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Research Article

Mechanism of Biofilm Formation by *E.coli*

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Abstract

E. coli is a gram-negative coliform of family Enterobacteraceae found in the intestine of human and animals. It is most studied intensively in genetics and molecular biology and it became a model for studying many essential cellular processes. It is a facultative anaerobe which is able to use alternative anaerobic electron acceptors depending on electron acceptor availability It is able to form biofilm on biotic and abiotic surface which provide them better condition for survival in adverse condition. Biofilm can be formed in the lower intestine by commensal, probiotic and pathogenic *E. coli*. Harsh environmental conditions such as, low temperature, stress and nutrient deprivation favour biofilm formation. It is made up of complex extracellular matrix which made them stable in diverse conditions. The main components of biofilm produced by *E. coli* are curli, cellulose, type I fimbriae, conjugative pili, autotransporter proteins and exopolysaccharide etc. A large number of genes involved in production of various surface appendages reveal the complexity of biofim. Quorum sensing and secondary messenger system regulate the expression of these genes. In this review, we summarize biofilm formation mechanisms and their cellular regulation in *E.coli*.

Keywords: *E. coli*, Biofilm, Components of Biofilm of *E. coli* Quorum sensing, Second messenger signalling

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Introduction

Escherichia coli is a facultative anaerobe that lives in the mammalian gut. It may be commensal or pathogenic. *E. coli* is habitually the first bacterium that colonizes in human infants and is a lifelong colonizer of adults which may be about 10^{21} E. coli cells [1]. *E. coli* was discovered by German bacteriologist Theodor Escherich in 1885. This was an important public health discovery. It can use various electron acceptors depending on electron acceptor availability which make them perfect for survival in adverse conditions [2]. It can follow different pathways such as Embden-Meyerh of Parnas glycolytic pathway (EMP), TCA cycle, pentose phosphate pathway (PP), Entner Doudoroff pathway (ED) and diverse fermentation pathways. It can use various substrates such as sugars, amino acids, dicarboxylate and a wide range of mono- and disaccharides, but cannot use complex polysaccharides because hydrolase enzymes is absent in *E. coli*. Its Central metabolic pathways are highly conserved constituting a significant part of the core *E. coli* genome [3].

Colonization refers to the indefinite persistence of a specific bacterial population in a particular microbiome without reintroduction. According to Freter's nutrient-niche hypothesis, the mammalian intestine is equivalent to a chemostat in which several species of bacteria can live in equilibrium. To co-colonize, each species must use at least one limiting nutrient better than all the other species [4]. In human gut, diversity of commensal strains of *E. coli* exists and the different strains may possess different strategies for utilizing growth-limiting nutrients [5]. *E coli* is a good colonizer because they have an ability to compete for nutrients,penetrate mucus layers, can avoid host defence [6].

Due to presence of large population of *E. coli* in colon of mammals it is used as an indicator of the sanitary quality of water and of the food-processing environment for faecal contamination. Some *E. coli* are beneficial, while some cause infections in gastrointestinal tract and urinary tract. More than 700 serotypes of *E. coli* have been identified on the basis of "O" and "H" antigens present on bacterial cell surface and their flagella. Some strains of *E. coli* are beneficial, while some strains cause infections in gastrointestinal tract and urinary tract. It can acquire specific virulence and become notorious pathogens causing a broad spectrum of diseases and posing a significant risk to human health world-wide. Some strains of *E. coli* can causes diarrheal diseases and a variety of extra intestinal infections which is similar to biofilm-associated infections [7].

Biofilm is a multicellular association of microorganisms that can be associated with biotic or abiotic surfaces. The formation of biofilm initiates with the slowing down of the movement of a single motile cell and ends with the active or passive dispersion of single cell or clusters from a mature three-dimensional biofilm [8]. A large number of adhesion factors and extracellular matrix components help in the development of biofilm from a single attached cell

and provide the shape of three-dimensional biofilms [9]. It is a model for well-structured community of bacterial cells surrounded by self-produced polymeric matrix and adherent to an inert or living surface [10]. Biofilm formation has been best studied in *Pseudomonas aeruginosa*, the causative agent of cystic fibrosis, and current models of bacterial biofilm formation and structure are derived from studies with this organism [11].

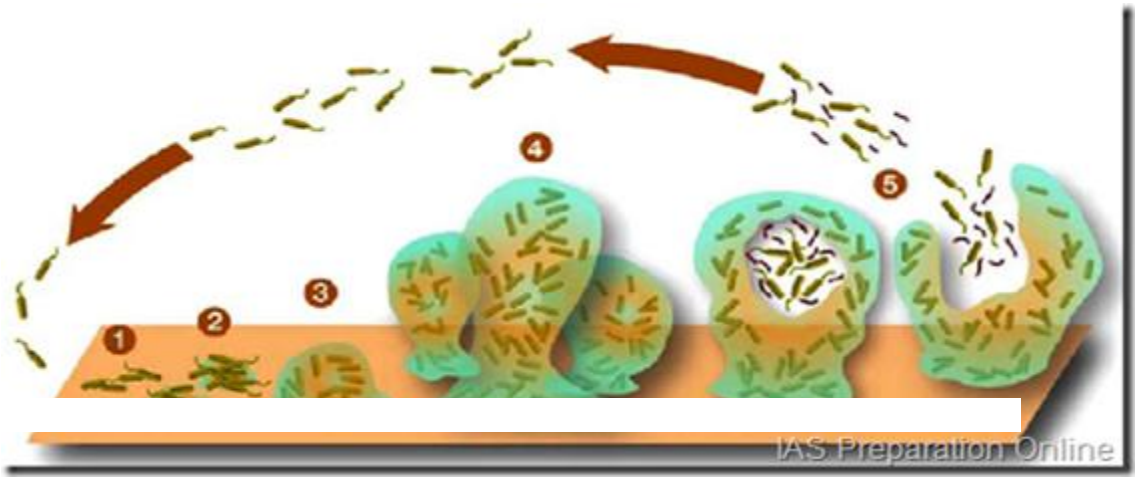

Figure 1 Developmental stages of biofilm [12]

This biofilm mode of growth can be seen as a specific lifestyle compared to the planktonic lifestyle because it involves a simple developmental process which results in differential gene expression and important physiological changes (**Figure 1**). A mature biofilm is a complex community exhibiting channels and pillars that may facilitate nutrient exchange and waste removal. Biofilms are of importance for many industrial activities which cause biofouling can reduce mass and heat transferred and increase corrosion. The formation of biofilms on food surfaces and in potable water distribution systems constitutes an increased risk for product contamination with pathogenic microflora. Composition of biofilm depends on environment. Microbial cells constitute only 2-5% of biofilm [13], rest are DNA, RNA, polysaccharide, proteins etc. (**Table 1**). From a medical perspective, biofilm-associated bacteria on implants or catheters are of great concern because they can cause serious infections [14]. Low temperature, stress and nutrient deprivation favour expression of biofilm. Biofilm formation occurs in the GI tract by commensal, probiotic and pathogenic *E. coli*. Biofilm formation by *E. coli* is a well-defined process but due to their large genetic variability and different environmental niches, variability in biofilm formation is difficult to explore among *E. coli* species [15].

Role of Biofilm

- Mean of microbial self-defence that increases survival
- Biofilm resists phagocytosis by predators
- Retards the penetration of toxic molecules
- Provides a favourable niche for microorganisms
- Facilitates cell-to-cell communication
- Facilitates nutrient and genetic exchange

S. No.	Components	Percentage of matrix
1	Microbial cells	2-5%
2	DNA and RNA	<1-2%
3	Polysaccharides	1-2%
4	Proteins	<1-2%
5	Water	Up to 97%

Table 1 Composition of biofilm [13]

A variety of extracellular molecules and surface organelles participate in biofilm development. In *E. coli*, flagella, type I pili, and curli fimbriae are involved in attachment and adherence

Strains of E. coli

E. coli is mainly divided into 3 groups: Commensal, Probiotic and pathogenic groups.

Commensal group

Commensal *E. coli* show large number of range of regulatory patterns of rdar (red, dry and rough) biofilm formation on agar plates [16] at 28°C and 37°C ranging from negligible rdar morphotype to a semi-constitutive rdar morphotype. In the colon, *E. coli* lies in the outer mucin layer, separated from the epithelial mono-cell barrier by the tighter inner mucin layer [17]. These strains grow on mucus lining of gut and use monosaccharide, disaccharides and other simple molecules [18]. Commensal *E. coli* K-12 possess seven additional pathways to use chaperone for fimbriae synthesis in addition to type I fimbriae [19].

Probiotic group

E. coli Nissle 1917 is a well-established probiotic strain of *E. coli* isolated in 1917 by the German physician Nissle. This strain form a specific rdar morphotype biofilm, distinct from other strains of *E. coli* which is characterized by CsgD and diguanylate cyclase YedQ independent 28/37°C cellulose expression uncoupled from ambient temperature-expressed curli [20]. It has an ability to significantly colonize in the mammalian gut and counteract colonization by pathogens [21]. It has been proved to be most efficient against the pathogenic isolates, i.e. ETEC and EPEC strains. They produce colicin H and microcin H47 and microcin M. It harbours a large number of genes encoding fitness/virulence factors. It can express several adhesins such as type I fimbriae, F I C fimbriae and Ag43, strongly produces curli and cellulose in a temperature independent manner, several proteases and an impressive array of iron acquisition systems [22].

Pathogenic group

pathogenic group are categorize into two groups i.e. intestinal pathogenic E. coli (InPEC) and Extraintestinal E. coli (ExPEC).

- Intestinal pathogenic *E. coli* (InPEC group): These group include the pathogens that infect and colonize the GI tract resulted into secretory or bloody diarrhea.The representative strains of this group are enteropathogenic *E. coli* (EPEC), enterotoxigenic *E. coli* (ETEC), enteroaggregative *E. coli* (EAEC), Shiga toxin (STEC)-producing *E. coli*, such as enterohemorrhagic (EHEC), enteroinvasive *E. coli* (EIEC), adherent-invasive *E. coli* (AIEC) and diffusely adherent *E. coli* (DAEC) [23]. Some pathovars cause infection on small intestine, while others cause infection in the large intestine [17]. Most of InPEC strains belong to phylogroup A and B1 (**Figure 2**).
- Extraintestinal *E. coli* (ExPEC) group: These groups include uropathogenic E. coli (UPEC) and newborn meningitis E. coli (NMEC), and mainly belong to the B2 and D phylogroups. The molecular mechanisms behind the pathogenicity of the *E. coli* pathovars have been reviewed extensively [24].

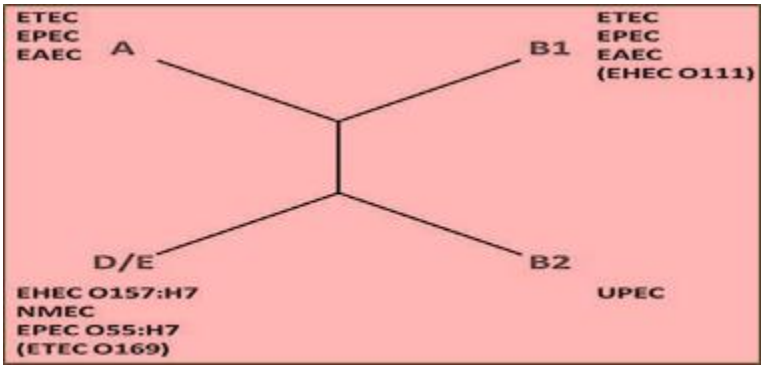

Figure 2 Phylogenetic tree of pathogenic group of *E. coli*

Mechanism of Biofilm Formation in E. coli

- Cell is reversibly attached to the biotic or abiotic surface by the help of flagella.

- Communications between cells start through quorum sensing system. Communication occurs through AI-2 and AI-3 system.
- Secretion of curli, type 1 fimbriae and cojugative pili started which result in permanent attachment of cells to the surface.
- Replication and growth of cells occur to increase the number of cells and biomass.
- Cells of biofilm start secreting a lot of polysaccharide matrix such as cellulose, curli, PGA colanic acid and autotransporter proteins.
- After sufficient secretion of polysaccharide matrix and other surface adhesion proteins the biofilm become mature and it is also resistant for antibiotics.
- When nutrient is present in excess, bacterial cells detached from the biofilm and dispersed.

Biofilm Forming Components of E. coli

- Curli
- Cellulose
- Type 1 Fimbriae
- Conjugative pili
- Autotransporter protein
- Exopolysaccharide

Curli

Curli is a collection of proteins such as fibronectin, laminin, and plasminogen of 6-12 nm in diameter which is present in ECM [25]. They are heteropolymeric filamentous appendages of proteinaceous nature which are composed of a major subunit (CsgA) and minor subunit (CsgB) That have adherence properties found in several biofilm-forming *E. coli* strains [26]. The genes for curli production are organized in the operons *csgBAC* and *csgDEFG* but only the function of some of these genes has been fully identified [27]. Curlin is produced by *csgA*, while *csgB* acts as a surface-exposed nucleator catalyzing forma-tion of insoluble curli at the bacterial surface [28]. CsgD is a transcriptional regulator belonging to the LuxR family and is required for the transcription of csgBAC [27]. It was reported that mutation in *omp*R234 increased the production of CsgD which cause over expression of *csgA* [29]. Mutation in the *ompR* gene resulted in leucine-to-arginine substitution at position 43 of the OmpR protein, leads to enhanced activation of the *csgDEFG* operon. The gene products of csgEFG are putative assembly factors and their role is just beginning to be elucidated. Curli is expressed by most of enterotoxigenic and enterohemorrhagic strains, but not expressed by enteropathogenic and enteroinvasive strains, suggest that it has a specific role in pathogenicity [30]. Pathogenic strains of *E. coli* isolated from human blood cultures show curli formation at 37^0C in vitro [31]. But commensal *E. coli* strains like *E. coli* K-12 can also synthesise curli which induce the production of pro-inflammatory cytokines in human macrophages and increase the release in human plasma of bradykinin, a potent inducer of fever, pain and hypotension [32].

Cellulose

It is one of the major polymer present in extracellular matrix of biofilm produced the *E. coli* which is made up of β-(1, 4)-linked glucose monomers [33]. Cellulose production by bacterial was first reported in 1887. *Gluconacetobacter xylinus* is the model organism for studies of bacterial cellulose synthesis [34]. CsgD is a regulator protein that regulate the genes for cellulose synthesis by activating transcription of *adr*A [35]. AdrA comprise a typical domain, GGDEF of diguanylate cyclases [36] which help in production of cyclic-di-GMP that binds to the PilZ domain of the cellulose synthase, BcsA, which stimulates cellulose synthesis [37] (**Figure 3**). BcsA linked to the UDP-D-glucose monomers by covalent bonds to produce a growing glucan chain. Diguanylate cyclase, other than AdrA also produce cyclic-di-GMP to drive BcsA activation. *E. coli* 1094 can produce CsgD independent cellulose via the YedQ (diguanylate cyclase) that works in place of AdrA [35].

Type I Fimbriae

Fimbriae are mainly attached with host tissue which is important for pathogenic *E. coli* strains strains [39]. The most common adhesins found in *E. coli* isolates and in other Enterobacteriaceae are Type 1 fimbriae, about 7-nm wide and 1-µm-long rod-shaped adhesive structure which influence on biofilm formation. The *fim* gene cluster encodes the structural components of the fimbriae and regulatory elements in which *fim*A encodes major unit of pilus and *fim*H

encodes mannose specific adhesins found at the tip of pilus [40]. The promoter of *fim* genes is located at upstream to the *fimA* which is a flip-flop type control system generating a phase variable expression of Type I fimbriae [41]. Physical contacts between Type I fimbriae and a solid surface cause structural changes in the outer membranes of attached cells due to reduction in the level of outer membrane proteins [42]. So, the deletion of one of these outer membrane proteins (OmpX) resulted in increased cell-solid surface contact [43]. In addition to Type I fimbriae, *E. coli* strains also produce other fimbriae that have adhesive, antigenic or physical properties in which the primary amino acid sequence of their major protein subunits are same [44].

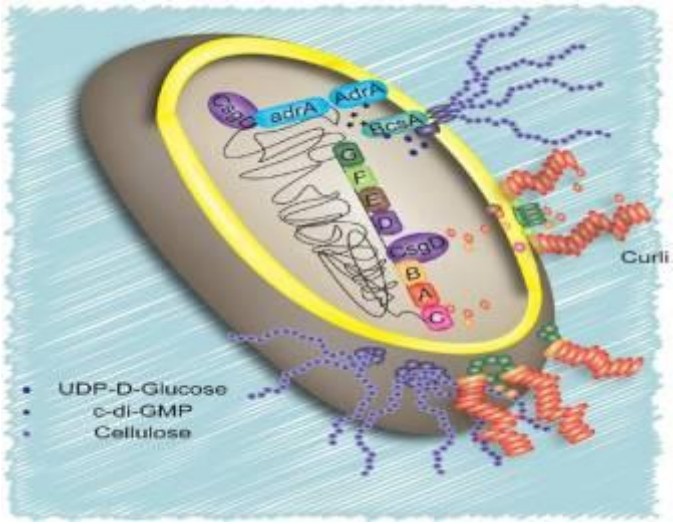

Figure 3 Regulatory pathway of cellulose and curli production [38]

Conjugative pili

Bacterial conjugation is a process of horizontal gene transfer that involves intimate cell-to-cell contact, in which a conjugative plasmid is transmitted from a donor to a recipient cell through a specialized conjugative pilus encoded on the plasmid [45]. *E. coli* strains carrying conjugative plasmids. Mixed *E. coli* communities having conjugative plasmids induce formation of a thick mature biofilm [46]. Mutational analysis of F plasmid demonstrates its importance not to mediate DNA transfer, but it requires a functional conjugative pilus for biofilm. Conjugative pilus acts as an adhesion factor supporting the three-dimensional growth of the biofilm.
The F-pilus promotes:

- Initial adhesion
- Cell-to-cell contacts
- Biofilm maturation through nonspecific attachment
- Stabilize the structure of the biofilm

Autotransporter protein

Autotransporter protein includes Ag43, Adhesin Involved in Diffused Adherence (AIDA), TibA.

Antigen 43

Ag43 is an abundant outer membrane protein encoded by *agn43* which influences auto-aggregation of *E. coli* in liquid culture [47]. OxyR-mediated regulation of *agn*43 expression is performed under stressful conditions to regulate biofilm formation. Antigen 43 establishes autoaggregation of cells through Ag43–Ag43 interactions by an intercellular handshake mechanism and promotes bacterial biofilm formation by its ability to induce micro-colony formation [48] and, on the basis of these properties, was proposed to potentially enhance colonization of the mammalian intestine. Capsulated strains of bacteria produce capsular polysaccharides of about 0.2–1.0 μm from the bacterial surface and form biofilm in Ag43-independent way [49]. Expression of Antigen 43 depends on DNA-methylating enzyme deoxyadenosine methylase (Dam) and the transcriptional regulator OxyR which is phase-variable also [50]. Dam is a specific methylase of *E. coli* that methylates the adenine residue of GATC sequences and OxyR is involved in the oxidative stress response and in monitoring the cellular thiol-disulfide status. If these sequences are

unmethylated, OxyR can repress transcription independently of its oxidation state, resulting in the OFF phase. If Dam methylates these sequences, OxyR does not bind and expression is ON [10].

Adhesin Involved in Diffused Adherence (AIDA)

Glycosylated adhesin associated with adherence of microbial cell to human cell [51]. AIDA interacts with Ag43 and enhances the aggregation. Its surface exposure can be blocked by the production of capsular polysaccharides, similar to the Ag43.

TibA

Glycosylated protein helps in adherence of microbial cell to epithelial cell. Production of the mature, glycosylated TibA protein requires additional sequences upstream of the *tibA* gene (*tibC* is thought to encode a glycosyltransferase) and this mature protein is associated with epithelial cell adherence and invasion. The glycosylated monomers of TibA are exposed to surface of cell having protein adherence properties, as non-glycosylated forms of TibA do not confer these effects [52].

Exopolysaccharide

It includes PNAG (Poly-1,6-N-Acetyl-D-Glucosamine or PGA) and Colanic acid.

PNAG (Poly-1,6-N-Acetyl-D-Glucosamine or PGA)

In *E. coli*, attachment of cell to abiotic surfaces and cell-to-cell adhesion depends on various surface organelles and PGA [53]. The production of PGA is encoded by the *pga*ABCD locus, which is also present in a variety of other bacterial pathogens such as *Staphylococcus aureus* and *S. epidermis*, where PGA is a virulence factor regulated by CsrA [54]. A *csr*A mutant attaches to substrate permanently due to overproduction of PGA. PGA is required for transition of cell attachment from temporary to permanent (**Figure 4**).

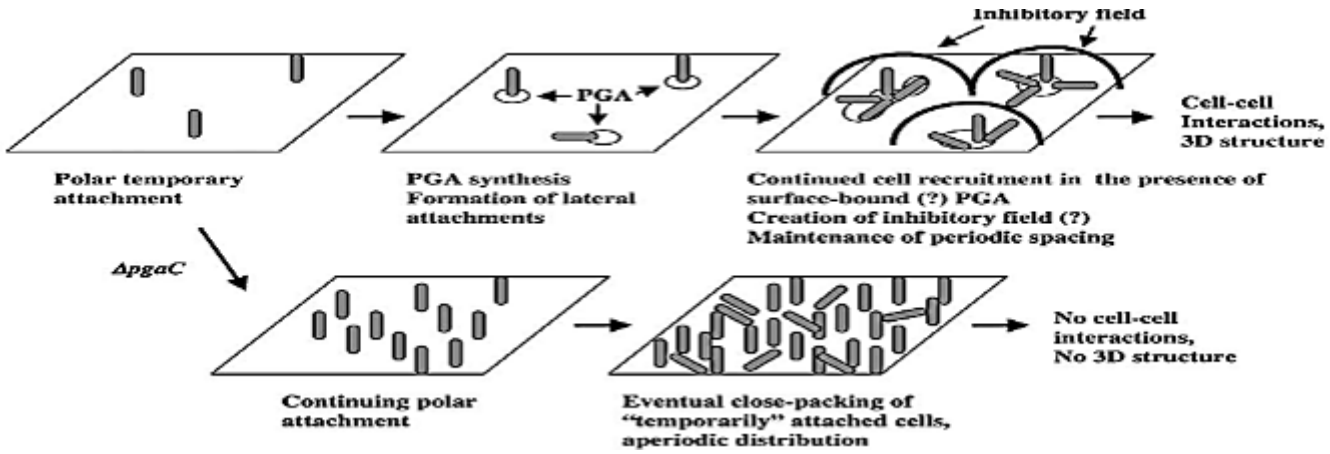

Figure 4 Production of PGA facilitates the biofilm formation [55]

β-1,6-N-acetyl-D-glucosamine (PGA) polysaccharide, a polymer involved in adhesion by staphylococci, was also involved in attachment to abiotic surfaces, intercellular adhesion, and biofilm formation in *E. coli* [56]. Furthermore, depolymerization of PGA led to dispersal of the biofilm. PGA dependent adhesion or biofilm formation may be an important virulence factor in diverse species.

Colanic acid

An extracellular polysaccharide produced by most of the *E. coli*. synthesis of colanic acid are grouped in the *wca* cluster, formerly called *cps* [57] and controlled by the two-component regulator of capsule synthesis Rcs system. But later it was realised that Rcs system is a multi-component regulatory system in which RcsC, RcsD and RcsB play key role in regulating *wca* gene cluster (**Figure 5**)

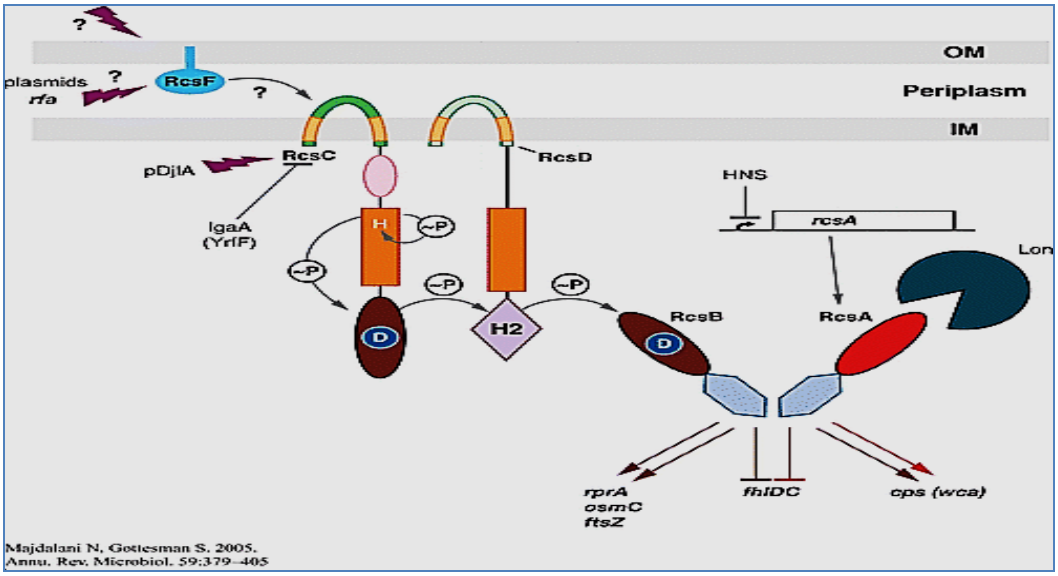

Figure 5 Regulatory system of *wca* gene [58]

Flagella

Flagellar mediated motility is important for establishing initial cell-to-surface contact. Gene *fliC* encodes flagellin, *motA* produces a proton conductor of motor energizing the flagellar rotation, *motB* encodes a part of basal body which anchors the peptidoglycan. Deletion of the chemotaxis genes *cheA* through *cheZ*, resulted in motile but non-chemotactic cells. The impact of these mutations was studied under stagnant culture conditions and it was determined that chemotaxis was dispensable and motility was critical for the initiation of *E. coli* biofilm formation [59]. Biofilm formation is regulated by two complex inversely affecting cascade namely FlhDC and σ^s which regulate various diguanylate cyclases and phosphodiesterases of cell (**Figure 6**). FlhDC mainly regulate the expression of flagellar gene [60].

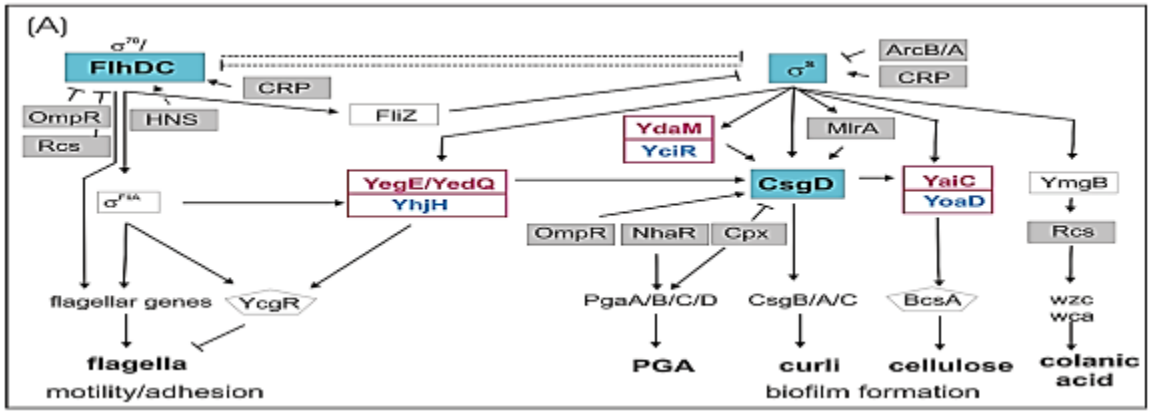

Figure 6 Two inversely affecting cascade involved in regulation of biofilm formation by *E. coli* [60]

Besides initial cell-to-surface contact, a possible role for flagella in the dispersal of the biofilm. The authors showed that the expression of *csr*A (encoding carbon storage regulator) fluctuates dynamically during biofilm formation, and that CsrA acts as repressor of biofilm formation and an activator of biofilm dispersal [61]. This effect of CsrA is primarily through its regulatory role in the metabolism of glycogen but could also possibly be related to a post-transcriptional regulation of *flhDC* gene expression by alteration of the decay rate of *flhDC* mRNA.

Quorum Sensing

Quorum sensing is the regulation of gene expression of bacterial community in response to cell population density. Quorum sensing allows control of behaviours on a community level. Bacterial cells detect the build-up of secreted autoinducers and respond with programmed changes in gene expression. Bacterial activities regulated by quorum sensing include biofilm development, virulence factor regulation, bioluminescence induction, antibiotic production,

sporulation, and competence initiation. The understanding that bacteria produce and respond to extracellular signals makes it possible to develop technologies aimed at obstructing bacterial communication systems.

Type of QS found in Gram negative

AI-1, AI-2 and AI-3 system but in *E. coli.*

- **AI-1 System**: Lux-I is absent in *E. coli*. SdiA can able to respond to AHLs secreted by other bacterial species [62]. Both deletion and overexpression of *sdiA* gene affects the curli production by transcriptional repression of curli operons [63]. It modulates the signalling system of c-di-GMP by controlling the expression of YhjH (c-di-GMP phosphodiesterase) and thus, affect the biofilm formation [64]. SdiA can bind to several molecules such as AI-1 1-octanoyl-rac-glycerol (OCL), a ubiquitous monoacylglycerol, present in both eukaryotes and prokaryotes [65], and the xylose in order to thus respond to different environmental and physiological cues. AI-1 system is rudimentary.
- **AI-2 System:** A universal bacterial signalling system which is able to mediate both inter- and intraspecies communication [66]. AI-2 is Furanosyl Borate Diester, the biological molecules having a boron atom as a constituent. It is synthesized by the LuxS protein starting from S-ribosylhomocysteine (SRH) as a non-toxic product of S-adenosylmethionine (SAM) metabolism into S-4, 5-Dihydroxy-2,3-Pentadione which later reorganised into AI-2 molecule [67]. Uptake of AI-2 occur through PTS and then phosphorylated by the AI-2 kinase (LsrK) present at basal levels, resulting into repression of LsrR on the *lsr* operon and initiating Lsr-dependent transport and depletion of AI-2 [68]. Genes responding to AI-2 are organized in two different operons, *lsr*ACDBFG, which synthesize proteins for uptake of AI-2- and two enzymes (LsrF and LsrG) involved in AI-2 degradation and recycling, and the regulatory *lsr*RK operon (**Figure 7**). When the AI-2 signalling system is activated, the signal molecule is promptly removed from the environment, preventing other bacteria to exploit the signal to regulate their behaviours [69]. As a consequence, and in contrast to other QS systems, AI-2 concentration in the culture medium of strains harbouring the *lsr* system peaks during late exponential phase and quickly declines during stationary phase [70]. In *E. coli*, AI-2 stimulates the expression of biofilm-related genes including flagella, cellular chemotaxis proteins, extracellular polysaccharides (PNAG adhesins and colanic acid), curli, type I fimbriae, antigen 43 and proteins involved in c-di-GMP turnover. Regardless of such variety of effects, biofilm stimulation by AI-2 chiefly depends on the regulation of flagellar activity with AI-2 directed chemotaxis to induce auto-aggregation [71].

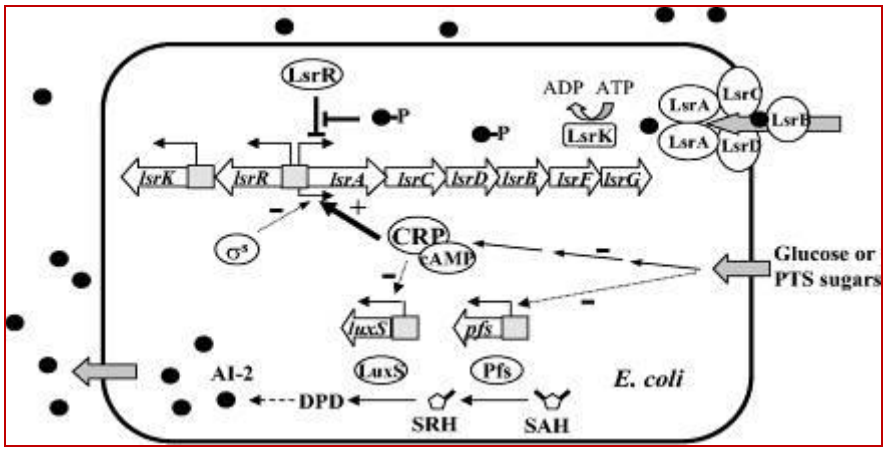

Figure 7 Mechanism of AI-2 system [72]

- **AI-3 system:** Both commensal and pathogenic strains of *E. coli* are able to synthesize a third quorum-sensing molecule independent of LuxS activity. This AI-3 is probably an aromatic amine, although its precise chemical structure has not been identified yet. AI-3 might resemble the mammalian hormones epinephrine (epi) and norepinephrine (NE), as these molecules can interact with some components of the AI-3 quorum-sensing system [73]. AI-3 signalling is produced by several pathogenic and commensal bacterial species that inhabit the human GI tract [74], thus suggesting that this molecule represents an important signal for bacteria in the gut. AI-3 was first described as a crucial player in the pathogenesis of EHEC O157:H7, where it controls the expression of different virulence loci [73]. QseBC forms a two component regulatory system where QseC acts as sensor kinase and QseB acts as response regulator (**Figure 8**). QseB controls biofilm formation mainly through modulation of the *FlhDC* regulon [75]. However, in pathogenic *E. coli* strains,

QseB can repress the pro- duction of different structures involved in cell adhesion, including EspA filaments, type 1 fimbriae and curli, thus impacting the ability of the bacterial cell to colonize gut epithelia [76].

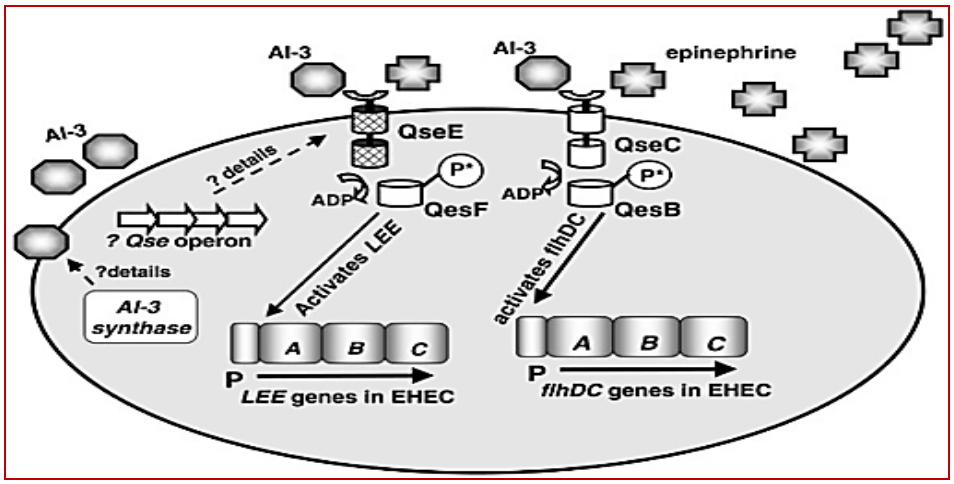

Figure 8 Mechenism of AI-3 system [77]

Second Messenger Signaling

The main components of second messanger signalling are ppGpp (guanosine tetraphosphate), cAMP (cyclic AMP) and cyclic-di-GMP [78]. c-di-GMP binding sites in receptors are diverse that bind different conformations of the nucleotide or even dimeric c-di-GMP aggregates [79]. This diversity of binding sites results in the development of class-specific c-di-GMP pathway. Out of these, cyclic di-GMP plays a major role in biofilm formation (**Figure 9**). Alteration in the level of ppGpp above a certain threshold leads to deviation of resources from active growth to promote rapid adaptation to stress and survival under harsh conditions [80]. ppGpp positively affect the biofilm formation by promoting the synthesis of type I fimbriae and inhibiting the activity of CsrA. cAMP signalling has a complex role in the biology of this major pathogen [81]. cAMP inhibit the activity of CsgD, a regulator of cellulose and curli synthesis which is a key factor in biofilm formation.

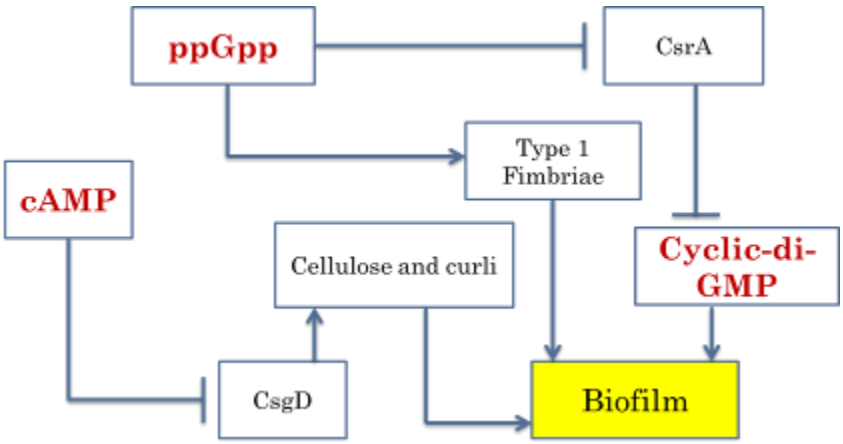

Figure 9 Secondary messenger signalling found in E. coli

Conclusion and Future Aspects

Biofilm formation provides resistance against variety of environmental stresses and immune system. The level of diversity of biofilm formation by *E. coli* reflects their adaptation in different niche and micro-environment. Different strains of *E. coli* have several quorum sensing system which regulate the bacterial metabolism and biofilm formation Numerous adhesive structures are still missing which are necessary for detailed study of complexity of biofilm and regulatory mechanisms. Large number of environmental signals that can affect adhesion production and formation of alternative biofilms are not yet investigated.Integrated analysis of *E. coli* in GI tract along with the microbiome can provide correlation between metabolic signals, gene expression and biofilm formation.Study of various ECM activation pathways of *E. coli* can lead to powerful therapeutic molecular targets for ECM related *E. coli* infection.

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