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E.coli quorum sensing: Appraisal towards curtailing pathogenesis

S.Adline Princy*, V.Praveen Krishna, Vinothkannan Ravichandiran, Surasikha Rangarajan
Quorum sensing Laboratory, School of Chemical and Biotechnology, SASTRA's Hub for Research and Innovation (SHRI), SASTRA University, Thanjavur, Tamil Nadu, (INDIA)
E-mail : adlineprinzy@biotech.sastra.edu

ABSTRACT

Lower organisms need to communicate with each other to perform various activities needed for their survival. Microbes have evolved in an astounding manner to develop tailor-made systems to enhance their chances of existence in harsh environmental conditions. They carry out these mechanisms by synchronizing their actions through cell-to-cell communications. These intra-cellular communications bring about a molecular chain of events to spread infection through biofilm formation and virulence. This review shines a spotlight on the entire *Escherichia coli* quorum sensing network, especially with regard to SdiA. As is apparent from earlier studies, SdiA acts as a global regulator for *E. coli* quorum sensing. We propose that by antagonizing SdiA, virulence and biofilm formation can be contained, thus suppressing pathogenesis of *E. coli*. As an extension, other Gram-negative microorganisms that have analogous circuits to *E. coli* can also be constrained.
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KEYWORDS

Quorum sensing;
E. coli;
SdiA;
Global regulator.

INTRODUCTION

Life's requirements must be accomplished by bacteria within the constantly changing environment they survive in, as a result of which they either have to adapt to the conditions or risk facing extinction. Adaptations stem from evolutionary or genetic changes that sustain a species over a long term. The preferred lifestyle of bacteria has evolved as one favoring composite biopolymer embedded microcolonies called biofilms. Biofilms are the major signals for the expression of virulence[1]. Bacteria communicate with one another through a mechanism called quorum sensing (QS). Various functions are regulated by the QS system of Gram-negative bacteria, that include plasmid conjugation in *Agrobacterium tumefacians*[2], virulence gene expression in *Vibrio cholerae*[3] and *Pseudomonas aeruginosa*[4], antibiotic production in *Erwinia carotovora*[5] and surface motility through swarming in *Serratia liquefacians*[6] and *Burkholderia cepacia*[7]. QS is achieved with the help of chemical molecules produced by the bacteria. The chemical molecules responsible for the signaling system in Gram-negative bacteria are N-Acyl Homoserine Lactones (AHLs). These molecules are utilized as autoinducers, by almost 7% of Gram-negative bacteria within the alpha, beta and gamma proteobacteria. These Gram-negative bacterial autoinducers differ from species to species, by varia-

tion in length of the carbon chain (ranging from 4 to 18) and the substitution at C-3 position of the acyl-side chain of the compound. The QS system present in *Vibrio fischeri* regulates bioluminescence produced by the organism under certain conditions[8]. Certain other Gram- negative bacteria possess LuxIR - type proteins which communicate with the AHL signals. There is a high specificity between the LuxR proteins and their corresponding AHL signals, and due to this reason, they are essentially used for intraspecies communication. The *luxI* homologous gene encodes for the AHL synthetase, and the *luxR* homologous gene encodes for the receptor, and together they compliment to establish the QS control mechanism. When the bacterial counts increases to a particular quorate, the signal molecule is released by LuxI homologue synthetase. Along with the LuxR homologous receptors, the signal molecules from LuxI homologue synthetase form an activated complex, which subsequently binds to specific regulator sites upstream of the promoter. This binding in turn results in the positive or negative regulation of target gene transcription. AHL pathway can be blocked either by the inhibition or antagonism of their receptors or by intervening with the biosynthesis of AHLs.

Bacterial quorum sensing is controlled via small signaling molecules, through which intra-cellular communication occurs, called autoinducers. Most autoinduction systems are known to possess three main characteristics: (i) LuxR homologue activates the transcription of genes indicating cell density dependence, (ii) for an earlier induction of LuxR homolog dependent transcription, conditioned medium is added to the culture, (iii) various LuxR homologues can react with a number of autoinducers. LuxI-type synthetase synthesizes the AHL signaling molecules which bind to the cognate receptor LuxR, which further regulates the expression of target genes. When bacterial population is low, compound synthesis occurs at a basal level and it is diluted after its diffusion into the surrounding medium. At higher density, AHL accumulation leads to a critical threshold concentration, and the AHL binds to its cognate receptor, and stimulation or repression of target genes takes place. LuxR proteins and other similar QS proteins : (a) cognate acyl-HSLs mediates specific binding, (b) binding of signal molecules leading to the conformational changes and alteration in protein multimerization, (c) attaching or releasing of sequence specific target genes, (d) regulation of transcription[8].

Unlike AHLs, many bacteria use a second regulatory system (AI-2) through an uncharacterized signal molecule, such as furanosyl borate diester. AI-2 autoinducers are found in both Gram-negative and Gram-positive bacteria. Some examples among Gram-positive bacteria include *Bacillus subtilis, Bacillus anthracis, Clostridium botulinum, Staphylococcus aureus* etc. In case of Gram-negative bacteria, the examples are *Haemophilus influenzae, Vibrio cholera, Vibrio harveyi, Escherichia coli, Salmonella typhimurium* etc. AI-2 molecules, unlike AHLs, participate in both intra-species and inter-species communication. AI-2 molecules are generally synthesized from 4, 5-dihydroxy-2, 3-pentadiene (DPD). AI-2 perception is dependent on an additional sensor kinase called LuxQ[9]. LuxQ and LuxN input sensor kinases are unified at the *lux* gene expression level itself, through a single repressor regulator LuxQ or additional regulator LuxR. The AI-2 network is found in nearly half the bacterial species, such as *Vibriyo harveyi luxS*/AI2 system, and the *E. coli* and *Salmonella* LuxS system. It begins with S-adenosylmethionine as the central metabolite, it acts as a precursor in both the AI-2/AHL pathways. LuxI-like enzymes processes SAM to produce AHL along with a toxic product, methyladenosine, which is then converted to non-toxic methylthioribose by 5'methylthioadenosine [Schauder and Bassler, 2001]. In the AI-2 synthetic pathway, methyltransferase enzyme converts SAM to S-adenosylhomocysteine, which is converted to dihydroxy 4, 5-dihydroxy 2, 3-pentadione through LuxS. This compound is responsible for the formation of AI-2 molecules such as a furanosyl borate diester[9].

A careful analysis of the genes responsible for imparting virulence and biofilm formation in *E. coli* shows that SdiA upregulates or downregulates them, as is the case, either directly or indirectly, ultimately leading to desired biofilm characteristics in the organism.

PATHOGENICITY ISLANDS – THE VIRULENCE FACTORY

The major virulence factors of *E. coli* are the set of proteins transcribed by the Locus of Enterocyte and Effacement (LEE) pathogenicity island (PAI)[10]. LEE PAI is known to secrete the type three secretion sys-

tem (TTSS) in *E. coli,* which culminates in the formation of attaching and effacing (AE) lesions in the intestine[11]. LEE1, LEE2, LEE3 and LEE4 operons are together responsible for AE phenotype expression in *E. coli.* It encodes for the proteins EscC (outer membrane protein), EscN (an ATPase) and EscF. LEE4 encodes for EspABD proteins. EspA is a monomer and by polymerization, it forms a filament over EscF, thereby manifesting the needle structure needed to inject the effector proteins into the host cell. Pores in the host plasma membrane are made by EspB and EspD, which are required for injection[12]. Then, EspF is injected into the cell which targets mitochondria leading to cell death pathway. LEE5 operon encodes for intimin proteins and Tir (translocated intimin receptor), an intimin receptor that helps in the in-depth attachment with the host. CesT, a chaperon for Tir is also encoded in the same operon[12]. The intimin receptor is translocated to the host and the binding of intimin helps in strong attachment of bacteria to the host epithelium. LEE1 operon consists of *ler* (LEE encoded regulator) genes, which on binding to autoinducers activate transcription of LEE3 and LEE5 operons[13]. LEE4 is constitutively expressed at high levels. LEE1 and LEE2 genes are expressed via normal transcription factors σ70 but LEE3 and LEE5 are transcribed by alternative σ factor, RpoS (σ34)[11].

From earlier studies, it has been found that all the LEE genes are regulated by QS mechanisms. LEE1 and LEE2 are directly QS regulated while LEE3 and LEE5 are QS regulated indirectly via Ler (a product of *ler* in LEE1). Other promoters transcribed by *rpoS* are also regulated by QS mechanism (like ftsQP1). Both enterohaemmoragic *E. coli* (EHEC) and enteropathogenic *E. coli* (EPEC) share all the above mechanisms, except for the fact that EHEC produces shiga toxin (stx) while EPEC do not. Quorum sensing regulator A of *E. coli* (QseA) activates the LEE genes, which are in turn activated by AI-2 molecules[12]. H-NS, which is important in gene silencing, can switch off the LEE genes by binding to *ler* of LEE1. H-NS is able to bridge or loop extended regions in the LEE genes thereby silencing it (H-NS is present only in EPEC). PerA (plasmid encoded regulator A) which is encoded in 70kb EAF virulence plasmid of *E. coli* positively regulates LEE5 operon. PerC, upon expression increases the transcription of LEE1 operon[12]. BipA also increases LEE expression by activating *ler* gene. GrlA and GrlR are encoded by *grlRA* operon present between the LEE genes. GrlA activates the LEE expression by acting upstream of *ler* genes during transcription. But, GrlR repress the LEE expression by acting at the same place of GrlA. SOS response genes, a common *E. coli* regulon used for its fundamental survival, also controls the LEE expression. LexA protein tends to repress the LEE operons. Therefore, presence of DNA damaging agents like mitomycinC (activates RecA that cleaves LexA) increases the LEE transcription[12]. Integration Host Factor also increases the LEE expression.

MOTILITY - THE BIOFILM TRIGGER

There are three quorum sensing *E. coli* regulators namely QseA, QseB and QseC. QseA previously called as b3243, is an activator of *ler* in LEE PAI[13]. QseA protein belongs to the LysR family, which is similar to AphB of *Vibrio fischeri* that encodes a master virulence factor. The AI-2 produced by the *luxS* gene activates the transcription of *qseA*. QseA then activates LEE PAI at *ler* gene. But QseA does not involve in the transcription of genes responsible for flagella and motility. It also doesn't influence AI-2 synthesis[13]. Thus, QseA, in a quorum dependent manner, regulates the LEE genes of *E. coli.* The other regulators QseB and QseC form a two component system. QseB encodes a response regulator and QseC encodes for a sensor kinase[11]. They do not involve directly in the regulation of LEE genes. They activate flhDC, a master regulon for motility genes. There are three classes of flagella regulons. Class1 is flhDC whose product upon expression activates Class2 regulon. FlgM and FliA belong to Class2 regulons which encode for hook and basal body of flagella. It is transcribed by an alternative σ factor RpoS (σ28). Class3 regulon consists of *fliC* that encodes for flagellin and mot operon that code for motility. Class3 regulon is activated by FliC[13]. This triggers the expression of genes involved in assembly of flagella and motility. Also, the activated flhDC increases the transcription of *luxS*/AI-2 QS system. The *luxS* transcription increases, thereby producing more AI-2 molecules[9]. AI-2 induces *mqsR* (motility quorumsensing regulator [b3022])[14] and MqsR again induces the QseBC two component system and *crl* genes[15]. Thus it is said to be positively autoregulated. AI-2 synthesized can also activate *qseA* thereby influencing LEE PAI indirectly[16].

lsR - COMMUNICATION SIGNALS TRANSPORTER

Lsr transporters are a kind of ABC transporters that import the AI-2 molecules inside the cell. *lsrABCD* encodes the proteins that help in AI-2 uptake and they have ATP binding cassettes[17]. It has been propsed that help in the AI-2 modification after the uptake[18]. Thus the genes *lsrABCDFG* constitutes the lsr operon. There are two other genes *lsrR* and *lsrK*, that belong to the same operon but are divergently transcribed[19]. They are present adjacent to the Lsr operon. The product of *lsrR* (LsrR) represses the transcription of the whole of *lsr* operon and itself. *lsrK* product (LsrK) helps in the phosphorylation of internalized AI-2 molecule, by utilizing one ATP molecule. Then the phosphorylated-AI-2 antagonizes LsrR thereby derepressing the lsr operon and blocking LsrR activity[20]. Thus, we can say that the *lsrR* and *lsrK* genes are regulators of *lsr* operon. But they also have many other roles in the cellular processes. It is proposed that AI-2 internalization can also be done by another transporter system that works with LsrK[19] or the AI-2 anomers which are the precursors have the capacity to bind LsrR that helps in various cellular processes. The genes regulated by AI-2 anomer-LsrR complex were found using *lsrR* and *lsrK* mutants (Li *et al.,* 2007). Their results showed that binding of AI-2 with LsrR activates the biofilm formation via flu genes that transcribes antigen 43 (Ag43) which helps in cell-cell aggregation. Ag 43 also plays a vital role in the initial recognition and binding to the host[19]. LsrR-AI-2 complex also activates *wza* genes that help in the production of colonic acid, thereby increasing the biosynthesis of extracellular polysaccharides (EPS) and capsular polysaccharides (CPS) that leads to biofilm formation. This complex plays a vital role in activation of small RNA (sRNA) regulators like DsrA that controls the acid resistance genes and other genes like *yheEF* responsible for type 2 secretion system[19]. It is hypothesized that LsrR, along with AI-2 is a global regulator of many genes responsible for biofilm formation, but with onset of late exponential phase, AI-2 uptake is done using Lsr operon and the phosphorylation by LsrK tends to block these cellular processes by binding with LsrR competitively[20], thus switching on and off the genes controlled by LsrR and LsrK[19].

HEMOLYSINS – THE JACK OF VIRULENCE

E. coli also encodes a major set of toxins called hemolysins, which can cleave the human coagulation factor and human red blood cells. It is encoded in a large virulence plasmid of 92kb with 100 ORF's (Open Reading Frames)[21]. In EHEC, the toxin is termed as EHEC-Hemolysin which is related to α-hemolysin but not identical to it. It is secreted from the *hly* operon of the plasmid. HlyA is injected into the plasma membrane of mammalian cells which results in atlered permeability. This is the major cause of toxicity. The other genes *hlyBCD* helps in the transport of HlyA and its injection into the mammalian cytoplasm. TolC is another protein encoded in chromosome that also helps in the HlyA transport. The HlyB and HlyD lie in the inner membrane and TolC lies in the outer membrane, thereby acting as transporters in the migration of HlyA. HlyC and majorly GrlA, the product of the gene *grlRA* induces the HlyA synthesis leading to hemolysin production[21].

katP is related to the hemolysin operon, whose product protects the bacteria from oxidative stress. It is a bifunctional periplasmic protein and it is also encoded in the same virulence plasmid (pO157 in EHEC). The *ler* gene that belongs to LEE PAI induces certain genes like *stcE* in pO157 which is silenced by H-NS[12]. In EPEC the plasmid pEAF has genes like *perABC* from which PerC is transcribed, and responsible for activation of LEE genes via *ler* induction. PerA also activates LEE5 operon and *bfp* (bundle forming pilus) genes that are present in the same plasmid. H-NS also silence the *perABC* and *bfp* genes[12]. DsrA, an sRNA regulator can block H-NS silencing of many genes by activating RcsA expression.

CELL DIVISION OPERON - *ftsQAZ*

In *E. coli, ftsQAZ* operon plays a major role in cell division[22]. ftsQP1 and ftsQP2 are two promoters located upstream of this operon. All the genes *ftsQ*, *ftsA* and *ftsZ* are transcribed in clockwise direction. The concentration of FtsZ protein increases during cell division[23]. This operon mainly helps in formation of septum during cell cycle. The ftsQP1 promoter is activated

by RpoS an alternative σ factor[22]. SdiA induces ftsQP2 promoter and activates the *ftsQAZ* operon thereby increasing cell division. Higher cell division rate also remains as a cause for biofilm formation. SdiA acts by simple recruitment factor to recruit RNA polymerase to ftsQP2 promoter but in contrast to class1 factors (interaction with α- C-Terminal Domain), σ-CTD is needed for the recruitment.

SdiA - THE MASTER CONTROLLER

sdiA, the gene that controls the cell division when amplified revealed many new genes controlled by it. Upon amplification, 62 genes, among which 41 are related to motility and chemotaxis are reduced 3 fold and in about 75 genes, the expression was elevated 3 fold. As the SdiA dosage increases the transcription of *ddlb-ftsQAZ-lpxC* operon increased. It also activates the efflux pump *acrABDEF* genes leading to multidrug resistance[24]. It highly induces genes responsible for DNA repair making the cell resistant to drugs and DNA damaging agents such as *uvrY*, *uvrC*, *mioC* and *yidAB*[25]. Additionally, SdiA is the usual suppressor of cell division inhibitors like MinC and MinD and helps in mini cell formation by increasing FtsZ yield[26]. Reduction in the SdiA expression by using conditioned medium decreased the activation of ftsQP2 promoter[27]. AHLs,which are synthesized by bacteria like *Vibrio harveyi*, potentiate the effects of SdiA. AHL which is normally called as AI-1 cannot be synthesized by *E. coli* due to the absence of a luxI homologue. In *E. coli*, indole is produced from tryptophan using tryptophanase enzyme coded by *tnaA* genes[28]. Though there is no clear evidence for indole binding to SdiA, it has been proved that indole reduces biofilm formation, represses the genes responsible for UMP synthesis (*carB, pyrCDF*) and also controls the expression of *qseB*, *flhD*, *fliA* etc., and reduces cell division in an SdiA dependent fashion[29]. MitomycinC (MMC), a DNA damaging agent, tends to induce the SOS response (activates *recA* to cleave LexA and helps transcription of DNA repair enzymes). High copy number of *sdiA* also makes the cell resistant to MMC. SdiA, by activating the efflux pump (*acr* genes), is mainly responsible for the multidrug resistance properties[30]. Figure 1 shows how SdiA controls the genetic circuit involved in biofilm formation and virulence.

THE BIGGER MOUTH – SdiA

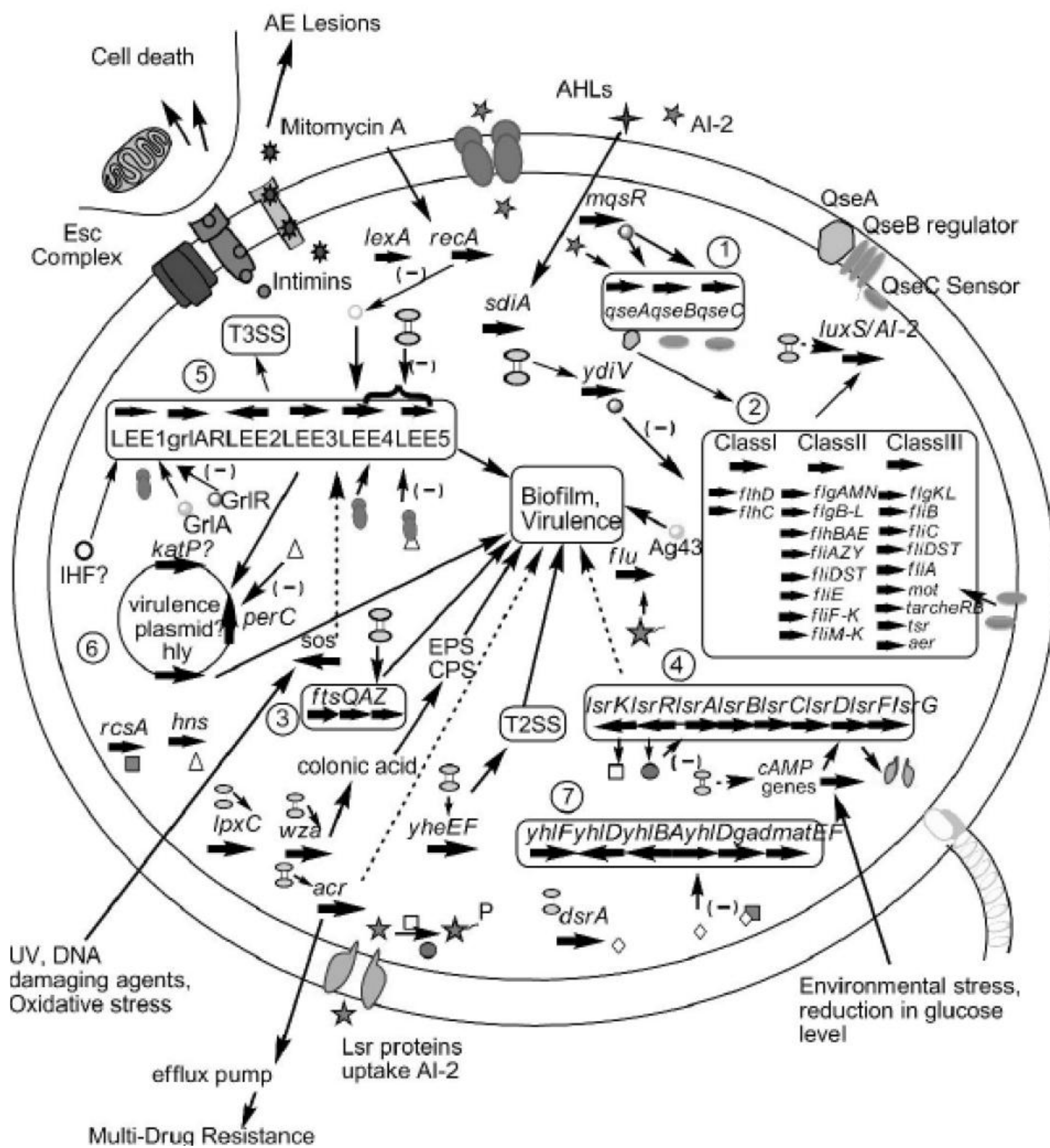
SdiA is a protein having 240 aminoacids with a molecular weight of 28117Da. Binding of AHLs potentiates SdiA activity by forming a favorable folding switch. It is a LuxR homologue which helps in AHL binding and activating the luminescence genes in *V.fischeri*[3]. Therefore initially it was thought to detect other bacterial populations (by AHL binding). Its DNA binding domain is composed of a helix-turn-helix motif at 197-216aa and AI binding domain at 1-171aa[31]. SdiA is found to be a global regulator of many genes responsible for cell division, biofilm formation, virulence factor expression etc. It acts as a link between QS system 1 and QS system 2. SdiA majorly constitutes the QS system 1 that responds to various cell signals[32]. The *lsr* operon with *lsrR* and *lsrK* induced by the CRP complex (cAMP receptor protein- cAMP complex also called as CAP [catabolite activated protein] - cAMP complex) forms the QS system 2. SdiA primarily induces the *ydiV* genes whose product contains an EAL domain protein. This activation by SdiA is potentiated by the presence of AI-1(AHL)[33]. YdiV with EAL domain and SdiA-AHL complex together controls the concentration of cAMP inside the cell leading to the inhibition of QS system 2 (Wei *et al.,,* 2001). The CAP-cAMP complex activates both the *lsr* operon and *lsrR* gene whose product inhibits the *lsr* genes. Thus, cAMP plays a vital role in this activation. Glucose controls the expression of both *sdiA* and *ydiV* genes. It is hypothesized that the QS system is activated during the cessation of glucose. It is known from the proofs that there are some factors which control the cAMP concentration inside the cell in a YdiV and SdiA-AHL dependent manner[34]. These factors can be IIA^{glc}, CpdA (hydrolysis adenylate cyclase responsible for cAMP synthesis), *cyaA* genes (induces adenylate cyclase synthesis) which may be related to YdiV thereby controlling cAMP concentration. Thus, we can say that YdiV and cAMP relates QS-1 and QS-2 systems, concluding that SdiA also controls the *lsr* expression indirectly[34].

CONCLUDING REMARKS

From the above proofs and hypotheses, it is clear

REVIEW

Direct activation ----> Indirect activation (−) Inhibition Genes



SdiA protein of *E. coli* acts as the nerve centre for stimulating various genes involved in biofilm formation such as 1) *qseABC* genes that transcribe QS regulator proteins, 2) flagella and motility genes, 3) cell division genes, 4) lsr operon that is involved in AI-2 uptake and luxS gene that produces AI-2. Additionally, virulence genes such as 5) LEE-PAI that forms T3SS, 6) hemolysins, 7) acid resistance genes and *yheEF* that forms T2SS are also activated. Besides these, SdiA enervates other genes such as *wza*, that forms EPS (exopolysaccharide) and CPS (capsular polysaccharide), the matrix of biofilm. During stress conditions, it also activates genes such as *sos*, that also augment biofilm formation and virulence.

Figure 1 : Global regulator protein SdiA acts as a master switch for biofilm formation and virulence in *E. coli*:

that SdiA activates and controls many genes directly and some operons indirectly. SdiA in presence of indole inhibits *qseB* expression[29], which inhibits the cascade for activating the flagella and motility genes[35] and reducing AI-2 synthesis by influencing on AI-2/*luxS* QS system affecting LEE PAI genes and other genes stimulated by *lsr*-AI-2 complex indirectly. It powers cell division and efflux pumps directly as given in the above statements. Thus, it is clear from all the evidences that SdiA acts as a master control protein of *E. coli.* There-

fore, we suggest that by antagonizing SdiA with suitable agents can control the virulence and biofilm thereby controlling the pathogenesis of *E. coli*. Future work can be targeted towards the bigger communication pathways like SdiA which relates and controls most of the virulence genes; henceforth diseases like cystitis and other major urinary tract infections (UTIs) can be treated.

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