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Indole as a regulator of bacterial physiology

Indol como regulador de la fisiología bacteriana

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Abstract:

During the last years, indole has been the subject of study, this is chemically defined as an aromatic bicyclic heterocycle, from the synthesis of tryptophan after its degradation by tryptophanase (*TnaA*); it has also been implicated as a signalling molecule used by several bacteria to establish multiple behaviours. Most studies have focused mainly on *Escherichia coli (E. coli)*, which produces a small amount of indole during the transition from exponential to stationary phase. According to the literature review, indole plays a fundamental role in many bacterial processes, mainly: biofilm formation, virulence, acid resistance, antibiotic resistance, and persistent cell formation; the concentrations of this molecule play an important role in regulating these mechanisms; on this basis, indole could control undesired bacterial physiological processes, offering us a new therapeutic alternative to different behaviours of microorganisms. The objective of this bibliographic review is to expand the area of knowledge about indole as a regulator of diverse bacterial mechanisms, and thus motivate further research on this molecule and its therapeutic use.

Keywords:

Indole, tryptophanase, bacteria, E. coli, regulator, signalling

Resumen:

Durante los últimos años, el indol ha sido objeto de estudio, este se define químicamente como un heterociclo bicíclico aromático, proveniente de la síntesis del triptófano tras su degradación con triptofanasa (*TnaA*); a su vez se le ha involucrado como molécula de señalización empleada por diversas bacterias para establecer múltiples comportamientos. La mayoría de estudios se han enfocado principalmente en *Escherichia coli (E. coli)*, dicha bacteria produce una pequeña cantidad de indol durante la transición de fase exponencial a fase estacionaria. De acuerdo a la revisión bibliográfica, el indol desempeña un papel fundamental en cuantiosos procesos fisiológicos bacterianos, principalmente: formación de biopelícula, virulencia, resistencia a los ácidos, resistencia antibiótica y formación de células persistentes; las concentraciones de esta molécula juegan un papel importante para regular dichos mecanismos; con base en esto, el indol podría controlar los procesos fisiológicos bacterianos indeseados, ofreciéndonos una nueva alternativa terapéutica a diversos comportamientos de los microorganismos. El objetivo de esta revisión bibliográfica es expandir el área de conocimiento acerca del indol como regulador de distintos mecanismos bacterianos, y de esta manera motivar a realizar más investigaciones sobre dicha molécula y su uso terapéutico.

Palabras Clave:

Indol, triptofanasa, bacteria, E. coli, regulador, señalización

INTRODUCTION

There are currently about 85 indole producing bacterial species, which are involved in multiple signalling processes.[1] Indole signalling is key communication pathway between mammalian gut species.[2] Several Gram-positive and Gram-negative bacteria encode a unique copy of *TnaA* gene on their chromosome and produce indole. Although most microorganisms have the ability to synthesize tryptophan, only bacteria encoding *TnaA* can synthesize indole.[3] It is important to mention that after induction with tryptophan, the molecular sensor (*tnaC*) controls indole biosynthesis for the correct functioning of the dynamics of macromolecules (RNA polymerase, ribosomes and transcription termination factors) during their transcription and translation.[2] This literature review aims to expand the knowledge on bacterial physiology by promoting interest in further research on this molecule.

INDOLE: DEFINITION AND SYNTHESIS

Chemically, indole is defined as an aromatic bicyclic heterocycle, where the benzene ring is fused to a pyrrole ring.[4] Indole is generated when tryptophan is taken up by bacteria

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through the environment and used as a source of carbon and nitrogen, after degradation with *TnaA*, pyruvate and ammonia are also obtained.[5]

TnaA is a pyridoxal phosphate dependent enzyme responsible for hydrolysing tryptophan to obtain indole.[6]

During the transition from exponential to stationary phase *E. coli* produces a significant amount of the indole molecule aromatic; in the Luria-Bertani (LB) medium the concentration of indole in the supernatant reaches a maximum of 0.5-1 mM.[1]

Indole production by *E. coli* is not constant during the growth of a culture, the indole concentration in the supernatant increases 5-fold in about 30 minutes; during this period, indole is produced faster than it can leave, and consequently there is a rapid but short-lived increase; at its peak the cell-associated concentration reaches 60 mM, a level that would otherwise require the addition of 4 mM to the culture supernatant, this phenomenon is described as "indole pulses".[1]

MAIN INDOLE-PRODUCING BACTERIA AND CONCENTRATIONS PRODUCED

Many bacteria excrete various metabolites within their natural habitat.[6] Indole production is widely used in the bacterial kingdom in more than 85 species of bacteria, both Gram negative and Gram positive.[5]

Since 1897, it has been demonstrated that *E. coli* a *Vibrio cholerae* produce indole during the stationary growth phase.[3]

This molecule is also produced by certain members of Proteeae such *Proteus vulgaris, Providencia spp, and Morganella spp.*[7]

Other bacteria of medical interest are also included such as: *Klebsiella oxytoca, Shigella dysenteriae*, and *Enterococcus faecalis.*[5] However, most studies have focused on indole production from *E. coli.* Commensal and pathogenic strains of *E. coli* produce approximately 500 μM in culture media.[8] The maximum indole concentration in a culture supernatant is usually 0.5-1 mM.[9]

At very high concentrations (5 mM) indole is harmful to *E. coli* because it causes changes in the membrane leading to superoxidation. However, the concentration at which indole is toxic is approximately 15 times higher than the physiological concentration observed in supernatants.[7]

INDOLE SIGNALING

Many bacteria use various intercellular signalling systems, such as quorum sensing (QS).[10] QS is a type of bacterial communication that depends on cell density.[6]

Intercellular signalling molecules include N-acyl-homoserine-lactones (AHLs) in Gram-negative bacteria, autoinducer 2 (AI-2) and indole in Gram-negative and Gram-positive bacteria, signal peptides in Gram-positive bacteria, and others.[10]

AHL molecules are synthesized by enzymes of the *LuxI* family and are sensed by regulatory proteins of the *LuxR* family, which bind the autoinducer molecule to cellular DNA; AI-2 is synthesized by the *LuxS* enzyme and the gene that encodes it, *LuxS*. *E. coli* possesses the ability to alter the expression pattern of some of its genes and their phenotypic properties in response to the AHL signal, but it isn´t able to synthesize this molecule, since the bacterium lacks the *LuxI* gene and a homologous gene that allows it to produce it; however, *E. coli* possesses a transcription receptor homologous to *LuxR*, called *SdiA*, which allows the bacterium to censor and respond to the accumulation of the AHL signal produced by other bacteria in the extracellular medium. Six groups of *E. coli* genes have been identified, which are transcriptionally activated when there is accumulation of signals in the medium; indole is the signal that regulates the expression of the genes: *astD, tnaB, and gabT*.[11]

The indole present in the extracellular medium is transported into the cell, through the cell membrane by means of Mtr permease to the cytosol, where in turn, indole is synthesized in the tryptophan metabolic pathway, by the enzyme tryptophanase, when the substance accumulates to a certain amount activates the regulator that allows the expression of the previously mentioned genes, and finally is expelled to the outside of the cell by the AcrEF pump.[11]

Most studies on indole signalling have been performed in *E. coli*, and have focused on concentrations of 0.5-1 mM, which are similar to those detected in a stationary-phase LB culture supernatant.[1]

The *E. coli LuxR* homologue, *SdiA*, has been considered to be related to indole signalling; in one study the addition of exogenous indole increased the expression of the *ppoR* gene, a homologue of *SdiA* in *Pseudomona putida*; thus it is speculated that indole may act as a signal through *ppoR*; although there is still no clear evidence that indole can bind to any homologue of *SdiA*, it has recently been argued that *SdiA* is unresponsive to indole in *E. coli* and *Salmonella enteric* serovar *Typhimurium (S. typhimurium).* Thus, the link between indole signalling and bacterial quorum sensing remains unclear.[12]

There are two forms of indole action: persistent or pulsed; in persistent signalling, indole is present in the culture for a prolonged period at a relatively low concentration (<1 mM). In pulsed signalling, intracellular indole reaches a high concentration (50 mM) for a short time (10-20 minutes) during entry into stationary phase.[13]

PROCESSES CONTROLLED BY INDOLE SIGNALING

A. BIOFILM

A biofilm is a community of microorganisms adhered to a surface, which play an important role in the persistence of infections; the biofilm is supported by a matrix composed of one or several extracellular polysaccharides, DNA and proteins; the biofilm channels allow water, oxygen and nutrients to reach the entire structure.[14]

According to several studies, this physiological function is affected by indole at a concentration of 0.5-2.0 mM, which is similar to the concentration of supernatants of *E. coli* cultures in stationary phase.[6]

Extracellular indole concentrations higher than 600 μM have been studied with wild-type *E. coli* K12 and 500 μM of indole was added in LB supplemented with 0. 2 % glucose, where biofilm formation decreased as a consequence of *TnaA* catabolite repression due to glucose, so such culture medium was chosen since the addition of exogenous indole would have a greater effect, this addition changed the biofilm architecture from dispersed tower to flat colony; i.e., it was reduced by 40%. In another study, 1000 μM of indole was added to 96 wells in LB at 30°C using crystal violet and it was found that the biofilm also decreased.[15]

In a two-species biofilm *E. coli* (indole-producing) and *Pseudomonas spp* (non-indole-producing) toluene o-monooxygenase is highly expressed and thus participates in indole oxidation. A change in indole concentration can affect the biofilm formation of two species; thus, bacteria that do not produce indole counteract the effects of exogenous indole and eliminate indole-induced stress.[6]

B. INHIBITION OF CELL DIVISION

Indole was recently found to inhibit *E. coli* cell division as part of the cell cycle caused by the accumulation of plasmid dimers. Plasmid dimers produce a regulatory RNA (Rcd) that stimulates indole synthesis by *TnaA*, but the mechanism by which indole prevents cell division is not yet well understood, although some ways by which it does so have been contemplated.[16]

Current studies suggest that cell division results because indole acts as a proton ionophore, reducing the electrical potential difference across the cytoplasmic membrane and preventing the proper functioning of the MinCD system that positions the FtsZ ring. This effect was not immediately obvious, since a concentration of indole approximately 10 times higher than that detected in the supernatants is required.[1] Concentrations of 3-6 mM indole added exogenously to an *E. coli* culture have shown reversible inhibition of growth and cell division.[16]

One study showed that adding 5 mM indole to *E. coli* culture medium immediately suppressed cell division; nondividing cells continued to grow slowly for up to approximately 2 hours, doubling in size.[17]

C. VIRULENCE REGULATION

Indole is produced by the microbiota of the lumen and absorbed by the cells of the intestinal epithelium, suggesting that a high concentration of indole may be present in the lumen; however, indole levels in the mammalian intestine are unknown. To evaluate the role of indole in the regulation of virulence genes, a mutant of enterohemorrhagic *E. coli* (EHEC) was constructed that lacked *TnaA* and therefore could not produce its own indole; an increasing concentration of indole was added to this mutant, which decreased the expression of virulence genes.[8]

Indole has also been shown to repress virulence genes in *Vibrio cholerae*, which produces several virulence factors such as pili regulatory toxin (TCP) and cholera toxin (CT). For testing, the effect of indole on TC and TCP production was quantified by culturing A Wyld type (WT) JB58 strain in the presence of increasing concentrations of indole starting at 0. 25 mM, with this, a dose-dependent indole decrease of TC and TCP was observed; TC production was reduced by 65% at 0.5 mM and 80% at 0.75 mM, TC and TCP production was repressed at 1 mM indole, thus concluding that indole is a virulence repressor.[18]

In another study performed with *Listeria monocytogenes* (non-indole-producing), which detects signals from the environment or host to regulate the transcription of virulence-associated genes, a concentration of 0.5 mM indole was used, bacteria were cultured with and without indole; the results showed a significant reduction of all genes associated with flagella and genes associated with biofilm formation.[19]

The decrease in indole concentrations favours bacterial pathogenesis, since it is produced by the intestinal microbiota to improve the function of the intestinal barrier; if damage occurs in this tissue, it would cause dissemination of pathogenic bacteria.[20]

D. RESISTENCE TO ACIDS

With respect to acid pH resistance, indole addition has been shown to repress the acid resistance gene of the glutamate decarboxylase *gadABCEX* 2 to 4-fold; *GadABC* is regulated by *GadE* that protect *E. coli* at a pH of 2 or below, allowing the bacterium to colonize the gastrointestinal tract. Also, the other known acid resistance genes and *hdeABD* (function as chaperones to prevent aggregation of periplasmic proteins in extremely acidic conditions) were repressed 3 to 5 times more than the rest. To test this, 2mM indole was added to a WT strain of *E. coli* K-12 at pH 2.5, in which a 350 to 650-fold decrease in survival was observed.[15]

E. ANTIBIOTIC RESISTENCE

It has been speculated that antibiotic-susceptible bacteria may acquire antibiotic resistance through activation of the expression of antibiotic defense genes against antibiotics, such as indole multidrug efflux pumps.[21]

When *E. coli* is exposed to antibiotics, some cells are lysed and protect most neighbouring cells by releasing indole as a defense signalling molecule. Indole-mediated antibiotic tolerance in *E. coli* and *S. typhimurium* may be due to the induction of oxidative stress and a phage shock response. Indole induces the expression of multidrug exporter genes and increases antibiotic resistance in *E. coli, Salmonella* and *Pseudomonas.*[6]

In one study they tested whether indole production increases in response to ciprofloxacin treatment, such an increase could be achieved both by increasing *TnaA* expression and by stimulation of indole production by the *TnaA* already present; an *E. coli* strain expressing *TnaA* GFP-tagged from its native promoter was used to explore the effect of the antibiotic on enzyme expression. Ciprofloxacin (100 × MIC) was added to an exponentially growing culture of *E. coli* WT and sampled during 5 hours of treatment for indole measurement in the supernatant. The indole concentration in the supernatant before addition of the antibiotic was about 25 μM and showed little change during treatment.[13]

microorganisms. Adapted from [3]

Table 1. Phenotypic changes affected by indole in

Bacterium	Phenotype changes
Aspergillus niger	Inhibited cell growth.
Escherichia coli spp.	Decreased biofilm formation and motility. Enhanced plasmid stability and delayed cell division. Acid resistance and drug. Activated *astD*, *gabT* and *tnaB* as an extracellular signalling molecule. Formation of quiescent cells (in genetically modified) and development of phenotypic diversity.[6]
Listeria monocytogenes	Suppression of virulence genes associated to flagella and biofilm formation.[19]
Pseudomona aeruginosa	Decreased virulence and increased antibiotic resistance. Biofilm formation affectation.[6]
Pseudomona putida	Inhibition of grow cells and promotion biofilm formation.[12]
Salmonella enteric	Drug resistance.[6]
Vibrio cholerae	Increased biofilm formation and grazing resistance to phagocytic eukaryote. Suppression of virulence genes (CT and TCP).[18]

Aspergillus niger, *Pseudomona aeruginosa and putida*, *Listeria monocytogenes* and *Salmonella enteric* did not produce indole.

F. PERSISTENT CELL FORMATION

Persistent cells are a subpopulation of genetically sensitive bacteria that survive antibiotics by entering a dormant state; the appearance of persistent cells after antibiotic withdrawal leads to recurrent infections.[13]

Indole and its functional metabolites are known to contribute to the regulation of persistent cells in *E. coli* at high concentrations (sub-mM to Mm).[22]

Incubation of *E. coli* with indole increased persistence by at least one order of magnitude after subsequent exposure to high concentrations of antibiotics from three different families: ofloxacin, kanamycin and ampicillin, suggesting that the protective effects of indole are a general phenomenon.[5]

In one study, bacterial communication through indole signalling was shown to induce persistence; to test this, indole-induced persistent cell formation was monitored by microfluidics and oxidative stress and shock pathways in phage's were identified; a model was proposed in which a bacterial subpopulation is "inoculated" against antibiotics by activating stress responses, leading to the formation of persistent cells.[23]

Similarly, it was shown that when grown on rich medium where indole signalling is expected to occur in the wild-type strain, a *TnaA* mutant showed a decrease in persistent cell formation by almost an order of magnitude, and that the addition of indole re-established this deficit; thus the authors proposed that the mechanism of indole mediated persistent cell formation involves the activation of the OxyR and Phage-Shock pathways; providing a clear example of bacterial communication through indole signalling, allowing the bacterial population to protect a subpopulation.[23]

CONCLUSION

Many bacteria are able to synthesize indole, a molecule that can control several bacterial functions such as: biofilm formation, acid and antibiotic resistance, inhibition of cell growth, virulence regulation, formation of persistent cells, among others (these functions are summarized in Table 1, which has been previously modified). To regulate these functions, the indole concentration plays an important role, as do the indole-responsive *SdiA* homologs, since upon detection of an amount of indole in the medium, the cells receive the "signal" to protect themselves from "stress" that may be caused by external factors.

Further molecular studies on the physiological functions of indole are needed to determine how it represses or protect cells, and thus to consider indole as a possible therapeutic agent.

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