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MINIREVIEW

Indole: a signaling molecule or a mere metabolic byproduct that alters bacterial physiology at a high concentration?

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Indole is an organic compound that is widespread in microbial communities inhabiting diverse habitats, like the soil environment and human intestines. Measurement of indole production is a traditional method for the identification of microbial species. *Escherichia coli* can produce millimolar concentrations of indole in the stationary growth phase under nutrient-rich conditions. Indole has received considerable attention because of its remarkable effects on various biological functions of the microbial communities, for example, biofilm formation, motility, virulence, plasmid stability, and antibiotic resistance. Indole may function as an intercellular signaling molecule, like a quorum-sensing signal. Nevertheless, a receptor system for indole and the function of this compound in coordinated behavior of a microbial population (which are requirements for a true signaling molecule) have not yet been confirmed. Recent findings suggest that a long-known quorum-sensing regulator, *E. coli*'s SdiA, cannot recognize indole and that this compound may simply cause membrane disruption and energy reduction, which can lead to various changes in bacterial physiology including unstable folding of a quorum-sensing regulator. Indole appears to be responsible for acquisition of antibiotic resistance via the formation of persister cells and activation of an exporter. This review highlights and summarizes the current knowledge about indole as a multitrophic molecule among bacteria, together with recently identified new avenues of research.

Keywords: indole, protein folding, antibiotic resistance, quorum sensing, SdiA, signaling

Introduction

Many bacteria excrete various metabolites into their natural habitat, such as soil or marine environments and human intestines (Helling *et al.*, 2002). *Escherichia coli* can secrete large amounts of indole during their stationary growth phase (Kobayashi *et al.*, 2006). Indole has received enormous attention because of its considerable effects on bacterial physiology. Indole is known to play important roles in biofilm formation, virulence, plasmid stabilization, spore formation, acid resistance, and formation of persister cells (Stamm *et al.*, 2005; Hirakawa *et al.*, 2010; Lee *et al.*, 2010b; Chu *et al.*, 2012; Field and Summers, 2012; Vega *et al.*, 2012; Kim *et al.*, 2013). Enteric bacteria can produce indole from tryptophan by means of tryptophanase (TnaA; Yanofsky *et al.*, 1991). TnaA converts tryptophan into indole, pyruvate, and ammonia (Newton and Snell, 1965). Indole is excreted to the extracellular medium, where indole concentration can typically reach 0.5–1.0 mM and even up to 5 mM (upper limit; Li and Young, 2013). The secreted indole is taken up and participates in the various above-mentioned physiological processes in *E. coli* even though indole cannot be metabolized by the bacterial cells (Wang *et al.*, 2001; Kobayashi *et al.*, 2006). Indole is produced by many bacterial species including *E. coli* and *Vibrio cholerae* (Lee and Lee, 2010). Bacteria coexist with other bacterial species or organisms in environmental niches. Therefore, indole-non-producing bacteria can encounter a considerable amount of indole excreted by indole-producing bacteria. The former type of bacteria can metabolize indole by means of several monooxygenases and dioxygenases and occasionally use indole or indole-related compounds as a source of carbon (Boyd *et al.*, 1997; Mordukhova *et al.*, 2000; Rui *et al.*, 2005; Yin *et al.*, 2005; Peng *et al.*, 2013).

Indole has been reported to act as an intercellular signaling molecule, such as a quorum sensing (QS) signal, in microbial communities (Ahmer, 2004; Lee and Lee, 2010). An *E. coli* homolog of the transcriptional activator LuxR (called SdiA) can interact with various signals, such as acyl-homoserine lactone (AHL), autoinducer 2 (AI-2), and indole (Lee *et al.*, 2009b). Some studies suggest that there may be a connection between indole-mediated and AHL-mediated signaling. On the other hand, it was recently reported that SdiA cannot respond to indole in *E. coli* and in *Salmonella enterica* serovar Typhimurium (Sabag-Daigle *et al.*, 2012). Most recently, it was shown that differential expression of diverse QS-controlled genes is attributable to inhibition of folding of a QS regulator in the presence of indole (Kim and Park, 2013). There is no direct evidence that indole can bind to any SdiA homolog (or SdiA itself). Thus, it remains unclear how SdiA and indole interact in regulating many bacterial

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cellular functions.

Even if a QS regulator cannot respond to indole as a signaling molecule, indole may be strongly involved in bacterial physiology. Recent studies showed that indole performs important functions in the acquisition of antibiotic resistance through formation of bacterial persister cells and induction of an exporter gene (Hirakawa *et al.*, 2005; Vega *et al.*, 2012; Molina-Santiago *et al.*, 2014). Indole may be responsible for formation of metabolically active quiescent *E. coli* cells both under aerobic and anaerobic conditions (Chen *et al.*, 2015). Thus, indole is widespread in natural environments in a broad range of concentrations, which can have harmful or beneficial effects on individual bacteria. This review highlights and summarizes the current knowledge about indole (Fig. 1) and discusses new perspectives on indole: this molecule participates in a broad spectrum of biological functions in the bacterial world and beyond.

Indole biosynthesis and toxic effects at a high concentration

Indole is generated by tryptophanase (TnaA), which catalyzes the synthesis of indole from tryptophan in bacteria. TnaA is a pyridoxal phosphate-dependent enzyme that hydrolyses tryptophan to generate indole, pyruvate, and ammonia (Newton and Snell, 1965). *E. coli* can use tryptophan as a sole carbon and nitrogen source because of the functional tryptophanase and tryptophan permease TnaB (Yanofsky *et al.*, 1991). Various factors can control the expression of TnaA. TnaA expression requires cyclic AMP and is increased by tryptophan, cysteine, alkaline stress, and heme depletion (Botsford, 1975; Rompf *et al.*, 1998; Saito and Kobayashi, 2003). TnaA is suppressed by glucose, pyruvate, and acetate (Beggs and Lichstein, 1965; Botsford and DeMoss, 1971; Botsford, 1975; Isaacs *et al.*, 1994). Tryptophanase can be a major stimulator of cellular L-cysteine desulfhydrase

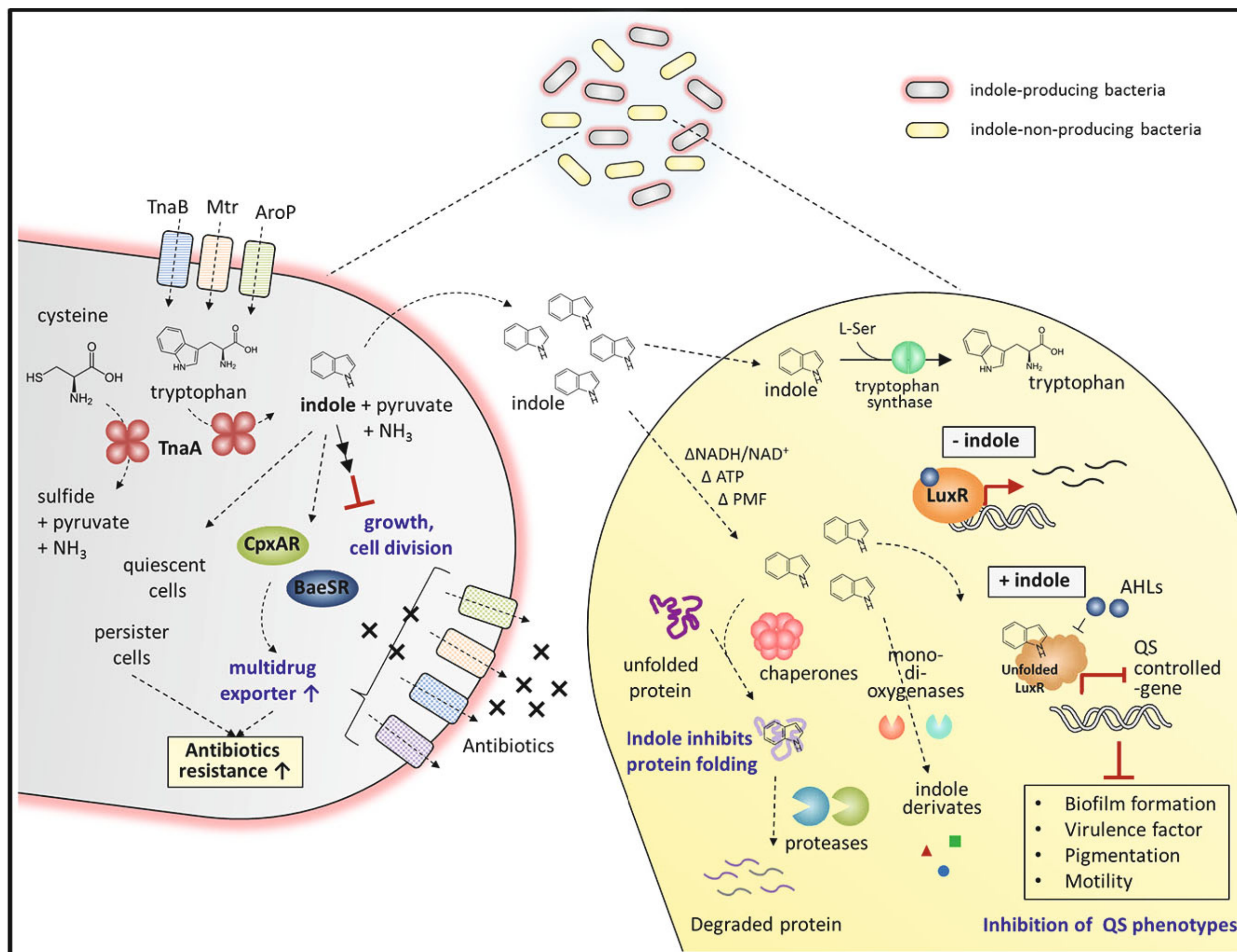

Fig. 1. An overview of diverse effects of indole in microbial communities. Indole-producing bacteria can synthesize indole from tryptophan by means of tryptophanase (TnaA). This enzyme also has a cysteine desulfhydrase activity. Indole freely diffuses across the cell membrane and increases antibiotic resistance by inducing a multidrug exporter and formation of persister cells. When indole is imported across the membrane, the bacterial cell can undergo a reduction in available energy and perturbations of the membrane potential. In addition, indole inhibits folding of some proteins related to quorum sensing (QS; e.g., the transcriptional activator LuxR not bound to acyl-homoserine lactone [AHL]) and promotes degradation of some proteins. The latter phenomenon alters the phenotype of many bacterial species. Indole-non-producing bacteria can oxidize and degrade indole by means of oxygenases.

activity (Snell, 1975; Awano *et al.*, 2005; Oguri *et al.*, 2012). Cysteine desulfhydrase catalyzes conversion of cysteine to pyruvate, ammonia, and sulfide (Snell, 1975). A high concentration of cysteine can be toxic due to effects on various cellular functions such as antibiotic resistance, oxidative stress resistance, and swarming motility (Oguri *et al.*, 2012). Thus, indole production that is mediated by TnaA should occur only under certain circumstances. Indole is likely to be exported by the AcrEF-TolC pump because indole excretion is decreased in the absence of *acrEF* (Kawamura-Sato *et al.*, 1999). The Mtr transporter may be responsible for indole import in *E. coli* (Yanofsky *et al.*, 1991). Recently, indole was shown to be freely diffusible across membranes, and AcrEF-TolC and Mtr are not required for the indole transport (Piñero-Fernandez *et al.*, 2011). Thus, indole-non-producing bacteria may directly import indole through their membrane from a natural habitat but further research is needed on how indole is transported. In *E. coli*, indole production is affected by temperature, pH, and the presence of antibiotics (Han *et al.*, 2011). Therefore, indole production should be affected by the microenvironment, and then indole will affect bacterial functions in the community. Research on the effects of indole on cells has been focused on indole-producing bacteria, such as *E. coli*, but the influence of exogenous indole on the physiological traits of indole-non-producing bacteria has not been studied well. The findings that indole enhances biofilm formation and decreases cell size in *Pseudomonas putida*, *P. aeruginosa*, and *Agrobacterium tumefaciens* are not consistent with the results obtained from *E. coli* studies (Lee *et al.*, 2009a, 2015; Kim *et al.*, 2013). The physiological roles of indole may differ between indole-non-producing and indole-producing bacteria.

According to many studies on biofilm formation, on virulence factor production, and on drug resistance, these physiological functions are affected by indole at 0.5–2.0 mM: concentrations that are similar to those in the culture supernatant of *E. coli* in the stationary phase (Lee *et al.*, 2009a, 2009b, 2010b, 2015; Kim *et al.*, 2013; Li and Young, 2013). Most recently, it was shown that the indole concentration in the culture supernatant rapidly increased (from 0.1 mM to approximately 0.8 mM) during the growth transition (for 30 min; optical density at 600 nm: 1.0–1.5) between the exponential phase and stationary phase (Gaimster *et al.*, 2014). The accumulation of indole is caused by enhanced production rather than simple increase of cell number (Gaimster *et al.*, 2014). The intracellular concentration of indole can reach a maximum of 60 mM transiently during entry into the stationary phase (Gaimster *et al.*, 2014).

The huge amount of intracellular indole, acting as a proton ionophore in *E. coli*, can inhibit growth and cell division (Chimerel *et al.*, 2012). The electrochemical potential decreases when indole is transported across the cytoplasmic membrane (Piñero-Fernandez *et al.*, 2011; Chimerel *et al.*, 2012). It is known that micromolar-to-millimolar concentrations of indole are also present in human intestines (Sabag-Daigle *et al.*, 2012). In that environment, indole should be neutralized regardless of whether its effects (which have been observed at high concentrations of indole) are biologically relevant or not.

In a dual-species biofilm of *E. coli* (indole producer) and *Pseudomonas* species (indole non-producer), toluene *o*-monooxygenase is highly expressed and involved in indole oxidation (Lee *et al.*, 2007). A change in the indole concentration can affect the dual-species biofilm formation. Thus, indole-non-producing bacteria counteract the effects of exogenous indole and eliminate or alleviate indole-induced stress. Degradation and incorporation of indole into a metabolic pathway is the first step in the reduction of stress caused by indole. Expression of *trpABCDE* (tryptophan operon) controls the tryptophan pathway, and overexpression of *trpAB* genes was observed in *P. putida* during indole treatment (Yanofsky *et al.*, 1991; Kim *et al.*, 2013). *P. aeruginosa* degrades tryptophan to anthranilate, not indole via a kynurenine pathway because of lack of *tnaA* (Kurnasov *et al.*, 2003). Indole could antagonize against the effect of anthranilate on biofilm formation because it activates genes involved in anthranilate degradation (Kim *et al.*, 2015). If indole-non-producing bacteria fail to degrade or metabolize the excess indole, defense mechanisms such as chaperones and proteases are turned on to protect the cell. A high concentration of indole can be toxic for and inhibit the growth of *P. putida* and *A. tumefaciens* (Kim *et al.*, 2013; Lee *et al.*, 2015). Indole increases the NADH/NAD^+ ratio and reduces the ATP concentration in *P. putida* because of perturbations in the membrane potential when indole is imported across the membrane (Fig. 1; Kim *et al.*, 2013). In addition, indole inhibits protein folding and promotes protein degradation, which may turn up the expression of many genes encoding molecular chaperones (*groEL*, *groES*, and *dnaK*) and proteases (*hslUV*, *lon*, and *clpB*) in *P. putida* and *Acinetobacter oleivorans* (Kim *et al.*, 2013; Kim and Park, 2013). Therefore, a large body of evidence suggests that indole may be a metabolic byproduct that at a high concentration can alter the physiology of many indole-non-producers.

The definition of "signal" and indole; simple metabolic cue or a signal molecule?

QS is a type of bacterial communication that depends on cell density (Fuqua *et al.*, 1994; Williams, 2007). In the stationary phase of growth, many gram-negative bacteria produce small diffusible signaling molecules and use those signals for QS (indole may be one of such molecules). One researcher recently suggested that indole is an intercellular signal in microbial communities (Ahmer, 2004). SdiA, which is an *E. coli* LuxR homolog, can interact with AHL and AI-2 (Lee *et al.*, 2009b). As described in several studies, there may be a relation between indole-based and AHL-based signaling in *E. coli*. For example, it was demonstrated that biofilm formation is decreased in the presence of indole, and that the SdiA protein influences indole production in *E. coli* (Lee *et al.*, 2009b). In addition, indole-producing *E. coli* can down-regulate QS-related virulence factors of *P. aeruginosa* (Chu *et al.*, 2012).

On the other hand, it was recently reported that SdiA cannot respond to indole, but SdiA activity can be inhibited by indole in *E. coli* and *S.* Typhimurium (Sabag-Daigle *et al.*, 2012). Biofilm formation and QS-controlled gene expression during indole treatment are not *sdiA* dependent (Sabag-

Daigle *et al.*, 2012). It was also observed that the AHL detection ability of a LuxR homolog is inhibited at a high concentration (1 mM) of indole in *E. coli*, *S.* Typhimurium, *A. oleivorans*, and *Chromobacterium violaceum* (Sabag-Daigle *et al.*, 2012; Kim and Park, 2013). QS-controlled formation of a pigment in *C. violaceum* (violacein), *Serratia marcescens* (prodigiosin), *P. aeruginosa* (pyocyanin), and in *P. chlororaphis* (phenazine) is disrupted by 0.5–1.0 mM indole (Lee *et al.*, 2009a; Chu *et al.*, 2012; Kim and Park, 2013; Hidalgo-Romano *et al.*, 2014). Differential expression of one QS-controlled gene is caused by inhibition of AqsR (a LuxR homolog) folding during indole treatment in *A. oleivorans* (Kim and Park, 2013). Indole upregulates many chaperone- and protease-encoding genes because indole inhibits protein stability and folding; the latter results were verified by an *in vitro* protein folding assay in the presence of indole (Kim *et al.*, 2013). QS regulators are subject to degradation without their cognate AHL signals, which are necessary for the correct folding of the QS regulators (Zhu and Winanas, 2001; Vannini *et al.*, 2002; Zhang *et al.*, 2002; Costa *et al.*, 2012). The GroEL/ES chaperonin system requires proper folding of a QS regulator, and this phenomenon in turn affects the expression of QS-dependent genes in *Sinorhizobium meliloti* and *A. tumefaciens* (Marketon and González, 2002; Chai and Winans, 2009). It has been suggested that inhibition of protein synthesis should have global effects within bacterial cells during indole treatment. Indole enhances the expression of many chaperones, including GroEL/ES, and proteases in *P. putida* and *A. oleivorans*; this finding indicates that these upregulated factors may be responsible for the LuxR stability in the presence of indole (Kim *et al.*, 2013; Kim and Park, 2013). When AHL is already bound to a QS regulator before treatment with indole, a QS-controlled phenotype or expression of genes can be activated normally. In the case of *P. putida* carrying an AHL expression plasmid, alterations of biofilm formation and motility are not affected by indole because the QS regulator PpoR is already bound to AHL (Lee *et al.*, 2010b). AHL-bound TraR of *Agrobacterium* can prevent the effects of indole, but AHL-free TraR cannot fold correctly in the presence of indole (Kim and Park, 2013). Taken together, the above data show that QS regulators require a cognate signal (AHL) for stabilization, but their folding can be affected by indole, which can cause rapid degradation of a QS regulator (Fig. 1). Indole should affect bacterial cells globally owing to the inhibition of synthesis of several unstable proteins, including the above-mentioned QS regulators.

Some researchers suggested that SdiA receives the indole signal in *E. coli*, but there is no direct evidence that indole can bind to any SdiA homolog (or SdiA itself). SdiA refolding activity is increased in the presence of the following AHLs: C6-HSL, C8-HSL, and 3-oxo-C8-HSL (Yao *et al.*, 2006). Most recently, it was shown that the endogenously produced ligand 1-octanoyl-*rac*-glycerol can bind to SdiA in the absence of AHL in *E. coli* (Nguyen *et al.*, 2015). 1-Octanoyl-*rac*-glycerol is a phospholipid precursor (membrane formation) and functions as a chemical chaperone placeholder that stabilizes SdiA (Nguyen *et al.*, 2015). LuxR homologs can sense dialkylresorcinols, cyclohexanediones, and α-pyrones instead of AHLs as signals in a human and insect pathogen, *Photorhabdus* (Brachmann *et al.*, 2013; Brameyer *et al.*, 2015). These findings confirm that LuxR orphans can detect both AHL and non-AHL signals, but the direct binding of indole to any SdiA homolog has not been demonstrated. Therefore, it would be premature to assume that indole is a QS signal mediated by SdiA.

Indole is accumulated at much higher concentration compared with other signal molecules that mostly work at micromolar range for their physiological effects (Han *et al.*, 2011; Vega *et al.*, 2012). Moreover, while indole production in *Paenibacillus alvei* and *E. coli* is inhibited by glucose, this was not the case in canonical QS systems (Vega *et al.*, 2012). Induced genes at low concentration of indole are involved in the tryptophan biosynthesis (Fig. 1) and metabolism of indole (e.g., *tnaB*, *astD*, and *gabT*) in *E. coli*. (Wang *et al.*, 2001; Ryan and Dow, 2008; Kim *et al.*, 2013). These findings suggest that indole cannot be a signal for SidA; therefore, the functions of indole as either a QS signal or a metabolic signal require further clarification.

Effects of indole on bacterial antibiotic resistance

Indole has received a great deal of attention because of the broad range of effects on bacterial physiology. Indole increases antibiotic resistance through the induction of stress resistance genes and formation of bacterial persister cells (Fig. 1; Hirakawa *et al.*, 2005; Vega *et al.*, 2012; Molina-Santiago *et al.*, 2014). Recent findings suggest that indole plays an important role in formation of persister cells in the presence of antibiotics (Vega *et al.*, 2012). A *tnaA* mutant of *E. coli* (indole-non-producer phenotype) shows a reduction in the formation of persister cells (Vega *et al.*, 2012). When *E. coli* cells are exposed to antibiotics, some cells are lysed and protect the majority of neighboring cells by releasing indole as a defense signaling molecule (Lee *et al.*, 2010a). It has been speculated that antibiotic-susceptible bacteria can acquire antibiotic resistance through turning on the expression of antibiotic-defense genes such as multidrug efflux pumps by indole (Lee *et al.*, 2010a). Stationary-phase cells of *E. coli* can also produce indole under nutrient-abundant conditions; this phenomenon may also be linked to stress defense (Vega *et al.*, 2012). Indole-mediated antibiotic tolerance in *E. coli* and *S.* Typhimurium may be due to induction of oxidative stress and a phage shock response (Vega *et al.*, 2012, 2013). It is unclear, however, whether indole can cause oxidative stress. Vega *et al.* (2012) used transcriptomic analysis to show that the OxyR regulon and phage shock pathway are activated by indole. Nevertheless, these researchers showed that only two genes (*oxyS* and *dps*) that are involved in the OxyR regulon are induced by indole in the stationary phase, and eight OxyR-controlled genes (*dps*, *katG*, *grxA*, *trxC*, *ahpF*, *sufS*, *flu*, and *hemH*) are moderately expressed in the exponential phase in the presence of indole except for the *oxyS* gene, which is upregulated approximately 15-fold. Therefore, the acquisition of antibiotic resistance in the presence of indole cannot fully explain activation of the oxidative-stress defense system. In addition, there are no changes in oxidative-stress defense

genes (including the OxyR and SoxR regulons) in *P. putida* in the presence of indole, and superoxide production was not detected during treatment with 1.0–3.0 mM indole (Kim *et al.*, 2013). Thus, further research is needed on whether indole can generate oxidative stress and induce the stress defense system, which may increase antibiotic tolerance.

Indole induces expression of multidrug exporter genes and increases antibiotic resistance in *E. coli*, *Salmonella*, and *Pseudomonas* (Hirakawa *et al.*, 2005; Nikaido *et al.*, 2012; Molina-Santiago *et al.*, 2014). Indole increases antibiotic resistance by enhancing expression of diverse xenobiotic exporter genes (*mdtAE*, *cusB*, *emrK*, and *yceL*) via two-component signal transduction systems (BaeSR and CpxAR) in *E. coli* (Fig. 1; Hirakawa *et al.*, 2005). In *Salmonella*, indole activates genes of efflux-mediated multidrug resistance by inducing *ramA* and *acrAB* (Nikaido *et al.*, 2012). During indole treatment, the plasmid-encoded TtgGHI efflux pump plays an important role in resistance to a bactericidal antibiotic (e.g., ampicillin) in the absence of TtgABC, which is the main antibiotic exporter and is located chromosomally in *P. putida* DOT-T1E (Molina-Santiago *et al.*, 2014). The *P. putida* KT2440 strain shows increased antibiotic resistance in the presence of indole (Kim and Park, unpublished data).

A novel mechanism controlling resistance to antibiotics and cationic antimicrobial peptides (e.g., protamine) in *E. coli* has been reported. As mentioned above, the CpxAR two-component regulatory system is necessary for multidrug transporters (MdtABC, AcrAB, and EmrAB) in the presence of indole (Hirakawa *et al.*, 2005; Weatherspoon-Griffin *et al.*, 2014). CpxAR activates the *marRAB* operon, which facilitates production of multidrug efflux transporters and increases transcription of *aroK* (gene of shikimate kinase; Weatherspoon-Griffin *et al.*, 2014). AroK produces aromatic metabolites including indole, salicylate, and 2,3-dihydroxybenzoate (Sulavik *et al.*, 1995). AroK drives production of indole (which is excreted) and upregulates CpxAR causing transcriptional activation of genes related to multiple antibiotic resistance (Weatherspoon-Griffin *et al.*, 2014). Other two aromatic compounds produced as a result of AroK activation can interact with MarR to release it from the *marRAB* transcription start site (Sulavik *et al.*, 1995; Weatherspoon-Griffin *et al.*, 2014). As a result, these regulatory cascades enhance antibiotic resistance by upregulating the multidrug efflux transporter in *E. coli* (Weatherspoon-Griffin *et al.*, 2014). These findings suggest that aromatic metabolites including indole may perform crucial functions in the antibiotic-mediated stress response.

New perspectives on indole: beyond microbial communities

Indole may globally affect various physiological functions via interaction with a variety of regulators in a number of bacterial species. It is believed that RpoS (RNA polymerase σS), which acts as the master regulator of the general stress response, is important for indole-mediated expression of multidrug exporter genes (*mdtEF*) in *E. coli* (Kobayashi *et al.*, 2006). Indole may affect activity of DksA (RNA polymerase-binding transcription factor) and the association between RNA polymerase and σ^{54} in *V. cholerae* (Mueller *et al.*, 2009). As discussed above, indole may inhibit folding of QS regulators (Kim and Park, 2013). Taken together, these data suggest that indole can have yet unknown global effects within bacterial cells.

Recently, it was suggested that direct supplementation of the culture medium with indole can cause formation of quiescent cells (a nongrowth but metabolically active state) in genetically modified *E. coli* (Chen *et al.*, 2015). Indole also plays an important role in the development of phenotypic diversity in *E. coli* (Saint-Ruf *et al.*, 2014). Indole accumulation can promote formation of cell clusters with different phenotypes in aged colonies by promoting cell death, and dead cells may be used as a nutrient source for the surviving cells (Saint-Ruf *et al.*, 2014). Thus, the indole production may be necessary for the prolonged survival of stationary phase cells.

Many enteric bacteria can secrete indole into the animal intestine, and a considerable amount of indole was detected in human fecal matter (DeMoss and Moser, 1969; Fujisawa *et al.*, 2006). In the human intestine, cytochrome P450 can oxidize indole, thus leading to formation of various indole derivatives and facilitating absorption of indole by the intestinal epithelium (Gillam *et al.*, 2000). When the gut microflora is changed, the concentration of indole-related metabolites is affected in the blood of mice (Wikoff *et al.*, 2009). Moreover, the expression of genes related to epithelial-cell tight junction and inflammation indicators are changed by indole (Bansal *et al.*, 2010). Indole can down-regulate virulence factors in *E. coli* O157:H7 and *P. aeruginosa* (Hirakawa *et al.*, 2009; Lee *et al.*, 2009a). These findings indicate that indole may be beneficial for the immune system of animals including humans.

Indole also plays an important role in the world of plants. Plants have a wide range of defense systems to reduce the damage caused by herbivore attacks. One of the defense mechanisms works as follows: a herbivore-attacked plant can emit blends of volatile organic compounds (Turlings *et al.*, 1990) that repel the herbivores. Indole is a fast-acting and potent volatile priming agent in maize and systemically prepares the tissues and neighboring plants for an imminent attack by a herbivore (Erb *et al.*, 2015). Therefore, indole may also be deeply involved in biological mechanisms and behavior outside microbial communities.

Conclusions

Indole controls various bacterial functions, such as biofilm formation, spore formation, plasmid stability, antibiotic resistance, virulence, and formation of quiescent cells in indole-producing bacteria, indole-non-producing bacteria, or in both (Stamm *et al.*, 2005; Lee *et al.*, 2010b; Chu *et al.*, 2012; Field and Summers, 2012; Vega *et al.*, 2012; Kim *et al.*, 2013; Chen *et al.*, 2015). More recent studies show universal effects of indole on bacterial behavior. Many bacterial and plant species secrete indole (Lee and Lee, 2010; Erb *et al.*, 2015). Significant amounts of indole appear to exist in human, pig, rat, and mouse intestines (up to 1,074 μM

in the human gut; Botsford and DeMoss, 1972; Sims and Renwick, 1983; Karlin *et al.*, 1985; Zuccato *et al.*, 1993; Hwang *et al.*, 2014). It appears that indole performs important functions in a microbial ecosystem beyond individual bacteria. It is still unclear how SidA homologs respond to indole and regulate a variety cellular functions in response to indole. According to current definition of "signal", it is not clear whether indole is a signal that requires a receptor or just a metabolic cue (a molecule naturally produced in a metabolic pathway). Studies involving various indole concentrations and time points of indole treatment are needed to understand the mode of action of indole. Indole-related compounds are known to have therapeutic effects. For instance, indole and indole derivatives are potent antioxidants and therefore hold promise as postbiotics (Wikoff *et al.*, 2009). Indirubin and indigo were successfully produced from tryptophan in an *E. coli* strain expressing oxygenases (Han *et al.*, 2012), and these indigoid compounds were found to be effective against cancer (e.g., leukemia) and Alzheimer's disease (Leclerc *et al.*, 2001; Han *et al.*, 2012). Indole-3-acetic acid appears to be a plant growth-promoting hormone and can regulate the central metabolic pathways in *E. coli* (Bianco *et al.*, 2006a, 2006b; Masciarelli *et al.*, 2013; Andrade *et al.*, 2014; Khan *et al.*, 2014) . Furthermore, indole is an effective volatile priming agent in maize (Erb *et al.*, 2015). Therefore, indole can perform important functions in microbial communities and may influence interactions between plant and microorganisms as well as the gut microbiota, and immune system because many plants and enteric bacteria excrete considerable amounts of indole. Further genetic and physiological studies along with ecological research on indole in the microbial world are necessary to clarify the role of indole as either a true signaling molecule or a metabolic byproduct that affects bacterial physiology at high concentrations.

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