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 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* cpaA, 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 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 signal 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 cpaA (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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mutants did occur, the estimated frequency was 10^{-3}. All three strains are
useful in these gene investigations, due to the availability of a mutant collection for it
MG1655 (for transcriptional profiling) and BW25113 (for the majority of
neous
During this study, we used several strains of
E. coli
exhibiting different colony morphologies from
Hi-Chrome ECC agar; Fluka) and so was used initially. How-
4°C for a maximum of 2 weeks until processed. RNA extraction, purifica-
37°C. All cultures were mixed 1:1 with RNAlater (Ambion) and stored at
were mixed 1:1 and subsequently incubated for an additional 45 min at
adjusted to an optical density at 600 nm (OD600) of 0.1 ([1.08 ± 0.15] × 10^{10} \text{ CFU/ml} \text{ (mean ± 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
[31]). All three strains are
E. coli K-12 derivatives (3, 6). During preliminary experiments, we tested several media to differentiate E. coli and P. aerugi-
osa during dilution plating. Strain ZK126 showed the most promise in
exhibiting different colony morphologies from
P. aeruginosa on chromo-
genic agar (Hi-Chrome ECC agar; Fluka) and so was used initially. How-
ever, due to concern about colony appearance being affected by mixed
growth, we developed selective media with LB plus ampicillin (100 µg/ml)
to select for
P. aeruginosa and LB plus cefsulodin (20 µg/ml) to select for E. coli (27). In preliminary experiments all three E. coli parent strains showed similar competition profiles in mixed culture. Although sponta-
neous
E. coli ampicillin-resistant and
P. aeruginosa cefsulodin-resistant mutants did occur, the estimated frequency was 10^{-9}, so we do not con-
sider 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
content, and thus account for a change in
indole could inhibit growth of
E. coli. 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. We used the protocol described by Kawamura-
Sato et al. (26).

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

**P. aeruginosa virulence factor assays.** Elastase activity was deter-
mied by using the Congo red elastin protocol (51). Colorimetric pyocy-
amin analysis was conducted using the chlorof orm extraction and HCl
acidification protocol (13).

**Data analysis.** Data calculations were conducted using Excel (Mi-
crosoft 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 cultures 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 (ap-
proximately 0.1%) were differentially expressed in the
P. aerugi-
no sar-1 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 una-
fected, 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), sug-
gesting that
E. coli purine upregulation in coculture is not strain or

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**TABLE 1 Bacterial strains used**

<table>
<thead>
<tr>
<th>Species and strain</th>
<th>Description</th>
<th>Source and/or reference(s)</th>
</tr>
</thead>
<tbody>
<tr>
<td>E. coli BW25113</td>
<td>wt</td>
<td></td>
</tr>
<tr>
<td>E. coli BW25113 cya::kan</td>
<td>Lacks adenylyl cyclase gene</td>
<td>3, 48</td>
</tr>
<tr>
<td>E. coli BW25113 cad::kan</td>
<td>Lacks cAMP-phosphodiesterase gene</td>
<td>3, 22</td>
</tr>
<tr>
<td>E. coli BW25113 cya::kan</td>
<td>Lacks cAMP receptor protein</td>
<td>3, 14</td>
</tr>
<tr>
<td>E. coli BW25113 mca::kan</td>
<td>Lacks luxR homologue (receptor for AHLs and indole)</td>
<td>3, 30, 43</td>
</tr>
<tr>
<td>E. coli MG1655</td>
<td>wt</td>
<td></td>
</tr>
<tr>
<td>E. coli MG1655 purH</td>
<td>Lacks IMP cyclohydrase (purine synthesis)</td>
<td>F. R. Blatter (25)</td>
</tr>
<tr>
<td>E. coli MG1655 purD</td>
<td>Lacks phosphoribosyltransferase (purine synthesis)</td>
<td>F. R. Blatter (25)</td>
</tr>
<tr>
<td>E. coli ZK126</td>
<td>wt</td>
<td></td>
</tr>
<tr>
<td>Pseudomonas aeruginosa PAO1</td>
<td>wt</td>
<td>D. A. Siegele (6)</td>
</tr>
<tr>
<td>P. aeruginosa PDO100 ΔrhlI::Tn501</td>
<td>Lacks AHL synthase for C4-HSL</td>
<td>V. Deretic (45)</td>
</tr>
<tr>
<td>P. aeruginosa PAO-MW1 Δrhl Δlas</td>
<td>Lacks AHL synthases for C4-HSL and 3-o-C12-HSL</td>
<td>E. P. Greenberg (50)</td>
</tr>
</tbody>
</table>

*E. coli BW2513 strains were obtained from the Genome Analysis Project in Japan.*
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 employed the competitiveness of *E. coli* in mixed culture by using strains deficient in cAMP production (adenylate cyclase [*cyaA*]), cAMP receptor gene (*crp*), or 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 ≥ 3).

![FIG 1](http://aem.asm.org/) 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 ≥ 3).

*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) 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). 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 μM 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*.
after 24 to 48 h in pure or mixed cultures and not by \textit{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 \textit{E. coli}. As shown in Fig. 3, mixed-culture growth induced indole production in \textit{E. coli}; however, this effect was only seen with the \textit{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 \textit{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 \textit{P. aeruginosa} cultures became nonpigmented during mixed-culture growth. We measured pyocyanin production by \textit{P. aerugi-
Indole Production in E. coli-P. aeruginosa Cocultures

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.

E. coli in monoculture, dropped from an OD 495 of 0.744 to 0.35, compared with log CFU/ml of 9.61 ± 0.07; \( P = 0.04 \). 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 (rhlI) or both (lasI rhlI) 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 (rhlI) or both (lasI rhlI) 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 rhlI mutant, but not in the lasI rhlI 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
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. **FIG 5** Interaction of *E. coli* tryptophanase (*tnaA*) and *P. aeruginosa* AHL synthesis (*lasI* and *rhlI*) 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.
induced gene (mecG) 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 cpaA 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 AHLLs 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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