

The Primary Physiological Roles of Autoinducer 2 in *Escherichia coli* Is Chemotaxis and Biofilm Formation

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Abstract: Autoinducer 2 (AI-2) is a ubiquitous metabolite but, instead of acting as a “universal signal,” relatively few phenotypes have been associated with it, and many scientists believe AI-2 is often a metabolic byproduct rather than a signal. Here, the aim is to present evidence that AI-2 influences both biofilm formation and motility (swarming and chemotaxis), using *Escherichia coli* as the model system, to establish AI-2 as a true signal with an important physiological role in this bacterium. In addition, AI-2 signaling is compared to the other primary signal of *E. coli*, indole, and it is shown that they have opposite effects on biofilm formation and virulence.

Keywords: AI-2; chemotaxis; aggregation; biofilm; motility; *Escherichia coli*

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1. Introduction

Quorum sensing (QS) is the process by which bacteria communicate via secreted signals (autoinducers); once the concentration of the autoinducers reaches a threshold, the signal is detected, and gene expression is altered [1]. The roles of QS are diverse and include population density detection, virulence, biofilm formation, and the maintenance of the stress response [2]. Although inhibitors of QS (quorum-quenching compounds) are still promoted as a means to reduce virulence without promoting resistance [3], these compounds will indubitably and unfortunately fail. The main problem is that the inhibition of QS leads to pleiotropic effects that affect growth; hence, lab strains and clinical isolates rapidly evolve resistance to these compounds [4–6]. Clearly, it is imperative to have a better understanding of QS in order to be in a position to better control bacteria to prevent diseases, such as stomach cancer and ulcers caused by *Helicobacter pylori* and Lyme disease by *Borrelia burgdorferi* [7], and to utilize them for synthetic biology applications. Therefore, in this opinion piece, we probe the physiological role of AI-2 by focusing on the best-studied bacterium, *Escherichia coli*.

2. Autoinducer-2

Commensal *E. coli* has several QS pathways, including one system based on indole (Figure 1) [8–10], which is produced by TnaA from tryptophan, and another system based on autoinducer 2 (AI-2) (Figure 1) [11], which is produced by LuxS from S-ribosylhomocysteine [12]. It appears AI-2 is used primarily for communication inside the gastrointestinal tract at 37 °C, while indole is used primarily at lower temperatures (30 °C and lower) when the bacterium is outside of its eucaryotic host [9]. Although *E. coli* can detect homoserine lactones through the autoinducer-1 sensor SdiA (a LuxR homolog), it lacks a homoserine lactone synthase to produce the homoserine lactone signal, so *E. coli* uses SdiA to eavesdrop on signals of other bacteria [13]. Moreover, there is an interaction between these systems in that SdiA has been shown to be important for indole signaling in *E. coli* [8].

Once produced by LuxS, the AI-2 precursor 4,5-dihydroxy-2,3-pentanedione is converted spontaneously into *R*-2-methyl-2,3,3,4-tetrahydroxytetrahydrofuran (*R*-THMF) in *E. coli* (Figure 1), and *R*-THMF is the active form of AI-2 [7]. Hydrophilic AI-2 is transported from the cell by the membrane protein TqsA [14]. Once a threshold concentration is reached in the late exponential phase, AI-2 is imported into *E. coli* through its recognition by the AI-2 receptor LsrB [15]. In addition to LsrB in *E. coli*, LuxP (e.g., *Vibrio harveyi*) and the dCACHE-domain proteins PctA/TlpQ (*Pseudomonas aeruginosa*) are receptors for AI-2 [15], so there are at least three forms of AI-2 receptors in different bacteria. Furthermore, upon import, AI-2 is phosphorylated by LsrK in *E. coli*, and phosphorylated AI-2 binds and inhibits the repressor LsrR, which leads to changes in gene expression primarily at 37 °C [9].

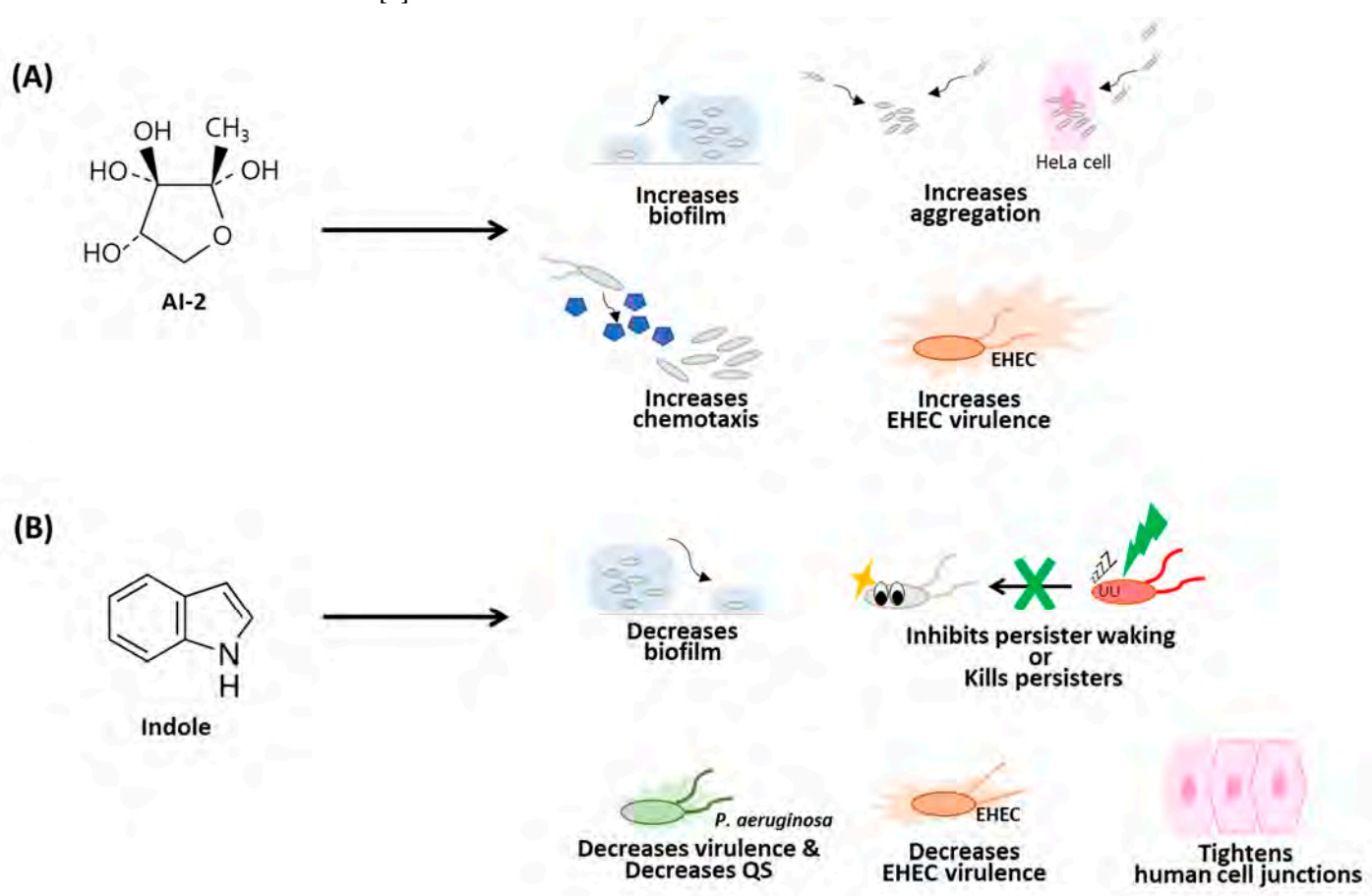


Figure 1. Comparison of the phenotypes affected by (A) autoinducer 2 (AI-2) and (B) indole. Curved black arrows indicate cell motility/movement, QS is quorum sensing, EHEC is *Escherichia coli* O157:H7, and flagella are indicated by two lines at one of the cell poles. Human cells are indicated by pink hexagons. Green lightning indicates the application of indole. The *R*-2-methyl-2,3,3,4-tetrahydroxytetrahydrofuran (*R*-THMF) form of AI-2 is shown.

3. AI-2 and Biofilm Formation

Although indole reduces both pathogenic [16] and non-pathogenic *E. coli* biofilm formation [17], AI-2 increases *E. coli* biofilm formation (Figure 1). Initially, QS was linked to biofilm formation using non-*E. coli* species and based on non-AI-2 signaling, specifically, for homoserine lactone increasing *Pseudomonas aeruginosa* [18]. Later studies, with *Vibrio cholerae* [19], *Serratia liquefaciens* [20], and *Streptococcus mutans* [21], confirmed the link of QS to biofilm formation.

The first report of AI-2 and biofilm formation was indirect and based on masking AI-2 signaling in *E. coli* with the QS inhibitor (5*Z*)-4-bromo-5-(bromomethylene)-3-butyl-2(5*H*)-furanone (henceforth furanone) from the alga *Delisea pulchra*; in this report, biofilm

formation was reduced by 60 µg/mL furanone [22]. Later reports of AI-2 influencing biofilm formation were based on *luxS* mutants rather than purified AI-2. For example, a *luxS* mutation in *Streptococcus gordonii* influenced mixed-species biofilm formation with *Porphyromonas gingivalis* [23], a *luxS* mutation had a small impact on the architecture of *Klebsiella pneumoniae* (although there was no effect for a *luxS* mutant for intestinal colonization and colonization on polystyrene) [24], and a *luxS* mutant increased biofilm formation in *Helicobacter pylori* [25]. Unfortunately, these early results related to AI-2 via *luxS* mutations do not provide compelling evidence due to pleiotropic changes resulting from the *luxS* mutations.

The first direct demonstration that AI-2 was responsible for influencing biofilm formation was the 4- to 24-fold increase in biofilm formation in microtiter plates for three *E. coli* strains upon the addition of 11 µM of purified AI-2 [11]. Moreover, AI-2 failed to stimulate biofilm formation for an *lsrK* AI-2 regulation mutant, and AI-2 stimulated biofilm formation five-fold in flow cells [11]. A decade later, the Sourjik group rediscovered that AI-2 increases *E. coli* biofilm formation and extended the original results to show AI-2 increases aggregation through the adhesin antigen 43 and curli [26]. They [26] also confirmed that the AI-2 Lsr uptake/processing pathway influences *E. coli* biofilm formation [27].

4. AI-2 and Chemotaxis

The first indication that AI-2 affects *E. coli* motility was that the QS inhibitor furanone at 13 µg/cm² inhibited *E. coli* swarming motility [22]; critically, the furanone also inhibited *E. coli* AI-2 signaling by 26,600-fold [22]. Next, furanone was shown to repress 44 of the 56 genes induced by AI-2, including those for chemotaxis (e.g., *aer*, *cheABRWYZ*, *tap*, *tsr*, *trg*) and motility (e.g., *motAB*, *flgABCDEFGHJKLMN*, *fliACDFHIKLMNOPQ*) [28]. Therefore, AI-2 induces chemotaxis and motility genes in *E. coli*, and masking AI-2 signaling with furanone reduces motility and biofilm formation.

The first direct report of AI-2 as a chemoattractant for any species was the 2008 discovery that *Escherichia coli* O157:H7 (EHEC) is attracted to purified AI-2 [29]. For EHEC, AI-2 also increases both swimming motility and attachment to HeLa cells [29]. For non-pathogenic *E. coli*, microfluidic devices were used a year later to show AI-2 is an attractant [30]. Later, similar to their studies on biofilm formation, the Sourjik group confirmed that AI-2 attracts *E. coli* [26]. Furthermore, as with biofilms, indole signaling is opposite that of AI-2 since indole repels enterohemorrhagic EHEC [31], whereas AI-2 attracts EHEC [29] (Figure 1).

The mechanism by which AI-2 is detected in *E. coli* was determined to be the chemotactic receptor Tsr, which previously was known for its recognition of L-serine [32]; LsrB, the AI-2 receptor, was also shown to be necessary [32]. As with chemotaxis and biofilm formation, chemotaxis through Tsr was corroborated by the Sourjik group [26]. Furthermore, the Manson group also verified that AI-2 increases biofilm formation in *E. coli* and found that biofilm formation in this strain is enhanced by chemotaxis to AI-2 [33]. Therefore, AI-2 stimulates biofilm formation in *E. coli* by increasing aggregation and chemotaxis (Figure 1).

5. AI-2 and Virulence

The two main *E. coli* signals influence pathogens in an opposite manner—indole decreases EHEC chemotaxis, motility, biofilm formation, and adherence to epithelial cells at the physiologically relevant concentration of primarily 0.5 mM [31]; these results that indole decreases EHEC virulence were largely confirmed 12 years later by the Sperandio group [34,35] (Figure 1). Indole from *E. coli* also reduces the virulence of *P. aeruginosa* by masking its QS [36], prevents *P. aeruginosa* from resuscitating [37] from the dormant persister state [38], and tightens the epithelial cell junctions of the human host [39]. Indole and its derivatives also kill persister cells [40,41]. In contrast, AI-2 at 100 µM to 500 µM

increases EHEC chemotaxis, motility, and adherence to epithelial cells and induces biofilm-related genes [29]. Moreover, AI-2 induces the expression of 23 genes of the locus of enterocyte effacement of EHEC [29]. Hence, in pathogenic *E. coli*, indole reduces pathogenicity, while AI-2 increases it.

6. Perspectives

The discovery that the *E. coli* AI-2 signal secreted by cells attracts other *E. coli* cells and leads to increased biofilm formation indicates that *E. coli* cells actively seek other *E. coli* cells to form communities [42]. Hence, it illustrates how bacteria can seek kin to increase their fitness, i.e., cells seek others to build communities (biofilms) to protect themselves from myriad stresses [43] and to increase their pathogenicity.

The chemoattractant property of AI-2 has also led to several synthetic biology applications. For example, biological nanofactories have been devised that detect and bind cancer cells and then produce AI-2 at the surface of the cancer cells, which attracts *E. coli* homing cells that internalize the synthesized AI-2 and then produce a biomarker or potentially an anti-cancer compound from an AI-2-induced promoter [44]. In this way, healthy cells could be discriminated from diseased ones. Therefore, the better understanding of the roles AI-2 and indole play in *E. coli* physiology has had a significant impact, both in our understanding of how communities are formed and in synthetic biology. Hence, AI-2 and indole are true and important signals in *E. coli*.

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References

1. Mukherjee, S.; Bassler, B.L. Bacterial quorum sensing in complex and dynamically changing environments. *Nat. Rev. Microbiol.* **2019**, *17*, 371–382, doi:10.1038/s41579-019-0186-5.
2. García-Contreras, R.; Nuñez-López, L.; Jasso-Chávez, R.; Kwan, B.W.; Belmont, J.A.; Rangel-Vega, A.; Maeda, T.; Wood, T.K. Quorum sensing enhancement of the stress response promotes resistance to quorum quenching and prevents social cheating. *ISME J.* **2015**, *9*, 115–125.
3. Ellermann, M.; Sperandio, V. Bacterial signaling as an antimicrobial target. *Curr. Opin. Microbiol.* **2020**, *57*, 78–86, doi:10.1016/j.mib.2020.08.001.
4. García-Contreras, R.; Martínez-Vázquez, M.; Velázquez-Guadarrama, N.; Villegas Pañeda, A.G.; Hashimoto, T.; Maeda, T.; Quezada, H.; Wood, T.K. Resistance to the quorum-quenching compounds brominated furanone C-30 and 5-fluorouracil in *Pseudomonas aeruginosa* clinical isolates. *Path. Dis.* **2013**, *68*, 8–11.
5. Maeda, T.; García-Contreras, R.; Pu, M.; Sheng, L.; García, L.R.; Tomás, M.; Wood, T.K. Quorum quenching quandary: Resistance to antivirulence compounds. *ISME J.* **2012**, *6*, 493–501, doi:10.1038/ismej.2011.122.
6. Kalia, V.C.; Wood, T.K.; Kumar, P. Evolution of Resistance to Quorum-Sensing Inhibitors. *Microb. Ecol.* **2014**, *68*, 13–23, doi:10.1007/s00248-013-0316-y.
7. Pereira, C.S.; Thompson, J.A.; Xavier, K.B. AI-2-mediated signalling in bacteria. *FEMS Microbiol. Rev.* **2013**, *37*, 156–181, doi:10.1111/j.1574-6976.2012.00345.x.
8. Lee, J.; Jayaraman, A.; Wood, T.K. Indole is an inter-species biofilm signal mediated by SdiA. *BMC Microbiol.* **2007**, *7*, 42, doi:10.1186/1471-2180-7-42.
9. Lee, J.; Zhang, X.-S.; Hegde, M.; Bentley, W.; Jayaraman, A.; Wood, T.K. Indole cell signaling occurs primarily at low temperatures in *Escherichia coli*. *ISME J.* **2008**, *2*, 1007–1023, doi:10.1038/ismej.2008.54.
10. Lee, J.-H.; Wood, T.K.; Lee, J. Roles of indole as an interspecies and interkingdom signaling molecule. *Trends Microbiol.* **2015**, *23*, 707–718.
11. Barrios, A.F.G.; Zuo, R.; Hashimoto, Y.; Yang, L.; Bentley, W.E.; Wood, T.K. Autoinducer 2 Controls Biofilm Formation in *Escherichia coli* through a Novel Motility Quorum-Sensing Regulator (MqsR, B3022). *J. Bacteriol.* **2006**, *188*, 305–316, doi:10.1128/jb.188.1.305-316.2006.
12. Chen, X.; Schauder, S.; Potier, N.; Van Dorsselaer, A.; Pelczar, I.; Bassler, B.L.; Hughson, F.M. Structural identification of a bacterial quorum-sensing signal containing boron. *Nat. Cell Biol.* **2002**, *415*, 545–549, doi:10.1038/415545a.

13. Soares, J.A.; Ahmer, B.M.M. Detection of acyl-homoserine lactones by *Escherichia* and *Salmonella*. *Curr. Opin. Microbiol.* **2011**, *14*, 188–193, doi:10.1016/j.mib.2011.01.006.
14. Herzberg, M.; Kaye, I.K.; Peti, W.; Wood, T.K. YdgG (TqsA) controls biofilm formation in *Escherichia coli* K-12 by enhancing autoinducer 2 transport. *J. Bacteriol.* **2006**, *188*, 587–598.
15. Zhang, L.; Li, S.; Liu, X.; Wang, Z.; Jiang, M.; Wang, R.; Xie, L.; Liu, Q.; Xie, X.; Shang, D.; et al. Sensing of autoinducer-2 by functionally distinct receptors in prokaryotes. *Nat. Commun.* **2020**, *11*, 1–13, doi:10.1038/s41467-020-19243-5.
16. Lee, J.; Bansal, T.; Jayaraman, A.; Bentley, W.E.; Wood, T.K. Enterohemorrhagic *Escherichia coli* Biofilms Are Inhibited by 7-Hydroxyindole and Stimulated by Isatin. *Appl. Environ. Microbiol.* **2007**, *73*, 4100–4109, doi:10.1128/aem.00360-07.
17. Domka, J.; Lee, J.; Wood, T.K. YliH (BssR) and YceP (BssS) Regulate *Escherichia coli* K-12 Biofilm Formation by Influencing Cell Signaling. *Appl. Environ. Microbiol.* **2006**, *72*, 2449–2459, doi:10.1128/aem.72.4.2449-2459.2006.
18. Davies, D.G.; Parsek, M.R.; Pearson, J.P.; Igilewski, B.H.; Costerton, J.W.; Greenberg, E.P. The Involvement of Cell-to-Cell Signals in the Development of a Bacterial Biofilm. *Science* **1998**, *280*, 295–298, doi:10.1126/science.280.5361.295.
19. Hammer, B.K.; Bassler, B.L. Quorum sensing controls biofilm formation in *Vibrio cholerae*. *Mol. Microbiol.* **2003**, *50*, 101–104, doi:10.1046/j.1365-2958.2003.03688.x.
20. Labbate, M.; Queck, S.Y.; Koh, K.S.; Rice, S.A.; Givskov, M.; Kjelleberg, S. Quorum Sensing-Controlled Biofilm Development in *Serratia liquefaciens* MG1. *J. Bacteriol.* **2004**, *186*, 692–698, doi:10.1128/jb.186.3.692-698.2004.
21. Li, Y.-H.; Lau, P.C.Y.; Lee, J.H.; Ellen, R.P.; Cvitekovich, D.G. Natural Genetic Transformation of *Streptococcus mutans* Growing in Biofilms. *J. Bacteriol.* **2001**, *183*, 897–908, doi:10.1128/jb.183.3.897-908.2001.
22. Ren, D.; Sims, J.J.; Wood, T.K. Inhibition of biofilm formation and swarming of *Escherichia coli* by (5Z)-4-bromo-5-(bromomethylene)-3-butyl-2(5H)-furanone. *Environ. Microbiol.* **2001**, *3*, 731–736, doi:10.1046/j.1462-2920.2001.00249.x.
23. McNab, R.; Ford, S.K.; El-Sabaeny, A.; Barbieri, B.; Cook, G.S.; Lamont, R.J.; Dietrich, G.; Kurz, S.; Hübner, C.; Aepinus, C.; et al. LuxS-Based Signaling in *Streptococcus gordonii*: Autoinducer 2 Controls Carbohydrate Metabolism and Biofilm Formation with *Porphyromonas gingivalis*. *J. Bacteriol.* **2003**, *185*, 274–284, doi:10.1128/jb.185.1.274-284.2003.
24. Balestrino, D.; Haagensen, J.A.J.; Rich, C.; Forestier, C. Characterization of Type 2 Quorum Sensing in *Klebsiella pneumoniae* and Relationship with Biofilm Formation. *J. Bacteriol.* **2005**, *187*, 2870–2880, doi:10.1128/jb.187.8.2870-2880.2005.
25. Cole, S.P.; Hardwood, J.; Lee, R.; She, R.; Guiney, D.G. Characterization of monospecies biofilm formation by *Helicobacter pylori*. *J. Bacteriol.* **2004**, *186*, 3124–3132.
26. Laganenka, L.; Colin, R.; Sourjik, V. Chemotaxis towards autoinducer 2 mediates autoaggregation in *Escherichia coli*. *Nature Commun.* **2016**, *7*, 12984.
27. Li, J.; Attila, C.; Wang, L.; Wood, T.K.; Valdes, J.J.; Bentley, W.E. Quorum Sensing in *Escherichia coli* Is Signaled by AI-2/LsrR: Effects on Small RNA and Biofilm Architecture. *J. Bacteriol.* **2007**, *189*, 6011–6020, doi:10.1128/jb.00014-07.
28. Ren, D.; Bedzyk, L.A.; Ye, R.W.; Thomas, S.M.; Wood, T.K. Differential gene expression shows natural brominated furanones interfere with the autoinducer-2 bacterial signaling system of *Escherichia coli*. *Biotechnol. Bioeng.* **2004**, *88*, 630–642, doi:10.1002/bit.20259.
29. Bansal, T.; Jesudhasan, P.; Pillai, S.; Wood, T.K.; Jayaraman, A. Temporal regulation of enterohemorrhagic *Escherichia coli* virulence mediated by autoinducer-2. *Appl. Microbiol. Biotechnol.* **2008**, *78*, 811–819, doi:10.1007/s00253-008-1359-8.
30. Englert, D.L.; Manson, M.D.; Jayaraman, A. Flow-Based Microfluidic Device for Quantifying Bacterial Chemotaxis in Stable, Competing Gradients. *Appl. Environ. Microbiol.* **2009**, *75*, 4557–4564, doi:10.1128/aem.02952-08.
31. Bansal, T.; Englert, D.; Lee, J.; Hegde, M.; Wood, T.K.; Jayaraman, A. Differential Effects of Epinephrine, Norepinephrine, and Indole on *Escherichia coli* O157:H7 Chemotaxis, Colonization, and Gene Expression. *Infect. Immun.* **2007**, *75*, 4597–4607, doi:10.1128/iai.00630-07.
32. Hegde, M.; Englert, D.L.; Schrock, S.; Cohn, W.B.; Vogt, C.; Wood, T.K.; Manson, M.D.; Jayaraman, A. Chemotaxis to the quorum-sensing signal AI-2 requires the Tsr chemo-receptor and the periplasmic LsrB AI-2-binding protein. *J. Bacteriol.* **2011**, *193*, 768–773.
33. Jani, S.; Seely, A.L.; Peabody, V., G.L.; Jayaraman, A.; Manson, M.D. Chemotaxis to self-generated AI-2 promotes biofilm formation in *Escherichia coli*. *Microbiology* **2017**, *163*, 1778–1790.
34. Kumar, A.; Sperandio, V. Indole Signaling at the Host-Microbiota-Pathogen Interface. *mBio* **2019**, *10*, e01031-19, doi:10.1128/mbio.01031-19.
35. Wood, T.K.; Lee, J. Precedence for the Role of Indole with Pathogens. *mBio* **2019**, *10*, 01599–01519, doi:10.1128/mbio.01599-19.
36. Lee, J.; Attila, C.; Cirillo, S.L.G.; Cirillo, J.D.; Wood, T.K. Indole and 7-hydroxyindole diminish *Pseudomonas aeruginosa* virulence. *Microb. Biotechnol.* **2008**, *2*, 75–90, doi:10.1111/j.1751-7915.2008.00061.x.
37. Zhang, W.; Yamasaki, R.; Song, S.; Wood, T.K. Interkingdom signal indole inhibits *Pseudomonas aeruginosa* persister cell waking. *J. Appl. Microbiol.* **2019**, *127*, 1768–1775, doi:10.1111/jam.14434.
38. Wood, T.K.; Song, S. Forming and waking dormant cells: The ppGpp ribosome dimerization persister model. *Biofilm* **2020**, *2*, 100018, doi:10.1016/j.biofilm.2019.100018.
39. Bansal, T.; Alaniz, R.C.; Wood, T.K.; Jayaraman, A. The bacterial signal indole increases epithelial-cell tight-junction resistance and attenuates indicators of inflammation. *Proc. Natl. Acad. Sci. USA* **2009**, *107*, 228–233, doi:10.1073/pnas.0906112107.
40. Song, S.; Gong, T.; Yamasaki, R.; Kim, J.; Wood, T.K. Identification of a potent indigoid persister antimicrobial by screening dormant cells. *Biotechnol. Bioeng.* **2019**, *116*, 2263–2274, doi:10.1002/bit.27078.

41. Song, S.; Wood, T.K. Combatting persister cells with substituted indoles. *Front. Microbiol.* **2020**, *11*, 1565, doi:10.3389/fmicb.2020.01565.
42. Defoirdt, T. Can bacteria actively search to join groups? *ISME J.* **2010**, *5*, 569–570, doi:10.1038/ismej.2010.147.
43. Zhang, X.-S.; García-ContrerasR.; Wood, T.K. YcfR (BhsA) Influences *Escherichia coli* Biofilm Formation through Stress Response and Surface Hydrophobicity. *J. Bacteriol.* **2007**, *189*, 3051–3062, doi:10.1128/jb.01832-06.
44. Wu, H.; Tsao, C.; Quan, D.N.; Cheng, Y.; Servinsky, M.D.; Carter, K.K.; Jee, K.J.; Terrell, J.L.; Zargar, A.; Rubloff, G.W.; et al. Autonomous bacterial localization and gene expression based on nearby cell receptor density. *Mol. Syst. Biol.* **2013**, *9*, 636, doi:10.1038/msb.2012.71.