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The Acyl Homoserine Lactone Receptor, SdiA, of *Escherichia coli* and *Salmonella enterica* Serovar Typhimurium Does Not Respond to Indole

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In this study, we tested the hypothesis that the SdiA proteins of *Escherichia coli* and *Salmonella enterica* serovar Typhimurium respond to indole. While indole was found to have effects on gene expression and biofilm formation, these effects were not *sdiA* dependent. However, high concentrations of indole did inhibit *N*-acyl-L-homoserine lactone (AHL) sensing by SdiA. We conclude that SdiA does not respond to indole but indole can inhibit SdiA activity in *E. coli* and *Salmonella*.

In prokaryotes, cell-to-cell signaling that allows bacteria to coordinate cellular processes within a larger population, or quorum, is called quorum sensing (9, 24). Bacteria secrete different molecules as intercellular signals, such as *N*-acyl-L-homoserine lactones (AHLs) and autoinducer 2 (AI-2) in the case of the *Proteobacteria* and small peptides in the case of the *Firmicutes* (2, 24). The paradigm for AHL signaling is the LuxR/LuxI system of *Vibrio fischeri* (23). LuxI, an AHL synthase, produces *N*-(3-oxo-hexanoyl)-L-homoserine lactone (oxoC6), which can diffuse passively across membranes (14). LuxR binds oxoC6 and responds by activating transcription of the *luxICDABEGH* operon, which encodes luciferase. Accumulation of AHL signals within a confined environ-

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TABLE 1 Strains and plasmids used in this work

Strain or plasmid	Genotype[a]	Source, reference, or description
Strains		
S. enterica serovar Typhimurium		
14028	Wild type	American Type Culture Collection
BA612	14028 *sdiA1*::mTn*3*	1
E. coli		
AL4001	BA4000 *gadW4001*::mTn*5-lux-kan2*	8
BA4000	Nal-resistant mutant of BW25113	8
BA760	MG1655 *sdiA*::Kan^r	P1 transduction of WX2 *sdiA*::Kan^r into MG1655
BA763	W3110 *sdiA*::Kan^r	P1 transduction of WX2 *sdiA*::Kan^r into W3110
BW25113	*lacI*^{q} *rrnB*$_{T14}$ Δ*lacZ*$_{WJ16}$ *hsdR514* Δ*araBAD*$_{AH33}$ Δ*rhaBAD*$_{LD78}$	6
JLD800	BA4000 *gadW4001*::mTn*5-lux-kan2 sdiA271*::*cam*	8
JNS3003	BW25113 *sdiA*$^+$-FRT-*cam*-FRT	BW25113 with insertion of FRT-cam-FRT cassette using λ Red recombination with primers BA1192 and BA1193 and template pKD3
JNS3212	BW25113 *sdiA*$^+$-*tnpR-lacZYA*	FRT-cam-FRT removed from JNS3003 using Flp recombinase encoded by pCP20; pCE70 inserted at FRT scar using Flp recombinase; pCP20 was subsequently cured by growth at 37°C
ME020	BA4000 *gadW4001*::mTn*5-lux-kan2 sdiA271*::*cam tnaA*::pGP704	*tnaA* disrupted by single crossover of pME017 suicide vector into JLD800 chromosome; verified insertion using PCR with primers BA685 and BA2421
ME021	BA4000 *gadW4001*::mTn*5-lux-kan2 tnaA*::pGP704	*tnaA* disrupted by single crossover of pME017 suicide vector into AL4001 chromosome; verified insertion using PCR with primers BA685 and BA2421
MG1655	F$^-$ lambda$^-$ *ilvG rfb-50 rph-1*	*E. coli* Genetic Stock Center
W3110	F$^-$ lambda$^-$ IN(*rrnD-rrnE*)1 *rph-1*	11
WX2	Δ*lac sdiA*::Kan^r	36
Plasmids		
pCE70	FRT-*tnpR-lacZY* oriR6K (Kan^r); contains wild-type *tnpR* Shine-Dalgarno; FRT orientation A	20
pCP20	*c*I857 λP$_R$ *flp* pSC101 oriTS (Amp^r Cam^r)	6
pGP704	Suicide vector, oriR6K (Amp^r)	22
pKD3	FRT-*cam*-FRT oriR6K (Amp^r)	6
pJNS25	P*srgE-luxCDABE* (Tet^r)	32
pME017	pGP704 carrying internal portion of *tnaA*	*tnaA* fragment amplified with PCR using primers BA2145 and BA2146, BW25113 as template, and *Taq* DNA polymerase (NEB); fragment cloned into pGEM T-Easy using T4 DNA ligase (Promega); fragment removed with XbaI SphI and ligated into pGP704 cut with XbaI SphI

[a] Nal, nalidixic acid.

TABLE 2 Primers used in this work

Primer	Sequence[a]	Description
BA685	AGATCTCTGGCGCGTCGTCGCCACCTACAGGC	*tnaA* insertion verification
BA1192	TGTTACGCGGCCGCTACTGGCTTAATTTGAgtgtaggctggagctgcttc	*sdiA*$^+$-FRT-*cam*-FRT
BA1193	TTGCATCTGGCACGCAGGACAGAAAAGAGAcatatgaatatcctccttag	*sdiA*$^+$-FRT-*cam*-FRT
BA2145	TCTAGACTGATTAAAAAACGCGAGCAGGAAAAAG	Internal *tnaA* portion
BA2146	GCATGCCATCGACCAGATACTGTACCTGCGCGATAC	Internal *tnaA* portion
BA2421	GTGGCACTTTTCGGGGAAATGTGCGCGGAACCCC	*tnaA* insertion verification

[a] Lowercase letters indicate the portion of the primer that binds the template pKD3.

ment leads to coordinate activation of light production (9, 10, 27, 28).

Escherichia coli and *Salmonella enterica* serovar Typhimurium (*S.* Typhimurium) encode a LuxR homolog, SdiA, but do not encode any type of AHL synthases and do not synthesize AHLs (21). Instead, these organisms respond to the AHLs produced by other species of bacteria, such as *Yersinia enterocolitica* (7, 21, 32–34). The structure of AHL bound to the N terminus of SdiA has been determined (37). SdiA upregulates two loci in *S.* Typhimurium, the *rck* (resistance to complement killing) operon, located on the *Salmonella* virulence plasmid, and *srgE* (*sdiA*-regulated gene), a horizontally acquired gene located on the chromosome (1, 21, 32). The function of SrgE is unknown, but computer algorithms suggest it may be a type III secreted effector (29). Neither of these loci is present in *E. coli*. In *E. coli* K-12, *sdiA* upregulates the acid fitness island (which is not present in *S.* Typhimurium) while downregulating flagellar genes (8, 13, 18, 25, 35). Enterohemorrhagic *E. coli* (EHEC) has an additional pathogenicity island, the locus of enterocyte effacement (LEE), which is also downregulated by *sdiA* (12, 13).

It has been reported that in addition to sensing AHLs, SdiA responds to indole (5, 16–18). Indole is an intermediate product in tryptophan biosynthesis and is produced by the tryptophan degradation enzyme, tryptophanase (TnaA). Similar to AHLs, indole has been shown to be freely diffusible across bacterial membranes (14, 26). While *E. coli* encodes TnaA and produces indole, *S.* Typhimurium does not. Indole was found to repress biofilm formation of *E. coli* (4, 16, 19, 30). Transcription profiling of biofilms indicated that *sdiA* was upregulated approximately 3-fold in the presence of indole (16). Subsequent work has shown that indole represses biofilm formation of *E. coli* at 30°C but not 37°C and that this repression is *sdiA* dependent (18). In this study, we attempted to replicate these findings.

SdiA has no effect on biofilm formation in *E. coli* K-12 or *S.* Typhimurium. Strains and primers are listed in Tables 1 and 2. The initial report linking *sdiA* and indole measured biofilm for-

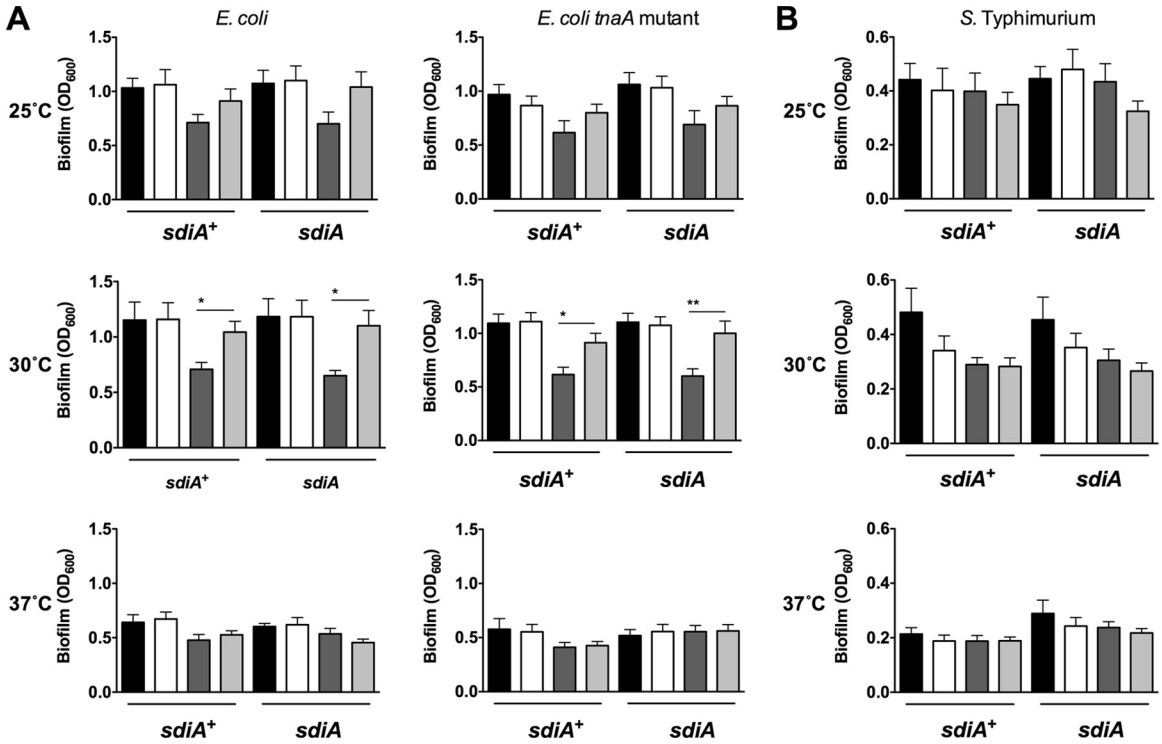

FIG 1 Biofilm formation in the presence of AHL and 500 μM indole in *E. coli* K-12 and *S.* Typhimurium. (A) Biofilm formation of *E. coli* K-12 BW25113 in either the wild-type (AL4001), *sdiA* mutant (JLD800), *tnaA* mutant (ME021), or *tnaA sdiA* double mutant (ME020) background. (B) Biofilm formation of *S.* Typhimurium 14028 or the isogenic *sdiA* mutant (BA612). Crystal violet absorbance at the optical density at 600 nm (OD_{600}) is indicated by bars for 1 μM AHL (black), 0.1% ethyl acetate (EA) (white), 500 μM indole (dark gray), and 0.1% dimethylformamide (DMF) (light gray). All data points are the averages for nine biological replicates, and error bars indicate SEM. Statistical significance in comparison to results for the solvent control is denoted by asterisks representing *t* test *P* values: *, <0.05; **, <0.005.

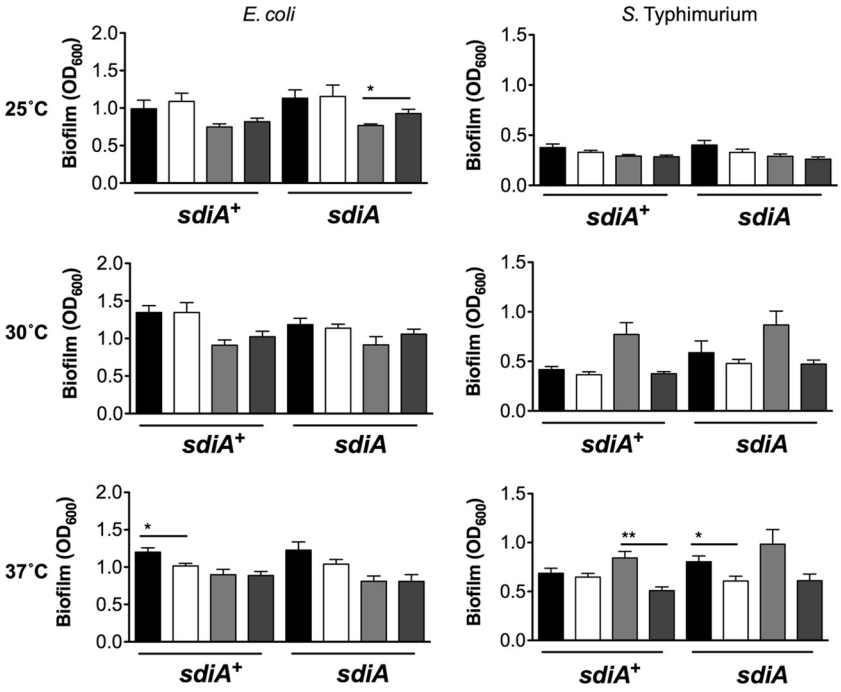

FIG 2 Biofilm formation in the presence of AHL and 1 mM indole in *E. coli* K-12 and *S.* Typhimurium. Biofilm formation of *E. coli* K-12 in either the wild type (AL4001) or the *sdiA* mutant (JLD800) and of *S.* Typhimurium 14028 or the isogenic *sdiA* mutant (BA612) was analyzed. Crystal violet absorbance at 600 nm is indicated by bars for 1 μM AHL (black), 0.1% EA (white), 1 mM indole (light gray), and 0.1% DMF (dark gray). All data points are the averages for nine biological replicates, and error bars indicate SEM. Statistical significance in comparison to results with the solvent control is denoted by asterisks representing *t* test *P* values: *, <0.05; **, <0.005.

mation of *E. coli* K-12 strain BW25113 grown in LB broth on polystyrene plates as measured by crystal violet staining (18). Therefore, we measured biofilm formation of *E. coli* K-12 strain BW25113 grown in LB broth on polystyrene in the presence of AHL (1 μM oxoC6), indole (500 μM), or solvent controls (acidified ethyl acetate for AHL [EA] and dimethyl formamide for indole [DMF]). We tested each of three growth temperatures, 25°C, 30°C, and 37°C. In either the *E. coli* wild-type or *tnaA* mutant

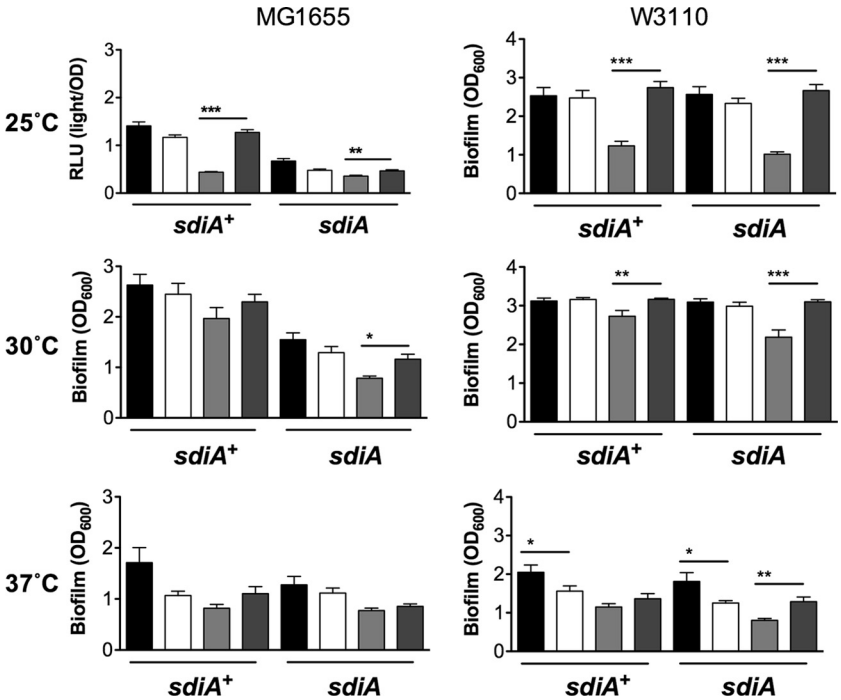

FIG 3 Biofilm formation in the presence of AHL and indole in other *E. coli* backgrounds. Biofilm formation of *E. coli* MG1655 and W3110 or the isogenic *sdiA* mutants, BA763 and BA760, is shown. Crystal violet absorbance at 600 nm is indicated by bars for 1 μM AHL (black), 0.1% EA (white), 1 mM indole (light gray), and 0.1% DMF (dark gray). All data points are the averages for nine biological replicates, and error bars indicate SEM. Statistical significance in comparison to results with the solvent control is denoted by asterisks representing *t* test *P* values: *, <0.05; **, <0.005; ***, <0.0005.

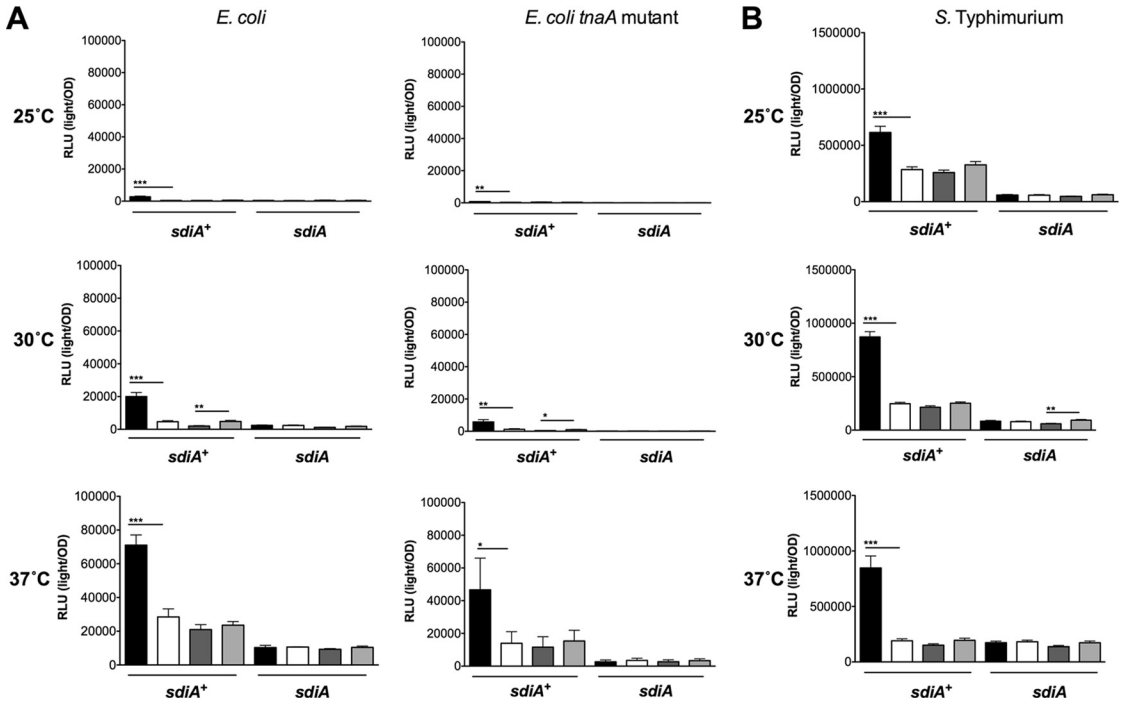

FIG 4 Response of SdiA to AHL and 500 μM indole in *E. coli* K-12 and *S.* Typhimurium during shaking growth conditions. (A) Expression of the *gadW*::Tn*5-luxCDABE* fusion in *E. coli* K-12 in either the wild-type (AL4001), *sdiA* mutant (JLD800), *tnaA* mutant (ME021), or *tnaA sdiA* double mutant (ME020) background. (B) Expression of the *srgE-luxCDABE* fusion in *S.* Typhimurium 14028 or the isogenic *sdiA* mutant (BA612). Relative light units (light/OD_{590}) after 9 h of growth with shaking are indicated by bars for 1 μM AHL (black), 0.1% ethyl acetate (EA) (white), 500 μM indole (dark gray), and 0.1% dimethylformamide (DMF) (light gray). All data points are the averages for nine biological replicates, and error bars indicate SEM. Statistical significance in comparison to results with the solvent control is denoted by asterisks representing *t* test *P* values: *, <0.05; **, <0.005; ***, <0.0005.

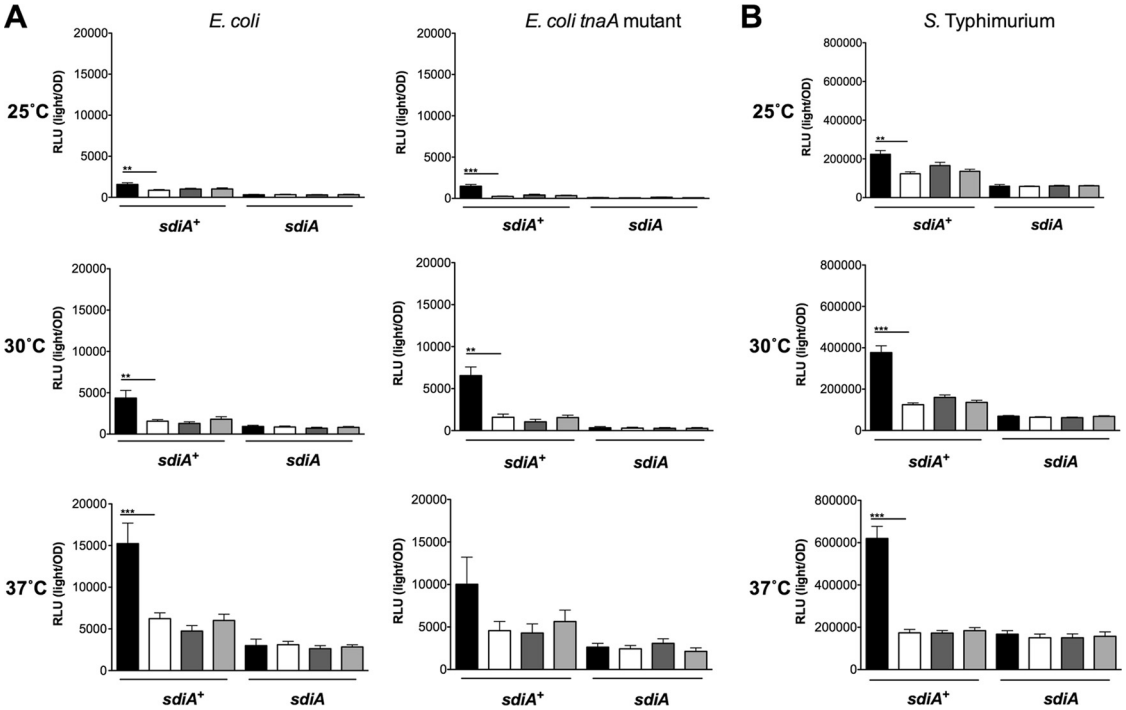

FIG 5 Response of SdiA to AHL and 500 μM indole in *E. coli* K-12 and *S.* Typhimurium during standing growth conditions. (A) Expression of the *gadW*::Tn*5-luxCDABE* fusion in *E. coli* K-12 in either the wild-type (AL4001), *sdiA* mutant (JLD800), *tnaA* mutant (ME021), or *tnaA sdiA* double mutant (ME020) background. (B) Expression of the *srgE-luxCDABE* fusion in *S.* Typhimurium 14028 or the isogenic *sdiA* mutant (BA612). Relative light units (light/OD_{590}) after 9 h of standing growth are indicated by bars for 1 μM AHL (black), 0.1% ethyl acetate (EA) (white), 500 μM indole (dark gray), and 0.1% dimethylformamide (DMF) (light gray). All data points are the averages for nine biological replicates, and error bars indicate SEM. Statistical significance in comparison to results with the solvent control is denoted by asterisks representing *t* test *P* values: **, <0.005; ***, <0.0005.

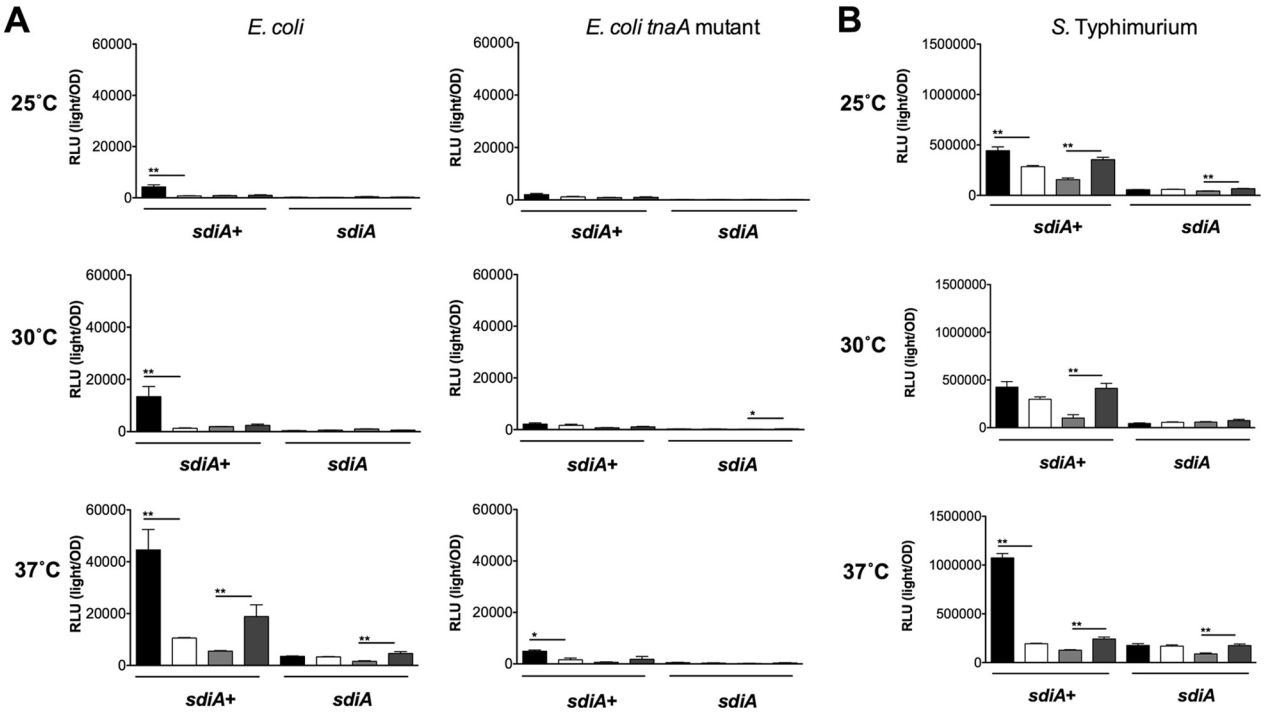

FIG 6 Response of SdiA to AHL and 1 mM indole in *E. coli* K-12 and S. Typhimurium during shaking growth conditions. (A) Expression of the *gadW*::Tn*5-luxCDABE* fusion in *E. coli* K-12 in either the wild-type (AL4001), *sdiA* mutant (JLD800), *tnaA* mutant (ME021), or *tnaA sdiA* double mutant (ME020) backgrounds. (B) Expression of the *srgE-luxCDABE* fusion in *S.* Typhimurium 14028 or the isogenic *sdiA* mutant (BA612). Relative light units (light/OD_{590}) after 9 h of growth with shaking are indicated by bars for 1 μM AHL (black), 0.1% ethyl acetate (EA) (white), 1 mM indole (dark gray), and 0.1% dimethylformamide (DMF) (light gray). All data points are the averages for nine biological replicates, and error bars indicate SEM. Statistical significance in comparison to results with the solvent control is denoted by asterisks representing *t* test *P* values: *, <0.05; **, <0.005.

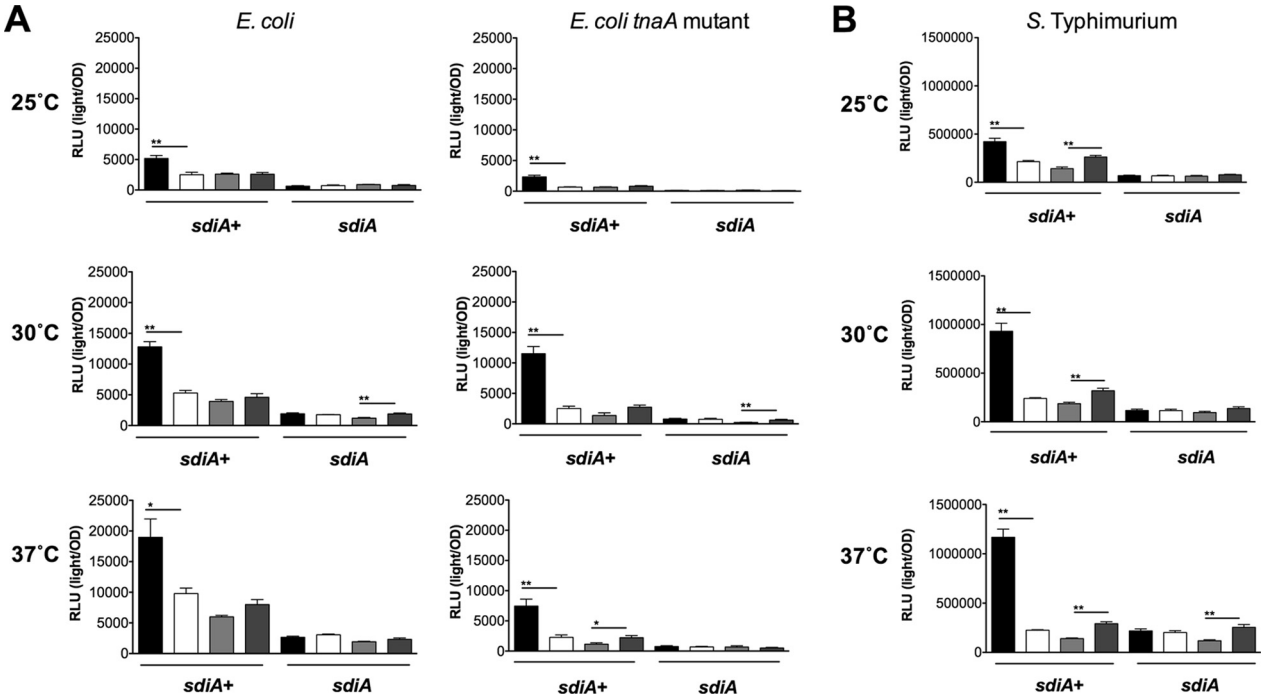

FIG 7 Response of SdiA to AHL and 1 mM indole in *E. coli* K-12 and *S.* Typhimurium during standing growth conditions. (A) Expression of the *gadW*::Tn*5-luxCDABE* fusion in *E. coli* K-12 in either the wild-type (AL4001), *sdiA* mutant (JLD800), *tnaA* mutant (ME021), or *tnaA sdiA* double mutant (ME020) background. (B) Expression of the *srgE-luxCDABE* fusion in *S.* Typhimurium 14028 or the isogenic *sdiA* mutant (BA612). Relative light units (light/OD_{590}) after 9 h of standing growth are indicated by bars for 1 μM AHL (black), 0.1% ethyl acetate (EA) (white), 1 mM indole (dark gray), and 0.1% dimethylformamide (DMF) (light gray). All data points are the averages for nine biological replicates, and error bars indicate SEM. Statistical significance in comparison to results with the solvent control is denoted by asterisks representing *t* test *P* values: *, <0.05; **, <0.005.

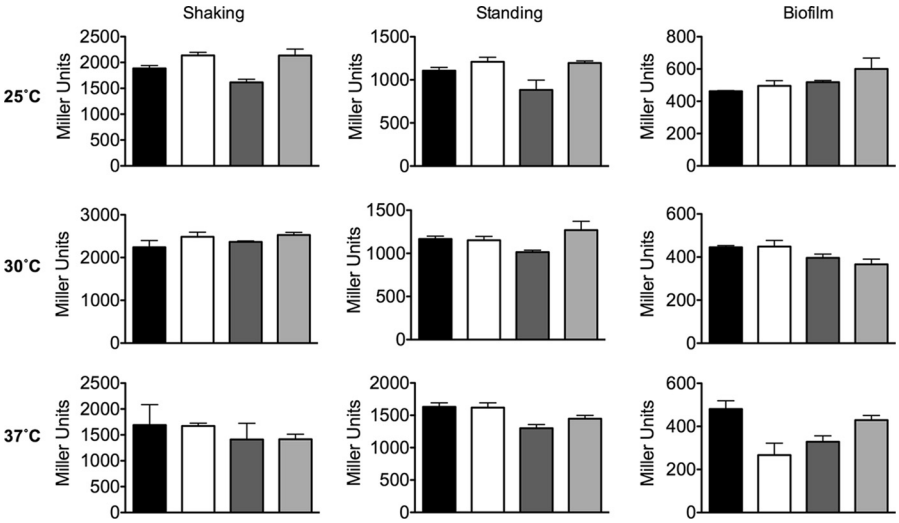

FIG 8 Expression of *E. coli sdiA* during growth in shaking or standing liquid cultures or in biofilms. Expression of the *sdiA-tnpR-lacZY* fusion in *E. coli* K-12 (JNS3212) is shown. Miller units after 9 h of growth in standing or shaking liquid, or 16 h of growth in biofilm, are indicated by bars for 1 μM AHL (black), 0.1% ethyl acetate (EA) (white), 500 μM indole (dark gray), and 0.1% dimethylformamide (DMF) (light gray). All data points are the averages for three biological replicates, and error bars indicate SEM.

background, the addition of AHL did not affect biofilm formation compared to results with the solvent control under any of the growth conditions tested (Fig. 1A). The addition of indole significantly repressed biofilm formation at 30°C compared to results with the solvent control. However, this decrease was not dependent upon *sdiA* (Fig. 1A). With *S.* Typhimurium we saw no significant effect of *sdiA*, AHL, or indole on biofilm formation (Fig. 1B). Similar results were observed using 1 mM indole (Fig. 2). Experiments performed using other *E. coli* K-12 backgrounds also failed to show an *sdiA*-dependent response to indole (Fig. 3). In-

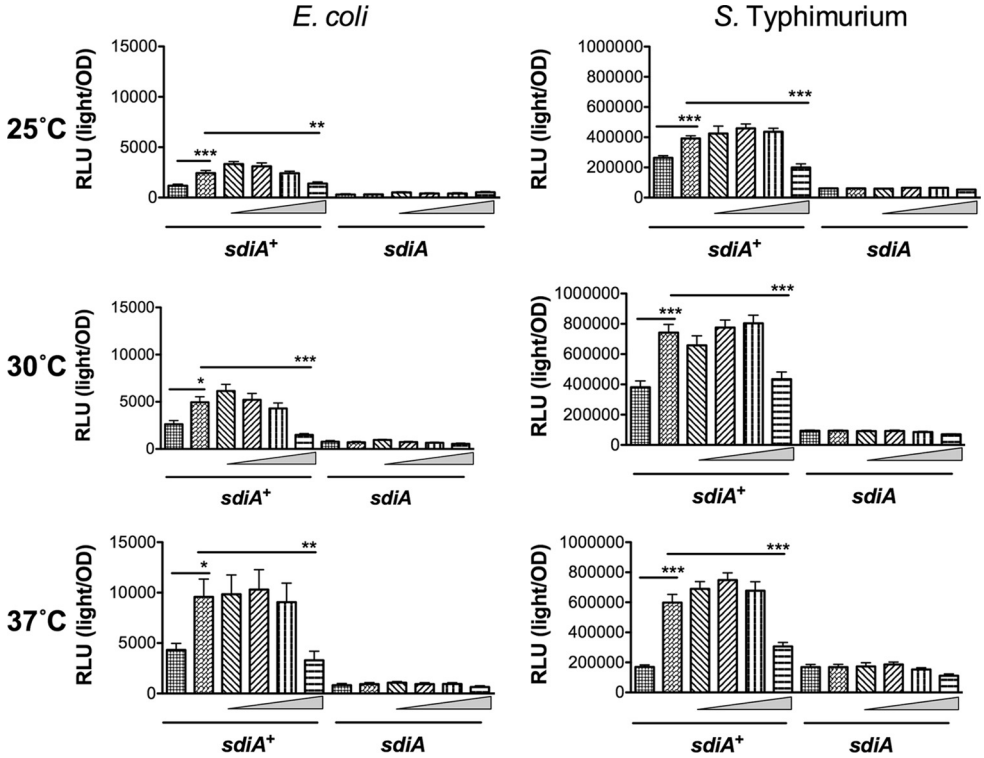

FIG 9 Indole inhibits AHL detection by SdiA in *E. coli* K-12 and *S.* Typhimurium during shaking growth conditions. (A) Expression of the *gadW*::Tn*5-luxCDABE* fusion in *E. coli* K-12 in either the wild-type (AL4001), *sdiA* mutant (JLD800), *tnaA* mutant (ME021), or *tnaA sdiA* double mutant (ME020) background. (B) Expression of the *srgE-luxCDABE* fusion in *S.* Typhimurium 14028 or *sdiA* mutant (BA612). Relative light units (light/OD_{590}) after 9 h of growth with shaking are indicated by bars for 0.1% ethyl acetate (EA) and 0.5% dimethylformamide (DMF) (hatched), 100 nM AHL + 0.5% DMF (bricked lines), 100 nM AHL + 1 μM indole (diagonal downward-slanting lines), 100 nM AHL + 10 μM indole (diagonal upward-slanting lines), 100 nM AHL + 100 μM indole (vertical lines), and 100 nM AHL + 1 mM indole (horizontal lines). All data points are the averages for nine biological replicates, and error bars indicate SEM. Statistical significance in comparison to results with the solvent control or the AHL + solvent control is denoted by asterisks representing *t* test *P* values: *, <0.05; **, <0.005; ***, <0.0005.

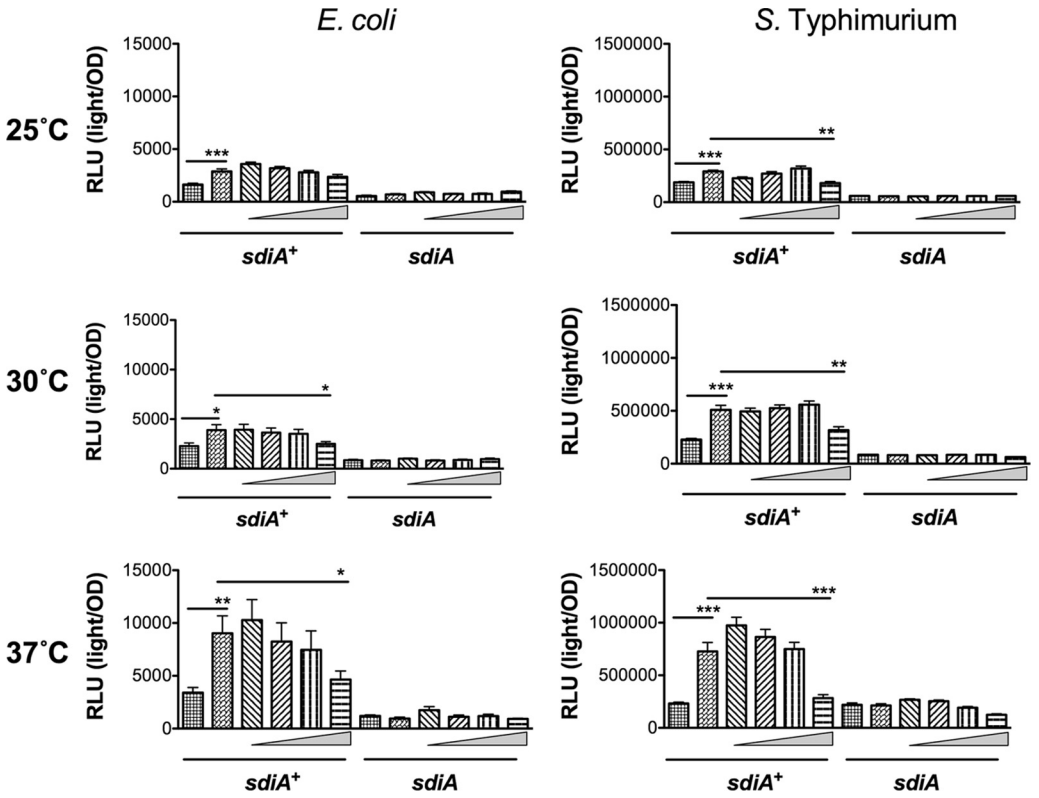

FIG 10 Indole inhibits AHL detection by SdiA of *E. coli* K-12 and *S.* Typhimurium during standing growth conditions. (A) Expression of the *gadW*::Tn*5-luxCDABE* fusion in *E. coli* K-12 in either the wild-type (AL4001), *sdiA* mutant (JLD800), *tnaA* mutant (ME021), or *tnaA sdiA* double mutant (ME020) background. (B) Expression of the *srgE-luxCDABE* fusion in *S.* Typhimurium 14028 or the *sdiA* mutant (BA612). Relative light units (light/OD_{590}) after 9 h of growth with shaking are indicated by bars for 0.1% ethyl acetate (EA) and 0.5% dimethylformamide (DMF) (hatched), 100 nM AHL + 0.5% DMF (bricked lines), 100 nM AHL + 1 μM indole (diagonal downward-slanting lines), 100 nM AHL + 10 μM indole (diagonal upward-slanting lines), 100 nM AHL + 100 μM indole (vertical lines), 100 nM AHL + 1 mM indole (horizontal lines). Each data point is the average for nine biological replicates, and error bars indicate SEM. Statistical significance in comparison to results with the solvent control or the AHL + solvent control is denoted by asterisks representing *t* test *P* values: *, <0.05; **, <0.005; ***, <0.0005.

terestingly, wild-type MG1655 makes more biofilm than its isogenic *sdiA* mutant, but this is not dependent upon AHL or indole. This is not seen in the BW25113, W3110, or *Salmonella* background.

SdiA reporter strains show little or no response to indole. To more thoroughly investigate SdiA activity in the presence of indole, we utilized previously described *sdiA*-dependent reporter strains of *E. coli* and *S.* Typhimurium (8, 32). In *E. coli* K-12, the most sensitive reporter of SdiA activity is a chromosomal *gadW*::Tn*5-luxCDABE* fusion (8). GadW is a transcription factor encoded within the acid fitness island. For *S.* Typhimurium, the most sensitive reporter is a plasmid-based *srgE-luxCDABE* fusion (32). We tested the *E. coli* K-12 and *S.* Typhimurium reporter strains grown in LB in the presence of AHL (1 μM oxoC6), indole (500 μM), or solvent controls during standing or shaking growth at each of three temperatures, 25°C, 30°C, and 37°C. Both fusions show *sdiA*-dependent activation only in the presence of AHL (Fig. 4 and 5). Indole had repressive effects on the fusions under some conditions, but in most instances this was not *sdiA* dependent. We believe the overall trend throughout the experiments is not *sdiA* dependent, although statistical significance is achieved with only the wild type or only with the *sdiA* mutant in some experiments. Experiments using 0.1 mM and 1 mM indole yielded similar conclusions (Fig. 6 and 7 and data not shown).

Indole does not regulate *sdiA* expression. Transcription profiling of *E. coli* biofilms grown in LB glucose in the presence and absence of indole has indicated that the *sdiA* gene is upregulated in the presence of indole (16). We constructed an *sdiA-tnpR-lacZY* chromosomal fusion in *E. coli* K-12 strain BW25113 to test the regulation of *sdiA* by indole. We saw no significant effect of AHL or indole on *sdiA* expression compared to results with the solvent control at 25°C, 30°C, or 37°C during shaking or standing growth conditions in broth cultures or during growth in biofilms (Fig. 8).

Indole inhibits detection of AHL by SdiA. In order to determine whether indole could alter AHL sensing by SdiA, we measured *gadW*::Tn*5-luxCDABE* and *srgE-luxCDABE* expression in *E. coli* and *S.* Typhimurium, respectively, in the presence of 100 nM AHL and increasing indole concentrations (1 μM, 10 μM, 100 μM, and 1 mM) during growth at 25°C, 30°C, and 37°C under shaking and standing conditions. Interestingly, at high concentrations, indole inhibited the detection of AHL by *E. coli* and *Salmonella* (Fig. 9 and 10). While SdiA activity was never reduced to the level of that of an *sdiA* mutant, the inhibition was significant at the highest concentration of 1 mM indole.

Based on the results obtained in this work, SdiA does not respond to indole. Indole has repressive effects on reporter gene expression in some instances, but these are not *sdiA* dependent. Indole also represses biofilm formation at lower temperatures, but

this is not *sdiA* dependent. We do not know why our result differs from previously published results. We obtained our results with three different *E. coli* backgrounds and with *S.* Typhimurium, suggesting that strain background is not the issue. However, we did find that high concentrations of indole inhibit the detection of AHL by SdiA. We see a gradation of inhibition between 100 μM and 1 mM indole, which may be physiologically relevant since indole is reported to be present in the mouse, rat, and human gut at ~140 μM, ~68 μM, and ~300 to 1,074 μM concentrations, respectively (3, 15, 31, 38).

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The content is solely the responsibility of the authors and does not necessarily represent the official views of the National Institute of Allergy and Infectious Diseases or the National Institutes of Health.

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