"It's a gut feeling" - Escherichia coli biofilm formation in the gastrointestinal tract environment

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Not all is shit - *Escherichia coli* biofilm formation with a focus on the gastrointestinal tract

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Abstract *Escherichia coli* is one of the most frequent commensals and pathogens of the human gastrointestinal tract. In this review, we discuss biofilm formation of commensal, probiotic and pathogenic strains of *E. coli.* Biofilm formation of *E. coli* is highly variable, although distinct regulatory pattern of *rdar* biofilm formation arise in certain pathovars. Interestingly, *E. coli* Nissle 1917 has its regulatory *rdar* biofilm expression pattern conserved for 100 years. Various environmental signals relevant for the gastrointestinal tract affect biofilm formation. Biofilm formation in the gastrointestinal tract has mainly been characterized in interaction with epithelial cells. Contribution of the vast number of core genome and pathovar specific proteinaceous surface appendages to biofilm formation has hardly been investigated.

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1 Introduction

The conventional view is that the natural habitat of the versatile species *Escherichia coli* is the intestine of humans and warm and cold-blooded animals, namely mammals, birds and reptiles. *E. coli* is one of the earliest colonizers of the gastrointestinal tract; although eventually, it is a minor component of the colonic gut microbiome in humans, where it represents less than 0.1% of the total bacterial cells (Eckburg et al., 2005). Nevertheless, due to the overall high cell density in the colon, this small percentage translates into a number around 10^8 cells/ml. Although generally defined as a commensal, *Escherichia coli* actually contributes to human physiology though the digestion of food and production of vitamin B and K with some strains even having probiotic features. On the other hand, *E. coli* is also one of the most prevalent human pathogens, causing various intestinal and extra-intestinal diseases, including being suspected to be the cause of sudden infant death syndrome (Bettelheim and Goldwater, 2015; Blount, 2015; Nataro and Kaper, 1998). Not surprisingly, due to its impact on human health, *E. coli* is one of the most well-investigated bacterial species, but nevertheless little is known about *E. coli* as there is a tremendous diversity within the species (Blount, 2015). Besides in its primary habitat, *E. coli* is also found in aquatic and terrestrial habitats as well as associated with plants (Meric et al., 2013). As a particular *E. coli* strain transmits through the gut in a time period varying from weeks to years, environmental and host associated strain populations are most probably tightly interconnected and shape each other.

The natural life style of microorganisms is considered to be biofilm formation, a tissue-like multicellular assembly with or without being associated with biotic or abiotic surfaces. The developmental process of biofilm formation conventionally initiates with the slow-down of the movement of a single motile cell in the liquid phase and restarts with the active or passive dispersion of cells from the mature three-dimensional biofilm with complex architecture. Adhesion factors and a variety of extracellular matrix components shape the development from the single attached cell over microcolony formation towards three-dimensional biofilms (Hobley et al., 2015). The predominance of this multicellular life style in nature has been attributed to survival advantages of multicellular assemblies compared to single cells such as tolerance to various forms of

stress including desiccation and tolerance against antimicrobial substances and the immune response of higher organisms.

E. coli is also capable to form biofilms. However, although biofilm formation is a well-defined process, the > 20% genome variability and the diversity of environmental niches suggest a hardly explored variability in biofilm formation within the different *E. coli* species. This review focuses mainly on biofilm formation of commensal, probiotic and pathogenic gastrointestinal *E. coli*. In the benign status, the microbiota, including *E. coli*, resides in the outer loose mucin layer, separated from the epithelial mono-cell barrier by an inner dense mucin barrier (Johansson et al., 2013). Upon infection and a disturbed immune status, pathogens penetrate and interact with the epithelial lining and beyond. Thereby, in some pathovars biofilm formation is integrally included in the virulence phenotype, while other pathovars only transiently form biofilms. Biofilm formation is thus tightly regulated against motility and virulence in response to gastrointestinal intrinsic signals.

2 Biofilm formation

2.1 Biofilm *in vitro* models

Model systems are needed to make biofilm formation molecularly investigable. The classical model species *Pseudomonas aeruginosa* has imprinted the view on mature biofilms as mushroom-like structures under the conditions of continuous flow (Klausen et al., 2003). A broad number of model systems exist to analyze biofilm formation of *E. coli*. A wide-spread popular system is the 'so-called' rdar (red, dry and rough) colony morphology, a distinct colony morphology type. This phenotype is indicative for the expression of biofilm extracellular matrix components curli fimbriae and cellulose concomitantly regulated by the major biofilm regulator CsgD (Fig. 1; (Hufnagel et al., 2015; Römling, 2005; Römling et al., 1998)). In a surprisingly high number of situations, structural and regulatory components of the 'rdar' biofilm contribute to biofilm formation, for example, in the colonization of plants and fruits, but also of tumor tissue (Pawar et al., 2014; Yaron and Römling, 2014). As 'rdar' is based on the binding of the azo-dye Congo Red semi-specific for amyloids and 1,4- and 1,3- beta glucans, colony morphology biofilms producing the exopolysaccharide poly-β-1,6-N-acetylglucosamine,

for example, can also be assessed. The rdar biofilm is the most intensively investigated biofilm with respect to structural components and regulation (see below 'Signals leading to biofilm formation'). At the center of biofilm regulatory network, lies the CsgD protein, transcribed from the *csgDEFG* locus characterized by an eukaryotic-like promoter that includes a 117 bp long leader sequence with regulatory sequences far up- and downstream of the transcriptional start site (Holmqvist et al., 2013). Complex transcriptional to post-translational regulatory mechanisms grossly affect and fine-tune bistable CsgD expression (Grantcharova, 2010; Holmqvist et al., 2010; Mika and Hengge, 2013; Simm et al., 2014). Of note, the green tea polyphenol epigallocatachin gallate (EGCG) downregulats *csgD* expression via the sRNA RybB and interferes with curli assembly (Serra et al., 2016).

Another frequently applied model system is biofilm formation on an abiotic surface, such as glass or plastic walls of an incubation tube under static or shaken culture conditions. A 'rdar' biofilm (Fig. 1), but also biofilms with alternative extracellular matrix components and regulation have been identified in this conditions. Molecular analysis of biofilm formation of *E. coli* under continuous flow is scarce, thus the components that built up the biofilm under constant nutrient flow require to be further investigated (Reisner et al., 2006).

Although residing in the mucus in distance from the intestinal cell layer, *E. coli* often forms biofilms on the epithelial lining under pathophysiological conditions. Therfore, factors that affect biofilm formation can be conveniently investigated in cell culture models (Fig. 1). Intracellular biofilms, so called intracellular pods, as formed by uropathogenic *E. coli*, are not the subject of this review (Anderson et al., 2003).

2.2 Biofilm formation in the environment

Outside the human body, *E. coli* is thriving in oligotrophic water and soil (Gordon and Cowling, 2003; van Elsas et al., 2011), an inherent feature of the *E. coli* species (Na et al., 2006). These observations are consistent with early studies that suggest low temperature, stress and nutrient deprivation to favour expression of biofilm genes (White-Ziegler et al., 2008). In these environmental habitats, cross-kingdom biofilms are rather the rule than the exception and consist of bacteria, fungi, algae and/or protozoa

(Costerton et al., 1999; Watnick and Kolter, 2000). Biofilms containing *E. coli* have been detected in fresh water streams and drinking water sources (Ahmed et al., 2013; Moreira et al., 2012). Thus, *E. coli* might integrate into preformed interkingdom biofilms, which serves as a vehicle for pathogen transmission (Alam et al., 2007). In any case, the presence of biofilms in water has been linked to epidemic peaks of diarrhea (Ahmed et al., 2013). Also, food borne transmission of commensal and pathovar *E. coli* has its basis in the attachment and biofilm formation on fresh produce such as lettuce, tomatoes and rocket (Jeter and Matthysse, 2005; Richter et al., 2014; Yaron and Römling, 2014).

3 Pathovar specific biofilm formation

Biofilm formation in *Escherichia coli* is highly variable, not surprising probably, considering the high genome variability and the various ecological niches *E. coli* is thriving in. Initial multi locus enzyme electrophoresis, MLEE, analysis (Selander and Levin, 1980) and subsequent multi locus sequence typing MLST (Maiden et al., 2013) indicated that many *E. coli* pathovars arose independently more than once throughout the phylogenetic tree with ETEC arousal most readily observed (Chaudhuri and Henderson, 2012). A simple, but surprisingly discriminative triplex PCR approach divided *E. coli* into five major phylogroups A, B1, B2, D, E, and less frequent C and F groups, still widely used until today (Clermont et al., 2013; Tenaillon et al., 2010). Subsequently, though, this classification is replaced by a next generation sequencing (NGS) based classification scheme with currently more than 2500 *E. coli* genomes available, with phylogrouping being congruent with genome comparison (Chaudhuri and Henderson, 2012). Currently the *E. coli* pangenome is estimated to be more than 16.000 genes, while the shared core genome is, with less than 1.500 genes, remarkably small (Rouli et al., 2015; Touchon et al., 2009). A significant portion of the accessory genome codes for virulence factors and metabolic genes and shapes the adaptation to various niches including the human gastrointestinal tract (Leimbach et al., 2013). Thus, it is not unexpected that genome variability grossly affects biofilm formation. On the other hand, even single nucleotide polymorphisms have been demonstrated to alter biofilm physiology dramatically (Römling et al., 1998).

3.1 Biofilm formation by commensal *E. coli*

Commensal *E. coli* isolates show the largest possible range of biofilm regulatory patterns with respect to 'rdar' biofilm formation on agar plates (Bokranz et al., 2005) ranging from a no rdar morphotype to a semi-constitutive rdar morphotype expressed at both 28°C and 37°C. In addition, expression variants include curli fimbriae or cellulose only (Fig. 1). The full biological impact of rdar morphotype variability is mostly unknown, although absence of rdar morphotype expression is associated with the invasive phenotype of *Shigella* and enteroinvasive *E. coli*. In general, bacterial cells expressing individual extracellular matrix components curli and cellulose, interact in a distinct and even opposite way with intestinal epithelial cells with respect to adhesion, invasion as well as induction of proinflammatory cytokine production (Monteiro et al., 2009; Saldaña et al., 2009; Wang et al., 2006). As a general pattern curli fimbriae stimulate these phenotypes, which are inhibited upon co-expression of cellulose. In the colon, *E. coli* resides in the outer mucin layer, separated from the epithelial mono-cell barrier by the tighter inner mucin layer (Johansson et al., 2013). Indeed, the sheer presence of mucin stimulates biofilm formation (Bollinger et al., 2006). Commensal *E. coli* growing in the mucus cannot use this nutrient source, but utilize mono-, disaccharides and other simple molecules derived from complex glycoprotein degradation by tightly interacting obligate anaerobes (Conway and Cohen, 2015; Ng et al., 2013). How nutrient provision in the gut affects biofilm formation is largely unknown, but, for example, monosaccharide availability and the PTS system have been shown to affect biofilm formation in various bacteria (Houot et al., 2010; Sutrina et al., 2007). In addition, secreted immunoglobulin A favors biofilm development of *E. coli* in the intestinal mucosa in a type 1 fimbriae-dependent manner (Bollinger et al., 2006). In total, the commensal model organism *Escherichia coli* K-12 has seven additional chaperone usher pathway fimbriae in addition to type 1 fimbriae (Korea et al., 2010), which have the potential to mediate biofilm formation.

3.2 Pathogenic *E. coli* have acquired different abilities to form biofilms

E. coli has evolved and adapted to a pathogenic lifestyle through the acquisition of virulence genes that confer advantages to colonize the host environment (Kaper et al.,

2004; Leimbach et al., 2013). Phenotypically pathogenic *E. coli* are divided into two groups, intestinal *E. coli* (InPEC) and extraintestinal *E. coli* (ExPEC). The differences in the pathogenic potential is reflected in the phylogeny as *E. coli* of phylogroup A, B1, C and E are commonly associated with intestinal disease, while *E. coli* of phylogroups B2, D and F are predominantly associated with extraintestinal diseases (Fig. 3), indicating that a specific genetic background (*i.e* phylogroup) provides pathogenic advances in distinct host environments. However, several exemptions from this general association of phylogroups to pathotypes exist indicating parallel evolution of *E. coli* pathotypes on multiple occasions and ready arise of certain pathovars in the *E. coli* population. (Bielecki et al., 2014; Chaudhuri and Henderson, 2012; Hazen et al., 2013; Reid et al., 2000; von Mentzer et al., 2014).

The InPEC group includes pathogens that infect and colonize the gastrointestinal tract and typically cause secretory or bloody diarrhea. The InPEC includes enterotoxigenic *E. coli* (ETEC), enteropathogenic *E. coli* (EPEC), enteroaggregative *E. coli* (EAEC), Shiga toxin (STEC) producing *E. coli* that includes enterohemorrhagic (EHEC), enteroinvasive *E. coli* (EIEC) and *Shigella* spp., adherent-invasive *E. coli* (AIEC) and diffusely adherent *E. coli* (DAEC) (Kaper et al., 2004; Leimbach et al., 2013). While some pathovars affect the small intestine, where the mucus in the small bowel is unattached to the epithelium, others cause infection in the large intestine (Fig. 2 and 3; (Johansson et al., 2013)). Extraintestinal *E. coli* (ExPEC) includes uropathogenic *E. coli* (UPEC) and newborn meningitis *E. coli* (NMEC) and mainly belongs to the B2 and D phylogroups. The molecular mechanisms behind the pathogenicity of the *E. coli* pathovars have been reviewed extensively (Croxen and Finlay, 2010; Croxen et al., 2013; Kaper et al., 2004; Nataro and Kaper, 1998).

The diversity of acquired pathogenicity islands/islet/genes in the pathovars including virulence factors and the unique chromosomal background of different *E. coli* pathotypes suggest that *E. coli* pathovars have unique regulatory and structural capacities to form biofilms. For examples, pathogenic *E. coli* express a wide array of pathovar specific fimbriae. On the other hand, though, there are biofilm components on the core genome of *E. coli* belonging to the 1500 common genes. Well-investigated amyloid curli fimbriae are involved in late stage biofilm formation (Farfan and Torres, 2012; Monteiro et al.,

2012). Curli expression, which, according to current knowledge is synonymous with expression of the major biofilm regulator CsgD, varies widely in expression pattern and is strain and/or pathovar dependent. For instance, sepsis and UTI (urinary tract infection) isolates frequently express curli fimbriae also at 37 °C (Bian et al., 2000), while in EPEC, ETEC and ETEC curli are often only expressed at ambient temperature (Szabó et al., 2005; Uhlich et al., 2001). In EIEC and *Shigella* spp., curli and *csgD* expression is often inactivated (Sakellaris et al., 2000).

Also type 1 fimbriae are expressed by the majority of *E. coli*, but their role in biofilm formation can differ between commensal and pathogenic *E. coli*. For instance, type 1 fimbriae are important for biofilm in the laboratory K-12 strain, but not in EHEC O157:H7 (Beloin et al., 2004; Roe et al., 2001). Interestingly, different *fimH* alleles confer different tissue tropism with respect to adhesion to epithelial cells via binding to mono-mannose receptors, affecting the ability of the bacteria to attach and invade epithelial cells (Khan et al., 2007; Martinez et al., 2000; Weissman et al., 2006). Similarly, during biofilm formation, different *fimH* alleles can result in different functionality and role of type 1 fimbriae. Finally, type 1 fimbriae exhibit a unique catch bond adhesion mechanism, which provides stronger attachment to the surface when tensile force is applied (Aprikian et al., 2011), which might be the initiation phase of biofilm formation.

Autotransporter adhesins are members of the type V secretion system and associated with autoaggregation and biofilms. Antigen 43 (Ag43) promotes cell-to-cell adhesion and aggregation at the initial stages of biofilm formation. Ag43 is present at higher frequency in non-O157 EHEC strains compared to O157:H7 (Verstraete et al., 2013).

Cellulose, a polysaccharidic component of the biofilm extracellular matrix, is frequently co-expressed with curli fibres, but can also be regulated independently (Beloin et al., 2008; Bokranz et al., 2005; Monteiro et al., 2009). Colanic acid is another core genome encoded exopolysaccharide. Although reported to promote biofilm formation, its contribution to this process, though, is not well defined (Matthysse et al., 2008; May and Okabe, 2008). The common exopolysaccharide poly-*N*-acetylglucosamine (PNAG) is expressed in *E. coli* K-12 upon deletion of the global carbon storage regulator CsrA, which represses translation of the *pga* operon. Although PNAG is, as cellulose, an

exopolysaccharide commonly produced in biofilms from diverse bacterial species, the *pga* operon is not present in all *E. coli* strains (Cerca et al., 2007).

Below we discuss the pathovar specific biofilm components. Besides investigated for biofilm formation *in vitro*, frequently, those surface components have been characterized in interaction with epithelial cells and gut colonization.

3.3 *ETEC specific biofilm mediators*

ETEC infections is a major cause of dehydrating diarrheal disease primarily affecting children less than five years of age and travellers to low- and middle-income countries with expression of the heat labile toxin (LT) and the heat stabile toxin (ST) as hallmark (Qadri et al., 2005). In addition, ETEC harbours distinct surface proteins, more than 27 identified and proposed colonization factors (CFs) and adhesins, which promote colonization of the small intestinal epithelium (Gaastra and Svennerholm, 1996). Biofilm production and adherence are associated with CFs and multiple CF genes located on the plasmid as well as the chromosome has been implicated to participate in biofilm development. For example, ETEC strains expressing the colonization factors CFA/I, CS1, CS2 or CS3 were reported to produce higher levels of biofilm than non-piliated ETEC strains (Liaqat and Sakellaris, 2012).

The toxigenic invasion loci A (Tia) and toxigenic invasion loci B (*tib*AB) (Elsinghorst and Weitz, 1994; Fleckenstein et al., 1996) are involved in adhesion and biofilm formation (Sherlock et al., 2005). The expression of TibA and Tia adhesins by 8% and 10% of ETEC strains is associated with certain colonization factor profiles (Sjöling et al., 2015). The presence of type IV pilus CS2, longus (Giron et al., 1994), is predominantly associated with expressing of CS1+CS3, CS2+CS3 and CFA/I colonization factors (von Mentzer et al., 2014) and associated with biofilm formation (Cruz-Cordova et al., 2014). ETEC isolates might vary considerably in their ability to form biofilms in the host and in the environment with consequences for dissemination and virulence properties. However, a systematic analysis of the biofilm formation capacity of ETEC strains has not been performed.

3.4 Biofilm formation by enterohemorrhagic *E. coli*

Enterohemorrhagic *E. coli* (EHEC) belong to the Shiga toxin-producing *E. coli* (STEC) where biofilm formation has been recently reviewed (Vogeleer et al., 2014). EHEC colonize both the distal ileum as well as of the colon. EHEC attaches to the epithelial cells via the use of pili, *E. coli* common pilus ECP and hemorrhagic pilus, HCP (Xicohtencatl-Cortes et al., 2009), as well as mucin binding flagella (Nagy et al., 2015; Xicohtencatl-Cortes et al., 2009). Initial attachment to and biofilm formation on spinach leaves and stainless steel surfaces is mediated by curli fimbriae in EHEC outbreak strains (Carter et al., 2016). Further, a multitude of adhesins are expressed in STEC (Klapproth et al., 2000; Nicholls et al., 2000), among them EspA, promoting attachment (Ebel et al., 1998; Sharma et al., 2016). Somewhat surprising, enterohemorrhagic *E. coli*, EHEC, strains show a characteristic pathovar dependent loss of rdar biofilm formation. Indeed, many strains have a disrupted or downregulated *mlrA* gene, which is required to transcriptionally elevate expression of the determinative biofilm regulator *csgD* (see below). Impaired *mlrA* activity is due to a *stx1* bearing phage insertion and/or abolished or attenuated RpoS activity. The latter finding is surprising as the stationary phase sigma factor RpoS is considered a major stress resistance determinant. Consequently, *E. coli* serotype O157:H7 strains usually do not display the rdar morphotype (Uhlich et al., 2013). Upon excision of the *stx1* prophage,though, rdar biofilm formation is restored (Uhlich et al., 2016).

3.5 *EPEC biofilm formation*

Enteropathogenic *E. coli* (EPEC) belong to the STEC type together with EHEC divided into typical EPEC expressing the bundle-forming pili (Bfp) and atypical EPEC (aEPEC) that do not express Bfp. Typical EPEC forms microcolonies, also known as localized adherence (LA), on epithelial cells in culture in a Bfp-dependent way (Bieber et al. 1998), while aEPEC show a looser LA-like adherence. Biofilm formation in EPEC is variable and depends on type 1 fimbriae, Ag43, curli and cyclic di-GMP (Moreira et al., 2006; Weiss-Muzkat et al., 2010; Nascimento et al., 2014). In addition, a secreted lipoprotein SslE mediates biofilm formation in EPEC (Baldi et al., 2012), encoded also by several other *E. coli* pathotypes and commensal *E. coli* (Moriel et al., 2012; Decanio et al., 2012; Luo et al., 2014; Sjöling et al., 2015).

Albeit generally low, adherence and pellicle formation in standing culture was more prevalent in aEPEC strains from patients compared to asymptomatic controls (Nascimento et al., 2014). The biofilm behaviour including rdar of aEPEC strains is generally mostly observed at 26°C (Weiss-Muzkat et al., 2010; Nascimento et al., 2014).

3.6 *DAEC*

DAEC are characterised by a diffuse pattern of adherence with little aggregation on epithelial cells (Fig. 2). The fimbrial adhesin F1845 (Bilge et al., 1989), Dr fimbrial, and afimbrial adhesins (Afa) bind to the brush-border associated receptors leading to brush border lesions and high adherence. The characteristics of DAEC have been extensively reviewed (Le Bouguenec and Servin, 2006; Servin, 2005, 2014).

3.7 *EAEC*

Enteroaggregative *E. coli* (EAEC) colonize the small and large intestine (Hebbelstrup Jensen et al., 2014), but can also cause extraintestinal infection. The single-layer aggregative adherence pattern on epithelial cells of the gut characteristic for this pathotype is referred to as "stacked-brick" configuration (Nataro et al., 1987). Major interbacterial and host cell adhesion components are pathovar specific aggregative adherence fimbriae (AAF), which belong to the Dr adhesins family (Czeczulin et al., 1997; Nataro et al., 1992) and afimbrial adhesins (reviewed in (Estrada-Garcia and Navarro-Garcia, 2012)), type 1 fimbriae and other components such as the secreted small 10-kDa protein dispersin. This small protein was shown to lead to dispersion of the cells along the intestinal epithelium (Sheikh et al., 2002) by shielding the negative charge of the bacterial outer membrane, allowing extension of the positively charged AAFs from the surface (Velarde et al., 2007). Biofilm formation has been suggested to be a major transmission and virulence factor as out-break strains on several occasions have been found to display enhanced biofilm formation including the EAEC/EHEC O104:H4 hybrid strain related to the recent major outbreak (Hebbelstrup Jensen et al., 2014; Karch et al., 2012; Kong et al., 2015).

3.8 *Crohn's disease associated* E. coli *biofilm formation*

In healthy human beings, mucus layers separate the epithelial lining from the gut microbiome. In Crohn's disease (CD), adherent-invasive *E. coli* (AIEC) with specific features are found in close association with epithelial cells (Darfeuille-Michaud et al., 2004). AIEC attachment is facilitated by type 1 fimbriae binding to specific host receptors upregulated in patients with CD (Barnich et al., 2007; Boudeau et al., 2001; Carvalho et al., 2009) and supported by flagella (Barnich et al., 2003). Further, adhesion is mediated by interaction of the bacterial chitinase ChiA with the host chitinase 3-like-1 (CHI3L1) via chitin binding domains (Low et al., 2013; Mizoguchi, 2006). Flagella and type 1 fimbriae are co-regulated in the Crohn's disease isolate *E. coli* strain LF82 with type 1 fimbriae production partially activated through downregulation of the second messenger cyclic di-GMP (Claret et al., 2007). The extracellular matrix component cellulose triggers induction of colitis in IL-10 deleted mice, which are prone to inflammation (Ellermann et al., 2015).

3.9 *Biofilm formation of Nissle 1917*

Escherichia coli Nissle 1917 is one of several established probiotic *E. coli* strains, isolated in 1917 by the German physician Nissle. *E. coli* Nissle 1917 expresses a particular rdar biofilm feature that distinguishes it from other *E. coli* strains with CsgD and di-guanylate cyclase YedQ independent cellulose expression uncoupled from ambient temperature expressed curli (Monteiro et al., 2009). This regulatory pattern is surprisingly stable as *E. coli* Nissle 1917 clonal strains isolated almost 100 years later from pigs show similar regulation (Kleta et al., 2006; Monteiro et al., 2009). The ecological impact of this regulation is not clear though. A screen of different fimbriae identified F1C fimbriae as being required for biofilm formation at 37°C on abiotic surfaces and intestinal colonization (Lasaro et al., 2009). Outcompetition of pathogenic *E. coli* strains and other species during biofilm formation might contribute to the probiotic features of Nissle 1917 (Hancock et al., 2010; Jiang et al., 2014; Lasaro et al., 2009; Rund et al., 2013).

4 Signals leading to biofilm formation

4.1 *Physiological and environmental signals linked to biofilm found in the*

gastrointestinal (GI) tract

Biofilm formation represents a very energy-costly process for bacterial cells, due to the production of the extrapolymeric substance and adhesion factors as described above; on the other hand, biofilms show increased resistance to environmental stresses and to the host immune system, as well as tolerance to antibiotics. In order to balance the pros and cons of the biofilm mode and transition to the planktonic life style, bacterial cells integrate diverse intra- and extracellular signals with a previously unprecedentedly observed complexity. Signals directly sensed are linked to the bacterial population density and composition, the presence of specific nutrients, light etc.

In the GI tract, commensal and pathogenic *E. coli* face a wide range of environmental signals (*e.g.,* temperature, oxygen, nutrients), as well as signals from the host (*e.g.*; bile salts, taurine, neuroendocrine hormones like norepinephrine and dopamine) and from other commensal bacteria (*e.g.,* quorum sensing (QS) signals, indole, polyamines). In this section, we will focus on a selection of environmental, host- and bacteria-related signals that *E. coli* can perceive in the GI tract and will describe how they regulate biofilm determinants. Some of these physiological and environmental signals are linked to second messenger signaling by modified nucleotides, such as cyclic-di-GMP (c-di-GMP), possibly the main effector for biofilm formation and maintenance in *E. coli*. The reader can find detailed descriptions of the mechanisms of c-di-GMP signaling in a number of extensive review articles recently published (Hengge, 2009; Jenal and Malone, 2006; Römling et al., 2013; Sondermann et al., 2012).

Quorum sensing

Quorum sensing molecules, also known as autoinducers (AI), are one of the most studied signals affecting biofilm formation in a broad range of bacteria. So far, several AI have been characterized that cluster in three classes: AI-1, AI-2, AI-3. Each signaling system relies on a specific autoinducer synthase and a cognate receptor. AI-1 molecules are synthesized by so called LuxI-type proteins and belong to the molecular class of acyl homoserine lactones (AHLs) showing a conserved homoserine lactone ring connected through an amide bond to a variable acyl chain (reviewed in (Reading et al.,

2009)). The acyl chains vary considerably in length (from 4 to 18 carbon atoms) and in chemical modifications: for instance, the third carbon of the chain is often derivatised to a carbonyl group, a hydroxyl group or a methylene moiety (Marketon et al., 2002; Whitehead et al., 2001). Such chemical variety confers these molecules properties of either intraspecies or interspecies signals: indeed, some AHLs are species-specific, while others are able to induce responses in a broader range of bacteria, mostly Gram-negative. The ability of AHL to act as signal molecules is mediated by their recognition by specific receptor proteins belonging to the LuxR family.

Unlike most Gram-negative bacteria, *E. coli* genome seems to lack LuxI synthases, and it does not produce AI-1. However, it possesses a LuxR homolog, SdiA (for Suppressor of cell Division Inhibitor (Wang et al., 1991)). SdiA can respond to AHLs secreted by other bacterial species (Dyszel et al., 2010a; Hughes et al., 2010; Lee et al., 2007; Smith et al., 2008; Soares and Ahmer, 2011) and even to synthetic AHLs (C6-HSL and oxo-C6-HSL) (Van Houdt et al., 2006). Thus, it appears that *E. coli* can sense AI-1 produced by other bacteria, probably as a mechanism to monitor their presence, but does not produce any own AHL. The precise role of SdiA, and how this regulator relays sensing of AI-1 from other bacterial species to gene expression, still remain elusive, and its involvement in *E. coli* biofilm formation is controversial. For instance, both deletion and overexpression of the *sdiA* gene result in inhibition of curli fibers production via transcriptional repression of the curli operons (Lee et al., 2009; Lee et al., 2008). The SdiA proteins can bind several ligands in addition to AI-1, including 1-octanoyl-*rac*-glycerol (OCL) (Nguyen et al., 2015), an ubiquitous monoacylglycerol, present in both eukaryotes and prokaryotes (Alvarez and Steinbüchel, 2002), and the sugar xylose (Yao et al., 2007; Yao et al., 2006), and can thus respond to different environmental and physiological cues. Likewise, the transcription of the *sdiA* gene is under the control of up to 15 regulators, again suggesting a very finely tuned control of intracellular SdiA concentrations in response to environmental conditions. Thus, it is likely that SdiA can integrate multiple signals, possibly having even opposite effects on gene expression depending on the conditions encountered by the bacterium. The most direct effect of SdiA on the transition between sessile and planktonic lifestyles is the repression of cellular motility, reported by several groups (Dyszel et al., 2010b;

Kanamaru et al., 2000; Lee et al., 2007; Lee et al., 2008), possibly via modulation of c-di-GMP signaling. Indeed, inactivation of SdiA affects the expression of YhjH, a c-di-GMP phosphodiesterase involved in regulation of curli fibres and flagella (Lindenberg et al., 2013; Pesavento et al., 2008), thus suggesting an effect of SdiA on the pivotal second messenger c-di-GMP.

Autoinducer-2 (AI-2) is widely conserved in both Gram-negative and Gram-positive bacteria and has therefore been proposed as a "universal" bacterial signal able to mediate both inter- and intraspecies communication (Surette et al., 1999). AI-2 is a furanosyl borate diester and one of the few biological molecules known to have a boron atom as a constituent (reviewed in (Pereira et al., 2012b)); it is synthesized by the LuxS protein starting from S-ribosylhomocysteine (SRH) as a non-toxic product of S-adenosylmethionine (SAM) metabolism (Pereira et al., 2012b; Schauder et al., 2001; Xavier and Bassler, 2003). In contrast to AI-1, *E. coli* possesses all the required machinery for synthesis and detection of AI-2. AI-2 synthesized by non-pathogenic *E. coli* strains seems to be perceived by colon cells inducing an inflammatory response that is quickly dampened after few hours, thus suggesting that AI-2 can also serve as an interkingdom signal (Zargar et al., 2015). Three different classes of receptors for AI-2 have been described, which belong to the LuxP, LsrB or RbsB families (Armbruster et al., 2011; Chen et al., 2002; Miller et al., 2004). AI-2 is synthesized by LuxS, and actively secreted into the extracellular environment throughout cell growth. AI-2 is not able to passively diffuse through the membrane (Zhu and Pei, 2008); initial uptake of a reduced amount of AI-2 takes place through the phosphoenolpyruvate phosphotransferase system (PTS) probably without substrate phosphorylation. The small pool of intracellular AI-2 obtained through uptake by PTS is then phosphorylated (P-AI-2) by basal level of the LsrK protein (AI-2 kinase), relieving LsrR repression on *lsr* operon and initiating Lsr-dependent, transport and depletion of AI-2 (Pereira et al., 2012a). Altogether, the genes responding to AI-2 are organized in two divergent operons, *lsrACDBFG*, which comprises genes encoding an AI-2-specific uptake system and two enzymes (LsrF and LsrG) involved in AI-2 degradation and recycling, and the regulatory *lsrRK* operon. The LsrK protein phosphorylates AI-2, which, in this form,

binds the LsrR protein relieving its repression of the *lsrACDBFG* operon. Thus, when the AI-2 signaling system is activated, the signal molecule is promptly removed from the environment, preventing other bacteria to exploit the signal to regulate their behaviors (Roy et al., 2010; Xavier and Bassler, 2005a). As a consequence, and in contrast to other QS systems, AI-2 concentration in the culture medium of strains harboring the *lsr* system peaks during late exponential phase and quickly declines during stationary phase (Xavier and Bassler, 2005b).

By way of its ability to sequester and degrade AI-2 present in the environment, *E. coli* probably interferes with AI-2 communication of other bacterial species. This strategy can represent a huge advantage in complex niches: indeed, AI-2 quenching seems to be a conserved strategy evolved in enteric bacteria in order to control colonization of GI tract by other bacteria, including pathogenic species (Hsiao et al., 2014; Xavier and Bassler, 2005a). For example, by quickly consuming AI-2, *E. coli* can potentially modulate virulence factor production, motility, cellular adhesion dynamically modifying persistence, pathogenicity and dissemination of bacteria such as *Vibrio cholerae* and *Salmonella* Typhimurium (reviewed in (Pereira et al., 2012b)).

In *E. coli*, AI-2 stimulates the expression of a large set of biofilm-related genes (González Barrios et al., 2006; Herzberg et al., 2006; Li et al., 2007; Ren et al., 2004; Sperandio et al., 2001), including cellular chemotaxis proteins, flagella (Domka et al., 2006), extracellular polysaccharides (colanic acid (Prigent-Combaret et al., 2000), poly-*N*-acetylglucosamine (Wang et al., 2004)), adhesins (type I fimbriae (Pratt and Kolter, 1998), antigen 43 (Klemm et al., 2004), curli fibers (Prigent-Combaret et al., 2000)), and proteins involved in c-di-GMP processing (YhjH (Lindenberg et al., 2013; Pesavento et al., 2008)). Despite such variety of effects, biofilm stimulation by AI-2 might mainly depend on the regulation of flagellar activity (González Barrios et al., 2006; Herzberg et al., 2006; Ren et al., 2004). Initially, it was suggested that AI-2 flagellar activation might depend on QseBC two-component regulatory system, in which the phosphorylated QseB response regulator can directly bind the *flhDC* promoter, in turn stimulating the expression of the flagellar activator FlhDC (Sperandio et al., 2002). Although the sensor protein QseC does not respond directly to AI-2, but rather to other signals originating from other bacteria or from the host ((Sperandio et al.,

2003); see also next section), data still support the notion that the QseB response regulator is required to relay AI-2 sensing to motility (González Barrios et al., 2006), thus suggesting a cross talk between AI-2 and other signaling systems. Through control of FlhDC expression, AI-2 can impact cell physiology and the motile/sessile cell switch, as FlhDC also regulates FliZ, a protein able to counteract the activity of the alternative sigma factor RpoS (Pesavento et al., 2008; Pesavento and Hengge, 2012), and of YhjH, a c-di-GMP phosphodiesterase promoting cellular motility while inhibiting curli fibers production (Lindenberg et al., 2013; Pesavento et al., 2008). QseBC expression also requires the activity of another component, MqsR, as mutants in the *mqsR* gene fail to regulate flagella and cellular motility in response to AI-2 in a QseBC-dependent manner (González Barrios et al., 2006). MsqR belongs to the MsqR/MsqA (*ygiU*/*ygiT*) toxin-antitoxin system, in which the MsqR toxin is a mRNA interferase that cleaves target RNAs at GC(A/U) sites, while MsqA is its cognate antitoxin (Christensen-Dalsgaard et al., 2010; Yamaguchi et al., 2009). In addition to inhibiting MqsR endoribonuclease activity, MsqA is able to bind DNA and to negatively regulate expression of its own operon; MsqA inhibition on its own promoter seems to be enhanced when the toxin/anti-toxin is formed (Yamaguchi et al., 2009). *In vitro* binding assays showed that MqsR does not interact directly with QseBC mRNA, raising the possibility that its effect could be indirect (Yamaguchi et al., 2009). Although TA systems are clearly involved in biofilm formation, as well as being crucial in many other cell processes, such as persistence (reviewed in (Wen et al., 2014)), they are often strictly interconnected with other signaling systems, and their effects may strongly be influenced by the genetic backgrounds and by the experimental conditions used (Kasari et al., 2010). It is interesting to note that most data on AI-2 signaling in *E. coli* are based on microarray or transcriptomics data performed comparing mutants in the AI-2 signaling system to their wild type. These experiments, despite providing a global picture of gene expression, fall short in the identification of direct targets for LsrR, the only specific responder to AI-2 known until now (Taga et al., 2001). So far, only one work carried out in *S.* Typhimurium using ChIP (Chromatin immunoprecipitation)-chip analysis has attempted to specifically identify LsrR regulon. The results of this work indicated that LsrR can directly bind the *lsr* operons, as expected; however, no other

LsrR-bound promoter was found (Thijs et al., 2010). It is conceivable that LsrR is not the only regulator directly responding to AI-2, and other unknown receptors can sense the signaling molecule, and relay it to biofilm formation.

Autoinducer 3/epinephrine (epi)/norepinephrine (NE)

Both commensal and pathogenic strains of *E. coli* are able to synthesize a third quorum-sensing molecule independent of LuxS activity but whose production can be affected by *luxS* disruption (Sperandio et al., 2003; Walters et al., 2006). This molecule, named AI-3, is probably an aromatic amine, although its precise chemical structure has not been identified yet. AI-3 might resemble the mammalian hormones ephinephrine and norepinephrine, as these molecules can interact with some components of the AI-3 quorum-sensing system (Sperandio et al., 2003). AI-3 signaling is conserved in several species that inhabit the human GI tract, including pathogenic and commensal strains (Clarke and Sperandio, 2005), and thus suggesting that this molecule represents an important signal for bacteria in the gut. AI-3 was firstly described as crucial player in pathogenesis of enterohemorrhagic *Escherichia coli* O157:H7 (EHEC), where it controls the expression of different virulence loci (Sperandio et al., 2003). This molecule is sensed by the QseC membrane histidine kinase, also potentially involved in AI-2 sensing, that undergoes autophosphorylation upon binding to AI-3. The cross-regulation between AI-2 and AI-3 sensing, as well as the signal transduction mechanisms coupling these signal molecules to regulation of gene expression, appear to be very intricate. For instance, although QseC forms a two component regulatory system with QseB, this response regulator can be present in a phosphorylated form even in the absence of the QseC sensor, suggesting cross talk between QseB and other sensor kinase(s) (Kostakioti et al., 2009). It is interesting that lack of QseB impairs AI-2-dependent expression of the *motA* gene, while the effect of QseC was not investigated (González Barrios et al., 2006). It would be important to understand whether, and how, AI-2 and AI-3 sensing can converge on QseB controlling its phosphorylation state in response to different signals. As already mentioned, QseB can control biofilm formation mainly through the modulation of FlhDC regulon (Clarke and Sperandio, 2005; González Barrios et al., 2006; Sperandio et al., 2002; Yang et al., 2014). However, in

pathogenic *E. coli* strains, QseB can repress the production of different structures involved in cell adhesion, including the *espA* gene, type 1 fimbriae (*fim*) and curli (Kostakioti et al., 2009; Sharma and Casey, 2014), thus impacting cell ability to colonize gut epithelia (Sharma and Casey, 2014). Another mechanism involving only a portion of the QseBC system is curli production, as it has been shown that QseC, but not QseB, affects regulation of curli genes (Sharma and Casey, 2014). It must be stressed that adaptation to a commensal vs. pathogenic lifestyle might have altered quite considerably the response of different *E. coli* strains to quorum sensing signals such as AI-2 and AI-3.

In addition to sensing the AI-3 signal, QseC is a bacterial adrenergic receptor, as it is able to bind and modulate its cognate response regulator in response to epinephrine and norepinephrine, two mammalian hormones present in the bloodstream and in the mucosa of the gastrointestinal tract, respectively (Lyte et al., 2011). Thus, in addition to AI-3, the QseBC system allows the bacteria to integrate these host signals with the control of biofilm determinants, contributing to bacterial community formation (Kostakioti et al., 2009; Sharma and Casey, 2014; Yang et al., 2014). It might be tempting to speculate that, as epinephrine and norepinephrine are able to trigger a sensor for AI-3, likewise this bacterial molecule might interact with the host's adrenergic receptors, thus representing an interkingdom signaling molecule. To our knowledge, however, no effects of AI-3 on eukaryotic cells are reported in literature yet.

Epinephrine/Norephinephrine sensing is not an exclusive feature of QseC histidine kinase, as QseEF, a secondary two-component regulatory system whose transcription depends on QseBC itself, was shown to respond to the two mammalian hormones in EHEC controlling strain virulence. Interestingly, QseEF does not sense bacterial AI-3 and is activated by other molecules such as sulfate and phosphate (Reading et al., 2009). QseEF is also conserved in non-pathogenic strains, thus controlling stress-related genes or adhesive structures could be its role in commensal bacteria.

Indole

Indole is an intermediate metabolic product of both biosynthesis and degradation of

the amino acid tryptophan. In tryptophan degradation, indole is produced by the enzyme tryptophanase (TnaA) together with ammonia and pyruvate, which are used as a source of carbon and nitrogen under starvation conditions (Newton and Snell, 1964). Although some reports suggested its active uptake by the protein complexes Mtr and AcrEF-TolC (Kawamura-Sato et al., 1999; Yanofsky et al., 1991), recent evidence indicates that indole can freely traverse the cell membrane (Piñero-Fernandez et al., 2011). Indole is produced by a large number of Gram-positive and Gram-negative bacteria, including *Escherichia coli*, and it might act as both an intra- and interspecies signal (reviewed in (Thijs et al., 2010)). A concentration ranging between 0.25 mM and 1.2 mM indole is found in human faeces; thus, it is likely that similar concentrations of this molecule are present in the GI tract, suggesting active degradation of tryptophan to indole by the bacterial gut flora. In addition to bacterial signaling, indole was shown to be an interkingdom molecule able to affect gene expression in epithelial cells (Bansal et al., 2010), mammalian cell metabolism and secretion of the incretin peptide GLP-1 (Chimerel et al., 2014), probably altering the lipid membrane potential (Chimerel et al., 2013; Wikoff et al., 2009).

In *E. coli*, the tryptophanase-encoding *tnaA* gene is under the control of the stationary phase sigma factor RpoS (Lacour and Landini, 2004); consistent with *tnaA* regulation by *rpoS*, indole can be detected in culture supernatants as cells approach stationary phase (Chant and Summers, 2007; Li and Young, 2013). Indole impacts a large number of cell processes, including transition to stationary phase (Lelong et al., 2007), plasmid stability (Chant and Summers, 2007), virulence factor in enterohemorrhagic *E. coli* (Hirakawa et al., 2009), biofilm formation (Domka et al., 2006; Lee et al., 2007; Martino et al., 2003; Wang et al., 2001). It has been reported that, at the onset of stationary phase, indole can reach a particularly high intracellular concentration (up to 60 mM), in comparison to a 4-5mM concentration in culture supernatants. This accumulation is only transient (20 minutes), but it appears to be crucial for bacterial survival in stationary phase (Gaimster et al., 2014). Finally, indole can work as an ionophore, controlling cell division through the modulation of membrane potential (Chimerel et al., 2012).

Early reports suggested that, in *E. coli*, indole can have a stimulatory effect on

biofilm formation as *tnaA* inactivation reduces biofilm formation and addition of the molecule restores the wild type (Martino et al., 2003). In contrast, however, later works suggested that indole represses biofilm development (Domka et al., 2006; Lee et al., 2007). As for other signal molecules, these discrepancies could be explained by the different conditions (e.g., presence or absence of glucose, a repressor of TnaA activity) and to the different *E. coli* strains used in these studies (Thijs et al., 2010).

Indole seems to have greater effects at 30°C than at 37°C; at lower temperatures its effect might be mediated by the AHLs sensor SdiA (see previous section), whose transcription is itself stimulated by indole (Lee et al., 2007; Lee et al., 2008). Indole can interfere with AHLs sensing by SdiA, as increasing concentrations of this molecule reduce transcription of SdiA-controlled gene *gadW* (Sabag-Daigle et al., 2012). However, indole also affects biofilm formation in a SdiA-independent fashion, as its inhibitory effect can be achieved both in wild type and *sdiA* mutant (Sabag-Daigle et al., 2012). Interestingly, indole production via tryptophanase is not very widespread in bacteria, and indole synthesis by *E. coli* can inhibit *P. aeruginosa* quorum sensing system increasing its competitiveness in mixed cultures (Chu et al., 2012). It is tempting to speculate that indole can be a *bona fide* QS molecule in *E. coli*, providing information on the population density of this bacterium while inhibiting the competition. Utilization of indole as an autoinducer might therefore be the reason for the lack of conservation of the AHL-dependent QS system in *E. coli*.

Short-chain fatty acids (SCFAs) and acetate/acetyl-CoA metabolism

In the caecum and in the large intestine, anaerobic bacteria ferment dietary fibers, producing a variety of metabolites, including short-chain fatty acids (SCFAs) such as propionate, butyrate, and acetate (den Besten et al., 2013). SCFAs are considered pivotal in the interactions between gut microbiota and the human host, as they seem to be a cofactor in important pathologies such as atherosclerosis, and neurological diseases such as autism (Galisteo et al., 2008; MacFabe, 2012). SCFAs absolute concentration and relative chemical composition vary with the diet and other factors (Macfarlane et al., 1992) creating an upward concentration gradient from the small towards the large intestine thus constituting a transit signal through the human gut (Herold et al., 2009).

Indeed, pathogenic EHEC regulate flagellar expression and motility based on relative composition and concentration of SFCAs by an unknown mechanism (Lackraj et al., 2016). Being an abundant and characteristic metabolite in the gut environment, SCFAs are also sensed by commensal *E. coli* through the histidine sensor kinase BarA, which in turn activates its cognate response regulator UvrY. The BarA/UvrY system controls the expression of the two non-coding RNAs CsrB and CsrC (Chavez et al., 2010), able to sequester the translational regulator CsrA, thus modulating carbon fluxes and inducing the expression of the PNAG polysaccharide. Moreover, expression of CsrB and CsrC is negatively regulated by cAMP-CRP (Pannuri et al., 2016). In addition to SCFAs, *E. coli* cells can also modulate biofilm formation in response to acetate; unlike other SCFAs, acetate is an endogenous product of *E. coli* fermentative metabolism, and is actively excreted from the cell to prevent its acidification. It has been proposed that, similar to SCFAs, extracellular acetate can induce the BarA/UvrY stimulating autophosphorylation of the BarA sensor protein. In addition, the UvrY regulatory protein can carry out autophosphorylation from acetyl phosphate, an intermediate of the mixed acid fermentation pathway in *E. coli* and other enterobacteria. Autophosphorylation of response regulators using acetyl phosphate might explain why *E. coli* strains mutated in the *ackA* genes, or *ackA pta* double mutants, both likely to accumulate acetyl phosphate, are more proficient in biofilm formation than wild type strain (Prüß et al., 2010). In addition to PNAG stimulation resulting from acetyl phosphate-dependent autophosphorylation of the UvrY regulator, this metabolite can trigger similar autophosphorylation activities in other response regulators, such as FimZ, RcsB and OmpR, independently of their cognate sensor proteins (Fredericks et al., 2006; Schwan et al., 2007; Wolfe et al., 2003), resulting in increased production of several biofilm determinants, namely, type 1 fimbriae and colanic acid (Fredericks et al., 2006; Wolfe et al., 2003). Thus, acetyl phosphate might represent a paramount example of how an intermediate of a metabolic pathway can relay a physiological cue to biofilm formation. It is noteworthy that acetyl phosphate can be produced in response to an excess of glucose or other sugars, as part of an overflow metabolism process, and could therefore serve as an indicator of the bacterial cell's energy state.

Polyamines

Polyamines, such as cadaverine, putrescine, spermine, spermidine, and norspermidine, are organic compounds that contain two or more amine groups and are found in abundance both in eukaryotic and prokaryotic cells. The major source of polyamines in the distal GI tract is the metabolism of the microbial flora, as polyamines are catabolites of basic amino acids such as arginine; polyamines synthesized by gut bacteria can be quickly absorbed by epithelial cells in the small intestine, providing yet another example of bacterial metabolites interacting with the host's cell (Milovic, 2001). In addition to *de novo* synthesis, bacteria have transporters for the uptake of extracellular polyamines. In *E. coli*, polyamines are essential for growth and viability, acting at various levels, from pH homeostasis, to stabilization of chromosomal DNA structure, to ribosomal activity: in particular, they appear to positively affect translation of several global regulators, including RpoS, Cya, H-NS, RelA and RpoZ (Terui et al., 2012). Thus, their physiological impact on the bacterial cell can be huge, and indeed they are connected with complex behaviors such as biofilm formation. While exogenous spermidine and norspermidine inhibit biofilm formed by potentially pathogenic *E. coli* in a concentration-dependent manner, putrescine greatly stimulates adhesion of nonpathogenic strains (Nesse et al., 2015; Sakamoto et al., 2012). Putrescine stimulation of biofilm is a concerted effect on different levels of the regulatory cascade. This polyamine promotes translation of UvrY and CpxR proteins, two response regulators belonging, respectively, to BarA/UvrY and CpxA/CpxR two-component response systems. Putrescine stimulates UvrY synthesis by promoting translation from the inefficient starting codon UUG of UvrY mRNA (Sakamoto et al., 2012) . In turns, UvrY positively controls the expression of CsrB and CsrC small RNAs (Suzuki et al., 2002), which can sequester the CsrA protein, a translational repressor of the *pga* locus, necessary for poly-*N*-acetyl-glucosamine (PNAG) exopolysaccharide production, an important biofilm determinant (Wang et al., 2005; Wang et al., 2004). At the same time, putrescine induces a conformational change around the Shine-Dalgarno (SD) region of *cpxR* mRNA increasing its translational rate (Sakamoto et al., 2012). CpxR is a known transcriptional repressor of CsgD, thus inhibiting biosynthesis of curli adhesin (Prigent-Combaret et al., 2001). Although curli are mainly produced at conditions not

encountered in the human host (e.g., at low growth temperature) (Olsén et al., 1993), there is evidence that they are produced in the intestinal tract (Bokranz et al., 2005). Thus, rather than being a straightforward activator of biofilm formation, putrescine might promote a curli-PNAG switch as the main components of the biofilm matrix. So far, very little is known about the precise contribution of different biofilm determinants and the possible ecological meaning of their re-modulations of the biofilm matrix in the human GI tract. It is possible that coordinated production of different adhesion factors and biofilm determinants might be aimed at the colonization of different niches within the human host, and sensing of compounds such as polyamines might be one of the presiding mechanisms to this adaptation.

Intra/extracellular nucleotides and nucleobases (pyrimidines and uracil)

Several works have highlighted the importance of nucleotides and nucleobases, in particular uracil, for biofilm formation in both *E. coli* and *P. aeruginosa* (Garavaglia et al., 2012; Lee et al., 2008; Ueda et al., 2009). In *E. coli* K-12, inactivation of genes belonging to the *de novo* pyrimidine and purine biosynthesis totally impairs biofilm formation, while mutations leading to increased nucleotide biosynthesis result in biofilm stimulation (Garavaglia et al., 2012). The link between biofilm and intracellular nucleotide concentrations appears to be particularly strong, as even slight perturbations in the *de novo* nucleotide synthesis, albeit not affecting primary metabolism or cell growth, completely abolish biofilm formation and maintenance. The importance of intracellular nucleotide pools in biofilm formation is also suggested by the fact that antimetabolite drugs inhibiting *de novo* purine or pyrimidines biosynthesis, such as 5-fluoruracil, azathioprine, and sulfathiazole, are strong inhibitors of biofilm formation in *E. coli* and *P. aeruginosa* (Antoniani et al., 2010; Antoniani et al., 2013; Ueda et al., 2009). Azathioprine and sulfathiazole have been shown to inhibit c-di-GMP biosynthesis, suggesting that even slight perturbations in intracellular GTP concentrations, *i.e.*, the substrate of the diguanylate cyclase (DGC) enzymes responsible for c-di-GMP production, might impair their activity. Whether biofilm inhibition due to reduction in pyrimidine levels might also be mediated by c-di-GMP or possibly by other pyrimidine-derived nucleotides acting as signal molecules for biofilm formation,

remains to be addressed.

Free nucleotides, nucleosides, and nucleobases in the gut derive primary from food degradation and up to 90% is absorbed by epithelial cells (Hess and Greenberg, 2012). However, fluctuations in their amounts can depend on bacterial metabolism: for example, uracil and hypoxanthine, both breakdown products of pyrimidine and purine nucleotide catabolism, accumulate in *E. coli* culture media during stationary phase (Rinas et al., 1995). Uracil was found to be actively excreted as a process to maintain pyrimidine homeostasis upon *de novo* synthesis dysregulation (Reaves et al., 2013), thus, accumulation of extracellular uracil might take place in response to changes in growth rate or carbon source availability, possibly acting as an indicator of metabolic activity. Moreover, it was recently shown that some gut pathogens secrete significant quantities of uracil while most commensal bacteria do not. In *Drosophila melanogaster* and other invertebrates used as model organisms for the study of innate immunity, uracil in the gut lumen strongly activates DUOX-dependent signaling, leading to reactive oxygen species release, as part of an immune response (Lee et al., 2013) Interestingly, exogenous uracil can specifically activate the YedQ diguanylate cyclase in *E. coli* K-12, resulting in stimulation of cellulose production (Garavaglia et al., 2012). These results suggest that pyrimidine sensing might indeed be tightly connected with c-di-GMP biosynthesis. In contrast, the YedQ homolog in the pathogenic bacterium *S.* Typhimurium, STM1987, was shown to modulate c-di-GMP synthesis in response to adenosine, L-arginine, L-lysine, *N*-acetyl-D-glucosamine, but not uracil (Mills et al., 2015). This specialization might reflect the different needs of a commensal versus an intracellular pathogen in the activation of c-di-GMP biosynthesis in response to specific cues.

N-acetyl-glucosamine

N-acetyl-glucosamine (GlcNAc) is an essential amino sugar necessary for peptidoglycan, lipopolysaccharide (LPS) and enterobacterial common antigen (ECA) biosynthesis in *E. coli*. GlcNAc is also the component of chitin, thought to be the biological polymer most abundant in nature, and is highly represented in glycosylated eukaryotic proteins and in glycoconjugates found on the surface of eukaryotic cells

(Varki and Varki, 2007). Due to its essentiality and its abundance, it is not surprising that most bacteria can obtain GlcNAc from the external environment through specialized uptake systems; however bacteria, including *E. coli*, can also synthesize GlcNAc using a *de novo* pathway. *E. coli* can also scavenge GlcNAc from recycling of its own extracellular peptidoglycan and LPS. Extracellular GlcNAc is turned into *N*-acetyl-glucosamine-6-phosphate (GlcNAc-6P) upon uptake and routed through an assimilatory pathway. Not surprisingly, such an important environmental signal is coupled to production of adhesion factors and biofilm formation: accumulation of GlcNAc-6P results in downregulation of curli *csg* operons by a mechanism independent of the NagC regulator (Barnhart et al., 2006), a repressor of the genes required for GlcNAc degradation. In contrast, GlcNAc-6P modulates NagC-dependent expression of FimB, a recombinase promoting the phase-variation switch necessary for the expression of genes encoding type I fimbriae (Pratt and Kolter, 1998). Accumulation of GlcNAc-6P turns off FimB activity, thus repressing type I fimbriae production (Sohanpal et al., 2004). Interestingly, FimB is controlled in a similar way by NanR and *N*-acetylneuraminate (Sohanpal et al., 2004), the predominant form of sialic acid present in humans. As *N*-acetylneuraminate is an environmental signal only found in the mammalian host, while GlcNAc/GlcNAc-6P levels affecting NagC activity can depend on the cell's energy state, *E. coli* can simultaneously regulate type I fimbriae and curli fibers production integrating both environmental and intracellular signals. Finally, the levels of both amino sugars increase in the host during inflammatory processes (Sillanaukee et al., 1999), and can therefore act as indicators for the activation of an immune reaction. Consequently, *E. coli* can turn off curli and type I fimbriae, thus reducing antigenic load on the immune system (Sohanpal et al., 2004), in what appears to be a host-driven adaptive response in *E. coli* gene regulation.

Bile

Bile is a mixture of water, biliary acids, mostly composed of cholic acids derived from cholesterol salts, which are linked either to the amino acid glycine or to the organosulfonate taurine to form bile salts. Bile is a disinfectant agent and a potent detergent necessary for food digestion. It is secreted in the lumen of the duodenum from

the gall bladder upon food ingestion. Although Gram-negative bacteria like *E. coli* are intrinsically resistant to bile salts as they do not readily cross the outer membrane (Begley et al., 2005), they have evolved the ability to recognize bile as a signal in order to modulate adhesive structures production. Bile, in particular bile salts deoxycholate (DC) and cholate (CC), stimulate cellular adhesion in pathogenic *V. cholerae* through the modulation of vibrio polysaccharide (VPS) (Hung et al., 2006). Glucocholate hydrate (GCH) and DC induce expression of ETEC colonization factor CS5 (Nicklasson et al., 2012). In contrast, taurocholate (TC), *i.e.*, the bile salt constituted by the cholate and taurine complex, promotes *V. cholerae* biofilm dispersion by a passive mechanism based on extracellular matrix degradation and has no effect of colonization factor expression in CS5 expressing ETEC (Hay and Zhu, 2015; Nicklasson et al., 2012). In the pathogen *E. coli* O157:H7, bile salts activate the expression of virulence factors, iron acquisition genes and putative adhesive fimbriae, while regulating either positively or negatively different classes of flagellar genes (Hamner et al., 2013). In the closely related bacterium *Salmonella enterica* serovar Typhi, bile stimulates biofilm on gallstones through the production of a specific exopolysaccharide (EPS) encoded by the *yihU-yshA* and *yihV-yihW* loci, independently of the AfgD (CsgD) regulator (Crawford et al., 2008). The same genomic region is conserved in *E. coli* K-12, and it was recently described as involved in sulphoglycolysis (Denger et al., 2014); however, no direct link between biofilm formation and these genes in this or other commensal strains has been reported yet. In addition to gene regulation and possible induction of adaptive responses, bile has also been proposed to drive a genetic diversification in gut-inhabiting *E. coli* strains, leading to a non-motile subpopulation characterized by deletion mutations in the *flhDC* operon (De Paepe et al., 2011). It was suggested that selection would benefit bacteria growing in an environment characterized by high bacterial cell densities, such as biofilms.

One of the main component of human bile is taurine, an amino sulphonic acid that *E. coli* can utilize as a sulphur source if neither inorganic sulphate nor cysteine are present. Though inorganic sulphate is likely to be present in significant amounts in the intestinal tract, both cysteine and taurine are likely to be utilized as sulphur source by *E. coli*. Preferential utilization of sulphur sources regulates the inorganic sulphate

assimilation pathway and affects the intracellular levels of its intermediate phosphoadenosine 5'-phosphosulphate, which has recently been shown to affect production of curli and other adhesion factors (Rossi et al., 2014).

Temperature

Besides living in association with warm-blooded animals, Enterobacteria can be found in other natural niches, mostly in water environments. Thus, it is not surprising that temperature represents a key signal for sensing the transition between water environments, where temperature is usually low and variable, to the warm-blooded hosts, where it is higher and constant. In *E. coli*, the production of several adhesion factors is affected by temperature: indeed, curli fibers, cellulose, colanic acid and poly-*N*-acetylglucosamine (PNAG) are produced at higher levels at temperature below 30°C (environmental temperature) than at 37°C (host temperature) (Beloin et al., 2008; Gualdi et al., 2008; Olsén et al., 1993; Wang et al., 2004), thus suggesting that the biofilm mode might play a role in adaptation to conditions typically found outside the host. However, expression of other adhesins is not temperature-dependent or it is even enhanced at 37°C; it must be also considered that temperature regulation of adhesion factors can vary greatly, depending on strains and genetic backgrounds. Indeed, a number of works showed that, unlike laboratory strains, the matrix of different *E. coli* isolated from the gut at 37°C can be composed of a mix of curli fibers, cellulose (Bokranz et al., 2005; Saldaña et al., 2009), PNAG (Cerca and Jefferson, 2008; Pieper et al., 2013), while antigen 43 (Danese et al., 2000; Ulett et al., 2007) and type I fimbriae can mediate cellular adhesion at host temperature. In addition, most colonization factors of ETEC are induced at 37°C indicating that these virulence factors are needed for colonization of the host (Sjöling et al., 2007).

To date, biofilm control by temperature has mostly been studied for curli fibers and cellulose, in *E. coli* and *Salmonella* strains showing temperature dependence for their expression (Gerstel and Römling, 2003; Olsén et al., 1993). Although the precise mechanism governing curli temperature-dependent expression has not yet been fully elucidated, it might require converging regulatory circuits. One proposed mechanism involves the Crl protein, which promotes the association of the alternative sigma factor

RpoS to RNA polymerase; Crl expression is increased during the transition from exponential to stationary phase and it accumulates at low temperature (30°C), thus stimulating expression of *rpoS*-dependent genes, including the curli-encoding *csgBAC* operon (Bougdour et al., 2004). The second regulatory system depends on the small regulatory RNA DsrA. DsrA is stabilized at low temperatures, and in turn promotes RpoS translation under these conditions (Repoila and Gottesman, 2001). However, it was reported that the *csgBAC* operon can be transcribed in a *crl*- and *rpoS*-independent fashion (Arnqvist et al., 1994; Gualdi et al., 2007; Provence and Curtiss, 1992) and that DsrA might act on *rpoS* translation also at 37°C (Mandin and Gottesman, 2010), suggesting further regulatory mechanisms for temperature-dependent curli production. Since curli and cellulose synthesis are exquisitely dependent on c-di-GMP accumulation both in *E. coli* and in *Salmonella* (Römling, 2005), and expression of several DGC-encoding genes only takes place at low growth temperature (Sommerfeldt et al., 2009), this signal molecule might be the main responsible for temperature-dependent regulation of curli and cellulose.

However, curli are not modulated in the same way in response to temperature in other Enterobacteria. Indeed, especially pathogenic strains are able to express this kind of adhesins at higher temperature (37°C) during host invasion and colonization (Bian et al., 2000). Therefore, current data clearly outline the complexity of temperature regulation of curli fibers in *E. coli*, stressing that different strains might have evolved various adaptive strategies even within the same ecological niches.

Besides specifically controlling curli fibers and cellulose, temperature stimulates or inhibits biofilm formation in *E. coli* acting on the whole cell physiology through global regulators. Indeed, after temperature downshift, cold-shock proteins such as CspA, CspB, CspF, CspG and PNPase are strongly induced. CspA and its paralogues destabilize mRNA secondary structures, a process that may be crucial for efficient mRNA translation at low temperatures (Jiang et al., 1997). Even though specific targets of Csp proteins have not been completely identified, cold-shock proteins CspA, CspB, CspF, CspG and CspI can all work as positive regulators of biofilm formation (Domka et al., 2007). The RNA degradosome component PNPase was recently described as a negative regulator of poly-*N*-acetylglucosamine, thus suggesting control of this EPS in

response to temperature at RNA level (Carzaniga et al., 2012). Although the nine Csp proteins are homologues, their expression patterns greatly vary in different temperature range with CspA mostly expressed between 10-24°C, CspB and CspG at 15°C, and CspI between 10-15°C (Etchegaray et al., 1996; Wang et al., 1999), thus suggesting a different contribution in modulating biofilm formation during temperature shifts. On the contrary, two Cps proteins, CspE and CspC, are almost expressed at 37°C. The two proteins were shown to promote *rpoS* mRNA stabilization and expression at high temperature (Phadtare and Inouye, 2001), thus providing yet another mechanism coupling low growth temperature with expression of *rpoS*-dependent genes, which include in addition the curli-encoding *csg* operons and several diguanylate cyclases (Sommerfeldt et al., 2009).

Oxygen availability

Oxygen concentration in the intestine is generally considered to be low and bacteria face a microaerophilic condition (range 0.1 - 2.3%) (Levitt, 1971). In particular, the lumen of the small intestine and colon is typically anaerobic, whereas the brush border of the small intestine is considered microaerophilic, probably due to diffusion of oxygen from the capillary network at the tips of the villi (Sengupta et al., 2014). Moreover, while the biofilm develops and becomes thicker, the innermost cells experience anaerobic condition due to oxygen consumption of the more exposed parts of the biofilms, resulting in a rich and stratified environment with different cell phenotypes (Serra and Hengge, 2014).

Oxygen represents a major signal for *E. coli* biofilm development and anaerobic conditions negatively affect bacterial adhesion (Colón-González et al., 2004; Landini and Zehnder, 2002). While strict anaerobiosis is in general a negative signal for biofilm formation in *E. coli*, microaerophilic conditions can play a more complex role. Production of curli fibers is impaired in anoxic conditions through a direct oxygen sensing system, DosP/DosC. DosP is a c-di-GMP phosphodiesterase, while DosC is a diguanylate cyclase that synthesizes the second messenger c-di-GMP. Both proteins bind a heme prosthetic group and co-purify in a single macromolecular complex whose c-di-GMP net output depends on oxygen tension (Delgado-Nixon et al., 2000;

Tuckerman et al., 2011; Tuckerman et al., 2009). Tuckerman and colleagues suggested that decreasing O_2 concentration switches on DosC protein, while DosP activity is reduced resulting in a DosC-dependent c-di-GMP biosynthesis. However, totally anoxic conditions also impair DosC activity, suggesting that biofilm determinants might be strongly expressed in response to microaerophilic conditions only to be switched off in the absence of oxygen (Tagliabue et al., 2010b). In *E. coli*, the DosC/DosP protein complex was shown to control at least two different adhesion factors, namely curli fibers and PNAG (Tagliabue et al., 2010a; Tagliabue et al., 2010b), thus allowing co-ordinate production of two important biofilm determinants. Interestingly, although the DosC/DosP system is missing in the closely related bacterium *Salmonella*, curli fibers are produced at higher levels in response to microaerophilic conditions also in this bacterium (Gerstel and Römling, 2001), suggesting that a similar response might be mediated by different mechanisms in *E. coli* and *Salmonella*.

Cysteine/sulphate

As already mentioned, *E. coli* can assimilate sulphur from a broad range of sources such as inorganic sulfate, cysteine, aliphatic sulphonate (*i.e.* taurine), with cysteine as its preferred source (van der Ploeg et al., 2001). The gut lumen is a complex environment where different sources of organic sulfur are made available from processing of dietary proteins (*e.g.* cysteine and methionine) or from compounds deriving from the host (*e.g* taurine, mucin). Taurine is very abundant in bile salts, as discussed in the section "*Bile*", thus, sensing this sulphur source might be part of an adaptation to exposure to bile. In the absence of cysteine, its preferred sulphur source, *E. coli* scavenges inorganic sulfate, which is reduced to H_2S through the sulfate assimilation pathway, and subsequently used to synthesize L-cysteine (van der Ploeg et al., 2001). In addition to its role as a proteinogenic amino acid, cysteine is also used in other cellular processes including biosynthesis of methionine, coenzyme A, and glutathione, the main agent in maintenance of the redox state in the bacterial cell (Sekowska et al., 2000). Cysteine and sulfate metabolism are directly linked to biofilm formation in *E.coli*: inactivation of *cysB* gene, which is required for expression of all genes belonging to the sulphate assimilation pathway, stimulates biofilm formation of *E. coli* grown in rich media (Ren

et al., 2005) while a faint to null effect has been observed in casamino acids-based media (Ren et al., 2005; Rossi et al., 2014). Likewise, inactivation of *cysE* gene stimulates biofilm formation accelerating its development independently of flagella, colanic acid and type 1 fimbriae (Sturgill et al., 2004). The CysE enzyme catalyzes the biosynthesis of the metabolic intermediate *O*-acetyl-L-serine that, upon spontaneous conversion to *N*-acetyl-L-serine, acts as a positive inducer for CysB-dependent transcription regulation. Thus, loss of the CysE enzyme would affect CysB activity, resulting in the perturbation of sulphate reduction pathway intermediates, in particular, phosphoadenosine-5'-phosphosulphate (PAPS). Indeed, this molecule has recently been identified as a signal molecule connecting the sulphate/cysteine availability with biofilm formation by positively regulating production of curli and other adhesion factors (Rossi et al., 2014). Although the molecular mechanism is not clear, PAPS seems to act at two different levels, enhancing and/or synergizing the activity of the cAMP-CRP complex, at the same time providing a feedback mechanism through a putative interaction with the global regulator HU (Longo et al., 2016). In addition to controlling expression of curli fibers (Thomason et al., 2012; Zheng et al., 2004), cAMP-CRP complex directs the hierarchical utilization of different carbon sources (Kolb et al., 1993; Stülke and Hillen, 1998). Thus, PAPS could connect sulfate sensing with sugar metabolism (Longo et al., 2016). Indeed, additional links between sulphur sources, carbon utilization and biofilm formation is provided by the GlrKR two component system (also known as QseEF). In addition to responding to epinephrine/norepinephrine in EHEC strains, GlrK can also sense sulfate (Reading et al., 2009). In *E. coli* K-12, the cognate response regulator GlrR stimulates the expression of the small RNA GlmY, a positive effector for translation of GlmS (Göpel et al., 2014), a key enzyme in amino sugar biosynthesis (Badet et al., 1987). As discussed in a previous section, amino sugars, in particular *N*-acetylglucosamine, play a dual role in biofilm, as they are the building blocks of PNAG and other EPS acting as adhesion factors, and since intracellular *N*-acetyl-glucosamine-6-phosphate concentrations work as regulatory molecules for curli and fimbriae production (Barnhart et al., 2006; Itoh et al., 2008).

The reason why extracellular sulphate signaling should be routed through production of amino sugars and connected with carbon source sensing remains elusive. Unlike

eukaryotes, *E. coli* is unable to synthesize sulfated polysaccharides, which are however found in great abundance in the gut epithelia (*e.g.* mucin and sulphated glycosaminglycans), where they carry out a protective function (Corfield et al., 2000). These carbohydrates can represent a valuable source of nutrients for bacteria, but usually their high sulfate content prevents their degradation; thus, expression of specific sulfatases is necessary to process host-derived sugars. Interestingly, YdeN and YdeM, two predicted sulfatases of *E. coli* K-12, are transcriptionally controlled by the level of *N*-acetyl-D-glucosamine-6-P (Oberto, 2010), another evidence for a connection between sulphate metabolism and amino sugars. Moreover, as mucin is composed of *N*-acetylgalactosamine, *N*-acetylglucosamine, fucose, galactose, and *N*-acetylneuraminic acid and traces of mannose and sulfate, it could be speculated that simultaneous presence of exogenous sulfate and *N*-acetylglucosamine (and/or *N*-acetylneuraminic acid) can be another indicator for *E. coli* to signal its location inside the host gut preparing the cell to utilization of host-derived sugars.

5 The host's defense against *E. coli* biofilm formation

Curli fimbriae are a virulence factor in several disease situations. Using synthetic molecules interfering with curli biogenesis (curlicides), curli were shown to promote the pathogenesis of urinary tract infection in a mouse model (Cegelski et al., 2009). Interestingly, the endogenous antimicrobial peptide LL-37 exhibits a similar function. Indeed, we could show that LL-37 interferes specifically with the formation of biofilms at subinhibitory concentrations (Kai-Larsen et al., 2010). By binding to CsgA monomers, polymerization and curli formation is inhibited *in vitro* and biofilm formation *ex vivo* is strongly reduced. In addition, curliated strains are protected from the antimicrobial activity of LL-37. As LL-37 binds to isolated curli, presumably, the peptide is trapped in the extracellular net of fibers and thus non-functional. However, LL-37 might also act through additional mechanisms in the cytoplasm. Engineered peptides related to LL-37 specifically inhibit biofilm formation by interference with ppGpp second messenger signaling in various bacterial species including *E. coli* (de la Fuente-Nunez et al., 2014).

Other consequences, potentially, include interference with the quorum sensing systems in *P. aeruginosa* (Overhage et al., 2008).

Besides interacting with LL-37, curli induce a strong host immune response and promoted early colonization, but also bacterial elimination by neutrophils at a later stage; in contrast, the biofilm extracellular matrix component cellulose opposed these effects (Kai-Larsen et al., 2010). Further, curli can trigger immune activation via Toll-like receptors (Tükel et al., 2010) and curli-DNA composites, at least from *S.* Typhimurium, stimulate autoimmunity in mice (Gallo et al., 2015).

Currently, it is now known whether LL-37 interferes with the functionality of additional fimbriae. As initiation of infection and immunostimulation are a general hallmark of curli fimbriae, interference with biofilm formation by antimicrobial peptides such as LL-37 might be an anti-virulence strategy also on other mucosal surfaces (Chromek et al., 2012).

6 Second messenger signaling regulating biofilm formation

The nucleotide second messengers cyclic AMP, cyclic di-GMP and ppGpp have been shown to regulate biofilm formation in *E. coli*. The environmental stimuli of these nucleotide-based second messengers are fundamentally different, as cyclic AMP is regulated in response to sugar availability, while cyclic di-GMP regulates sessile/motile and commensal/virulence life style transition through a multitude of extra- and intracellular signals, whereas adaptation of ppGpp concentration occurs through nutrient starvation. Nevertheless, though, these signaling systems are interconnected at several knots (Fig. 5).

Catabolite repression of rdar and other biofilm types is partially mediated through the cAMP/CRP system by different sugars at high concentration, while low concentration of the same molecules has a biofilm-stimulating effect (Jackson et al., 2002; Prüß et al., 2006; Sutrina et al., 2015). Although some additional receptors have been identified, the cAMP signaling system is essentially linear with one cAMP synthase, the cAMP receptor protein, a transcriptional regulator, which regulates over 100 genes, and one cAMP phosphodiesterase.

In contrast, the cyclic di-GMP signaling system consists of over 30 membrane standing or cytoplasmic c-di-GMP metabolizing proteins plus a number of strain/genomic island/pathovar specific enzymes (Povolotsky and Hengge, 2015; Römling, 2005). The main biofilm activator CsgD is a major target of transcriptional regulation by cyclic di-GMP signaling and cyclic di-GMP subsequently activates production of downstream extracellular matrix components curli fimbriae and cellulose (Povolotsky and Hengge, 2012). The fundamental sessility/motility life style transition of cyclic di-GMP as in other bacteria exists also in *E. coli*, thus inversely regulated with biofilm formation is motility (Römling, 2012; Römling et al., 2013). Besides in rdar biofilm formation, ETEC strains show elevated cyclic di-GMP in interaction with intestinal epithelial cells (Kansal et al., 2013). In the AIEC strain LF82, cyclic di-GMP inhibits type 1 pili synthesis as well as adhesion, and invasion through expression of the FliA dependent phosphodiesterase YhjH (Claret et al., 2007). Interestingly, type 1 fimbriae are repressed by cyclic di-GMP, as indirectly concluded by the effect of the metabolizing enzymes, in this strain, while, conventionally, cyclic di-GMP elevates production of type 1 fimbriae in the UTI strain background as would be expected (Spurbeck et al., 2012). Also synthesis of the commonly produced exopolysaccharide PNAG, involved in attachment (Agladze et al., 2005), depends on c-di-GMP levels (Steiner et al., 2013). The diguanylate cyclase YdeH (DgcZ) regulates PNAG production (Boehm et al., 2009). Transcription of *ydeH* depends on the two component system CpxAR (Raivio et al., 2013) activated by the lipoprotein NlpE upon surface recognition (Otto and Silhavy, 2002). Thereby, YdeH connects surface sensing with subsequent adhesion (Lacanna et al., 2016).

ppGpp, long called an alarmone, is actually a second messenger responding to nutrient deprivation thereby activating the ‘so-called’ stringent response. One pathway of ppGpp mediated activation of biofilm formation, is enhancing expression of the small RNAs CsrB and CsrC, thereby inhibiting CsrA (Fig. 5; (Dalebroux and Swanson, 2012)). CsrA subsequently regulates the expression of cyclic di-GMP metabolizing proteins, thereby connecting ppGpp and cyclic di-GMP signalling (Jonas et al., 2008). In a *csrA* deletion background, though, ppGpp inhibits biofilm formation (Boehm et al., 2009), indicating counteracting regulatory mechanisms by ppGpp.

7 Perspectives and future questions

In summary, *Escherichia coli* commensal strains and the various *E. coli* pathovars frequently form biofilms. Thereby biofilm formation is highly variable. On the other hand, the probiotic strain Nissle 1917 shows a highly stable biofilm pattern. Thus, what are the evolutionary forces in commensalism and disease, but also outside the human body, which shape biofilm regulation? And, more specifically, what is the physiological impact of the different rdar biofilm regulatory patterns seen e.g. among commensal *E. coli* strains?

Indeed, we have discovered few biofilm phenotypes; can we define additional phenotypes and the circumstances, when they are required? The commensal model organism *Escherichia coli* K-12 has seven additional chaperone usher pathway fimbriae in addition to type 1 fimbriae (Korea et al., 2010), which seem to be functional and mediate biofilm formation. Under which biofilm conditions are these fimbriae expressed? For example, does *E. coli* interact with other microorganisms of the gut microflora through biofilm formation?

Also, how are the different biofilm phenotypes, as they exist, differentially regulated? Adhesion and biofilm factors specific to *E. coli* pathovars exist. How many biofilms are regulated by the ubiquitous second messenger cyclic di-GMP network? And if regulated, how is differential regulation achieved? Horizontally acquired fimbriae such as Pap and Saf fimbriae specific for certain *E. coli* pathovars, adjacently include regulon specific cyclic di-GMP turnover proteins (Sjöstrom et al., 2009). Besides cyclic di-GMP signalling, also other secondary messenger signalling systems are involved in biofilm regulation such as the ppGpp and cAMP signalling system. Also, phosphotransfer signalling through two component systems and the phosphoenolpyruvate phosphotransferase systems (PTS) that differentially phosphorylate proteins in response to carbohydrate transport over the cytoplasmic membrane are involved in biofilm formation. Are these systems relevant, and if so, how are these systems interconnected in *E. coli*?

Although not covered extensively in this review, we have to state that cyclic di-GMP signaling is a major regulator of biofilm-motility and prospectively biofilm-virulence transition in *E. coli* (Spurbeck et al., 2012), as it is in other bacteria (Römling, 2012). Can

we define specific cyclic di-GMP metabolizing enzymes involved in biofilm versus virulence regulation in the different pathovars? How many of the described signals affecting biofilm formation interact with the cyclic di-GMP signalling pathway, either directly, by being the ligand of sensor domains, or indirectly?

In general, few phenotypes mediated by cyclic di-GMP signaling have been identified in *E. coli*; how can we identify additional phenotypes regulated by cyclic di-GMP signaling? Acute virulence phenotypes are generally inhibited in the biofilm life style and by cyclic di-GMP signaling, is this also the case in *E. coli*? In addition, we know very little about the contribution of biofilm formation and cyclic di-GMP signaling to chronic infection phenotypes.

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References

Agladze, K., Wang, X., and Romeo, T. (2005). Spatial periodicity of *Escherichia coli* K-12 biofilm microstructure initiates during a reversible, polar attachment phase of development and requires the polysaccharide adhesin PGA. J Bacteriol *187*, 8237-8246.

Ahmed, D., Islam, M.S., Begum, Y.A., Janzon, A., Qadri, F., and Sjöling, A. (2013). Presence of enterotoxigenic *Escherichia coli* in biofilms formed in water containers in poor households coincides with epidemic seasons in Dhaka. J Appl Microbiol *114*, 1223-1229.

Alam, M., Sultana, M., Nair, G.B., Siddique, A.K., Hasan, N.A., Sack, R.B., Sack, D.A., Ahmed, K.U., Sadique, A., Watanabe, H.*, et al.* (2007). Viable but nonculturable *Vibrio cholerae* O1 in biofilms in the aquatic environment and their role in cholera transmission. Proc Natl Acad Sci U S A *104*, 17801-17806.

Alvarez, H.M., and Steinbüchel, A. (2002). Triacylglycerols in prokaryotic microorganisms. Applied microbiology and biotechnology *60*, 367-376.

Anderson, G.G., Palermo, J.J., Schilling, J.D., Roth, R., Heuser, J., and Hultgren, S.J. (2003). Intracellular bacterial biofilm-like pods in urinary tract infections. Science *301*, 105-107.

Antoniani, D., Bocci, P., Maciag, A., Raffaelli, N., and Landini, P. (2010). Monitoring of diguanylate cyclase activity and of cyclic-di-GMP biosynthesis by whole-cell assays suitable for high-throughput screening of biofilm inhibitors. Applied microbiology and biotechnology *85*, 1095-1104.

Antoniani, D., Rossi, E., Rinaldo, S., Bocci, P., Lolicato, M., Paiardini, A., Raffaelli, N., Cutruzzolà, F., and Landini, P. (2013). The immunosuppressive drug azathioprine inhibits biosynthesis of the bacterial signal molecule cyclic-di-GMP by interfering with intracellular nucleotide pool availability. Applied microbiology and biotechnology *97*, 7325-7336.

Aprikian, P., Interlandi, G., Kidd, B.A., Le Trong, I., Tchesnokova, V., Yakovenko, O., Whitfield, M.J., Bullitt, E., Stenkamp, R.E., Thomas, W.E.*, et al.* (2011). The bacterial fimbrial tip acts as a mechanical force sensor. PLoS Biol *9*, e1000617.

Armbruster, C.E., Pang, B., Murrah, K., Juneau, R.A., Perez, A.C., Weimer, K.E.D., and Swords, W.E. (2011). RbsB (NTHI_0632) mediates quorum signal uptake in nontypeable *Haemophilus influenzae* strain 86-028NP. Molecular microbiology *82*, 836-850.

Arnqvist, A., Olsén, A., and Normark, S. (1994). Sigma S-dependent growth-phase induction of the *csgBA* promoter in *Escherichia coli* can be achieved *in vivo* by sigma 70 in the absence of the nucleoid-associated protein H-NS. Molecular microbiology *13*, 1021-1032.

Badet, B., Vermoote, P., Haumont, P.Y., Lederer, F., and LeGoffic, F. (1987). Glucosamine synthetase from *Escherichia coli*: purification, properties, and glutamine-utilizing site location. Biochemistry *26*, 1940-1948.

Bansal, T., Alaniz, R.C., Wood, T.K., and Jayaraman, A. (2010). The bacterial signal indole increases epithelial-cell tight-junction resistance and attenuates indicators of inflammation. Proceedings of the National Academy of Sciences of the United States of America *107*, 228-233.

Barnhart, M.M., Lynem, J., and Chapman, M.R. (2006). GlcNAc-6P levels modulate the expression of curli fibers by *Escherichia coli*. Journal of bacteriology *188*, 5212-5219.
Barnich, N., Boudeau, J., Claret, L., and Darfeuille-Michaud, A. (2003). Regulatory and functional co-operation of flagella and type 1 pili in adhesive and invasive abilities of AIEC strain LF82 isolated from a patient with Crohn's disease. Mol Microbiol *48*, 781-794.
Barnich, N., Carvalho, F.A., Glasser, A.L., Darcha, C., Jantscheff, P., Allez, M., Peeters, H., Bommelaer, G., Desreumaux, P., Colombel, J.F.*, et al.* (2007). CEACAM6 acts as a receptor for adherent-invasive *E. coli*, supporting ileal mucosa colonization in Crohn disease. J Clin Invest *117*, 1566-1574.
Begley, M., Gahan, C.G.M., and Hill, C. (2005). The interaction between bacteria and bile. FEMS microbiology reviews *29*, 625-651.
Beloin, C., Roux, A., and Ghigo, J.M. (2008). *Escherichia coli* biofilms. Curr Top Microbiol Immunol *322*, 249-289.
Beloin, C., Valle, J., Latour-Lambert, P., Faure, P., Kzreminski, M., Balestrino, D., Haagensen, J.A., Molin, S., Prensier, G., Arbeille, B.*, et al.* (2004). Global impact of mature biofilm lifestyle on *Escherichia coli* K-12 gene expression. Mol Microbiol *51*, 659-674.
Bettelheim, K.A., and Goldwater, P.N. (2015). *Escherichia coli* and sudden infant death syndrome. Front Immunol *6*, 343.
Bian, Z., Brauner, A., Li, Y., and Normark, S. (2000). Expression of and cytokine activation by *Escherichia coli* curli fibers in human sepsis. J Infect Dis *181*, 602-612.
Bielecki, P., Muthukumarasamy, U., Eckweiler, D., Bielecka, A., Pohl, S., Schanz, A., Niemeyer, U., Oumeraci, T., von Neuhoff, N., Ghigo, J.M.*, et al.* (2014). *In vivo* mRNA profiling of uropathogenic *Escherichia coli* from diverse phylogroups reveals common and group-specific gene expression profiles. MBio *5*, e01075-01014.
Bilge, S.S., Clausen, C.R., Lau, W., and Moseley, S.L. (1989). Molecular characterization of a fimbrial adhesin, F1845, mediating diffuse adherence of diarrhea-associated *Escherichia coli* to HEp-2 cells. J Bacteriol *171*, 4281-4289.
Blount, Z.D. (2015). The unexhausted potential of *E. coli*. eLife *4*.
Boehm, A., Steiner, S., Zaehringer, F., Casanova, A., Hamburger, F., Ritz, D., Keck, W., Ackermann, M., Schirmer, T., and Jenal, U. (2009). Second messenger signalling governs *Escherichia coli* biofilm induction upon ribosomal stress. Mol Microbiol *72*, 1500-1516.
Bokranz, W., Wang, X., Tschäpe, H., and Römling, U. (2005). Expression of cellulose and curli fimbriae by *Escherichia coli* isolated from the gastrointestinal tract. Journal of medical microbiology *54*, 1171-1182.
Bollinger, R.R., Everett, M.L., Wahl, S.D., Lee, Y.H., Orndorff, P.E., and Parker, W. (2006). Secretory IgA and mucin-mediated biofilm formation by environmental strains of *Escherichia coli*: role of type 1 pili. Mol Immunol *43*, 378-387.
Boudeau, J., Barnich, N., and Darfeuille-Michaud, A. (2001). Type 1 pili-mediated adherence of *Escherichia coli* strain LF82 isolated from Crohn's disease is involved in bacterial invasion of intestinal epithelial cells. Mol Microbiol *39*, 1272-1284.
Bougdour, A., Lelong, C., and Geiselmann, J. (2004). Crl, a low temperature-induced protein in *Escherichia coli* that binds directly to the stationary phase sigma subunit of RNA polymerase. Journal of Biological Chemistry *279*, 19540-19550.

Carter, M.Q., Louie, J.W., Feng, D., Zhong, W., and Brandl, M.T. (2016). Curli fimbriae are conditionally required in *Escherichia coli* O157:H7 for initial attachment and biofilm formation. Food Microbiol *57*, 81-89.
Carvalho, F.A., Barnich, N., Sivignon, A., Darcha, C., Chan, C.H., Stanners, C.P., and Darfeuille-Michaud, A. (2009). Crohn's disease adherent-invasive *Escherichia coli* colonize and induce strong gut inflammation in transgenic mice expressing human CEACAM. J Exp Med *206*, 2179-2189.
Carzaniga, T., Antoniani, D., Dehò, G., Briani, F., and Landini, P. (2012). The RNA processing enzyme polynucleotide phosphorylase negatively controls biofilm formation by repressing poly-N-acetylglucosamine (PNAG) production in *Escherichia coli* C. BMC Microbiology *12*, 270.
Cegelski, L., Pinkner, J.S., Hammer, N.D., Cusumano, C.K., Hung, C.S., Chorell, E., Aberg, V., Walker, J.N., Seed, P.C., Almqvist, F.*, et al.* (2009). Small-molecule inhibitors target *Escherichia coli* amyloid biogenesis and biofilm formation. Nat Chem Biol *5*, 913-919.
Cerca, N., and Jefferson, K.K. (2008). Effect of growth conditions on poly-N-acetylglucosamine expression and biofilm formation in *Escherichia coli*. FEMS microbiology letters *283*, 36-41.
Cerca, N., Maira-Litran, T., Jefferson, K.K., Grout, M., Goldmann, D.A., and Pier, G.B. (2007). Protection against *Escherichia coli* infection by antibody to the *Staphylococcus aureus* poly-N-acetylglucosamine surface polysaccharide. Proc Natl Acad Sci U S A *104*, 7528-7533.
Chant, E.L., and Summers, D.K. (2007). Indole signalling contributes to the stable maintenance of *Escherichia coli* multicopy plasmids. Molecular microbiology *63*, 35-43.
Chaudhuri, R.R., and Henderson, I.R. (2012). The evolution of the *Escherichia coli* phylogeny. Infect Genet Evol *12*, 214-226.
Chavez, R.G., Alvarez, A.F., Romeo, T., and Georgellis, D. (2010). The physiological stimulus for the BarA sensor kinase. Journal of bacteriology *192*, 2009-2012.
Chen, X., Schauder, S., Potier, N., Van Dorsselaer, A., Pelczer, I., Bassler, B.L., and Hughson, F.M. (2002). Structural identification of a bacterial quorum-sensing signal containing boron. Nature *415*, 545-549.
Chimerel, C., Emery, E., Summers, D.K., Keyser, U., Gribble, F.M., and Reimann, F. (2014). Bacterial metabolite indole modulates incretin secretion from intestinal enteroendocrine L cells. Cell Reports *9*, 1202-1208.
Chimerel, C., Field, C.M., Piñero-Fernandez, S., Keyser, U.F., and Summers, D.K. (2012). Indole prevents *Escherichia coli* cell division by modulating membrane potential. Biochimica et biophysica acta *1818*, 1590-1594.
Chimerel, C., Murray, A.J., Oldewurtel, E.R., Summers, D.K., and Keyser, U.F. (2013). The effect of bacterial signal indole on the electrical properties of lipid membranes. Chemphyschem : a European journal of chemical physics and physical chemistry *14*, 417-423.
Christensen-Dalsgaard, M., Jørgensen, M.G., and Gerdes, K. (2010). Three new RelE-homologous mRNA interferases of *Escherichia coli* differentially induced by environmental stresses. Molecular microbiology *75*, 333-348.

Chromek, M., Arvidsson, I., and Karpman, D. (2012). The Antimicrobial Peptide Cathelicidin Protects Mice from *Escherichia coli* O157:H7-Mediated Disease. Plos One *7*.
Chu, W., Zere, T.R., Weber, M.M., Wood, T.K., Whiteley, M., Hidalgo-Romano, B., Valenzuela, E., and McLean, R.J.C. (2012). Indole production promotes *Escherichia coli* mixed-culture growth with *Pseudomonas aeruginosa* by inhibiting quorum signaling. Applied and environmental microbiology *78*, 411-419.
Claret, L., Miquel, S., Vieille, N., Ryjenkov, D.A., Gomelsky, M., and Darfeuille-Michaud, A. (2007). The flagellar sigma factor FliA regulates adhesion and invasion of Crohn disease-associated *Escherichia coli* via a cyclic dimeric GMP-dependent pathway. The Journal of biological chemistry *282*, 33275-33283.
Clarke, M.B., and Sperandio, V. (2005). Transcriptional regulation of *flhDC* by QseBC and sigma (FliA) in enterohaemorrhagic *Escherichia coli*. Molecular microbiology *57*, 1734-1749.
Clermont, O., Christenson, J.K., Denamur, E., and Gordon, D.M. (2013). The Clermont *Escherichia coli* phylo-typing method revisited: improvement of specificity and detection of new phylo-groups. Environ Microbiol Rep *5*, 58-65.
Colón-González, M., Méndez-Ortiz, M.M., and Membrillo-Hernández, J. (2004). Anaerobic growth does not support biofilm formation in Escherichia coli K-12. Research in microbiology *155*, 514-521.
Conway, T., and Cohen, P.S. (2015). Commensal and pathogenic *Escherichia coli* metabolism in the gut. Microbiol Spectr *3*.
Corfield, A.P., Myerscough, N., Longman, R., Sylvester, P., Arul, S., and Pignatelli, M. (2000). Mucins and mucosal protection in the gastrointestinal tract: new prospects for mucins in the pathology of gastrointestinal disease. Gut *47*, 589-594.
Costerton, J.W., Stewart, P.S., and Greenberg, E.P. (1999). Bacterial biofilms: a common cause of persistent infections. Science *284*, 1318-1322.
Crawford, R.W., Gibson, D.L., Kay, W.W., and Gunn, J.S. (2008). Identification of a bile-induced exopolysaccharide required for *Salmonella* biofilm formation on gallstone surfaces. Infection and immunity *76*, 5341-5349.
Croxen, M.A., and Finlay, B.B. (2010). Molecular mechanisms of *Escherichia coli* pathogenicity. Nat Rev Microbiol *8*, 26-38.
Croxen, M.A., Law, R.J., Scholz, R., Keeney, K.M., Wlodarska, M., and Finlay, B.B. (2013). Recent advances in understanding enteric pathogenic *Escherichia coli*. Clin Microbiol Rev *26*, 822-880.
Cruz-Cordova, A., Espinosa-Mazariego, K., Ochoa, S.A., Saldana, Z., Rodea, G.E., Cazares-Dominguez, V., Rodriguez-Ramirez, V., Eslava-Campos, C.A., Navarro-Ocana, A., Arrellano-Galindo, J.*, et al.* (2014). CS21 positive multidrug-resistant ETEC clinical isolates from children with diarrhea are associated with self-aggregation, and adherence. Front Microbiol *5*, 709.
Czeczulin, J.R., Balepur, S., Hicks, S., Phillips, A., Hall, R., Kothary, M.H., Navarro-Garcia, F., and Nataro, J.P. (1997). Aggregative adherence fimbria II, a second fimbrial antigen mediating aggregative adherence in enteroaggregative *Escherichia coli*. Infect Immun *65*, 4135-4145.
Dalebroux, Z.D., and Swanson, M.S. (2012). ppGpp: magic beyond RNA polymerase. Nat Rev Microbiol *10*, 203-212.

Danese, P.N., Pratt, L.A., Dove, S.L., and Kolter, R. (2000). The outer membrane protein, antigen 43, mediates cell-to-cell interactions within *Escherichia coli* biofilms. Molecular microbiology *37*, 424-432.
Darfeuille-Michaud, A., Boudeau, J., Bulois, P., Neut, C., Glasser, A.L., Barnich, N., Bringer, M.A., Swidsinski, A., Beaugerie, L., and Colombel, J.F. (2004). High prevalence of adherent-invasive *Escherichia coli* associated with ileal mucosa in Crohn's disease. Gastroenterology *127*, 412-421.
de la Fuente-Nunez, C., Reffuveille, F., Haney, E.F., Straus, S.K., and Hancock, R.E.W. (2014). Broad-Spectrum Anti-biofilm Peptide That Targets a Cellular Stress Response. PLoS pathogens *10*.
De Paepe, M., Gaboriau-Routhiau, V., Rainteau, D., Rakotobe, S., Taddei, F., and Cerf-Bensussan, N. (2011). Trade-off between bile resistance and nutritional competence drives *Escherichia coli* diversification in the mouse gut. PLoS Genetics *7*, e1002107.
Delgado-Nixon, V.M., Gonzalez, G., and Gilles-Gonzalez, M.A. (2000). Dos, a heme-binding PAS protein from *Escherichia coli*, is a direct oxygen sensor. Biochemistry *39*, 2685-2691.
den Besten, G., van Eunen, K., Groen, A.K., Venema, K., Reijngoud, D.J., and Bakker, B.M. (2013). The role of short-chain fatty acids in the interplay between diet, gut microbiota, and host energy metabolism. J Lipid Res *54*, 2325-2340.
Denger, K., Weiss, M., Felux, A.-K., Schneider, A., Mayer, C., Spiteller, D., Huhn, T., Cook, A.M., and Schleheck, D. (2014). Sulphoglycolysis in *Escherichia coli* K-12 closes a gap in the biogeochemical sulphur cycle. Nature *507*, 114-117.
Domka, J., Lee, J., Bansal, T., and Wood, T.K. (2007). Temporal gene-expression in *Escherichia coli* K-12 biofilms. Environmental microbiology *9*, 332-346.
Domka, J., Lee, J., and Wood, T.K. (2006). YliH (BssR) and YceP (BssS) regulate *Escherichia coli* K-12 biofilm formation by influencing cell signaling. Applied and environmental microbiology *72*, 2449-2459.
Dyszel, J.L., Smith, J.N., Lucas, D.E., Soares, J.A., Swearingen, M.C., Vross, M.A., Young, G.M., and Ahmer, B.M.M. (2010a). *Salmonella enterica* serovar Typhimurium can detect acyl homoserine lactone production by *Yersinia enterocolitica* in mice. Journal of bacteriology *192*, 29-37.
Dyszel, J.L., Soares, J.A., Swearingen, M.C., Lindsay, A., Smith, J.N., and Ahmer, B.M.M. (2010b). *E. coli* K-12 and EHEC genes regulated by SdiA. PloS one *5*, e8946.
Ebel, F., Podzadel, T., Rohde, M., Kresse, A.U., Kramer, S., Deibel, C., Guzman, C.A., and Chakraborty, T. (1998). Initial binding of Shiga toxin-producing *Escherichia coli* to host cells and subsequent induction of actin rearrangements depend on filamentous EspA-containing surface appendages. Mol Microbiol *30*, 147-161.
Eckburg, P.B., Bik, E.M., Bernstein, C.N., Purdom, E., Dethlefsen, L., Sargent, M., Gill, S.R., Nelson, K.E., and Relman, D.A. (2005). Diversity of the human intestinal microbial flora. Science *308*, 1635-1638.
Ellermann, M., Huh, E.Y., Liu, B., Carroll, I.M., Tamayo, R., and Sartor, R.B. (2015). Adherent-Invasive *Escherichia coli* Production of Cellulose Influences Iron-Induced Bacterial Aggregation, Phagocytosis, and Induction of Colitis. Infect Immun *83*, 4068-4080.

Elsinghorst, E.A., and Weitz, J.A. (1994). Epithelial cell invasion and adherence directed by the enterotoxigenic *Escherichia coli tib* locus is associated with a 104-kilodalton outer membrane protein. Infect Immun *62*, 3463-3471.
Estrada-Garcia, T., and Navarro-Garcia, F. (2012). Enteroaggregative *Escherichia coli* pathotype: a genetically heterogeneous emerging foodborne enteropathogen. FEMS Immunol Med Microbiol *66*, 281-298.
Etchegaray, J.P., Jones, P.G., and Inouye, M. (1996). Differential thermoregulation of two highly homologous cold-shock genes, *cspA* and *cspB*, of *Escherichia coli*. Genes to cells : devoted to molecular & cellular mechanisms *1*, 171-178.
Farfan, M.J., and Torres, A.G. (2012). Molecular mechanisms that mediate colonization of Shiga toxin-producing *Escherichia coli* strains. Infect Immun *80*, 903-913.
Fleckenstein, J.M., Kopecko, D.J., Warren, R.L., and Elsinghorst, E.A. (1996). Molecular characterization of the *tia* invasion locus from enterotoxigenic *Escherichia coli*. Infect Immun *64*, 2256-2265.
Fredericks, C.E., Shibata, S., Aizawa, S.-I., Reimann, S.A., and Wolfe, A.J. (2006). Acetyl phosphate-sensitive regulation of flagellar biogenesis and capsular biosynthesis depends on the Rcs phosphorelay. Molecular microbiology *61*, 734-747.
Gaastra, W., and Svennerholm, A.M. (1996). Colonization factors of human enterotoxigenic *Escherichia coli* (ETEC). Trends Microbiol *4*, 444-452.
Gaimster, H., Cama, J., Hernández-Ainsa, S., Keyser, U.F., and Summers, D.K. (2014). The Indole Pulse: A New Perspective on Indole Signalling in *Escherichia coli*. PloS one *9*, e93168.
Galisteo, M., Duarte, J., and Zarzuelo, A. (2008). Effects of dietary fibers on disturbances clustered in the metabolic syndrome. The Journal of nutritional biochemistry *19*, 71-84.
Gallo, P.M., Rapsinski, G.J., Wilson, R.P., Oppong, G.O., Sriram, U., Goulian, M., Buttaro, B., Caricchio, R., Gallucci, S., and Tukel, C. (2015). Amyloid-DNA Composites of Bacterial Biofilms Stimulate Autoimmunity. Immunity *42*, 1171-1184.
Garavaglia, M., Rossi, E., and Landini, P. (2012). The pyrimidine nucleotide biosynthetic pathway modulates production of biofilm determinants in *Escherichia coli*. PloS one *7*, e31252.
Gerstel, U., and Römling, U. (2001). Oxygen tension and nutrient starvation are major signals that regulate *agfD* promoter activity and expression of the multicellular morphotype in *Salmonella typhimurium*. Environmental microbiology *3*, 638-648.
Gerstel, U., and Römling, U. (2003). The *csgD* promoter, a control unit for biofilm formation in *Salmonella typhimurium*. Research in microbiology *154*, 659-667.
Giron, J.A., Levine, M.M., and Kaper, J.B. (1994). Longus: a long pilus ultrastructure produced by human enterotoxigenic *Escherichia coli*. Mol Microbiol *12*, 71-82.
González Barrios, A.F., Zuo, R., Hashimoto, Y., Yang, L., Bentley, W.E., and Wood, T.K. (2006). Autoinducer 2 controls biofilm formation in *Escherichia coli* through a novel motility quorum-sensing regulator (MqsR, B3022). Journal of bacteriology *188*, 305-316.

Göpel, Y., Khan, M.A., and Görke, B. (2014). Ménage à trois: post-transcriptional control of the key enzyme for cell envelope synthesis by a base-pairing small RNA, an RNase adaptor protein, and a small RNA mimic. RNA biology *11*, 433-442.
Gordon, D.M., and Cowling, A. (2003). The distribution and genetic structure of *Escherichia coli* in Australian vertebrates: host and geographic effects. Microbiology *149*, 3575-3586.
Grantcharova, N., Peters, V., Monteiro, C., Zakikhany, K., and Römling, U. (2010). Bistable expression of CsgD in biofilm development of Salmonella enterica serovar Typhimurium. Journal of bacteriology *192*, 456-466.
Gualdi, L., Tagliabue, L., Bertagnoli, S., Ieranò, T., De Castro, C., and Landini, P. (2008). Cellulose modulates biofilm formation by counteracting curli-mediated colonization of solid surfaces in *Escherichia coli*. Microbiology (Reading, England) *154*, 2017-2024.
Gualdi, L., Tagliabue, L., and Landini, P. (2007). Biofilm formation-gene expression relay system in *Escherichia coli*: modulation of sigmaS-dependent gene expression by the CsgD regulatory protein via sigmaS protein stabilization. Journal of bacteriology *189*, 8034-8043.
Hamner, S., McInnerney, K., Williamson, K., Franklin, M.J., and Ford, T.E. (2013). Bile Salts Affect Expression of *Escherichia coli* O157:H7 Genes for Virulence and Iron Acquisition, and Promote Growth under Iron Limiting Conditions. PloS one *8*, e74647.
Hancock, V., Dahl, M., and Klemm, P. (2010). Probiotic *Escherichia coli* strain Nissle 1917 outcompetes intestinal pathogens during biofilm formation. Journal of medical microbiology *59*, 392-399.
Hay, A.J., and Zhu, J. (2015). Host intestinal signal-promoted biofilm dispersal induces *Vibrio cholerae* colonization. Infection and immunity *83*, 317-323.
Hazen, T.H., Sahl, J.W., Fraser, C.M., Donnenberg, M.S., Scheutz, F., and Rasko, D.A. (2013). Refining the pathovar paradigm via phylogenomics of the attaching and effacing *Escherichia coli*. Proc Natl Acad Sci U S A *110*, 12810-12815.
Hebbelstrup Jensen, B., Olsen, K.E., Struve, C., Krogfelt, K.A., and Petersen, A.M. (2014). Epidemiology and clinical manifestations of enteroaggregative *Escherichia coli*. Clin Microbiol Rev *27*, 614-630.
Hengge, R. (2009). Principles of c-di-GMP signalling in bacteria. Nat Rev Microbiol *7*, 263-273.
Herold, S., Paton, J.C., Srimanote, P., and Paton, A.W. (2009). Differential effects of short-chain fatty acids and iron on expression of iha in Shiga-toxigenic *Escherichia coli*. Microbiology (Reading, England) *155*, 3554-3563.
Herzberg, M., Kaye, I.K., Peti, W., and Wood, T.K. (2006). YdgG (TqsA) Controls Biofilm Formation in *Escherichia coli* K-12 through Autoinducer 2 Transport. Journal of bacteriology *188*, 587-598.
Hess, J.R., and Greenberg, N.A. (2012). The role of nucleotides in the immune and gastrointestinal systems: potential clinical applications. Nutrition in clinical practice : official publication of the American Society for Parenteral and Enteral Nutrition *27*, 281-294.
Hirakawa, H., Kodama, T., Takumi-Kobayashi, A., Honda, T., and Yamaguchi, A. (2009). Secreted indole serves as a signal for expression of type III secretion system

translocators in enterohaemorrhagic *Escherichia coli* O157:H7. Microbiology *155*, 541-550.
Hobley, L., Harkins, C., MacPhee, C.E., and Stanley-Wall, N.R. (2015). Giving structure to the biofilm matrix: an overview of individual strategies and emerging common themes. FEMS Microbiol Rev *39*, 649-669.
Holmqvist, E., Reimegård, J., Sterk, M., Grantcharova, N., Römling, U., and Wagner, E.G. (2010). Two antisense RNAs target the transcriptional regulator CsgD to inhibit curli synthesis. EMBO J *29*, 1840-1850.
Holmqvist, E., Reimegård, J., and Wagner, E.G. (2013). Massive functional mapping of a 5'-UTR by saturation mutagenesis, phenotypic sorting and deep sequencing. Nucleic Acids Res *41*, e122.
Houot, L., Chang, S., Pickering, B.S., Absalon, C., and Watnick, P.I. (2010). The phosphoenolpyruvate phosphotransferase system regulates *Vibrio cholerae* biofilm formation through multiple independent pathways. J Bacteriol *192*, 3055-3067.
Hsiao, A., Ahmed, A.M.S., Subramanian, S., Griffin, N.W., Drewry, L.L., Petri, W.A., Haque, R., Ahmed, T., and Gordon, J.I. (2014). Members of the human gut microbiota involved in recovery from *Vibrio cholerae* infection. Nature *515*, 423-426.
Hufnagel, D.A., Depas, W.H., and Chapman, M.R. (2015). The Biology of the *Escherichia coli* Extracellular Matrix. Microbiol Spectr *3*.
Hughes, D.T., Terekhova, D.A., Liou, L., Hovde, C.J., Sahl, J.W., Patankar, A.V., Gonzalez, J.E., Edrington, T.S., Rasko, D.A., and Sperandio, V. (2010). Chemical sensing in mammalian host-bacterial commensal associations. Proceedings of the National Academy of Sciences of the United States of America *107*, 9831-9836.
Hung, D.T., Zhu, J., Sturtevant, D., and Mekalanos, J.J. (2006). Bile acids stimulate biofilm formation in *Vibrio cholerae*. Molecular microbiology *59*, 193-201.
Itoh, Y., Rice, J.D., Goller, C., Pannuri, A., Taylor, J., Meisner, J., Beveridge, T.J., Preston, J.F., and Romeo, T. (2008). Roles of *pgaABCD* genes in synthesis, modification, and export of the *Escherichia coli* biofilm adhesin poly-beta-1,6-N-acetyl-D-glucosamine. . Journal of bacteriology *190*, 3670-3680.
Jackson, D.W., Simecka, J.W., and Romeo, T. (2002). Catabolite repression of *Escherichia coli* biofilm formation. J Bacteriol *184*, 3406-3410.
Jenal, U., and Malone, J. (2006). Mechanisms of cyclic-di-GMP signaling in bacteria. Annu Rev Genet *40*, 385-407.
Jeter, C., and Matthysse, A.G. (2005). Characterization of the binding of diarrheagenic strains of *E. coli* to plant surfaces and the role of curli in the interaction of the bacteria with alfalfa sprouts. Mol Plant Microbe Interact *18*, 1235-1242.
Jiang, W., Hou, Y., and Inouye, M. (1997). CspA, the major cold-shock protein of *Escherichia coli*, is an RNA chaperone. Journal of Biological Chemistry *272*, 196-202.
Jiang, Y., Kong, Q., Roland, K.L., Wolf, A., and Curtiss, R., 3rd (2014). Multiple effects of *Escherichia coli* Nissle 1917 on growth, biofilm formation, and inflammation cytokines profile of *Clostridium perfringens* type A strain CP4. Pathogens and disease *70*, 390-400.
Johansson, M.E., Sjövall, H., and Hansson, G.C. (2013). The gastrointestinal mucus system in health and disease. Nature reviews. Gastroenterology & hepatology *10*, 352-361.

Jonas, K., Edwards, A.N., Simm, R., Romeo, T., Römling, U., and Melefors, Ö. (2008). The RNA binding protein CsrA controls cyclic di-GMP metabolism by directly regulating the expression of GGDEF proteins. Mol Microbiol *70*, 236-257.
Kai-Larsen, Y., Lüthje, P., Chromek, M., Peters, V., Wang, X., Holm, A., Kadas, L., Hedlund, K.O., Johansson, J., Chapman, M.R.*, et al.* (2010). Uropathogenic *Escherichia coli* modulates immune responses and its curli fimbriae interact with the antimicrobial peptide LL-37. PLoS pathogens *6*, e1001010.
Kanamaru, K., Kanamaru, K., Tatsuno, I., Tobe, T., and Sasakawa, C. (2000). SdiA, an *Escherichia coli* homologue of quorum-sensing regulators, controls the expression of virulence factors in enterohaemorrhagic *Escherichia coli* O157:H7. Molecular microbiology *38*, 805-816.
Kansal, R., Rasko, D.A., Sahl, J.W., Munson, G.P., Roy, K., Luo, Q., Sheikh, A., Kuhne, K.J., and Fleckenstein, J.M. (2013). Transcriptional modulation of enterotoxigenic *Escherichia coli* virulence genes in response to epithelial cell interactions. Infect Immun *81*, 259-270.
Kaper, J.B., Nataro, J.P., and Mobley, H.L. (2004). Pathogenic *Escherichia coli*. Nat Rev Microbiol *2*, 123-140.
Karch, H., Denamur, E., Dobrindt, U., Finlay, B.B., Hengge, R., Johannes, L., Ron, E.Z., Tonjum, T., Sansonetti, P.J., and Vicente, M. (2012). The enemy within us: lessons from the 2011 European *Escherichia coli* O104:H4 outbreak. EMBO Mol Med *4*, 841-848.
Kasari, V., Kurg, K., Margus, T., Tenson, T., and Kaldalu, N. (2010). The *Escherichia coli mqsR* and *ygiT* genes encode a new toxin-antitoxin pair. Journal of bacteriology *192*, 2908-2919.
Kawamura-Sato, K., Shibayama, K., Horii, T., Iimuma, Y., Arakawa, Y., and Ohta, M. (1999). Role of multiple efflux pumps in *Escherichia coli* in indole expulsion. FEMS microbiology letters *179*, 345-352.
Khan, N.A., Kim, Y., Shin, S., and Kim, K.S. (2007). FimH-mediated *Escherichia coli* K1 invasion of human brain microvascular endothelial cells. Cell Microbiol *9*, 169-178.
Klapproth, J.M., Scaletsky, I.C., McNamara, B.P., Lai, L.C., Malstrom, C., James, S.P., and Donnenberg, M.S. (2000). A large toxin from pathogenic *Escherichia coli* strains that inhibits lymphocyte activation. Infect Immun *68*, 2148-2155.
Klausen, M., Aaes-Jorgensen, A., Molin, S., and Tolker-Nielsen, T. (2003). Involvement of bacterial migration in the development of complex multicellular structures in *Pseudomonas aeruginosa* biofilms. Mol Microbiol *50*, 61-68.
Klemm, P., Hjerrild, L., Gjermansen, M., and Schembri, M.A. (2004). Structure-function analysis of the self-recognizing Antigen 43 autotransporter protein from *Escherichia coli*. Molecular microbiology *51*, 283-296.
Kleta, S., Steinruck, H., Breves, G., Duncker, S., Laturnus, C., Wieler, L.H., and Schierack, P. (2006). Detection and distribution of probiotic *Escherichia coli* Nissle 1917 clones in swine herds in Germany. J Appl Microbiol *101*, 1357-1366.
Kolb, A., Busby, S., Buc, H., Garges, S., and Adhya, S. (1993). Transcriptional Regulation by cAMP and Its Receptor Protein. Annual Review of Biochemistry *62*, 749-797.

Kong, H., Hong, X., and Li, X. (2015). Current perspectivesin pathogenesis and antimicrobial resistance of enteroaggregative *Escherichia coli*. Microb Pathog *85*, 44-49.
Korea, C.G., Badouraly, R., Prevost, M.C., Ghigo, J.M., and Beloin, C. (2010). *Escherichia coli* K-12 possesses multiple cryptic but functional chaperone-usher fimbriae with distinct surface specificities. Environ Microbiol *12*, 1957-1977.
Kostakioti, M., Hadjifrangiskou, M., Pinkner, J.S., and Hultgren, S.J. (2009). QseC-mediated dephosphorylation of QseB is required for expression of genes associated with virulence in uropathogenic *Escherichia coli*. Molecular microbiology *73*, 1020-1031.
Lacanna, E., Bigosch, C., Kaever, V., Boehm, A., and Becker, A. (2016). Evidence for *Escherichia coli* Diguanylate Cyclase DgcZ Interlinking Surface Sensing and Adhesion via Multiple Regulatory Routes. J Bacteriol *198*, 2524-2535.
Lackraj, T., Kim, J.I., Tran, S.-L.L., and Barnett Foster, D. (2016). Differential Modulation of Flagella Expression in Enterohemorrhagic *Escherichia coli* O157:H7 by Intestinal Short Chain Fatty Acid Mixes. Microbiology (Reading, England).
Lacour, S., and Landini, P. (2004). SigmaS-dependent gene expression at the onset of stationary phase in *Escherichia coli*: function of sigmaS-dependent genes and identification of their promoter sequences. Journal of bacteriology *186*, 7186-7195.
Landini, P., and Zehnder, A.J.B. (2002). The global regulatory *hns* gene negatively affects adhesion to solid surfaces by anaerobically grown *Escherichia coli* by modulating expression of flagellar genes and lipopolysaccharide production. Journal of bacteriology *184*, 1522-1529.
Lasaro, M.A., Salinger, N., Zhang, J., Wang, Y., Zhong, Z., Goulian, M., and Zhu, J. (2009). F1C fimbriae play an important role in biofilm formation and intestinal colonization by the *Escherichia coli* commensal strain Nissle 1917. Appl Environ Microbiol *75*, 246-251.
Le Bouguenec, C., and Servin, A.L. (2006). Diffusely adherent *Escherichia coli* strains expressing Afa/Dr adhesins (Afa/Dr DAEC): hitherto unrecognized pathogens. FEMS Microbiol Lett *256*, 185-194.
Lee, J., Jayaraman, A., and Wood, T.K. (2007). Indole is an inter-species biofilm signal mediated by SdiA. BMC Microbiology *7*, 42.
Lee, J., Maeda, T., Hong, S.H., and Wood, T.K. (2009). Reconfiguring the quorum-sensing regulator SdiA of *Escherichia coli* to control biofilm formation via indole and N-acylhomoserine lactones. Applied and environmental microbiology *75*, 1703-1716.
Lee, J., Zhang, X.-S., Hegde, M., Bentley, W.E., Jayaraman, A., and Wood, T.K. (2008). Indole cell signaling occurs primarily at low temperatures in *Escherichia coli*. The ISME journal *2*, 1007-1023.
Lee, K.-A., Kim, S.-H., Kim, E.-K., Ha, E.-M., You, H., Kim, B., Kim, M.-J., Kwon, Y., Ryu, J.-H., and Lee, W.-J. (2013). Bacterial-derived uracil as a modulator of mucosal immunity and gut-microbe homeostasis in *Drosophila*. Cell *153*, 797-811.
Leimbach, A., Hacker, J., and Dobrindt, U. (2013). *E. coli* as an all-rounder: the thin line between commensalism and pathogenicity. Curr Top Microbiol Immunol *358*, 3-32.

Lelong, C., Aguiluz, K., Luche, S., Kuhn, L., Garin, J., Rabilloud, T., and Geiselmann, J. (2007). The Crl-RpoS regulon of *Escherichia coli*. Molecular & cellular proteomics : MCP *6*, 648-659.
Levitt, M.D. (1971). Volume and composition of human intestinal gas determined by means of an intestinal washout technic. The New England journal of medicine *284*, 1394-1398.
Li, G., and Young, K.D. (2013). Indole production by the tryptophanase TnaA in *Escherichia coli* is determined by the amount of exogenous tryptophan. Microbiology *159*, 402-410.
Li, J., Attila, C., Wang, L., Wood, T.K., Valdes, J.J., and Bentley, W.E. (2007). Quorum sensing in *Escherichia coli* is signaled by AI-2/LsrR: effects on small RNA and biofilm architecture. Journal of bacteriology *189*, 6011-6020.
Liaqat, I., and Sakellaris, H. (2012). Biofilm formation and binding specificities of CFA/I, CFA/II and CS2 adhesions of enterotoxigenic *Escherichia coli* and Cfae-R181A mutant. Braz J Microbiol *43*, 969-980.
Lindenberg, S., Klauck, G., Pesavento, C., Klauck, E., and Hengge, R. (2013). The EAL domain protein YciR acts as a trigger enzyme in a c-di-GMP signalling cascade in *E. coli* biofilm control. EMBO J *32*, 2001-2014.
Longo, F., Motta, S., Mauri, P., Landini, P., and Rossi, E. (2016). Interplay of the modified nucleotide phosphoadenosine 5'-phosphosulfate (PAPS) with global regulatory proteins in *Escherichia coli*: modulation of cyclic AMP (cAMP)-dependent gene expression and interaction with the HupA regulatory protein. Chem Biol Interact.
Low, D., Tran, H.T., Lee, I.A., Dreux, N., Kamba, A., Reinecker, H.C., Darfeuille-Michaud, A., Barnich, N., and Mizoguchi, E. (2013). Chitin-binding domains of *Escherichia coli* ChiA mediate interactions with intestinal epithelial cells in mice with colitis. Gastroenterology *145*, 602-612 e609.
Lyte, M., Vulchanova, L., and Brown, D.R. (2011). Stress at the intestinal surface: catecholamines and mucosa-bacteria interactions. Cell and tissue research *343*, 23-32.
MacFabe, D.F. (2012). Short-chain fatty acid fermentation products of the gut microbiome: implications in autism spectrum disorders. Microbial ecology in health and disease *23*.
Macfarlane, G.T., Gibson, G.R., and Cummings, J.H. (1992). Comparison of fermentation reactions in different regions of the human colon. The Journal of applied bacteriology *72*, 57-64.
Maiden, M.C., Jansen van Rensburg, M.J., Bray, J.E., Earle, S.G., Ford, S.A., Jolley, K.A., and McCarthy, N.D. (2013). MLST revisited: the gene-by-gene approach to bacterial genomics. Nat Rev Microbiol *11*, 728-736.
Mandin, P., and Gottesman, S. (2010). Integrating anaerobic/aerobic sensing and the general stress response through the ArcZ small RNA. The EMBO journal *29*, 3094-3107.
Marketon, M.M., Gronquist, M.R., Eberhard, A., and Gonzalez, J.E. (2002). Characterization of the *Sinorhizobium meliloti sinR/sinI* locus and the production of novel N-acyl homoserine lactones. Journal of bacteriology *184*, 5686-5695.

Martinez, J.J., Mulvey, M.A., Schilling, J.D., Pinkner, J.S., and Hultgren, S.J. (2000). Type 1 pilus-mediated bacterial invasion of bladder epithelial cells. EMBO J *19*, 2803-2812.
Martino, P.D., Fursy, R., Bret, L., Sundararaju, B., and Phillips, R.S. (2003). Indole can act as an extracellular signal to regulate biofilm formation of *Escherichia coli* and other indole-producing bacteria. Canadian journal of microbiology *49*, 443-449.
Matthysse, A.G., Deora, R., Mishra, M., and Torres, A.G. (2008). Polysaccharides cellulose, poly-beta-1,6-n-acetyl-D-glucosamine, and colanic acid are required for optimal binding of *Escherichia coli* O157:H7 strains to alfalfa sprouts and K-12 strains to plastic but not for binding to epithelial cells. Appl Environ Microbiol *74*, 2384-2390.
May, T., and Okabe, S. (2008). *Escherichia coli* harboring a natural IncF conjugative F plasmid develops complex mature biofilms by stimulating synthesis of colanic acid and Curli. J Bacteriol *190*, 7479-7490.
Meric, G., Kemsley, E.K., Falush, D., Saggers, E.J., and Lucchini, S. (2013). Phylogenetic distribution of traits associated with plant colonization in *Escherichia coli*. Environ Microbiol *15*, 487-501.
Mika, F., and Hengge, R. (2013). Small Regulatory RNAs in the Control of Motility and Biofilm Formation in *E. coli* and *Salmonella*. Int J Mol Sci *14*, 4560-4579.
Miller, S.T., Xavier, K.B., Campagna, S.R., Taga, M.E., Semmelhack, M.F., Bassler, B.L., and Hughson, F.M. (2004). *Salmonella typhimurium* recognizes a chemically distinct form of the bacterial quorum-sensing signal AI-2. Molecular cell *15*, 677-687.
Mills, E., Petersen, E., Kulasekara, B.R., and Miller, S.I. (2015). A direct screen for c-di-GMP modulators reveals a *Salmonella* Typhimurium periplasmic L-arginine-sensing pathway. Sci Signal *8*, ra57.
Milovic, V. (2001). Polyamines in the gut lumen: bioavailability and biodistribution. European journal of gastroenterology & hepatology *13*, 1021-1025.
Mizoguchi, E. (2006). Chitinase 3-like-1 exacerbates intestinal inflammation by enhancing bacterial adhesion and invasion in colonic epithelial cells. Gastroenterology *130*, 398-411.
Monteiro, C., Papenfort, K., Hentrich, K., Ahmad, I., Le Guyon, S., Reimann, R., Grantcharova, N., and Römling, U. (2012). Hfq and Hfq-dependent small RNAs are major contributors to multicellular development in *Salmonella enterica* serovar Typhimurium. RNA Biol *9*, 489-502.
Monteiro, C., Saxena, I., Wang, X., Kader, A., Bokranz, W., Simm, R., Nobles, D., Chromek, M., Brauner, A., Brown, R.M., Jr.*, et al.* (2009). Characterization of cellulose production in *Escherichia coli* Nissle 1917 and its biological consequences. Environ Microbiol *11*, 1105-1116.
Moreira, S., Brown, A., Ha, R., Iserhoff, K., Yim, M., Yang, J., Liao, B., Pszczolko, E., Qin, W., and Leung, K.T. (2012). Persistence of *Escherichia coli* in freshwater periphyton: biofilm-forming capacity as a selective advantage. FEMS Microbiol Ecol *79*, 608-618.
Na, S.H., Miyanaga, K., Unno, H., and Tanji, Y. (2006). The survival response of *Escherichia coli* K12 in a natural environment. Appl Microbiol Biotechnol *72*, 386-392.

Nagy, A., Mowery, J., Bauchan, G.R., Wang, L., Nichols-Russell, L., and Nou, X. (2015). Role of Extracellular Structures of *Escherichia coli* O157:H7 in Initial Attachment to Biotic and Abiotic Surfaces. Appl Environ Microbiol *81*, 4720-4727.
Nataro, J.P., Deng, Y., Maneval, D.R., German, A.L., Martin, W.C., and Levine, M.M. (1992). Aggregative adherence fimbriae I of enteroaggregative *Escherichia coli* mediate adherence to HEp-2 cells and hemagglutination of human erythrocytes. Infect Immun *60*, 2297-2304.
Nataro, J.P., and Kaper, J.B. (1998). Diarrheagenic *Escherichia coli*. Clin Microbiol Rev *11*, 142-201.
Nataro, J.P., Kaper, J.B., Robins-Browne, R., Prado, V., Vial, P., and Levine, M.M. (1987). Patterns of adherence of diarrheagenic *Escherichia coli* to HEp-2 cells. Pediatr Infect Dis J *6*, 829-831.
Nesse, L.L., Berg, K., and Vestby, L.K. (2015). The effect of norspermidine and spermidine on biofilm formation by potentially pathogenic *Escherichia coli* and *Salmonella enterica* wild type strains. Applied and environmental microbiology *81*, AEM.03518-03514-02232.
Newton, W.A., and Snell, E.E. (1964). Catalytic properties of tryptophanase, a multifunctional pyridoxal phosphate enzyme. Proceedings of the National Academy of Sciences of the United States of America *51*, 382-389.
Ng, K.M., Ferreyra, J.A., Higginbottom, S.K., Lynch, J.B., Kashyap, P.C., Gopinath, S., Naidu, N., Choudhury, B., Weimer, B.C., Monack, D.M.*, et al.* (2013). Microbiota-liberated host sugars facilitate post-antibiotic expansion of enteric pathogens. Nature *502*, 96-99.
Nguyen, Y., Nguyen, N.X., Rogers, J.L., Liao, J., MacMillan, J.B., Jiang, Y., and Sperandio, V. (2015). Structural and mechanistic roles of novel chemical ligands on the SdiA quorum-sensing transcription regulator. mBio *6*, e02429-02414.
Nicholls, L., Grant, T.H., and Robins-Browne, R.M. (2000). Identification of a novel genetic locus that is required for in vitro adhesion of a clinical isolate of enterohaemorrhagic *Escherichia coli* to epithelial cells. Mol Microbiol *35*, 275-288.
Nicklasson, M., Sjöling, A., von Mentzer, A., Qadri, F., and Svennerholm, A.M. (2012). Expression of colonization factor CS5 of enterotoxigenic *Escherichia coli* (ETEC) is enhanced in vivo and by the bile component Na glycocholate hydrate. PLoS One *7*, e35827.
Oberto, J. (2010). FITBAR: a web tool for the robust prediction of prokaryotic regulons. BMC Bioinformatics *11*, 554.
Olsén, A., Arnqvist, A., Hammar, M., and Normark, S. (1993). Environmental regulation of curli production in *Escherichia coli*. Infectious agents and disease *2*, 272-274.
Otto, K., and Silhavy, T.J. (2002). Surface sensing and adhesion of *Escherichia coli* controlled by the Cpx-signaling pathway. Proc Natl Acad Sci U S A *99*, 2287-2292.
Overhage, J., Campisano, A., Bains, M., Torfs, E.C., Rehm, B.H., and Hancock, R.E. (2008). Human host defense peptide LL-37 prevents bacterial biofilm formation. Infect Immun *76*, 4176-4182.
Pannuri, A., Vakulskas, C.A., Zere, T., McGibbon, L.C., Edwards, A.N., Georgellis, D., Babitzke, P., and Romeo, T. (2016). Circuitry linking the catabolite repression and Csr global regulatory systems of *Escherichia coli*. J Bacteriol.

Pawar, V., Crull, K., Komor, U., Kasnitz, N., Frahm, M., Kocijancic, D., Westphal, K., Leschner, S., Wolf, K., Loessner, H*., et al.* (2014). Murine solid tumours as a novel model to study bacterial biofilm formation *in vivo*. Journal of internal medicine.
Pereira, C.S., Santos, A.J.M., Bejerano-Sagie, M., Correia, P.B., Marques, J.C., and Xavier, K.B. (2012a). Phosphoenolpyruvate phosphotransferase system regulates detection and processing of the quorum sensing signal autoinducer-2. Molecular microbiology *84*, 93-104.
Pereira, C.S., Thompson, J.A., and Xavier, K.B. (2012b). AI-2-mediated signalling in bacteria. FEMS microbiology reviews *37*, 156-181.
Pesavento, C., Becker, G., Sommerfeldt, N., Possling, A., Tschowri, N., Mehlis, A., and Hengge, R. (2008). Inverse regulatory coordination of motility and curli-mediated adhesion in *Escherichia coli*. Genes & Development *22*, 2434-2446.
Pesavento, C., and Hengge, R. (2012). The global repressor FliZ antagonizes gene expression by σ S-containing RNA polymerase due to overlapping DNA binding specificity. Nucleic acids research *40*, 4783-4793.
Phadtare, S., and Inouye, M. (2001). Role of CspC and CspE in regulation of expression of RpoS and UspA, the stress response proteins in *Escherichia coli*. Journal of bacteriology *183*, 1205-1214.
Pieper, R., Zhang, Q., Clark, D.J., Parmar, P.P., Alami, H., Suh, M.-J., Kuntumalla, S., Braisted, J.C., Huang, S.-T., and Tzipori, S. (2013). Proteomic View of Interactions of Shiga Toxin-Producing *Escherichia coli* with the Intestinal Environment in Gnotobiotic Piglets. PloS one *8*, e66462.
Piñero-Fernandez, S., Chimerel, C., Keyser, U.F., and Summers, D.K. (2011). Indole transport across *Escherichia coli* membranes. Journal of bacteriology *193*, 1793-1798.
Povolotsky, T.L., and Hengge, R. (2012). 'Life-style' control networks in *Escherichia coli*: signaling by the second messenger c-di-GMP. J Biotechnol *160*, 10-16.
Povolotsky, T.L., and Hengge, R. (2015). Genome-based comparison of c-di-GMP signaling in pathogenic and commensal *Escherichia coli* strains. J Bacteriol.
Pratt, L.A., and Kolter, R. (1998). Genetic analysis of *Escherichia coli* biofilm formation: roles of flagella, motility, chemotaxis and type I pili. Molecular microbiology *30*, 285-293.
Prigent-Combaret, C., Brombacher, E., Vidal, O., Ambert, A., Lejeune, P., Landini, P., and Dorel, C. (2001). Complex regulatory network controls initial adhesion and biofilm formation in *Escherichia coli* via regulation of the *csgD* gene. Journal of bacteriology *183*, 7213-7223.
Prigent-Combaret, C., Prensier, G., Le Thi, T.T., Vidal, O., Lejeune, P., and Dorel, C. (2000). Developmental pathway for biofilm formation in curli-producing *Escherichia coli* strains: role of flagella, curli and colanic acid. Environmental microbiology *2*, 450-464.
Provence, D.L., and Curtiss, R. (1992). Role of *crl* in avian pathogenic *Escherichia coli*: a knockout mutation of *crl* does not affect hemagglutination activity, fibronectin binding, or curli production. Infection and immunity *60*, 4460-4467.

Prüß, B.M., Besemann, C., Denton, A., and Wolfe, A.J. (2006). A complex transcription network controls the early stages of biofilm development by *Escherichia coli*. J Bacteriol *188*, 3731-3739.
Prüß, B.M., Verma, K., Samanta, P., Sule, P., Kumar, S., Wu, J., Christianson, D., Horne, S.M., Stafslien, S.J., Wolfe, A.J.*, et al.* (2010). Environmental and genetic factors that contribute to *Escherichia coli* K-12 biofilm formation. Archives of microbiology *192*, 715-728.
Qadri, F., Svennerholm, A.M., Faruque, A.S., and Sack, R.B. (2005). Enterotoxigenic *Escherichia coli* in developing countries: epidemiology, microbiology, clinical features, treatment, and prevention. Clin Microbiol Rev *18*, 465-483.
Raivio, T.L., Leblanc, S.K., and Price, N.L. (2013). The *Escherichia coli* Cpx envelope stress response regulates genes of diverse function that impact antibiotic resistance and membrane integrity. J Bacteriol *195*, 2755-2767.
Reading, N.C., Rasko, D.A., Torres, A.G., and Sperandio, V. (2009). The two-component system QseEF and the membrane protein QseG link adrenergic and stress sensing to bacterial pathogenesis. Proceedings of the National Academy of Sciences of the United States of America *106*, 5889-5894.
Reaves, M.L., Young, B.D., Hosios, A.M., Xu, Y.-F., and Rabinowitz, J.D. (2013). Pyrimidine homeostasis is accomplished by directed overflow metabolism. Nature *500*, 237-241.
Reid, S.D., Herbelin, C.J., Bumbaugh, A.C., Selander, R.K., and Whittam, T.S. (2000). Parallel evolution of virulence in pathogenic *Escherichia coli*. Nature *406*, 64-67.
Reisner, A., Krogfelt, K.A., Klein, B.M., Zechner, E.L., and Molin, S. (2006). *In vitro* biofilm formation of commensal and pathogenic *Escherichia coli* strains: impact of environmental and genetic factors. J Bacteriol *188*, 3572-3581.
Ren, D., Bedzyk, L.A., Ye, R.W., Thomas, S.M., and Wood, T.K. (2004). Differential gene expression shows natural brominated furanones interfere with the autoinducer-2 bacterial signaling system of *Escherichia coli*. Biotechnology and bioengineering *88*, 630-642.
Ren, D., Zuo, R., González Barrios, A.F., Bedzyk, L.A., Eldridge, G.R., Pasmore, M.E., and Wood, T.K. (2005). Differential gene expression for investigation of *Escherichia coli* biofilm inhibition by plant extract ursolic acid. Applied and environmental microbiology *71*, 4022-4034.
Repoila, F., and Gottesman, S. (2001). Signal transduction cascade for regulation of RpoS: temperature regulation of DsrA. Journal of bacteriology *183*, 4012-4023.
Richter, A.M., Povolotsky, T.L., Wieler, L.H., and Hengge, R. (2014). Cyclic-di-GMP signalling and biofilm-related properties of the Shiga toxin-producing 2011 German outbreak *Escherichia coli* O104:H4. EMBO Mol Med *6*, 1622-1637.
Rinas, U., Hellmuth, K., Kang, R., Seeger, A., and Schlieker, H. (1995). Entry of *Escherichia coli* into stationary phase is indicated by endogenous and exogenous accumulation of nucleobases. Applied and environmental microbiology *61*, 4147-4151.
Roe, A.J., Currie, C., Smith, D.G., and Gally, D.L. (2001). Analysis of type 1 fimbriae expression in verotoxigenic *Escherichia coli*: a comparison between serotypes O157 and O26. Microbiology *147*, 145-152.

Römling, U. (2005). Characterization of the rdar morphotype, a multicellular behaviour in Enterobacteriaceae. Cellular and molecular life sciences : CMLS *62*, 1234-1246.
Römling, U. (2012). Cyclic di-GMP, an established secondary messenger still speeding up. Environ Microbiol *14*, 1817-1829.
Römling, U., Galperin, M.Y., and Gomelsky, M. (2013). Cyclic di-GMP: the first 25 years of a universal bacterial second messenger. Microbiology and molecular biology reviews : MMBR *77*, 1-52.
Römling, U., Sierralta, W.D., Eriksson, K., and Normark, S. (1998). Multicellular and aggregative behaviour of *Salmonella typhimurium* strains is controlled by mutations in the *agfD* promoter. Mol Microbiol *28*, 249-264.
Rossi, E., Motta, S., Mauri, P., and Landini, P. (2014). Sulfate assimilation pathway intermediate phosphoadenosine 5′ -phosphosulfate acts as a signal molecule affecting production of curli fibres in *Escherichia coli*. Microbiology (Reading, England) *160*, 1832-1844.
Rouli, L., Merhej, V., Fournier, P.E., and Raoult, D. (2015). The bacterial pangenome as a new tool for analysing pathogenic bacteria. New Microbes New Infect *7*, 72-85.
Roy, V., Fernandes, R., Tsao, C.-Y., and Bentley, W.E. (2010). Cross species quorum quenching using a native AI-2 processing enzyme. ACS chemical biology *5*, 223-232.
Rund, S.A., Rohde, H., Sonnenborn, U., and Oelschlaeger, T.A. (2013). Antagonistic effects of probiotic *Escherichia coli* Nissle 1917 on EHEC strains of serotype O104:H4 and O157:H7. Int J Med Microbiol *303*, 1-8.
Sabag-Daigle, A., Soares, J.A., Smith, J.N., Elmasry, M.E., and Ahmer, B.M.M. (2012). The acyl homoserine lactone receptor, SdiA, of *Escherichia coli* and *Salmonella enterica* serovar Typhimurium does not respond to indole. Applied and environmental microbiology *78*, 5424-5431.
Sakamoto, A., Terui, Y., Yamamoto, T., Kasahara, T., Nakamura, M., Tomitori, H., Yamamoto, K., Ishihama, A., Michael, A.J., Igarashi, K.*, et al.* (2012). Enhanced biofilm formation and/or cell viability by polyamines through stimulation of response regulators UvrY and CpxR in the two-component signal transducing systems, and ribosome recycling factor. The international journal of biochemistry & cell biology *44*, 1877-1886.
Sakellaris, H., Hannink, N.K., Rajakumar, K., Bulach, D., Hunt, M., Sasakawa, C., and Adler, B. (2000). Curli loci of *Shigella* spp. Infect Immun *68*, 3780-3783.
Saldaña, Z., Xicohtencatl-Cortes, J., Avelino, F., Phillips, A.D., Kaper, J.B., Puente, J.L., and Girón, J.A. (2009). Synergistic role of curli and cellulose in cell adherence and biofilm formation of attaching and effacing *Escherichia coli* and identification of Fis as a negative regulator of curli. Environmental microbiology *11*, 992-1006.
Schauder, S., Shokat, K., Surette, M.G., and Bassler, B.L. (2001). The LuxS family of bacterial autoinducers: biosynthesis of a novel quorum-sensing signal molecule. Molecular microbiology *41*, 463-476.
Schwan, W.R., Shibata, S., Aizawa, S., and Wolfe, A.J. (2007). The two-component response regulator RcsB regulates type 1 piliation in *Escherichia coli*. J Bacteriol *189*, 7159-7163.

Sekowska, A., Kung, H.F., and Danchin, A. (2000). Sulfur metabolism in *Escherichia coli* and related bacteria: facts and fiction. Journal of molecular microbiology and biotechnology *2*, 145-177.
Selander, R.K., and Levin, B.R. (1980). Genetic diversity and structure in *Escherichia coli* populations. Science *210*, 545-547.
Sengupta, C., Ray, S., and Chowdhury, R. (2014). Fine tuning of virulence regulatory pathways in enteric bacteria in response to varying bile and oxygen concentrations in the gastrointestinal tract. Gut Pathogens *6*, 38.
Serra, D.O., and Hengge, R. (2014). Stress responses go three dimensional - the spatial order of physiological differentiation in bacterial macrocolony biofilms. Environmental microbiology *16*, 1455-1471.
Serra, D.O., Mika, F., Richter, A.M., and Hengge, R. (2016). The green tea polyphenol EGCG inhibits *E. coli* biofilm formation by impairing amyloid curli fibre assembly and downregulating the biofilm regulator CsgD via the sigma(E) -dependent sRNA RybB. Mol Microbiol *101*, 136-151.
Servin, A.L. (2005). Pathogenesis of Afa/Dr diffusely adhering *Escherichia coli*. Clin Microbiol Rev *18*, 264-292.
Servin, A.L. (2014). Pathogenesis of human diffusely adhering *Escherichia coli* expressing Afa/Dr adhesins (Afa/Dr DAEC): current insights and future challenges. Clin Microbiol Rev *27*, 823-869.
Sharma, V.K., and Casey, T.A. (2014). *Escherichia coli* O157:H7 lacking the *qseBC*-encoded quorum-sensing system outcompetes the parental strain in colonization of cattle intestines. Applied and environmental microbiology *80*, 1882-1892.
Sharma, V.K., Kudva, I.T., Bearson, B.L., and Stasko, J.A. (2016). Contributions of EspA Filaments and Curli Fimbriae in Cellular Adherence and Biofilm Formation of Enterohemorrhagic *Escherichia coli* O157:H7. PLoS One *11*, e0149745.
Sheikh, J., Czeczulin, J.R., Harrington, S., Hicks, S., Henderson, I.R., Le Bouguenec, C., Gounon, P., Phillips, A., and Nataro, J.P. (2002). A novel dispersin protein in enteroaggregative *Escherichia coli*. J Clin Invest *110*, 1329-1337.
Sherlock, O., Vejborg, R.M., and Klemm, P. (2005). The TibA adhesin/invasin from enterotoxigenic *Escherichia coli* is self recognizing and induces bacterial aggregation and biofilm formation. Infect Immun *73*, 1954-1963.
Sillanaukee, P., Pönniö, M., and Jääskeläinen, I. (1999). Occurrence of sialic acids in healthy humans and different disorders. European Journal of Clinical Investigation *29*, 413-425.
Simm, R., Ahmad, I., Rhen, M., Le Guyon, S., and Römling, U. (2014). Regulation of biofilm formation in *Salmonella enterica* serovar Typhimurium. Future microbiology *9*, 1261-1282.
Sjöling, A., von Mentzer, A., and Svennerholm, A.M. (2015). Implications of enterotoxigenic *Escherichia coli* genomics for vaccine development. Expert Rev Vaccines *14*, 551-560.
Sjöling, A., Wiklund, G., Savarino, S.J., Cohen, D.I., and Svennerholm, A.M. (2007). Comparative analyses of phenotypic and genotypic methods for detection of enterotoxigenic *Escherichia coli* toxins and colonization factors. J Clin Microbiol *45*, 3295-3301.

Sjöstrom, A.E., Sonden, B., Müller, C., Rydström, A., Dobrindt, U., Wai, S.N., and Uhlin, B.E. (2009). Analysis of the *sfaX*(II) locus in the *Escherichia coli* meningitis isolate IHE3034 reveals two novel regulatory genes within the promoter-distal region of the main S fimbrial operon. Microb Pathog *46*, 150-158.
Smith, J.N., Dyszel, J.L., Soares, J.A., Ellermeier, C.D., Altier, C., Lawhon, S.D., Adams, L.G., Konjufca, V., Curtiss, R., Slauch, J.M., *et al.* (2008). SdiA, an N-acylhomoserine lactone receptor, becomes active during the transit of *Salmonella enterica* through the gastrointestinal tract of turtles. PloS one *3*, e2826.
Soares, J.A., and Ahmer, B.M.M. (2011). Detection of acyl-homoserine lactones by *Escherichia* and *Salmonella*. Current opinion in microbiology *14*, 188-193.
Sohanpal, B.K., El-Labany, S., Lahooti, M., Plumbridge, J.A., and Blomfield, I.C. (2004). Integrated regulatory responses of *fimB* to N-acetylneuraminic (sialic) acid and GlcNAc in *Escherichia coli* K-12. Proceedings of the National Academy of Sciences *101*, 16322-16327.
Sommerfeldt, N., Possling, A., Becker, G., Pesavento, C., Tschowri, N., Tschowri, N., and Hengge, R. (2009). Gene expression patterns and differential input into curli fimbriae regulation of all GGDEF/EAL domain proteins in *Escherichia coli*. Microbiology (Reading, England) *155*, 1318-1331.
Sondermann, H., Shikuma, N.J., and Yildiz, F.H. (2012). You've come a long way: c-di-GMP signaling. Curr Opin Microbiol *15*, 140-146.
Sperandio, V., Torres, A.G., Girón, J.A., and Kaper, J.B. (2001). Quorum sensing is a global regulatory mechanism in enterohemorrhagic *Escherichia coli* O157:H7. Journal of bacteriology *183*, 5187-5197.
Sperandio, V., Torres, A.G., Jarvis, B., Nataro, J.P., and Kaper, J.B. (2003). Bacteria-host communication: the language of hormones. Proceedings of the National Academy of Sciences *100*, 8951-8956.
Sperandio, V., Torres, A.G., and Kaper, J.B. (2002). Quorum sensing *Escherichia coli* regulators B and C (QseBC): a novel two-component regulatory system involved in the regulation of flagella and motility by quorum sensing in *E. coli*. Molecular microbiology *43*, 809-821.
Spurbeck, R.R., Tarrien, R.J., and Mobley, H.L. (2012). Enzymatically active and inactive phosphodiesterases and diguanylate cyclases are involved in regulation of Motility or sessility in *Escherichia coli* CFT073. MBio *3*.
Steiner, S., Lori, C., Boehm, A., and Jenal, U. (2013). Allosteric activation of exopolysaccharide synthesis through cyclic di-GMP-stimulated protein-protein interaction. EMBO J *32*, 354-368.
Stülke, J., and Hillen, W. (1998). Coupling Physiology and Gene Regulation in Bacteria: The Phosphotransferase Sugar Uptake System Delivers the Signals. Naturwissenschaften *85*, 583-592.
Sturgill, G., Toutain, C.M., Komperda, J., O'Toole, G.A., and Rather, P.N. (2004). Role of CysE in production of an extracellular signaling molecule in *Providencia stuartii* and *Escherichia coli*: loss of CysE enhances biofilm formation in *Escherichia coli*. Journal of bacteriology *186*, 7610-7617.
Surette, M.G., Miller, M.B., and Bassler, B.L. (1999). Quorum sensing in *Escherichia coli*, *Salmonella typhimurium*, and *Vibrio harveyi*: a new family of genes responsible

for autoinducer production. Proceedings of the National Academy of Sciences of the United States of America *96*, 1639-1644.
Sutrina, S.L., Daniel, K., Lewis, M., Charles, N.T., Anselm, C.K., Thomas, N., and Holder, N. (2015). Biofilm Growth of *Escherichia coli* Is Subject to cAMP-Dependent and cAMP-Independent Inhibition. J Mol Microbiol Biotechnol *25*, 209-225.
Sutrina, S.L., McGeary, T., and Bourne, C.A. (2007). The phosphoenolpyruvate:sugar phosphotransferase system and biofilms in gram-positive bacteria. J Mol Microbiol Biotechnol *12*, 269-272.
Suzuki, K., Wang, X., Weilbacher, T., Pernestig, A.-K., Melefors, Ö., Georgellis, D., Babitzke, P., and Romeo, T. (2002). Regulatory circuitry of the CsrA/CsrB and BarA/UvrY systems of *Escherichia coli*. Journal of bacteriology *184*, 5130-5140.
Szabó, E., Skedsmo, A., Sonnevend, A., Al-Dhaheri, K., Emody, L., Usmani, A., and Pál, T. (2005). Curli expression of enterotoxigenic *Escherichia coli*. Folia Microbiol (Praha) *50*, 40-46.
Taga, M.E., Semmelhack, J.L., and Bassler, B.L. (2001). The LuxS-dependent autoinducer AI-2 controls the expression of an ABC transporter that functions in AI-2 uptake in *Salmonella typhimurium*. Molecular microbiology *42*, 777-793.
Tagliabue, L., Antoniani, D., Maciag, A., Bocci, P., Raffaelli, N., and Landini, P. (2010a). The diguanylate cyclase YddV controls production of the exopolysaccharide poly-N-acetylglucosamine (PNAG) through regulation of the PNAG biosynthetic *pgaABCD* operon. Microbiology (Reading, England) *156*, 2901-2911.
Tagliabue, L., Maciag, A., Antoniani, D., and Landini, P. (2010b). The *yddV-dos* operon controls biofilm formation through the regulation of genes encoding curli fibers' subunits in aerobically growing *Escherichia coli*. FEMS immunology and medical microbiology *59*, 477-484.
Tenaillon, O., Skurnik, D., Picard, B., and Denamur, E. (2010). The population genetics of commensal *Escherichia coli*. Nat Rev Microbiol *8*, 207-217.
Terui, Y., Akiyama, M., Sakamoto, A., Tomitori, H., Yamamoto, K., Ishihama, A., Igarashi, K., and Kashiwagi, K. (2012). Increase in cell viability by polyamines through stimulation of the synthesis of ppGpp regulatory protein and ω protein of RNA polymerase in *Escherichia coli*. The international journal of biochemistry & cell biology *44*, 412-422.
Thijs, I.M., Zhao, H., De Weerdt, A., Engelen, K., De Coster, D., Schoofs, G., McClelland, M., Vanderleyden, J., Marchal, K., and De Keersmaecker, S.C.J. (2010). The AI-2-dependent regulator LsrR has a limited regulon in *Salmonella Typhimurium*. Cell research *20*, 966-969.
Thomason, M.K., Fontaine, F., De Lay, N., and Storz, G. (2012). A small RNA that regulates motility and biofilm formation in response to changes in nutrient availability in *Escherichia coli*. Molecular microbiology *84*, 17-35.
Touchon, M., Hoede, C., Tenaillon, O., Barbe, V., Baeriswyl, S., Bidet, P., Bingen, E., Bonacorsi, S., Bouchier, C., Bouvet, O*., et al.* (2009). Organised genome dynamics in the *Escherichia coli* species results in highly diverse adaptive paths. PLoS Genet *5*, e1000344.

Tuckerman, J.R., Gonzalez, G., and Gilles-Gonzalez, M.-A. (2011). Cyclic di-GMP activation of polynucleotide phosphorylase signal-dependent RNA processing. Journal of Molecular Biology *407*, 633-639.
Tuckerman, J.R., Gonzalez, G., Sousa, E.H.S., Wan, X., Saito, J.A., Alam, M., and Gilles-Gonzalez, M.-A. (2009). An oxygen-sensing diguanylate cyclase and phosphodiesterase couple for c-di-GMP control. Biochemistry *48*, 9764-9774.
Tükel, C., Nishimori, J.H., Wilson, R.P., Winter, M.G., Keestra, A.M., van Putten, J.P., and Bäumler, A.J. (2010). Toll-like receptors 1 and 2 cooperatively mediate immune responses to curli, a common amyloid from enterobacterial biofilms. Cell Microbiol *12*, 1495-1505.
Ueda, A., Attila, C., Whiteley, M., and Wood, T.K. (2009). Uracil influences quorum sensing and biofilm formation in *Pseudomonas aeruginosa* and fluorouracil is an antagonist. Microbial biotechnology *2*, 62-74.
Uhlich, G.A., Chen, C.Y., Cottrell, B.J., Hofmann, C.S., Dudley, E.G., Strobaugh, T.P., Jr., and Nguyen, L.H. (2013). Phage insertion in *mlrA* and variations in *rpoS* limit curli expression and biofilm formation in *Escherichia coli* serotype O157: H7. Microbiology *159*, 1586-1596.
Uhlich, G.A., Chen, C.Y., Cottrell, B.J., Hofmann, C.S., Yan, X., and Nguyen, L. (2016). Stx1 prophage excision in *Escherichia coli* strain PA20 confers strong curli and biofilm formation by restoring native *mlrA*. FEMS Microbiol Lett *363*.
Uhlich, G.A., Keen, J.E., and Elder, R.O. (2001). Mutations in the *csgD* promoter associated with variations in curli expression in certain strains of *Escherichia coli* O157:H7. Appl Environ Microbiol *67*, 2367-2370.
Ulett, G.C., Valle, J., Beloin, C., Sherlock, O., Ghigo, J.-M., and Schembri, M.A. (2007). Functional analysis of antigen 43 in uropathogenic *Escherichia coli* reveals a role in long-term persistence in the urinary tract. Infection and immunity *75*, 3233-3244.
van der Ploeg, J.R., Eichhorn, E., and Leisinger, T. (2001). Sulfonate-sulfur metabolism and its regulation in *Escherichia coli*. Archives of Microbiology *176*, 1-8.
van Elsas, J.D., Semenov, A.V., Costa, R., and Trevors, J.T. (2011). Survival of *Escherichia coli* in the environment: fundamental and public health aspects. ISME J *5*, 173-183.
Van Houdt, R., Aertsen, A., Moons, P., Vanoirbeek, K., and Michiels, C.W. (2006). N-acyl-L-homoserine lactone signal interception by *Escherichia coli*. FEMS Microbiol Lett *256*, 83-89.
Varki, N.M., and Varki, A. (2007). Diversity in cell surface sialic acid presentations: implications for biology and disease. Laboratory investigation; a journal of technical methods and pathology *87*, 851-857.
Velarde, J.J., Varney, K.M., Inman, K.G., Farfan, M., Dudley, E., Fletcher, J., Weber, D.J., and Nataro, J.P. (2007). Solution structure of the novel dispersin protein of enteroaggregative *Escherichia coli*. Mol Microbiol *66*, 1123-1135.
Verstraete, K., K, D.E.R., S, V.A.N.W., Pierard, D., L, D.E.Z., Herman, L., Robyn, J., and Heyndrickx, M. (2013). Genetic characteristics of Shiga toxin-producing *E. coli* O157, O26, O103, O111 and O145 isolates from humans, food, and cattle in Belgium. Epidemiol Infect *141*, 2503-2515.

Vogeleer, P., Tremblay, Y.D., Mafu, A.A., Jacques, M., and Harel, J. (2014). Life on the outside: role of biofilms in environmental persistence of Shiga-toxin producing *Escherichia coli*. Front Microbiol *5*, 317.
von Mentzer, A., Connor, T.R., Wieler, L.H., Semmler, T., Iguchi, A., Thomson, N.R., Rasko, D.A., Joffre, E., Corander, J., Pickard, D.*, et al.* (2014). Identification of enterotoxigenic *Escherichia coli* (ETEC) clades with long-term global distribution. Nat Genet *46*, 1321-1326.
Walters, M., Sircili, M.P., and Sperandio, V. (2006). AI-3 synthesis is not dependent on *luxS* in *Escherichia coli*. J Bacteriol *188*, 5668-5681.
Wang, D., Ding, X., and Rather, P.N. (2001). Indole can act as an extracellular signal in *Escherichia coli*. Journal of bacteriology *183*, 4210-4216.
Wang, N., Yamanaka, K., and Inouye, M. (1999). CspI, the ninth member of the CspA family of *Escherichia coli*, is induced upon cold shock. Journal of bacteriology *181*, 1603-1609.
Wang, X., Dubey, A.K., Suzuki, K., Baker, C.S., Babitzke, P., and Romeo, T. (2005). CsrA post-transcriptionally represses *pgaABCD*, responsible for synthesis of a biofilm polysaccharide adhesin of *Escherichia coli*. Molecular microbiology *56*, 1648-1663.
Wang, X., Preston, J.F., and Romeo, T. (2004). The *pgaABCD* locus of *Escherichia coli* promotes the synthesis of a polysaccharide adhesin required for biofilm formation. Journal of bacteriology *186*, 2724-2734.
Wang, X., Rochon, M., Lamprokostopoulou, A., Lünsdorf, H., Nimtz, M., and Römling, U. (2006). Impact of biofilm matrix components on interaction of commensal *Escherichia coli* with the gastrointestinal cell line HT-29. Cellular and molecular life sciences : CMLS *63*, 2352-2363.
Wang, X.D., de Boer, P.A., and Rothfield, L.I. (1991). A factor that positively regulates cell division by activating transcription of the major cluster of essential cell division genes of *Escherichia coli*. The EMBO journal *10*, 3363-3372.
Watnick, P., and Kolter, R. (2000). Biofilm, city of microbes. J Bacteriol *182*, 2675-2679.
Weissman, S.J., Chattopadhyay, S., Aprikian, P., Obata-Yasuoka, M., Yarova-Yarovaya, Y., Stapleton, A., Ba-Thein, W., Dykhuizen, D., Johnson, J.R., and Sokurenko, E.V. (2006). Clonal analysis reveals high rate of structural mutations in fimbrial adhesins of extraintestinal pathogenic *Escherichia coli*. Mol Microbiol *59*, 975-988.
Wen, Y., Behiels, E., and Devreese, B. (2014). Toxin-Antitoxin systems: their role in persistence, biofilm formation, and pathogenicity. Pathogens and disease *70*, 240-249.
White-Ziegler, C.A., Um, S., Perez, N.M., Berns, A.L., Malhowski, A.J., and Young, S. (2008). Low temperature (23 degrees C) increases expression of biofilm-, cold-shock- and RpoS-dependent genes in *Escherichia coli* K-12. Microbiology *154*, 148-166.
Whitehead, N.A., Barnard, A.M.L., Slater, H., Simpson, N.J.L., and Salmond, G.P.C. (2001). Quorum‐sensing in Gram‐negative bacteria. FEMS microbiology reviews *25*, 365-404.
Wikoff, W.R., Anfora, A.T., Liu, J., Schultz, P.G., Lesley, S.A., Peters, E.C., and Siuzdak, G. (2009). Metabolomics analysis reveals large effects of gut microflora on mammalian

blood metabolites. Proceedings of the National Academy of Sciences of the United States of America *106*, 3698-3703.
Wolfe, A.J., Chang, D.-E., Walker, J.D., Seitz-Partridge, J.E., Vidaurri, M.D., Lange, C.F., Prüß, B.M., Henk, M.C., Larkin, J.C., and Conway, T. (2003). Evidence that acetyl phosphate functions as a global signal during biofilm development. Molecular microbiology *48*, 977-988.
Xavier, K.B., and Bassler, B.L. (2003). LuxS quorum sensing: more than just a numbers game. Current opinion in microbiology *6*, 191-197.
Xavier, K.B., and Bassler, B.L. (2005a). Interference with AI-2-mediated bacterial cell-cell communication. Nature *437*, 750-753.
Xavier, K.B., and Bassler, B.L. (2005b). Regulation of uptake and processing of the quorum-sensing autoinducer AI-2 in *Escherichia coli*. Journal of bacteriology *187*, 238-248.
Xicohtencatl-Cortes, J., Monteiro-Neto, V., Saldana, Z., Ledesma, M.A., Puente, J.L., and Giron, J.A. (2009). The type 4 pili of enterohemorrhagic *Escherichia coli* O157:H7 are multipurpose structures with pathogenic attributes. J Bacteriol *191*, 411-421.
Yamaguchi, Y., Park, J.-H., and Inouye, M. (2009). MqsR, a crucial regulator for quorum sensing and biofilm formation, is a GCU-specific mRNA interferase in *Escherichia coli*. The Journal of biological chemistry *284*, 28746-28753.
Yang, K., Meng, J., Huang, Y.-c., Ye, L.-h., Li, G.-j., Huang, J., and Chen, H.-m. (2014). The role of the QseC quorum-sensing sensor kinase in epinephrine-enhanced motility and biofilm formation by *Escherichia coli*. Cell biochemistry and biophysics *70*, 391-398.
Yanofsky, C., Horn, V., and Gollnick, P. (1991). Physiological studies of tryptophan transport and tryptophanase operon induction in *Escherichia coli*. Journal of bacteriology *173*, 6009-6017.
Yao, Y., Dickerson, T.J., Hixon, M.S., and Dyson, H.J. (2007). NMR detection of adventitious xylose binding to the quorum-sensing protein SdiA of *Escherichia coli*. Bioorganic & medicinal chemistry letters *17*, 6202-6205.
Yao, Y., Martinez-Yamout, M.A., Dickerson, T.J., Brogan, A.P., Wright, P.E., and Dyson, H.J. (2006). Structure of the *Escherichia coli* quorum sensing protein SdiA: activation of the folding switch by acyl homoserine lactones. Journal of Molecular Biology *355*, 262-273.
Yaron, S., and Römling, U. (2014). Biofilm formation by enteric pathogens and its role in plant colonization and persistence. Microbial biotechnology *7*, 496-516.
Zargar, A., Quan, D.N., Carter, K.K., Guo, M., Sintim, H.O., Payne, G.F., and Bentley, W.E. (2015). Bacterial secretions of nonpathogenic *Escherichia coli* elicit inflammatory pathways: a closer investigation of interkingdom signaling. mBio *6*, e00025.
Zheng, D., Constantinidou, C., Hobman, J.L., and Minchin, S.D. (2004). Identification of the CRP regulon using in vitro and in vivo transcriptional profiling. Nucleic acids research *32*, 5874-5893.
Zhu, J., and Pei, D. (2008). A LuxP-based fluorescent sensor for bacterial autoinducer II. ACS chemical biology *3*, 110-119.

Figure legends

Fig. 1 Typical biofilm models as formed by commensal *E. coli* strains. The commensal strains were previously isolated from the faeces of healthy humans (Bokranz *et al*., 2005). A: Macrocolony biofilm appearance on agar plates. Upper panel: Rdar (red, dry and rough) biofilm morphotype of strain Fec51 (left) and saw (smooth and white) non-biofilm forming Fec75 (right) grown at 28°C for 72 h on plates supplemented with 40 µg/ml Congo Red and 20 µg/ml Coomassie Brilliant Blue G. Fec51 rdar biofilm formation is due to the production of an extracellular matrix consisting of curli fimbriae and cellulose (Römling, 2005). In contrast, Fec75 does not express these components and expresses a saw morphotype. Expression of cellulose can also be visualized on Calcofluor supplemented (50 µg/ml) LB agar plates without salt (lower panel). Fec51 fluoresces after excitation with 365 nm UV light. CR: Congo Red; CF: Calcofluor (Fluorescent brightener 28).
B: Pellicle formation at the air-liquid interface in standing culture. Top: pellicle of commensal strain Fec101, down: commensal strain Fec75 (negative control). Bacterial strains were grown in a 96 well plate in LB without salt medium at 28°C for 48h.
C: Biofilm formation in liquid culture as exemplified by clumping and adherence to abiotic glass surface. Fec101 was grown in LB without salt medium at 28°C for 18h with shaking at 200rpm. Under these growth conditions, extensive clumping and adherence to wall of well is observed (left). To stain the attached cells, the liquid was removed and the biofilm stained with 0.4% crystal violet for 10 min (right).
D: Biofilm formation of commensal *E. coli* TOB1 on HT-29 epithelial cells; left, adherence of a biofilm producing mutant; right, adherence of a non-biofilm producing mutant ((Wang et al., 2006), picture: Heinrich Lünsdorf).

Fig 2 Biofilm formation of *E. coli* in the gastrointestinal tract. A. *E. coli* forms biofilms in the environment potentially following the common biofilm developmental pathway as depicted. Attachment and biofilm formation to produce causes food-borne outbreaks. A motile cell (A) is attaching reversibly (B) to a surface. After becoming irreversibly attached (C) a microcolony is formed, expressing an extracellular matrix (D). After full maturation of the biofilm (E), dispersion takes places in that motile cells (F) or microcolonies (G) leave the biofilm. B. Biofilm formation of *E. coli* on the intestinal epithelium. Commensal *E. coli* are mainly present in the mucus layer of the colon establishing biofilms by formation of an extracellular matrix consisting of curli and cellulose. Enterohemorrhagic *E. coli* (EHEC) attach to the epithelial cells via the use *E. coli* common pilus ECP, hemorrhagic pilus HCP, as well as mucin binding flagella and other adhesins. Intimate attachment is facilitated by interaction of intimin with the translocated Tir receptor or intimin-host nucleolin interaction, followed by pedestal formation and attaching and effacing (A/E) lesions. Enteroinvasive *E. coli* (EIEC) pass both mucus layers and invade the epithelial cells. Enteroaggregative *E. coli* EAEC form a biofilm using aggregative adherence Dr-fimbriae (AAF) as well as dispersin. Typical enteropathogenic *E. coli* tEPEC form A/E lesions as well as pedestals similar to EHEC. The EspA filament promotes initial attachment, the adhesin LifA facilitates attachment along with bundle-forming pili (BFP), supported by *E. coli* common pilus (ECP) and mucin binding flagella. Intimate attachment is mediated by Tir-intimin interaction.

Enterotoxigenic *E. coli* (ETEC) adhere to the epithelial lining via the adhesin EtpA exposed at the flagellar tip and colonization factors (CFs) of different kinds. Further, the secreted protein CexE might have a similar function as dispersin in EAEC. Intimate attachment occurs by protein based adhesion via Tia and TibA. Diffusely adherent *E. coli* DAEC adhere non-localized to the epithelial cells through (Dr-) fimbrial and afimbrial (Afa) adhesins. Adherent-invasive *E. coli* (AIEC) attach by type I pili supported by flagella. Further, adhesion is mediated by interaction of the bacterial chitinase ChiA with the host chitinase 3-like-1 (CHI3L1) via chitin binding domains. Long polar fimbriae promote invasion.

Fig 3 The phylogroups of *E. coli* and their correlation to pathotypes. The phylogroups A and B1 are sister groups with a common origin. Pathogens that induce watery diarrhea such as ETEC, EPEC and EAEC often belong to these phylogroups. The apparently most successful lineages of UPEC and EHEC O157/H7 belong to phylogroups B2 and E, respectively. There are numerous exceptions and individual ETEC, EPEC EAEC, and UPEC strains, as well as successful lineages such as ETEC O169 and EHEC O111 and O26 may belong to different phyologroups. However, there might be advantages assigned with certain chromosomal backgrounds that fit better with certain virulence factors than others and thus shape virulence including biofilm formation, transmission pathways and infection capabilities. The lines are not to scale and do not represent the genetic differences between phylogroups.

Fig 4 The regulatory network involved in adhesive structure production in the gastrointestinal (GI) tract. Extracellular and intracellular signals that are involved in biofilm formation of commensal *E. coli* strains in the GI tract. The figure only represents a selection of the many cues that might affect biofilm formation. For clarity, some regulatory mechanisms, such as those depending on small RNAs, have been omitted. A. Bacterial-derived signals. *E. coli* senses and communicates using auto-released signals, known as quorum sensing (QS) molecules: AI-1 can be perceived but not synthesized, while AI-2 and AI-3 are also synthesized by specific pathways. B. Environmental and metabolic signals deriving from metabolism of either *E. coli* or gut microbiota or eukaryotic cells. Solid lines: experimentally verified; dashed lines: unknown mechanism if connecting two regulators, or intermediate reactions omitted if connecting metabolites. green lines: positive effectors, red lines: negative effectors, orange lines: post-transcriptional stimulatory effect. Cellulose and PNAG production are represented by dotted lines as not all *E. coli* isolates synthesize them.

Fig 5 Interconnection of second messenger signalling cAMP, c-di-GMP and ppGpp signalling to regulate biofilm formation in *E. coli*. For more detailed description, see text. TA=Toxin/Antitoxin system.

Figure 1

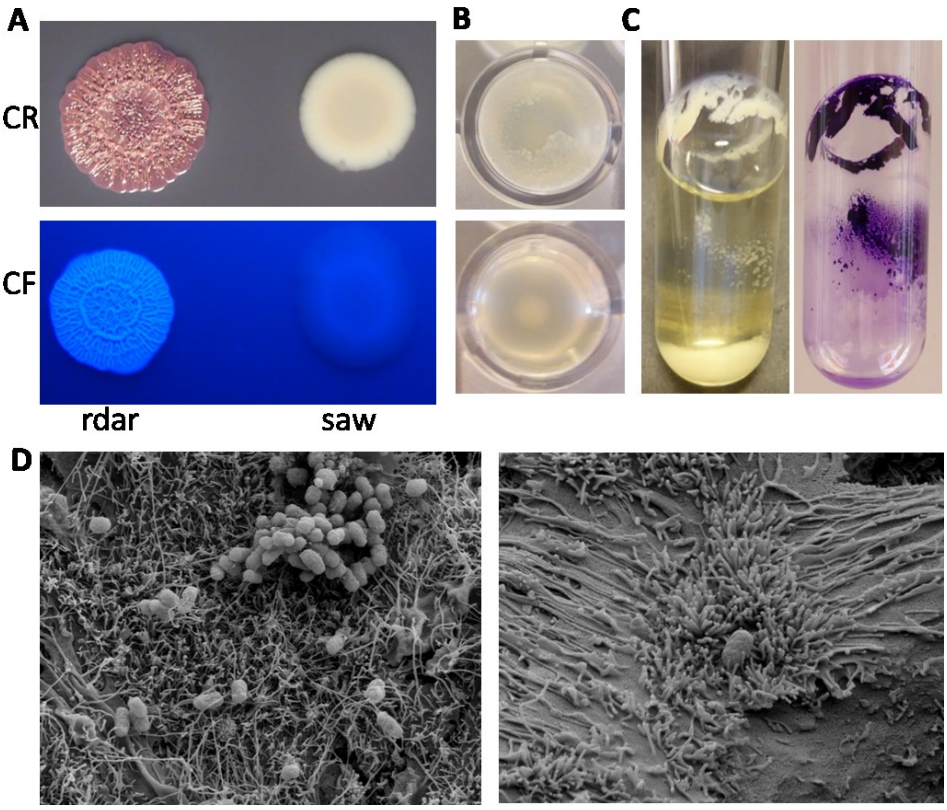


Figure 2

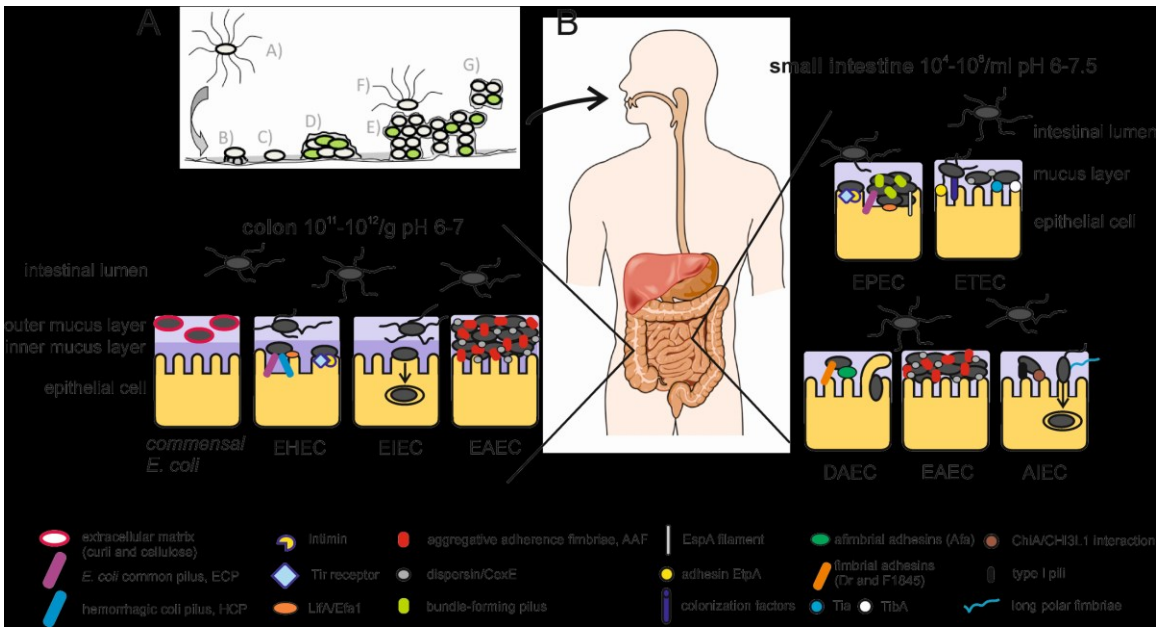

A
A)
B)
C)
D)
E)
F)
G)
B
small intestine 10⁴-10⁵/ml pH 6-7.5
intestinal lumen
mucus layer
epithelial cell
EPEC
ETEC
colon 10¹¹-10¹²/g pH 6-7
intestinal lumen
outer mucus layer
inner mucus layer
epithelial cell
commensal E. coli
EHEC
EIEC
EAEC
DAEC
EAEC
AIEC
extracellular matrix (curli and cellulose)
E. coli common pilus, ECP
hemorrhagic coli pilus, HCP
intimin
Tir receptor
Lifa/Efa1
aggregative adherence fimbriae, AAF
dispersin/CexE
bundle-forming pilus
EspA filament
adhesin EtpA
colonization factors
afimbrial adhesins (Afa)
fimbrial adhesins (Dr and F1845)
Tia
TibA
ChiA/CHI3L1 interaction
type I pili
long polar fimbriae

Figure 3

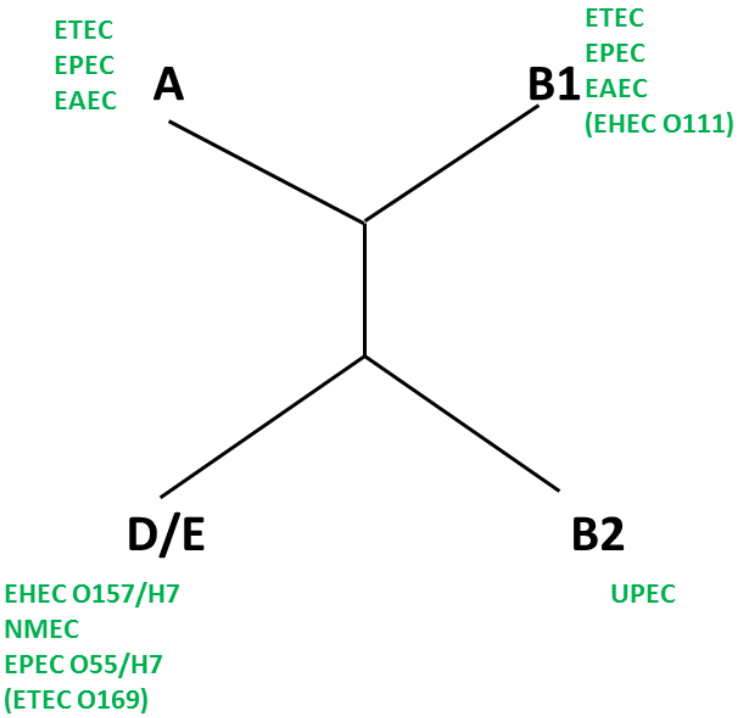

ETEC
EPEC
EAEC
A
B1
ETEC
EPEC
EAEC
(EHEC O111)
D/E
B2
EHEC O157/H7
NMEC
EPEC O55/H7
(ETEC O169)
UPEC

Figure 4

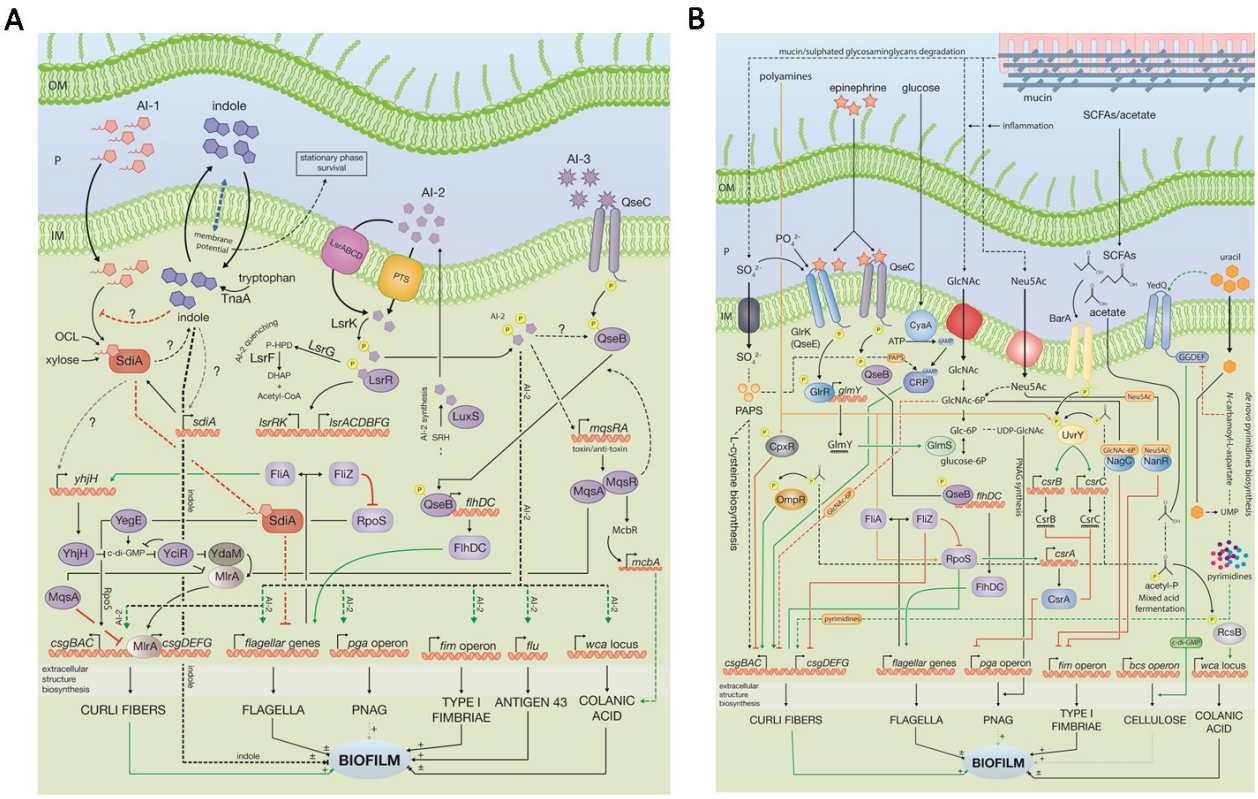
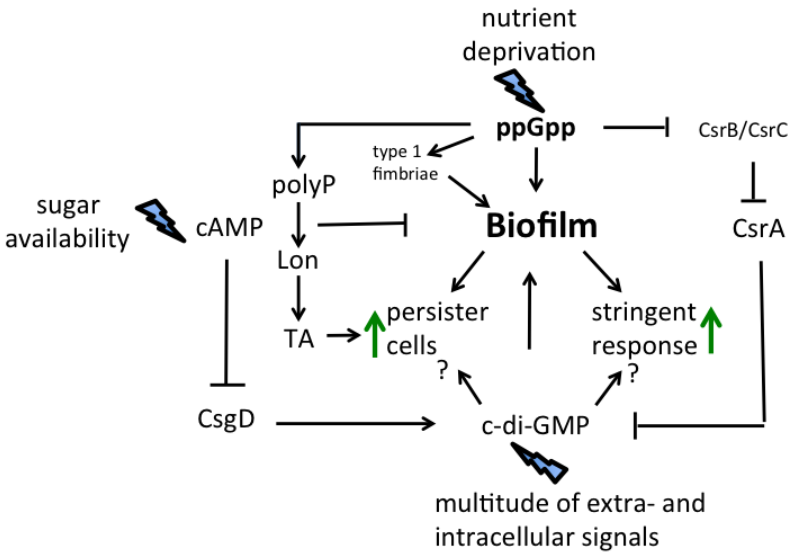

A
OM
IM
P
AI-1
indole
stationary phase survival
AI-2
AI-3
QseC
membrane potential
LsrABCD
PTS
tryptophan
TnaA
indole
OCL
xylose
SdiA
LsrK
LsrG
LsrF
LsrR
AI-2 quenching
P-HPD
DHAP
Acetyl-CoA
AI-2 synthesis
LuxS
SRH
QseB
sdiA
lsrRK
lsrACDBFG
mqsRA
toxin/anti-toxin
MqsA
MqsR
McbR
mcbA
yhjH
FliA
FliZ
YegE
RpoS
flhDC
YhjH
c-di-GMP
YciR
YdaM
MlrA
csgBAC
csgDEFG
flagellar genes
pga operon
fim operon
flu
wca locus
extracellular structure biosynthesis
CURLI FIBERS
FLAGELLA
PNAG
TYPE I FIMBRIAE
ANTIGEN 43
COLANIC ACID
BIOFILM
B
mucin/sulphated glycosaminglycans degradation
polyamines
epinephrine
glucose
mucin
inflammation
SCFAs/acetate
PO4 2-
SO4 2-
QseC
GlcNAc
Neu5Ac
SCFAs
uracil
YedQ
acetate
BarA
GlrK (QseE)
ATP
CyaA
cAMP
CRP
PAPS
QseB
GlrR
glmY
GlcNAc-6P
Neu5Ac
GGDEF
Glc-6P
UDP-GlcNAc
UvrY
CpxR
GlmY
GlmS
glucose-6P
NagC
NanR
OmpR
FliA
FliZ
RpoS
FlhDC
csrB
csrC
csrA
CsrA
acetyl-P
Mixed acid fermentation
pyrimidines
RcsB
c-di-GMP
L-cysteine biosynthesis
PAPS synthesis
de novo pyrimidines biosynthesis
N-carbamoyl-L-aspartate
UMP
csgBAC
csgDEFG
flagellar genes
pga operon
fim operon
bcs operon
wca locus
CURLI FIBERS
FLAGELLA
PNAG
TYPE I FIMBRIAE
CELLULOSE
COLANIC ACID
BIOFILM

Figure 5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polyP
sugar
availability
cAMP
Biofilm
CsrA
Lon
TA
persister
cells
stringent
response
?
?
CsgD
c-di-GMP
multitude of extra- and
intracellular signals