamide, respectively, and sterilized by filtration (0.2- μ m pore size) prior to use. To investigate whether exogenous addition of cAMP or indole could restore competitiveness of the mutant strains of *E. coli* in mixed culture growth with PAO1, these compounds were added to LB prior to experimentation at different concentrations (0.2, 0.5, and 1 mM), and the competition experiments were conducted as described above. We also investigated if indole could inhibit growth of *P. aeruginosa* or promote growth of *E. coli* and thus account for a change in *E. coli* competitiveness. Here, *E. coli* BW25113 and *P. aeruginosa* PAO1 were grown (37°C, 100 rpm) as monocultures in LB broth in 125-ml sidarm flasks, in the presence (1 mM) or absence of indole, and growth was monitored by turbidity (OD₆₀₀).

Indole assay. Bacterial cultures were tested for indole production in pure and mixed cultures by using the protocol described by Kawamura-Sato et al. (26).

***P. aeruginosa* virulence factor assays.** Elastase activity was determined by using the Congo red elastin protocol (51). Colorimetric pyocyanin analysis was conducted using the chloroform extraction and HCl acidification protocol (13).

Data analysis. Data calculations were conducted using Excel (Microsoft Office 2007). Statistical analysis and graphing were performed using Sigma Plot v. 11 (Systat Software Inc.).

Microarray sequence accession number. The gene array results have been deposited in Gene Expression Omnibus (GEO) under accession number GSE26931.

RESULTS

Microarray. We conducted microarray analysis on planktonic cocultures of *P. aeruginosa* PAO1 and *E. coli* ZK126 (Table 1) grown in LB. As shown in Table S1 of the supplemental material, a total of 162 genes (approximately 3% of the genome) were differentially expressed in the *E. coli* mixed culture, whereas only 10 genes (approximately 0.1%) were differentially expressed in the *P. aeruginosa* coculture. Interestingly, many genes upregulated in *E. coli* mixed cultures were involved in *de novo* purine biosynthesis (see Table S1). In contrast, pyrimidine synthesis genes were unaffected, which rules out a requirement for DNA or RNA synthesis. Similar upregulation in *de novo* purine synthesis genes was seen in microarray analysis of mixed culture *E. coli* MG1655 and *P. aeruginosa* PAO1 grown for 5 h in a chemically defined minimal medium (38) with vitamin supplementation (10) containing 1 mM *N*-acetylglucosamine as a carbon source (see Table S2 in the supplemental material) (GEO accession number GSE26932), suggesting that *E. coli* purine upregulation in coculture is not strain or

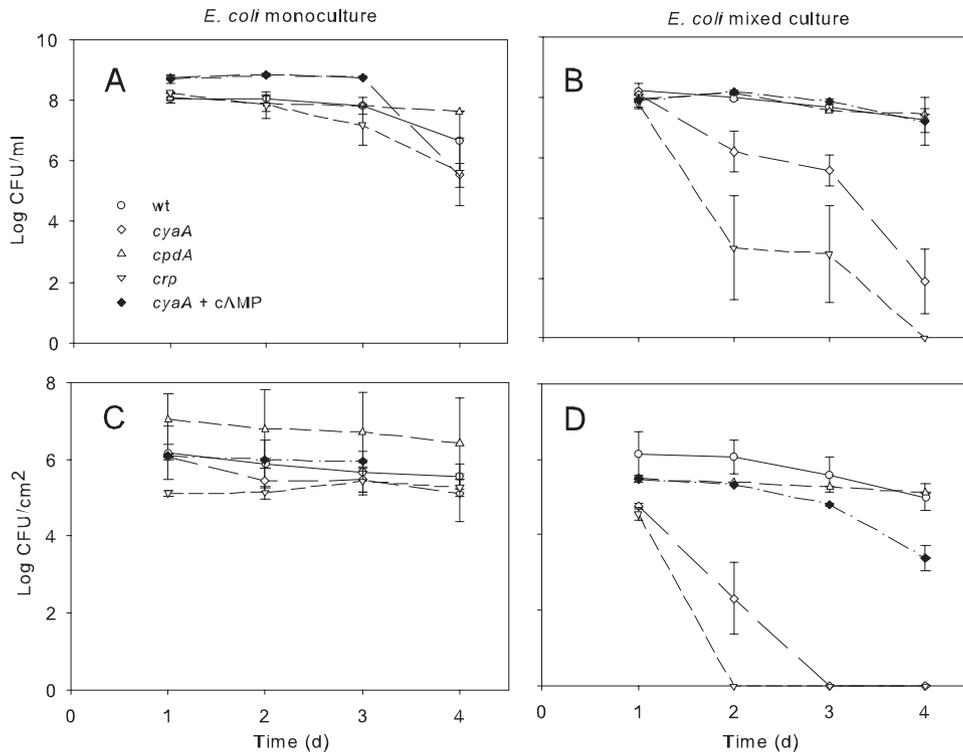


FIG 1 Influence of mutations in *E. coli* cAMP production (adenylate cyclase [*cyaA*]), cAMP receptor gene ([*crp*]), and cAMP phosphodiesterase, encoded by *cpdA* on *E. coli* monoculture planktonic (A) and biofilm (C) cultures and *E. coli* populations in mixed-planktonic (B) and biofilm cultures (D) with *P. aeruginosa*. The growth decline in *E. coli cyaA* mixed cultures was reversed by the addition of 1 mM cAMP. The growth medium in all figures is LB. Error bars in all figures represent standard errors of the means ($n \geq 3$).

medium dependent. Nevertheless, we cannot rule out cross-hybridization of *E. coli* cDNA on *P. aeruginosa* gene chips and vice versa.

Competitiveness of purine mutants. We tested the competitiveness of *E. coli* strains lacking two different genes in *de novo* purine synthesis (*purD* and *purH*) in mixed culture but observed no significant changes in planktonic or biofilm cultures of either *E. coli* or *P. aeruginosa* (see Fig. S1 in the supplemental material). It is conceivable that the salvage pathway for purine synthesis (36) allowed *E. coli purD* and *purH* strains to compensate for these mutations.

cAMP. Aside from being a nucleic acid component, the purine adenine is a component of the signal molecule cAMP. To explore this possibility, we explored the competitiveness of *E. coli* in mixed culture by using strains deficient in cAMP production (*cyaA*), reception (*crp*), or intracellular regulation (*cpdA*). As shown in Fig. 1, *E. coli* cAMP mutants were not affected in monoculture planktonic (Fig. 1A) or biofilm (Fig. 1C) cultures. In coculture, significant loss of *E. coli* competitiveness occurred in both planktonic (Fig. 1B) and biofilm (Fig. 1D) populations in mutants unable to produce cAMP (*cyaA*) or lacking the cAMP receptor gene (*crp*) (14). Competitiveness in the *cyaA* but not *crp* mutants could be restored to wild-type (wt) levels for 24 h by addition of 1 mM cAMP (Fig. 1C and D); however, after 48 h these effects had disappeared, likely due to cAMP depletion. Surprisingly, there was no change in the cAMP degradation (*cpdA*) mutant (22) due to cAMP supplementation in monoculture (Fig. 1A and C) or mixed culture (Fig. 1B and D), suggesting that the levels of cAMP present

were not inhibitory to *E. coli* under our growth conditions. *P. aeruginosa* planktonic and biofilm populations were unaffected by cAMP disruption in *E. coli* (see Fig. S2 in the supplemental material). Based on these results, we conclude that cAMP plays a key role in *E. coli* competitiveness in mixed culture.

Indole. As shown previously (23), we found that indole production was absent in *E. coli crp* and reduced in *cyaA* mutants. We tested mutants deficient in indole production (*tnaA*) and the indole receptor (*sdiA*) (30). As shown in Fig. 2, the *E. coli tnaA* mutant was notably less competitive than its wild-type counterparts in both planktonic (Fig. 2A) and biofilm (Fig. 2C) mixed cultures. In both cases, supplementation with 1 mM indole restored mutant populations to wild-type levels for 24 h (Fig. 2A and C). Population levels of the *sdiA* mutant were not significantly different from the wild type, but its competitive ability was enhanced by indole supplementation (Fig. 2A and C). *P. aeruginosa* populations were not affected (see Fig. S2 in the supplemental material). Indole supplementation also restored the competitiveness of the cAMP mutants *cyaA* and *crp* (Fig. 2B and D). In order to determine whether indole altered growth, we grew *E. coli* and *P. aeruginosa* monocultures in the presence and absence of 1 mM indole and observed a slight indole-mediated inhibition of growth of both cultures (Fig. 2E). The most prominent effects were seen at physiologically relevant concentrations between 500 μ M and 1 mM (47) (Fig. 2E shows the effects in 1 mM indole). At higher concentrations (≥ 5 mM), indole was toxic to both *E. coli* and *P. aeruginosa* and no growth occurred. With *P. aeruginosa*, the growth inhibition caused by indole disappeared between 24 and 48 h. As shown in Fig. 2F, indole was degraded by *P. aeruginosa*

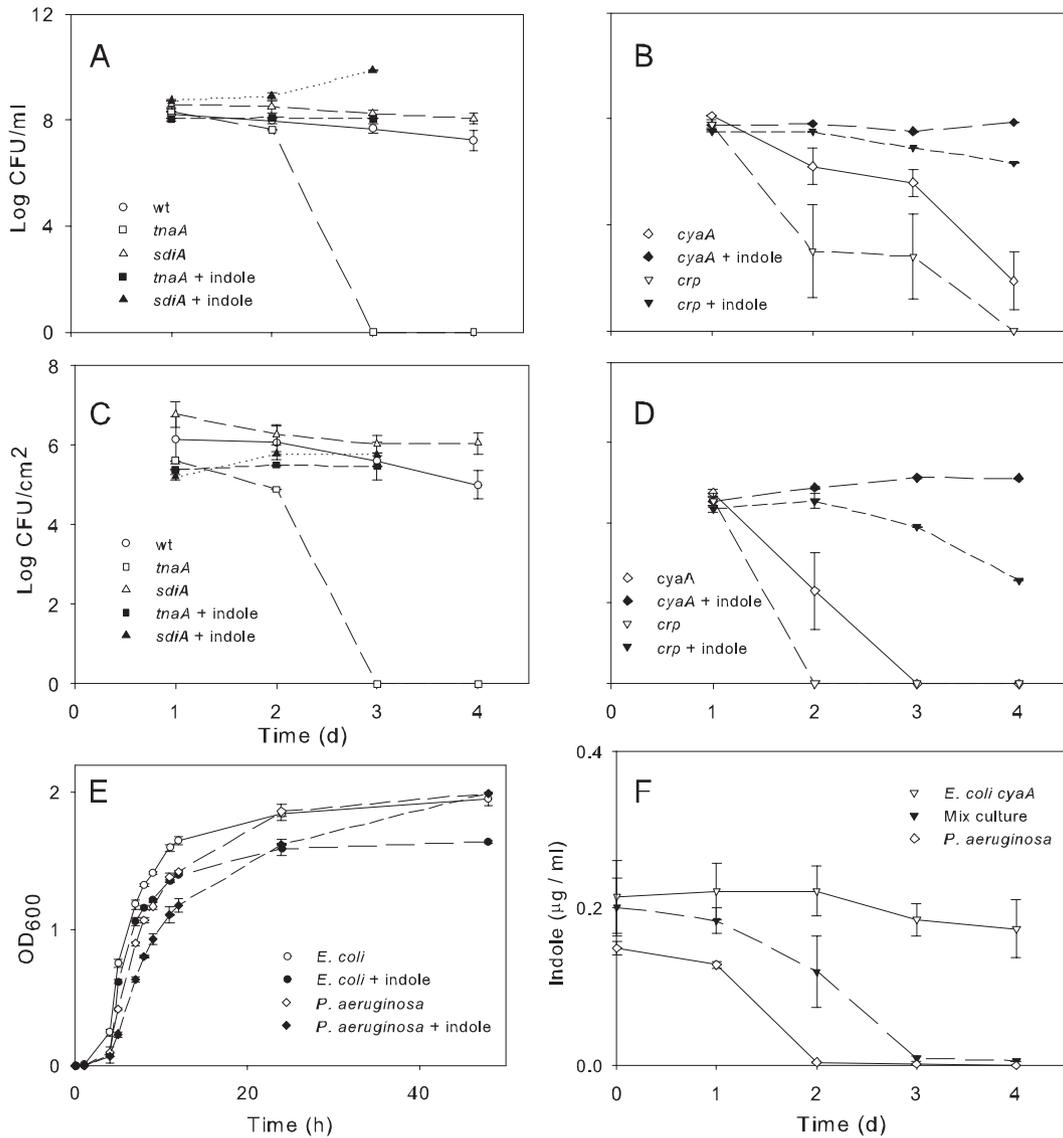


FIG 2 Influence of mutations in *E. coli* indole production (tryptophanase [*tnaA*] and receptor gene (*sdiA*) on growth in mixed culture with *P. aeruginosa* in planktonic (A) and biofilm (C) cultures. Addition of 1 mM indole restored competitiveness of *E. coli* tryptophanase mutants (*tnaA*) to wt levels (A and C) as well as strains lacking adenylate cyclase (*cyaA*) and the cAMP receptor gene (*crp*) (B and D). (E) Monoculture growth of *E. coli* and *P. aeruginosa* in the presence and absence of 1 mM indole. Supplemented indole was metabolized by *P. aeruginosa* over 48 h in pure or mixed culture with an *E. coli* strain unable to produce indole (such as *E. coli cyaA* [panel F]).

after 24 to 48 h in pure or mixed cultures and not by *E. coli*, explaining the transient effects of indole supplementation seen in Fig. 2A to E.

Coculture stimulates indole formation. We investigated whether coculture influenced indole production in *E. coli*. As shown in Fig. 3, mixed-culture growth induced indole production in *E. coli*; however, this effect was only seen with the *E. coli cpdA* strain. These findings suggest that mixed culture growth can enhance cAMP, which in turn promotes indole production. However, this effect did not occur in *E. coli* populations in which cAMP levels were moderated through *cpdA*-mediated homeostasis (22).

Mixed-culture growth inhibits pyocyanin and other AHL-regulated virulence factors. One consistent observation was that *P. aeruginosa* cultures became nonpigmented during mixed-culture growth. We measured pyocyanin production by *P. aeruginosa*

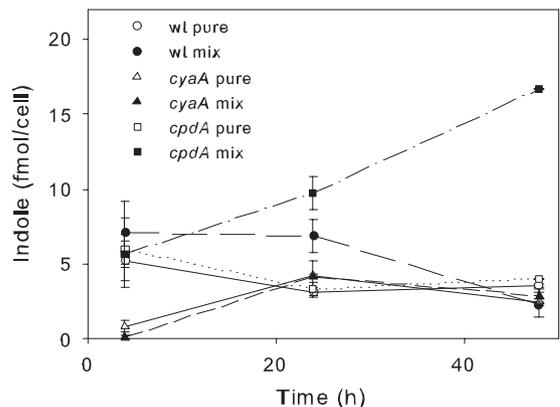


FIG 3 Influence of pure and mixed-culture growth on indole production in wt and *cyaA* and *cpdA* mutants. Indole concentrations were calculated per *E. coli* cell.

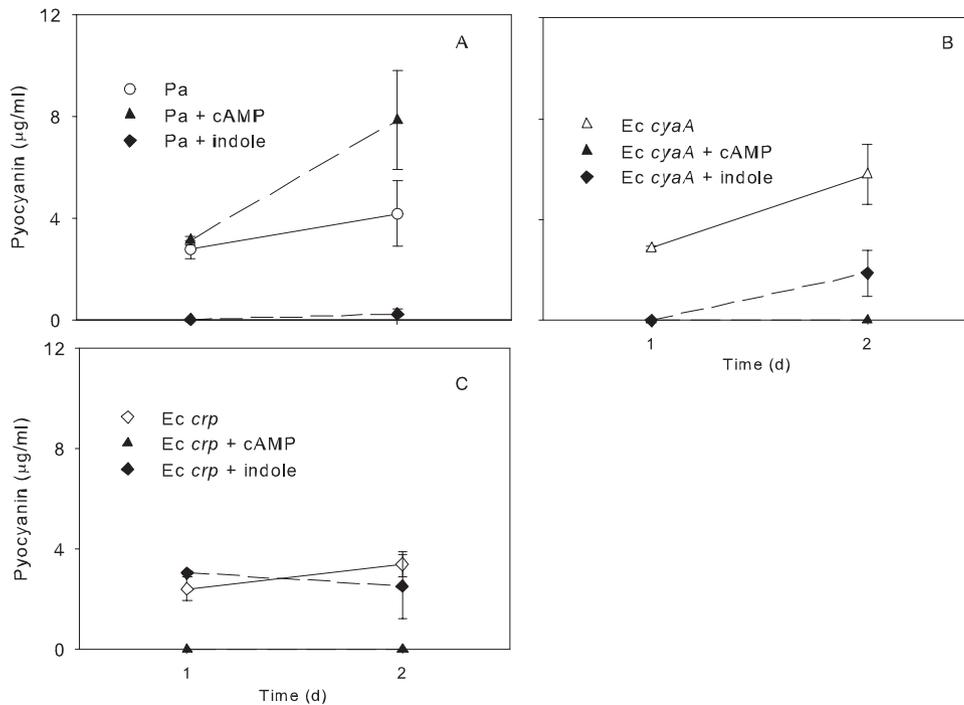


FIG 4 Effects of 1 mM cAMP and 1 mM indole supplementation on pyocyanin production by *P. aeruginosa* in pure culture (A) and mixed culture with *E. coli cyaA* (B) or *E. coli crp* (C). The two *E. coli* strains are unable to produce indole.

nosa in pure and mixed cultures (Fig. 4). Under all conditions tested, pyocyanin production was abolished in the presence of indole. In pure culture, addition of cAMP enhanced pyocyanin production (Fig. 4A). However, in mixed culture with *E. coli cyaA* (Fig. 4B), addition of cAMP decreased pyocyanin, likely due to indole formation. In mixed culture with the *E. coli crp* mutant (Fig. 4C), there was no significant change in pyocyanin formation by *P. aeruginosa* regardless of the presence or absence of cAMP. As pyocyanin production is controlled by quorum signaling (41), we investigated the influence of indole on other AHL-regulated virulence factors of *P. aeruginosa* as well as *P. aeruginosa* strains deficient in AHL production. As measured colorimetrically by dye release with the Congo red elastin protocol (51), elastase production in 24-h *P. aeruginosa* cultures, normalized to *P. aeruginosa* cell density in monoculture, dropped from an OD_{495} of 0.744 ± 0.027 in pure cultures to a 0.023 ± 0.011 ($P < 0.001$) when *P. aeruginosa* was grown in mixed culture with indole-producing *E. coli* strains. In the same time frame, mixed-culture growth with wt *E. coli* caused a small reduction in *P. aeruginosa* cell density (log CFU/ml for pure culture, 9.87 ± 0.35 , compared with log CFU/ml of the mixed culture, 8.64 ± 0.21 ; $P = 0.04$). There was a statistically insignificant reduction in *P. aeruginosa* cell density when it was grown with the *E. coli tnaA* strain (log CFU/ml, 9.61 ± 0.07 ; $P = 0.57$). While the lower *P. aeruginosa* cell density in mixed culture could certainly contribute to a reduction in quorum-regulated elastase, two other studies by Lee et al. (29, 33) have shown indole to inhibit *P. aeruginosa* quorum-regulated virulence factors, including elastase and pyocyanin. On the basis of work reported by Lee et al. (29, 33) and our own studies, we attribute the reductions in elastase and pyocyanin to *E. coli* indole production.

In order to further explore the connection between *E. coli* indole production and *P. aeruginosa* AHL production, we tested the

competitiveness of *E. coli* wt and *tnaA* strains with *P. aeruginosa* strains defective in one (*rhII*) or both (*lasI rhII*) AHL synthase genes (50). As shown in Fig. 5, *E. coli* competitiveness in planktonic (Fig. 5A) and biofilm (Fig. 5C) cultures was restored in *tnaA* mutants when cocultured against *P. aeruginosa* strains deficient in one (*rhII*) or both (*lasI rhII*) AHL synthase genes. In comparison to coculture with wt *E. coli*, *P. aeruginosa* cell numbers increased in mixed planktonic (Fig. 5B) and biofilm (Fig. 5D) populations during coculture with *E. coli tnaA*. Interestingly, there was a small but significant decline in the *P. aeruginosa rhII* mutant, but not in the *lasI rhII* mutant, in biofilm populations (Fig. 5D) during coculture with *E. coli*. One explanation may relate to the inability of *P. aeruginosa* AHL synthesis mutants to degrade indole (Fig. 5E) and reduce its growth inhibition (Fig. 2E). Based on our results, we conclude that the major protective effect of indole during *E. coli* mixed-culture growth is due to direct inhibition of pyocyanin and other quorum-regulated competition factors of *P. aeruginosa*.

DISCUSSION

In nature most bacteria exist within mixed populations. In terms of broad-based genetic studies (involving transcriptomic or proteomic approaches), most mixed-culture investigations have explored a Gram-positive and a Gram-negative organism grown in coculture. Because of fundamental differences in their cell wall architecture (5), one can selectively lyse one member of the consortium and extract desired cellular materials for analysis. These studies have been useful in highlighting some of the novel aspects of mixed-culture interactions (10, 34, 40). When the Gram-negative dental pathogen *Aggregatibacter actinomycetemcomitans* is grown in mixed culture with the Gram-positive commensal *Streptococcus gordonii*, it shifts its energy source to lactate from glucose (10), but it also gains enhanced serum protection that can

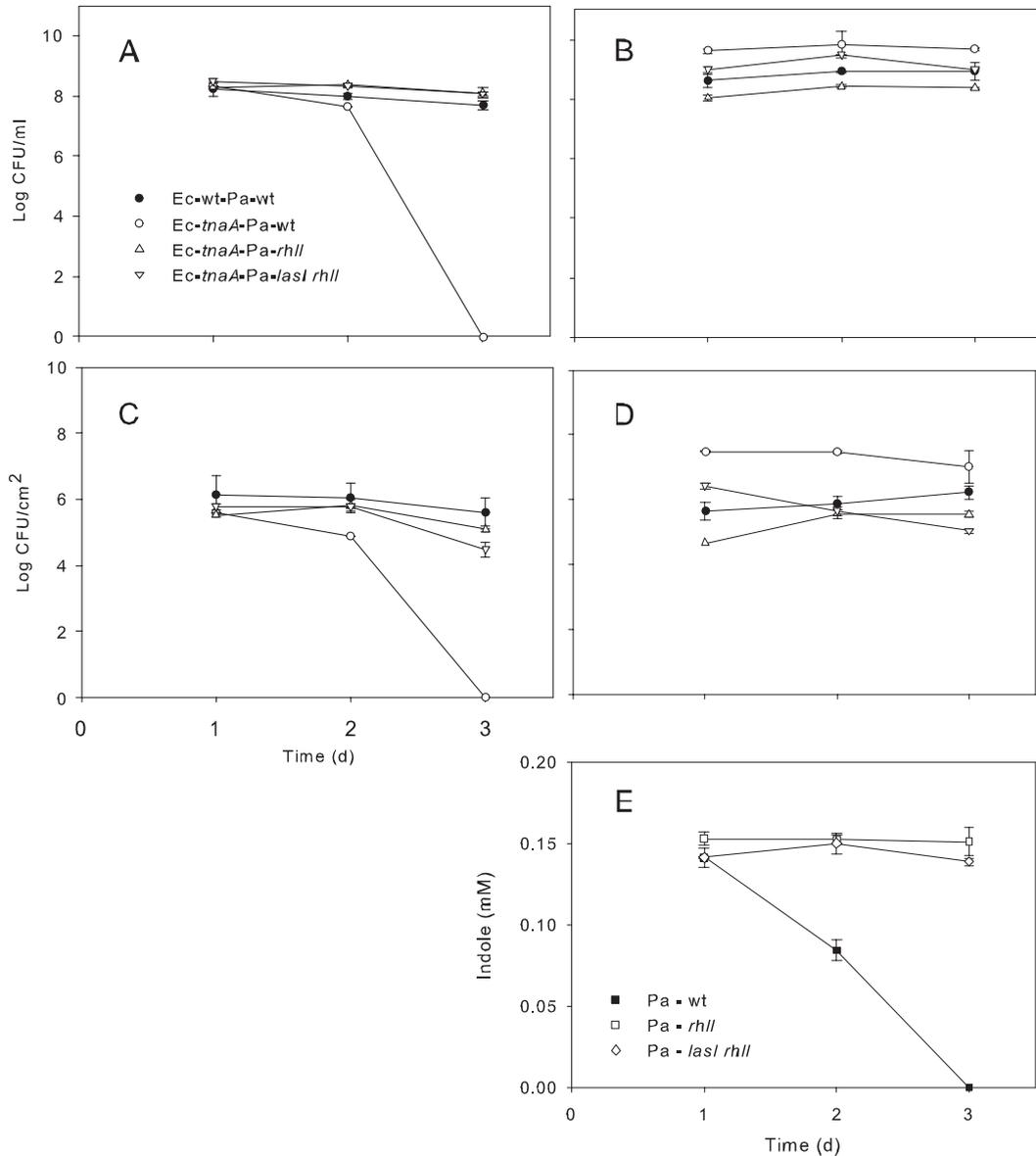


FIG 5 Interaction of *E. coli* tryptophanase (*tnaA*) and *P. aeruginosa* AHL synthesis (*lasI* and *rhII*) on *E. coli* (A [planktonic] and C [biofilm]) and *P. aeruginosa* (B [planktonic] and D [biofilm]) growth in mixed culture. Symbols for panels A to D are shown in panel A. Data for growth of *E. coli* wt with *P. aeruginosa* wt and of *E. coli tnaA* with *P. aeruginosa* wt from Fig. 2 are shown again for comparison. (E) In contrast to the wild type, *P. aeruginosa* AHL synthesis mutants were unable to degrade indole.

enable it to enter the circulatory system (40). In another example, coculture of *P. aeruginosa* and *Staphylococcus aureus* resulted in *P. aeruginosa* lysing *S. aureus* as an iron source (34), a process mediated by outer membrane vesicles produced in *P. aeruginosa* through the action of the *Pseudomonas* quinolone signal (PQS) (35). PQS is a quorum signal in *P. aeruginosa* under the control of the *las* and *rhl* AHL quorum system (39). In one study involving two Gram-negative organisms, An et al. (2) showed motility genes, associated with biofilm formation and quorum signal regulation, enhanced *P. aeruginosa* competition with *Agrobacterium tumefaciens* in this mixed-culture system. Of relevance to the current study, *P. aeruginosa* mutants deficient in AHL-mediated quorum signaling were less competitive against *A. tumefaciens* than the wild type. While *A. tumefaciens* also has an AHL-regulated

quorum-signaling system (2), this system did not appear to play a role during its interactions with *P. aeruginosa*. In the An et al. study (2), the enhanced growth rate of *P. aeruginosa* relative to *A. tumefaciens* also provided a competitive advantage to *P. aeruginosa*. In our study, the growth rates of *P. aeruginosa* and *E. coli*, at least in monoculture, were similar (Fig. 2E), so we do not consider the growth rate differences to be important. One common theme from these aforementioned mixed-culture studies involving *P. aeruginosa* (2, 35) appears to be a role of quorum-regulated competition factors. We also found *P. aeruginosa* AHL-regulated quorum signaling to be important in its interactions with *E. coli* (Fig. 5), and we address this issue below. In general, microorganisms appear to adopt several strategies for mixed-culture survival, including nutritional and metabolic flexibility, mixed-culture-

induced gene (*mcg*) expression, and signaling. These strategies must be considered in context with previously identified mechanisms, including bacteriocins, resource competition, and generation of harmful metabolites, that are employed during competition (9, 12).

Based on transcriptional profiling results showing elevated *E. coli* purine synthesis in mixed culture (see Table S1 in the supplemental material), we investigated the growth of *E. coli purD* and *purH* strains in mixed culture and found no loss of competitiveness (see Fig. S1 in the supplemental material), possibly due to the action of the purine salvage pathway (36). Pyrimidine synthesis genes were unaffected by mixed culture (see Table S1), which discounts a requirement for enhanced nucleic acid synthesis. Although ATP and GTP levels were not measured, we saw no evidence that *E. coli* growth was inhibited in purine synthesis mutants (*purD* and *purH*) either during growth in pure culture or in mixed culture (see Fig. S1), as might be expected from energy depletion. Aside from cAMP, purines are also involved in other signaling systems in *E. coli*, notably c-di-GMP and ppGpp (17). In contrast to wt, *E. coli relA* and *relA spoT* stringent response mutants that are defective in ppGpp synthesis show inhibited growth in pure culture (4) and so were not investigated in coculture, as growth inhibition would mask any competitive disadvantage. The effect of c-di-GMP on mixed-culture growth remains to be investigated. However, the most significant changes in *E. coli* competition were due to cAMP disruption (Fig. 1), which became the focus of this study.

In *E. coli*, cAMP and its receptor protein, Crp, have been traditionally associated with glucose-mediated catabolite repression (8). Transcriptome analyses (15) have shown that approximately 200 operons are regulated directly or indirectly by cAMP. Aside from carbon catabolism, many other cell functions are now recognized as components of the Crp regulon, including some genes associated with stress responses, cell division, and amino acid metabolism, including the tryptophanase gene *tnaA* (23). During mixed-culture growth, *E. coli* would need to be flexible for nutrient utilization as individual carbon sources are depleted and metabolites accumulate, including some that may induce stress. From both a nutrition and stress response perspective, a central role of cAMP in *E. coli* mixed-culture growth is logical (Fig. 1).

During competition with other species, *P. aeruginosa* produces a number of toxic compounds that are regulated by quorum signaling. These include pyocyanin and other phenazine compounds (16), rhamnolipids (24), and membrane vesicles (35). Pyocyanin enhances oxidative stress for competing organisms (16). Pyocyanin and membrane vesicles are toxic against *Staphylococcus aureus* during pulmonary infections in cystic fibrosis (35), and rhamnolipids have been shown to be toxic against eukaryotic cells (24). As well, rhamnolipids enhance swarming motility in *P. aeruginosa* (28). It was quite notable that coculture with wild-type *E. coli* caused a decrease in several AHL-regulated features in *P. aeruginosa*. These included pyocyanin production (Fig. 4) and elastase (described above). Inhibition of protease and pyocyanin was not present in the cAMP mutants *cyaA* and *cpdA* or in the *tnaA* mutant (data not shown), but it could be restored upon supplementation with 1 mM indole. Our observations of indole-based inhibition of *P. aeruginosa* AHL-regulated elastase and pyocyanin are in agreement with those reported by Lee et al. (29). *E. coli tnaA* strains regained competitive fitness during coculture with *P.*

aeruginosa rhlI and *lasI rhlI* AHL synthesis mutants (Fig. 5A and C). As well, *P. aeruginosa* AHL synthesis mutants were unable to degrade exogenously supplied indole (Fig. 5E), giving further support to the interactions between AHL signaling and indole. The mechanism of indole degradation is not known but is under investigation. Based on our results and those of Lee et al. (29), it would appear that *rhl*-regulated genes are involved in indole degradation (Fig. 5E), but both *las*- and *rhl*-regulated genes in *P. aeruginosa* are affected by indole. In contrast to the culture data, we did not observe any alterations in AHL-mediated gene expression during transcriptome studies. The changes in competition (Fig. 1 and 2) occurred after 24 h, whereas the transcriptome analyses were done in early-log-phase cultures (45 min in LB and 3 to 4 h in defined medium). Quorum signaling typically begins in late-log-phase and stationary-phase cultures (37), which occurred after 12 h (Fig. 2E), and was therefore not detected. Indole and related compounds have been shown to inhibit pyocyanin and other quorum-regulated virulence factors in *P. aeruginosa* (29), although the role of indole in microbial competition has not been previously described.

Indole has been shown to be a cell signal molecule (30, 47) in *E. coli*. Functions associated with indole regulation in *E. coli* include amino acid catabolism (47), acid resistance, biofilm inhibition, and motility (30). In this context, it was important to determine whether any competition-enhancing effect was due to a direct influence of indole on *P. aeruginosa* or whether it was due to an indirect effect via indole-mediated gene expression in *E. coli*. Deletion of the *E. coli* indole receptor, *sdiA* (30), had no effect on *E. coli* competition in either planktonic (Fig. 2A) or biofilm (Fig. 2C) populations, which discounts an indirect effect. Although *E. coli* does not produce AHLs (it lacks a *luxI* homologue), it has a gene, *sdiA*, that functions as a *luxR* homologue (30, 31) and could conceivably use and deplete AHLs produced by other organisms. Our results (Fig. 2A and C) do not support *sdiA*-mediated quorum quenching as a factor in *E. coli* competitiveness under our experimental conditions. With respect to indole signaling, our investigations were performed at 37°C, whereas *E. coli* indole signaling is only prominent at 25 to 30°C and largely absent at 37°C (30, 32). Interestingly, the loss of competition of cAMP mutants *cyaA* and *crp* could be reversed by supplementation with physiologically relevant concentrations of indole (47) (Fig. 2B and D). As shown in Fig. 3, we saw that mixed-culture growth had the potential to enhance indole production via increasing cAMP levels in *E. coli cpdA* cells but not in other strains. As *cpdA*-encoded phosphodiesterase removes excess intracellular cAMP levels in *E. coli* (22), this observation shows that exposure to *P. aeruginosa* has the potential to increase indole production via enhanced cAMP levels, but any stimulation is buffered by cAMP reduction by the *cpdA* gene product (22). *P. aeruginosa* produces a number of metabolites during growth (46), and further investigation is needed to identify the metabolites that may impact *E. coli* indole production.

In summary, we conclude that cAMP and indole enhance *E. coli* during mixed-culture growth with *P. aeruginosa* through the indole-based inhibition of several *las*- and *rhl*-regulated *P. aeruginosa* virulence factors, notably pyocyanin. *P. aeruginosa* indole degradation appears to require *rhl*-mediated quorum signaling. Similar beneficial effects of indole on *E. coli* competition were seen in both biofilm and planktonic populations, and so we interpreted

this phenomenon as a global effect, rather than a planktonic- or biofilm-specific effect, as reported in other studies (27, 49). While indole production by bacteria has been known for over a century (19), this study shows that this metabolite provides a key mechanism to explain the natural ecological success of *E. coli* in mixed communities.

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