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Valentin Ageorges, Ricardo Monteiro, Sabine Leroy, Catherine Burgess, Mariagrazia Pizza, et al.. Molecular Determinants of Surface Colonisation in Diarrhoeagenic *Escherichia coli* (DEC): from Bacterial Adhesion to Biofilm Formation. *FEMS Microbiology Reviews*, 2020, 175, pp.105996. 10.1093/femsre/fuaa008 . hal-02532777

HAL Id: hal-02532777

<https://hal.inrae.fr/hal-02532777v1>

Submitted on 30 Aug 2023

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Molecular Determinants of Surface Colonisation in Diarrhoeagenic *Escherichia coli* (DEC): from Bacterial Adhesion to Biofilm Formation

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Runnig title: Surface colonisation factors in diarrhoeagenic *Escherichia coli*

Keywords: Biofilm formation, Pathogenic *Escherichia coli*, Surface colonisation, Bacterial adhesion, Surface proteins, Exopolymers.

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1 **Abstract**

2 *Escherichia coli* is primarily known as a commensal colonising the
3 gastrointestinal tract of infants very early in life but some strains being responsible for
4 diarrhoea, which can be especially severe in young children. Intestinal pathogenic
5 *E. coli* include six pathotypes of diarrhoeagenic *E. coli* (DEC), namely the (i)
6 enterotoxigenic *E. coli*, (ii) enteroaggregative *E. coli*, (iii) enteropathogenic *E. coli*, (iv)
7 enterohemorrhagic *E. coli*, (v) enteroinvasive *E. coli*, and (vi) diffusely-adherent *E. coli*.
8 Prior to human infection, DEC can be found in natural environments, animal reservoirs,
9 food processing environments and contaminated food matrices. From an
10 ecophysiological point of view, DEC thus deal with very different biotopes and
11 biocoenoses all along the food chain. In this context, this review focuses on the wide
12 range of surface molecular determinants acting as surface colonisation factors (SCFs)
13 in DEC. In the first instance, SCFs can be broadly discriminated into (i) extracellular
14 polysaccharides, (ii) extracellular DNA, and (iii) surface proteins. Surface proteins
15 constitute the most diverse group of SCFs broadly discriminated into (i) monomeric
16 SCFs, such as autotransporter (AT) adhesins, inverted ATs, heat-resistant agglutinins
17 or some moonlighting proteins, (ii) oligomeric SCFs, namely the trimeric ATs, and (iii)
18 supramolecular SCFs, including flagella and numerous pili, e.g. the injectisome, type 4
19 pili, curli chaperone-usher pili or conjugative pili. This review also details the gene
20 regulatory network of these numerous SCFs at the various stages as it occurs from pre-
21 transcriptional to post-translocational levels, which remains to be fully elucidated in
22 many cases.

23 **One-sentence summary**

24 Diarrhoeagenic *Escherichia coli* (DEC) express numerous surface colonisation
25 factors contributing to their contamination of the food chain, from natural
26 environments, animal reservoirs, food processing environments to food matrices, and
27 ultimately, human infection.

PRE-PRINT

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88 **Introduction**

89 Most recent phylogenetic analyses have revealed that the *Escherichia* genus is
90 subdivided into eight groups containing three species, namely *Escherichia coli*,
91 *E. fergusonii*, and *E. albertii*, as well as five clades numbered from I to V (Lawrence &
92 Hartl, 1991, Walk *et al.*, 2009). *E. coli* is undoubtedly the most investigated bacterial
93 species and is used as a model organism in microbiology. This lipopolysaccharidic
94 (LPS) diderm bacterium (archetypical Gram-negative bacterium) is primarily known as
95 a harmless commensal of the gastrointestinal tract (GIT) (Mason & Richardson, 1981,
96 Chagnot *et al.*, 2013). While *E. coli* is prevalently an inhabitant of the gut of warm-
97 blooded animals, especially mammals but also birds, it is worth mentioning this
98 bacterial species can also be isolated from fish, frogs or reptiles, such as crocodiles,
99 turtles or snakes, but also insects, such as flies (Janisiewicz *et al.*, 1999, Souza *et al.*,
100 1999, Gordon & Cowling, 2003, Escobar-Paramo *et al.*, 2006, Blazar *et al.*, 2011);
101 *E. coli* generally appears more prevalent in herbivores and omnivores than carnivores.
102 In humans, *E. coli* colonises the GIT of young children early in life and usually
103 represents less than 1 % of the human intestinal microbiota in adults (Eckburg *et al.*,
104 2005).

105 Nevertheless, some *E. coli* species possess some virulence factors that enable
106 them to cause a broad range of human extraintestinal and intestinal infections. On one
107 side extraintestinal pathogenic *E. coli* (ExPEC) mainly comprises the uropathogenic
108 *E. coli* (UPEC), neonatal meningitis *E. coli* (NMEC), necrotoxic *E. coli* (NTEC) and
109 sepsis-associated *E. coli* (SEPEC). On the other side, and in addition to the adherent
110 invasive *E. coli* (AIEC) associated with Crohn's disease (Mann & Saeed, 2012), the
111 intestinal pathogenic *E. coli* (InPEC) essentially encompasses six pathotypes of
112 diarrhoeagenic *E. coli* (DEC), namely the (i) enterotoxigenic *E. coli* (ETEC), (ii)

113 enteroaggregative *E. coli* (EAEC), (iii) enteropathogenic *E. coli* (EPEC), (iv)
114 enterohemorrhagic *E. coli* (EHEC), (v) enteroinvasive *E. coli* (EIEC), and (vi) diffusely-
115 adherent *E. coli* (DAEC) (Kaper *et al.*, 2004, Croxen & Finlay, 2010); of note, EHEC
116 belong to the larger group of shigatoxin-encoding *E. coli* (STEC), or shigatoxin-
117 producing *E. coli*, which are not all considered as pathogenic as they can exhibit very
118 various virulence levels ranging from avirulence to hyper-virulence (Karmali *et al.*,
119 2003, Laing *et al.*, 2009, Monteiro *et al.*, 2016). The pathogenicity of DEC strains is
120 well documented and their main virulence factors are also well defined (Croxen &
121 Finlay, 2010). Some of these pathotypes are not restricted to human infections, but can
122 be responsible for diarrhoea in animals, for instance (i) ETEC in porcines (piglets),
123 bovines (calves) or ovines (lambs), (ii) EPEC in rabbits, dogs, cats, pigs, calves, lambs
124 and goats, and (iii) STEC in calves and piglets (Beutin, 1999, DebRoy & Maddox,
125 2001); to date, EAEC, EIEC and DAEC have not been reported as etiological agents of
126 diarrhoea in animals. Despite the high genome plasticity demonstrating intensive gene
127 flow, the population structure of *E. coli* remains mostly clonal (Touchon *et al.*, 2009),
128 with a clear delineation into seven principal phylogenetic groups (A, B1, B2, C, D, E
129 and F) (Jauregui *et al.*, 2008, Walk *et al.*, 2009, Tenaillon *et al.*, 2010, Clermont *et al.*,
130 2013, Beghain *et al.*, 2018). Commensal *E. coli* strains generally belong to phylogroup
131 A, whereas DEC usually belong to phylogroups A, B1, C, D and E (Jauregui *et al.*,
132 2008, Okeke *et al.*, 2010, Croxen *et al.*, 2013, Hazen *et al.*, 2016, Rossi *et al.*, 2018):
133 (i) ETEC can be found in phylogroups A and B1 and to lesser extent in D, (ii) EAEC
134 are found within phylogroup A but also B1, D and to a smaller extend in B2, (iii) EPEC
135 can belong to phylogroups E and B2, (iv) EHEC strains are mostly found in
136 phylogroups B1 and D but also in E (with the with serotype O157:H7 or O104:H4), (v)
137 EIEC are mainly present in phylogroups A, B1 and E, together with *Shigella*, which are

138 essentially *E. coli* species from phylogenetic and taxonomic perspectives (Brenner *et*
139 *al.*, 1972, Lan & Reeves, 2002, Chaudhuri & Henderson, 2012, Pettengill *et al.*, 2015),
140 and (vi) DAEC which mostly belong to phylogroups B2 and D (Servin, 2014, Mosquito
141 *et al.*, 2015, Walczuk *et al.*, 2019). This distinct grouping suggests a parallel evolution
142 of the different pathotypes on multiple occasions, possibly with the intervention of
143 mobile elements enabling the acquisition of specific combinations of virulence factors
144 (Chaudhuri & Henderson, 2012, Croxen *et al.*, 2013).

145 DEC can be found all along the food chain (Giaouris *et al.*, 2014, Kim *et al.*,
146 2017). They can have various environmental reservoirs, such as ruminants for EHEC,
147 and are mainly transmitted to humans by the faecal-oral route through the consumption
148 of contaminated food, including water, or contact with contaminated surfaces (Croxen
149 *et al.*, 2013). Besides anthroozoonosis, transmission can also occur from host to host
150 between humans. In any case, the colonisation of the food chain by DEC is a major
151 issue for the agri-food and public health sectors alike. The surface colonisation process
152 can occur *via* bacterial adhesion and/or biofilm formation to various biotic or abiotic
153 surfaces. When the reversible adhesion to the surface by low energy linkages (e.g.
154 electrostatics, Van der Waals interactions) is overcome, some bacteria can grow at the
155 surface. As such, biofilm formation can be broadly defined as the sessile development
156 of microorganisms at a surface or interface (Azeredo *et al.*, 2017). Biofilm can be
157 monospecies but are more generally multispecies in the natural environment, forming
158 a complex multicellular community, which is often embedded in an exopolymeric
159 matrix (EPM) (Costerton, 1995, Costerton *et al.*, 1999). It confers to bacterial cells an
160 increased resistance against environmental stress, antibiotics and/or immunological
161 defences of the host. Once the reversible adhesion is overcome, the bacterial biofilm
162 formation is *per se* divided in several steps: (i) initial and irreversible adhesion of

163 bacterial cells to the surface, (ii) bacterial division at the site of adhesion resulting in
164 the formation of microcolonies, (iii) maturation of the biofilm architecture into a three-
165 dimensional structure, and (iv) bacterial dispersion enabling the colonisation of other
166 sites (O'Toole *et al.*, 2000, Hall-Stoodley & Stoodley, 2002). Biofilm formation can
167 thus plays a key role in DEC ecophysiology by enabling colonisation of various
168 environmental niches (soil, water, vegetables, agri-food surfaces, *etc...*), the
169 asymptomatic and direct colonisation of some hosts, as well as contributing to
170 transmission through the food chain and ultimately human infection (Ahmed *et al.*,
171 2013).

172 Most information about the colonisation process in *E. coli* is focused on the
173 domesticated laboratory strain K12, commonly considered as representative of the
174 *E. coli* species (Beloin *et al.*, 2008). However, this notion is biased due to the numerous
175 and very significant genotypic and/or phenotypic differences with commensal and
176 pathogenic *E. coli* isolates (Hobman *et al.*, 2007). Indeed, *E. coli* K12 has one of the
177 smallest genomes compared to other genome-sequenced strains of *E. coli* due to the
178 loss of a large variety of genes during its domestication (Lenski, 2017). With regards
179 to the selective pressures that shapes the genome evolution, *E. coli* K12 have been
180 replicated and studied for a long time under laboratory conditions, far from those
181 encountered in natural environments (Hobman *et al.*, 2007); some molecular
182 determinants, including some surface colonisation factors (SCFs), could thus be lacking
183 or misregulated in domesticated laboratory strains of *E. coli* compared to commensal
184 and pathogenic *E. coli* isolates. As the interface between the bacterial cell and its
185 surroundings, the molecular surface determinants are key players in the initial adhesion
186 and sessile development processes and this review aims at summarising exhaustively
187 the SCFs present in DEC. The complexity of the regulation network occurring at

188 various stages, from pre-transcriptional to post-translocational levels, is also
189 highlighted. A greater understanding of the parameters that influence adhesion and
190 biofilm formation may inform the development of interventions to minimise DEC
191 dissemination in the food chain, from the environment, animal, food, to human.

192 **1. Molecular determinants involved in surface colonisation by** 193 **DEC**

194 The colonisation processes along the food chain, from natural environments,
195 such as soil, plants and animals, to food environments, including the industrial
196 processing food chain and food matrices, and ultimately infection or asymptomatic
197 carriage in human, are very complex and involves many molecular determinants.
198 Sessile development at a surface or interface is generally accompanied by the formation
199 of an EPM embedding the bacterial cells in biofilms (Figure 1). These exopolymers can
200 act as glue for adherence of the bacterial cell to the support and shape the architecture
201 of the biofilm (Hobley *et al.*, 2015). Furthermore, the EPM provides protection by
202 shielding the bacteria from desiccation and antimicrobial compounds but also
203 participates in the channelling of nutrients and signalling molecules (Sutherland, 2001,
204 Starkey *et al.*, 2004, Beloin *et al.*, 2008). As such, the EPM contribute to the survival
205 strategy and persistence of bacteria in various environmental conditions (Branda *et al.*,
206 2005). Molecular determinants participating in the surface colonisation by DEC can
207 either be closely associated with the bacterial cell surface and form the cell-associated
208 EPM (caEPM) or present in the extracellular milieu, namely the interstitial EPM
209 (iEPM) (Figure 1) (van Houdt & Michiels, 2005).

210 At a biochemical level, EPM components can be broadly discriminated between
211 (i) extracellular polysaccharides (EPS), (ii) extracellular DNA (eDNA), and (iii) surface
212 proteins. Depending on the different DEC pathotypes, these various determinants can
213 be either present or absent (Table 1). Outer membrane vesicles (OMVs) have been
214 reported to be components of the EPM in *E. coli* K12 (Schooling & Beveridge, 2006)
215 and their presence in biofilm from DEC is likely, although it remains to be
216 demonstrated. To date, there is no report of their contribution to biofilm formation in
217 DEC, as observed in *Pseudomonas aeruginosa* or *Helicobacter pylori* (Yonezawa *et*
218 *al.*, 2009, Wang *et al.*, 2015), but it is an aspect that would deserve further investigation
219 in DEC. Of note, poly- γ -glutamate (PGA) can be found as a component of the EPM of
220 numerous bacteria, especially parietal monoderm bacteria (archetypical Gram-positive
221 bacteria) and only a few LPS-diderm bacteria, where it can either be released or cell-
222 surface attached to form a capsule (Candela & Fouet, 2006, Ogunleye *et al.*, 2015,
223 Radchenkova *et al.*, 2018) but, to date, this has never been reported in any *E. coli* strain.

224 **1.1. Exopolysaccharides (EPS)**

225 EPS are one of the main components of the EPM in *E. coli* biofilms (Beloin *et*
226 *al.*, 2008). DEC can biosynthesise a variety of EPS, namely (i) lipopolysaccharide
227 (LPS), (ii) poly- β -1,6-N-acetyl-D-glucosamine (PNAG), (iii) colanic acid, and (iv)
228 cellulose. Because of their intimate association with the bacterial cell surface, several
229 of these EPS can contribute to the caEPM and the formation of a so-called capsule.
230 Actually, *E. coli* harbours some serotype-specific polysaccharides, namely
231 lipopolysaccharides (LPS) (O antigen) and capsular polysaccharides (K antigen).
232 *E. coli* capsules are composed of high-molecular weight polysaccharides embedding
233 the bacterial cells and linked to the cell-surface *via* covalent attachments (Whitfield,

234 2006). More than 80 capsular antigens have been reported in *E. coli*, which are divided
235 into four groups, from G1 to G4 (Whitfield, 2006, Yaron & Romling, 2014). DEC
236 (including EPEC, ETEC and EHEC) produce G1 and G4 capsules that share a common
237 assembly system and can be associated with the lipid A of LPS (K_{LPS}) or be structurally
238 similar to the O-polysaccharides of the LPS (O-antigen capsules). During an infection,
239 these capsules allow bacteria to be protected from opsonophagocytosis and
240 complement-mediated killing (Whitfield, 2006). In EHEC O104:H4, the capsule has
241 been shown to play a role in bacterial survival in the environment and in direct bacterial
242 interaction with plants (Jang & Matthews, 2018).

243 **1.1.1. Lipopolysaccharide (LPS)**

244 LPS is located at the outer leaflet of the outer membrane (OM) and part of the
245 caEPM (Raetz & Whitfield, 2002). This glycolipidic polymer is formed around a toxic
246 component, lipid A, and for this reason is also considered an endotoxin; the LPS is
247 further composed of the core region linked to the lipid A (divided into an inner and
248 outer part) and the O-antigen that is linked to the outer part of the core region (Raetz &
249 Whitfield, 2002). Biosynthesis and assembly pathways of LPS have been fully
250 described and involve more than 50 genes encoded in operons or monocistrons
251 scattered on the bacterial chromosome (Sandkvist, 2001, Szalo *et al.*, 2006). The
252 structures of lipid A and its core region are highly conserved in *E. coli* but the core
253 region has five basic structures, called R1, R2, R3, R4 and K12. Among these, R1 is
254 the most prevalent in non-STEC clinical isolates of *E. coli* and R3 is more associated
255 with STEC strains (Gibb *et al.*, 1992, Appelmelk *et al.*, 1994, Currie & Poxton, 1999,
256 Amor *et al.*, 2000). In *E. coli* clinical isolates, R1 is most prevalent, whilst the K12 core
257 is not detected (Gibb *et al.*, 1992, Appelmelk *et al.*, 1994). More than 170 O-antigens

258 have been identified and consist of 10–25 repeating units containing one to eight sugar
259 residues (Stenutz *et al.*, 2006). The O-antigen can be present (smooth LPS, also called
260 S-LPS or LPS I, resulting in colonies with a smooth phenotype) or absent (rough LPS,
261 also called R-LPS or LPS II, resulting in colonies with a rough phenotype) depending
262 on the *E. coli* strain; if the core region is also absent, it is called deep-rough LPS
263 (Hitchcock *et al.*, 1986). Smooth strains are the most commonly found in nature,
264 including in DEC, whereas the rough phenotype is more commonly found in laboratory
265 strains (Whitfield & Keenleyside, 1995, Nataro & Kaper, 1998). For smooth strains,
266 the LPS length is positively correlated with the force of adhesion (Strauss *et al.*, 2009).
267 The O-antigen assists adhesion through hydrogen binding (Tomme *et al.*, 1996). For
268 example, it has been demonstrated that the O-antigen enables EHEC O157:H7 strains
269 to colonise animal hosts (Sheng *et al.*, 2008). Mutations in LPS biosynthesis genes have
270 been shown to affect the adhesion of *E. coli* to abiotic surfaces and its biofilm formation
271 ability (Bilge *et al.*, 1996, Genevaux *et al.*, 1999, Landini & Zehnder, 2002, Beloin *et*
272 *al.*, 2006). Additionally, LPS can promote or inhibit biofilm formation by two distinct
273 mechanisms, mainly by interacting with cell-surface-exposed adhesion factors. It has
274 been shown that alteration of LPS synthesis can impair type 1 pili and colanic acid
275 expression as well as bacterial motility, whereas the reduction in LPS expression may
276 unmask *E. coli* adhesins and thus promote adhesion or biofilm formation as observed
277 for EHEC O157:H7 strain (Bilge *et al.*, 1996, Beloin *et al.*, 2006, Beloin *et al.*, 2008).

278 **1.1.2. Poly-N-acetyl glucosamine (PNAG)**

279 PNAG is an EPS attached to the bacterial surface and is involved in biofilm
280 formation on abiotic surfaces (Wang *et al.*, 2004). The biosynthetic pathway for PNAG
281 is encoded by the *pgaABCD* locus (formerly *ycdSRQP*). Initiation of PNAG production

282 occurs with the PgaDC, a glycosyl transferase localised on the cytoplasmic side of the
283 inner membrane that uses the UDP-N-acetyl-D-glucosamine as substrate (Wang *et al.*,
284 2004, Itoh *et al.*, 2005, 2008). The PNAG polymer is exported and anchored to the
285 bacterial surface through the β -barrel formed by two outer membrane proteins (OMPs),
286 namely PgaB and PgaA. Although PNAG forms a surface capsule and is one of the
287 main components of the caEPM in diverse bacterial biofilm, the *pga* locus is not present
288 in all *E. coli* strains (Cerca *et al.*, 2007, Cimdins *et al.*, 2017). In DEC, PNAG plays a
289 role in the stabilisation of biofilm architecture (Wang *et al.*, 2004, Al Safadi *et al.*,
290 2012). It has been demonstrated to be important for biofilm formation of EHEC on
291 sprouts and tomato roots (Matthyse *et al.*, 2008). *In vivo* expression of *pgaA* during
292 infection by EHEC O104:H4 suggests that biofilm formation is a key step in
293 pathogenesis (Al Safadi *et al.*, 2012). PNAG is also expressed by some ETEC strains
294 and often induced by conditions found in the environment (Gonzales-Siles & Sjolting,
295 2016).

296 **1.1.3. Colanic acid**

297 Colanic acid is a negatively charged polymer of glucose, galactose, fucose, and
298 glucuronic acid produced by most *E. coli* strains, including DEC (Obadia *et al.*, 2007).
299 The *wca* operon (or *cps*) encodes 19 proteins including polymerases involved in colanic
300 acid synthesis from sugar residues (Stevenson *et al.*, 1996). Colanic acid actually forms
301 the G1 capsule but a significant portion of the colanic acid produced can also be
302 released into the extracellular milieu to contribute to the iEPM (Whitfield & Roberts,
303 1999, Beloin *et al.*, 2008, Beloin *et al.*, 2008). The exact contribution of colanic acid to
304 biofilm formation is still unclear (Matthyse *et al.*, 2008, May & Okabe, 2008).
305 Nonetheless, it forms a physical barrier that helps bacteria to survive outside the host

306 with the formation of a protective capsule around the bacterial cell. This capsule allows
307 *E. coli* biofilms to resist osmotic and oxidative stresses as well as to temperature
308 variations (Whitfield & Roberts, 1999, Chen *et al.*, 2004). In EHEC O157:H7, it has
309 been shown to play a role in the bacterial survival in simulated GIT fluids (Mao *et al.*,
310 2006). In EAEC, the presence of colanic acid has been linked with the formation of
311 large biofilm structures on the surface of sprouts (Borgersen *et al.*, 2018). In contrast,
312 the production of colanic acid could also mask some cell-surface adhesins and
313 consequently impair initial adhesion to some supports (Hanna *et al.*, 2003, Schembri *et*
314 *al.*, 2004, Beloin *et al.*, 2008).

315 **1.1.4. Cellulose**

316 Cellulose is a linear homopolysaccharide composed of D-glucopyranose units
317 linked by β -1 \rightarrow 4 glycosidic bonds. While this widespread biopolymer is generally
318 related to plant biology, it is also present in the iEPM in some bacterial species where
319 it plays a role in protection, maturation and structure of the biofilm (Solano *et al.*, 2002,
320 Ude *et al.*, 2006). In *E. coli*, cellulose biosynthesis genes are located in two operons,
321 namely *bcsQABZC* and *bcsEFG* (Zogaj *et al.*, 2001, Solano *et al.*, 2002, Le Quere &
322 Ghigo, 2009). The cellulose synthase is formed by BcsAB, which catalyses cellulose
323 biosynthesis from UDP-glucose subunits and forms a transmembrane pore across the
324 inner membrane for cellulose export prior to secretion across the OM *via* a β -barrel
325 pore formed by BcsC (Keiski *et al.*, 2010, Omadjela *et al.*, 2013). The role of the
326 *bcsEFG* operon is still unclear but its presence is necessary for cellulose production
327 (Solano *et al.*, 2002). These genes are found in both commensal and pathogenic *E. coli*
328 strains (Beloin *et al.*, 2008). Although cellulose production is essential for biofilm
329 maturation, over-production negatively impacts biofilm formation and bacterial

330 aggregation, possibly by coating and thus masking the adhesive properties of surface
331 proteins such as curli (Gualdi *et al.*, 2008). In EHEC O157:H7 and EPEC O127:H6
332 cellulose production has been shown to contribute to biofilm formation, and
333 consequently, host colonisation and survival in different environments (Saldana *et al.*,
334 2009). The involvement of cellulose in *E. coli* colonisation of plant materials has also
335 been demonstrated but it depends on the vegetable, as its presence seems dispensable
336 for biofilm formation by *E. coli* O157:H7 to spinach leaves, but it is required for
337 bacterial adhesion to alfalfa sprouts (Matthysse *et al.*, 2008, Macarisin *et al.*, 2012).
338 Expression of these genes in some ETEC strains is often induced at ambient
339 temperatures, low ionic strength and nutrient limitation (Bokranz *et al.*, 2005, Szabo *et*
340 *al.*, 2005).

341 **1.2. Extracellular DNA (eDNA)**

342 The importance of eDNA in biofilm maturation has been demonstrated in
343 numerous bacterial species (Muto & Goto, 1986, Kadurugamuwa & Beveridge, 1995,
344 Steinberger *et al.*, 2002), including *E. coli* (Xi & Wu, 2010, Nakao *et al.*, 2012). As a
345 component of the iEPM, eDNA serves as structural component of the biofilm but can
346 also contribute to a cation gradient, as a nutrient source, induce antibiotic resistance,
347 and aid horizontal gene transfer (Bockelmann *et al.*, 2006, Palchevskiy & Finkel, 2006,
348 Sanchez-Torres *et al.*, 2011). However, the role of eDNA in DEC strains remains to be
349 elucidated. The molecular mechanism explaining the presence of eDNA has been a
350 subject of investigation for some time but essentially results from the release of
351 genomic DNA upon cell lysis, following the bacteriophage lytic cycle or bacterial cell
352 apoptosis (Palmen & Hellingwerf, 1995, Steinmoen *et al.*, 2002, Qin *et al.*, 2007).
353 Nonetheless, the lysis of outer membrane vesicles (OMVs) containing DNA

354 (Kadurugamuwa & Beveridge, 1996, Whitchurch *et al.*, 2002), as well as DNA
355 secretion through the conjugative Type IV, subtype b, secretion system (T4bSS)
356 (Hamilton *et al.*, 2005, Chagnot *et al.*, 2013) could also contribute to the presence of
357 eDNA. The extent and respective contribution of these different mechanisms to the
358 presence of eDNA would undoubtedly require further investigations, especially in
359 DEC, also considering the impact of the apparent presence of pancreatic nuclease in the
360 intestine (Maturin & Curtiss, 1977).

361 **1.3. Cell-surface proteins**

362 The cell surface of LPS-diderm bacteria can display a number of proteins
363 associated with the OM. Proteinaceous determinants found at the bacterial cell surface
364 and acting as SCFs can be broadly discriminated into (i) monomeric proteins, (ii)
365 multimeric proteins (Figure 2).

366 In the scientific literature, *E. coli* adhesins have generally been discriminated
367 between fimbrial and afimbrial (or non-fimbrial). However, and as with animal
368 classification, a group is much better defined by features that are present rather than by
369 the absence of some features. As such, the term afimbrial adhesins does not tell
370 anything about the nature of these adhesins. In addition, some afimbrial adhesins later
371 appeared to be atypical fimbriae secreted by the same family of protein secretion
372 system, e.g. the CS31A (coli surface associated 31a antigen) pili (Adams *et al.*, 1997).
373 For these reasons, we here propose to regroup those cell-surface proteins under the term
374 of monomeric proteinaceous adhesins, or monomeric proteinaceous colonisation
375 factors. Besides, the term fimbriae is not very well defined across the Bacteria kingdom
376 when considering different bacterial species. On the contrary, the term pili can be used
377 as a generic term encompassing the various type of pili and fimbriae, including curli or

378 injectisome. In addition, some cell-surface appendages contributing to surface
379 colonisation in bacteria cannot be categorised as fimbrial adhesins *per se*, e.g. the
380 flagella and the trimeric autotransporters. To avoid any ambiguity, these different cell-
381 surface appendages are proposed to be regrouped under the term of multimeric
382 proteinaceous colonisation factors.

383 **1.3.1. Monomeric proteinaceous surface colonisation factors**

384 In *E. coli*, monomeric protein acting as SCFs include some autotransporters
385 (ATs), inverted autotransporters (IATs), and some OMPs, but also the surface-exposed
386 lipoprotein SslE, Efa-1 (*E. coli* factor adherence 1), dispersin, as well as some
387 moonlighting proteins. Of note, the ATs (also sometimes called classical ATs) only
388 belong to the Type V, subtype a, secretion system (T5aSS) and correspond to
389 monomeric polypeptides with modular organisation into at least three main regions, i.e.
390 (i) a N-terminal signal peptide, (ii) a central passenger, and (iii) a translocator at the C-
391 terminus (Desvaux *et al.*, 2003, Desvaux *et al.*, 2004, Leo *et al.*, 2012). ATs (T5aSS)
392 should not be mistaken with the trimeric ATs, hybrid ATs and inverted ATs, which
393 belong the T5sSS, T5dSS and T5eSS, respectively.

394 *1.3.1.1. Autotransporters (ATs)*

395 Classical ATs acting as SCFs comprise the autotransporter adhesins (ATAs),
396 the self-associating autotransporters (SAATs), and some serine protease
397 autotransporters from Enterobacteriaceae (SPATES) (Henderson & Desvaux, 2004,
398 Henderson *et al.*, 2004, Desvaux *et al.*, 2006, Rojas-Lopez *et al.*, 2017).

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1.3.1.1.1. Autotransporter adhesins (ATAs)

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ATAs enable direct adhesion to abiotic supports, e.g. glass, stainless steel or plastic ware, and/or biotic surface, e.g. mammalian cells or extracellular matrix (ECM) components such as collagens (Vo *et al.*, 2017). As such, they can also belong to MSCRAMM (microbial surface components recognizing adhesive matrix molecules) proteins (Chagnot *et al.*, 2012).

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In EHEC, several enterohaemorrhagic *E. coli* autotransporters (Eha) have been identified (Wells *et al.*, 2008). Among them, EhaB has been shown to promote bacterial cells binding to laminin and collagen I (Wells *et al.*, 2008, Wells *et al.*, 2009), whereas EhaJ causes strong adherence to fibronectin, fibrinogen, collagens II, III and V, and laminin (Easton *et al.*, 2011). EhaB has also been identified in EPEC and ETEC (Zude *et al.*, 2014). Immediately adjacent to the *eha* gene, *egtA* encodes a glycosyltransferase. EhaJ requires glycosylation to mediate strong biofilm formation but not for adhesion to ECM components (Easton *et al.*, 2011). Following genomic analysis, *ehaJ* appears to be also present in EAEC, EIEC and ETEC where its function is still unknown. In EPEC, its exact function in the colonisation process remains unclear, as it does not seem to be required for bacterial adhesion and biofilm formation (Easton *et al.*, 2011). While EhaD has been shown to mediate biofilm formation, its role in bacterial adhesion has not been determined yet and its contribution to sessile development in DEC would require more in-depth investigation (Wells *et al.*, 2008). In the laboratory strain *E. coli* K12, the EhaD homologue YpjA has been shown to promote adhesion to glass and polyvinyl chloride (PVC), as well as biofilm formation together with the EhaC homologue YfaL and YcgV (Roux *et al.*, 2005). In EHEC, however, EhaC was not shown to promote biofilm formation (Wells *et al.*, 2008). A homologue of *ycgV* has been genetically identified in several DEC, namely EPEC, ETEC, EAEC and EIEC (Wells *et al.*, 2010,

424 Zude *et al.*, 2014). Altogether, this information emphasises the need for further
425 experimental characterisation of the adhesive functions of Eha, particularly considering
426 the diversity of DEC.

427 Some ATs originally identified in UPEC and acting as adhesins have been
428 identified in DEC, namely UpaB (uropathogenic *E. coli* autotransporter B) and UpaI
429 (Zude *et al.*, 2014). From UPEC investigations, these proteins appeared to promote
430 adhesion to a wide range of ECM components (Allsopp *et al.*, 2012, Zude *et al.*, 2014),
431 whilst UpaI was further demonstrated to mediate biofilm formation (Zude *et al.*, 2014).
432 Although the genes are found in EPEC and STEC, none of them have been functionally
433 characterised in any DEC to date (Zude *et al.*, 2014).

434 Following genomic analysis, AatA (avian pathogenic *E. coli* autotransporter A)
435 appears to be also present in some DEC strains (Zude *et al.*, 2014). In APEC (avian
436 pathogenic *E. coli*), AatA is important for pathogenesis as it enhanced adhesion to
437 chicken fibroblast cells (Dai *et al.*, 2010, Li *et al.*, 2010, Wang *et al.*, 2011). However,
438 its role and contribution in DEC is still unknown.

439 1.3.1.1.2. Self-associating autotransporters (SAATs)

440 SAATs are primarily able to associate to one another resulting in bacterial
441 cell autoaggregation (Klemm, 2006). In *E. coli*, the SAATs regroup ATs from the Ag43
442 (antigen 43), AIDA-I (adhesin involved in diffuse adherence phenotype) and TibA
443 (toxigenic invasion locus b) families (Trunk *et al.*, 2018). Of note, SAATs differentiate
444 from ATAs as they do not necessarily play a role in direct adhesion to biotic or abiotic
445 surfaces but can nonetheless contribute directly or indirectly to surface colonisation.

446 Ag43 is probably the SAAT which has triggered the most research to date, with
447 most of the information resulting from investigations in the *E. coli* K12 laboratory

448 strain (van der Woude & Henderson, 2008). Besides autoaggregation, Ag43 has been
449 demonstrated to increase biofilm formation on abiotic surfaces (Kjaergaard *et al.*, 2000)
450 and adhesion to epithelial cells (Sherlock *et al.*, 2006, de Luna *et al.*, 2008) but to
451 decrease bacterial motility (Ulett *et al.*, 2007). The gene encoding Ag43 has been shown
452 to be highly expressed during the early stage of biofilm formation (Schembri *et al.*,
453 2003) but not in mature biofilms (Beloin *et al.*, 2004). While biofilm formation is
454 favoured by the autoaggregation phenomenon (van der Woude & Henderson, 2008),
455 Ag43 is not involved in gut colonisation (de Luna *et al.*, 2008). It is also known that
456 the expression of pili would shield the interaction between Ag43 and thus prevent the
457 autoaggregation (Korea *et al.*, 2010). Phylogenetic analysis revealed the *agn43* gene is
458 distributed into two subfamilies, namely subfamily I (SF-I) and SF-II, and is only found
459 among, but not all, *E. coli* (including some *Shigella* spp.) (van der Woude &
460 Henderson, 2008). It has been suggested that *agn43* is more prevalent in pathogenic
461 *E. coli* strains than in commensal *E. coli* strains (van der Woude & Henderson, 2008).
462 It can be detected as a single gene copy, like in *E. coli* K12, or in multiple alleles, like
463 in EHEC O157:H7 EDL933 where two identical copies are found in two different
464 pathogenicity islands, namely the O-island 43 (OI-43) and OI-48 (Torres *et al.*, 2002).
465 In UPEC CFT073, Ag43 is encoded by two different alleles, namely *agn43a* and
466 *agn43b* (Ulett *et al.*, 2007). Compared to the Ag43 encoded by the first allele, Ag43
467 from allele b had a slower autoaggregation kinetics and lower propensity for biofilm
468 formation.

469 Autoaggregation results from the L-shape structure of Ag43 passenger region,
470 which drives molecular interaction *via* salt bridges and hydrogen bonds along the β -
471 helix structure in a molecular Velcro-like handshake mechanism (Heras *et al.*, 2014).
472 In *E. coli* O157:H7 EDL933, Ag43 was shown to promote autoaggregation, calcium

473 binding and biofilm formation but was unable to mediate adhesion to epithelial cells
474 (Torres *et al.*, 2002). While present in other DEC, such as EPEC, ETEC and EAEC
475 (Zude *et al.*, 2014, Vo *et al.*, 2017), functional characterisation of Ag43 in these
476 different pathotypes has not been examined in detail to date. Most recently, phylogenetic
477 network analysis revealed the Ag43 passengers were distributed into four distinct
478 classes, namely C1, C2, C3 and C4 (Ageorges *et al.*, 2019). Structural alignment and
479 modelling analyses indicated the N-terminal and C-terminal regions of the passengers
480 belonged to two different subtypes which gave rise to these four distinct Ag43 classes
481 upon domain shuffling. Functional analyses demonstrated that expression of Ag43^{C3}
482 (which both *agn43a* and *agn43b* from UPEC CFT073 belong to) induced a slower
483 sedimentation kinetics of bacterial cells and smaller aggregates compared to the three
484 other Ag43 classes (Ageorges *et al.*, 2019). Using prototypical Ag43^{C1} from *E. coli*
485 K12 MG1655, Ag43^{C2} from EHEC EDL933, Ag43^{C3} from UPEC CFT073 (allele
486 *agn43b*) and Ag43^{C4} from ETEC H10407, it appeared that heterotypic interactions
487 occurred in a very limited number of cases compared to homotypic interactions. This
488 ability of Ag43 variants to specifically identify genetic copies of themselves in other
489 bacterial cells through Ag43-Ag43 interactions further suggests a greenbeard effect
490 (Gardner & West, 2010, Wall, 2016), the ecophysiological relevance of which
491 undoubtedly requires further investigation (Ageorges *et al.*, 2019).

492 AIDA-I is involved in the diffuse adherence of DEC strains (Benz & Schmidt,
493 1989, Benz & Schmidt, 1992) and also in bacterial autoaggregation, biofilm formation
494 and adherence to a wide range of human and non-human cells (Benz & Schmidt, 1989,
495 Sherlock *et al.*, 2006). While the function of AIDA-I is quite similar to Ag43, they
496 clearly belong to different protein families (Vo *et al.*, 2017). The gene encoding AIDA-
497 I is especially prevalent in ETEC and STEC strains from porcine origin, which suggests

498 pork as a main animal reservoir for this gene (Niewerth *et al.*, 2001, Ha *et al.*, 2003).
499 In EPEC, the AIDA-I gene (*aidA*) is associated with *aah* which encodes a 45-KDa
500 heptosyltransferase (Benz & Schmidt, 2001). These genes are plasmid located and
501 transcribed as bicistronic mRNA, but their expression seems to be restricted to a small
502 number of DEC strains (Owen *et al.*, 1996, Sherlock *et al.*, 2004). Aah (adhesin
503 associated heptosyltransferase) modifies the AIDA-I by addition of 19 heptose residues
504 on average, which enables EPEC to adhere to human cells (Benz & Schmidt, 1992,
505 Benz & Schmidt, 2001, Laarmann & Schmidt, 2003, Schembri *et al.*, 2004). In EHEC
506 O157:H7, though, AIDA-I does not play a role in adherence to cultured cells or to pig
507 intestinal epithelial cells (Yin *et al.*, 2009). This suggests different subfamilies or
508 classes of AIDA-I could exist as observed for Ag43, which would require further in-
509 depth investigation.

510 TibA has been found to self-aggregate, promote biofilm formation and facilitate
511 colonisation of the intestinal epithelia (Sherlock *et al.*, 2005, Cote & Mourez, 2011). In
512 ETEC, TibA is encoded by the *tib* operon, which also encodes the glycosyltransferase
513 TibC (Lindenthal & Elsinghorst, 1999). Glycosylation of TibA is important for its
514 function since its unglycosylated form is less stable and cannot oligomerise properly
515 and in turn cannot promote bacterial adhesion to epithelial cells (Cote *et al.*, 2013);
516 nonetheless, it can autoaggregate, promote biofilm formation and cell invasion.
517 Interestingly, TibA, AIDA-I and Ag43 have been reported to interact with one another
518 resulting in the formation of mixed bacterial aggregates (Klemm, 2006). These
519 interesting findings deserve further in-depth characterisation, especially with regards to
520 recent findings where the interactions between Ag43 variants appears quite specific
521 (Ageorges *et al.*, 2019).

522 In *E. coli* O157:H7, EhaA has been shown to mediate autoaggregation and
523 adhesion to primary epithelial cells derived from the bovine terminal rectum, as well as
524 biofilm formation (Wells *et al.*, 2008). As such, EhaA can be considered as an
525 additional member of SAAT also found in EAEC, EPEC and ETEC (Vo *et al.*, 2017).
526 Similarly, UpaC was reported to promote autoaggregation, as well as biofilm formation
527 (Zude *et al.*, 2014). UpaC is found in a wide range of InPEC (Zude *et al.*, 2014). Of
528 note, some ATAs such as UpaI can further promote autoaggregation to some extent
529 (Zude *et al.*, 2014).

530 1.3.1.1.3. Serine protease autotransporters from enterobacteriaceae
531 (SPATEs)

532 SPATEs correspond to a subfamily of protease autotransporters that specifically
533 exhibit a serine protease domain (IPR034061) in the passenger region (Rojas-Lopez *et*
534 *al.*, 2017). While their primary function is associated with the degradation of various
535 proteins, such as mucin or haemoglobin, they can contribute to bacterial virulence *via*
536 their cytotoxic effect, and some can even be involved in bacterial colonisation (Dautin,
537 2010).

538 In EHEC, EspP (extracellular serine protease plasmid-encoded), also known as
539 PssA (protein secreted by Stx-producing *E. coli*), contributes to biofilm formation,
540 bacterial adherence to intestinal epithelial cells, including bovine primary rectal cells,
541 and colonisation of the bovine intestine (Dziva *et al.*, 2007, Puttamreddy *et al.*, 2010,
542 Farfan & Torres, 2012). EspP is encoded on the pO157 plasmid and can be found in
543 diverse STEC isolates (van Diemen *et al.*, 2005, Dziva *et al.*, 2007, Ruiz-Perez &
544 Nataro, 2014). At the bacterial cell surface, EspP passenger domains self-assemble to
545 form supramolecular structures, called ropes (Xicohtencatl-Cortes *et al.*, 2010). Besides
546 cytopathic activities, the EspP ropes have strong adhesive properties to host epithelial

547 cells and can further serve as a substratum for bacterial adherence and biofilm
548 formation. Similar observations have also been made for EspC from EPEC
549 (Xicohtencatl-Cortes *et al.*, 2010).

550 In EAEC, Pic (protein involved in colonisation) is involved in mucin
551 degradation but also directly in mucin binding (Gutierrez-Jimenez *et al.*, 2008, Andrade
552 *et al.*, 2017). It thus participates in intestinal colonisation and may also be involved in
553 bacterium-mucus biofilm (Navarro-Garcia & Elias, 2011). Pic is also expressed by the
554 hybrid EHEC/EAEC *E. coli* O104:H4 but its exact contribution to the colonisation
555 process in this genetic background remains to be ascertained (Henderson *et al.*, 1999,
556 Harrington *et al.*, 2009, Abreu *et al.*, 2015, Abreu *et al.*, 2016). Of note, Shmu is a
557 mucinase identical to Pic found in *Shigella* (Rajakumar *et al.*, 1997).

558 1.3.1.2. Inverted autotransporters (IATs)

559 In IATs, which correspond to the Type V, subtype e, secretion system (T5eSS),
560 the translocator is located in the N-terminal region and the passenger at the C-terminal,
561 which is the opposite of the modular organisation found in ATs (Tsai *et al.*, 2010,
562 Oberhettinger *et al.*, 2012). In DEC, there are several IATs acting as SCFs, namely
563 intimin, FdeC (Factor adherence of *E. coli*) and YeeJ. More recently, additional IATs
564 have been identified in *E. coli*, where *iata* appeared quite prevalent but the functional
565 characterisation of the gene product is still awaited (Goh *et al.*, 2019). IatB, IatC and
566 IatD from an environmental *E. coli* strain were further shown to be involved in strong
567 biofilm formation when overexpressed in a recombinant *E. coli* K12 background, but
568 not in autoaggregation nor adhesion to ECM proteins (Goh *et al.*, 2019). While
569 identified in several DEC, their role and contribution in their native genetic background
570 is still unknown.

571 1.3.1.2.1. Intimin

572 Intimin is the prototypical member of IATs (Leo *et al.*, 2015). In EPEC and
573 EHEC, the intimin is encoded by the *eae* (for *E. coli* attachment effacement) gene in
574 the locus of enterocyte effacement (LEE) (Nataro & Kaper, 1998). This protein
575 interacts specifically with its receptor Tir (translocated intimin receptor) allowing the
576 establishment of the intimate attachment of the bacteria with the host cell, pedestal
577 formation and attaching/effacing lesions (A/E) (Schmidt, 2010). In addition, intimin
578 contributes to intestinal colonisation in a Tir-independent manner (Mallick *et al.*, 2012).
579 Intimin may also bind to alternative receptors such as β_1 integrins or nucleolin but this
580 remains to be clarified (Liu *et al.*, 1999, Leo *et al.*, 2015).

581 1.3.1.2.2. Factor adherence of *E. coli* (FdeC)

582 FdeC is a widespread IAT in *E. coli* and present in all DEC pathotypes (Nesta
583 *et al.*, 2012, Easton *et al.*, 2014). In EHEC O26:H11, FdeC was shown to contribute to
584 biofilm formation and potentially in colonisation of the terminal rectum of cattle
585 (Easton *et al.*, 2014).

586 1.3.1.2.3. YeeJ

587 More recently, the gene encoding YeeJ has been reported to be present in some
588 DEC, namely EHEC, EPEC, ETEC and EIEC (Martinez-Gil *et al.*, 2017). In *E. coli*
589 K12, this IAT has been shown to participate in biofilm formation. While YeeJ exists
590 into two distinct variants of different lengths, no functional difference could be detected
591 between them. However, the contribution of YeeJ to biofilm formation in DEC remains
592 to be established.

593 *1.3.1.3. Other outer membrane proteins (OMPs)*

594 Besides ATs and IATs, several additional monomeric OMPs can act as SCFs in
595 DEC, namely OmpA, Hra (Heat-resistant agglutinin), and Iha (Iron-regulated protein
596 A homologue adhesin). OMPs are integrated to the OM *via* the β -barrel assembly
597 machinery (Bam) complex (Leyton *et al.*, 2015, Botos *et al.*, 2017, Schiffrin *et al.*,
598 2017).

599 1.3.1.3.1. Outer membrane protein A (OmpA)

600 While originally considered as a pore forming protein (Sugawara & Nikaido,
601 1992), whether the OmpA β -barrel offers a channel for the continuous passage of water
602 or solutes remains controversial (Smith *et al.*, 2007). Nowadays, OmpA is rather
603 viewed as a multifaceted protein with functions of an adhesin as well as an invasin. In
604 EHEC O157:H7, OmpA is involved in adhesion to intestinal epithelial cells (Torres &
605 Kaper, 2003, Kudva *et al.*, 2015). OmpA further appears to be the key molecular
606 determinant for bacterial adhesion to plant surfaces, such as alfalfa sprouts (Torres *et*
607 *al.*, 2005). The role of OmpA as an invasin was demonstrated in NMEC (Prasadarao *et*
608 *al.*, 1996) but remains to be established in DEC. Interestingly, OmpA can be encoded
609 by at least two different alleles, namely *ompA1* and *ompA2* (Power *et al.*, 2006). Many
610 of the interaction properties of OmpA emanate from protein loops external to the OM,
611 which are displayed on the bacterial cell surface (Smith *et al.*, 2007); in the two alleles,
612 differences in these regions could influence the adhesin and/or invasin properties of the
613 protein. Of note, OmpA further serves as a receptor for bacteriophages and bacteriocins
614 (Smajs *et al.*, 1997, Power *et al.*, 2006). Regarding biofilm formation, the direct
615 contribution of OmpA remains controversial; while OmpA from *E. coli* K12 has been
616 shown to bind to abiotic surfaces and to significantly influence biofilm formation
617 (Lower *et al.*, 2005, Barrios *et al.*, 2006), the role of OmpA in EHEC O157:H7 biofilm

618 formation appears to be minor and it acts rather as a modulator than a contributor to
619 sessile development (Torres *et al.*, 2005, Kudva *et al.*, 2015). Keeping in mind that
620 OmpA is an important contributor to the structural integrity of the bacterial cell
621 envelope by bridging the OM and cell wall, along with lipoproteins (Wang, 2002), the
622 interpretations of phenotypes from OmpA mutants must be considered with caution due
623 to possible pleiotropic effects that can be confounding. Further investigations on these
624 various aspects are clearly needed, and in particular the allelic variation of OmpA
625 should also be more carefully considered to decipher their exact role.

626 1.3.1.3.2. Heat-resistant agglutinin (Hra)

627 The Hra family of OMPs were first described with Hek (haemagglutinin from
628 *E. coli* K1) in NMEC, where it was reported to promote autoaggregation, interactions
629 with human erythrocytes and epithelial cells, as well as adhesion to, and invasion of
630 epithelial cells (Fagan & Smith, 2007). Hek was originally identified because of its
631 homology with Tia (toxigenic invasion protein A) (Bhargava *et al.*, 2009). In ETEC,
632 Tia mediates attachment to intestinal epithelial cells as well as their invasion
633 (Fleckenstein *et al.*, 1996, Sjolting *et al.*, 2015). It also appears to bind several
634 mammalian heparan sulphate binding proteins suggesting, that ETEC use these
635 ubiquitous cell surface heparan sulphate proteoglycans as receptors to adhere and
636 invade host epithelial cells (Fleckenstein *et al.*, 2002).

637 In EAEC O42, Hra1 (heat-resistant agglutinin 1) was demonstrated to be
638 responsible for autoaggregation and aggregative adherence, as well as biofilm
639 formation (Bhargava *et al.*, 2009). While these observations were made upon protein
640 expression in nonadherent and nonpathogenic laboratory *E. coli* strains, an EAEC 042
641 *hral* deletion mutant was not deficient in these phenotypes, indicating that Hra1 is an
642 accessory colonisation factor in this genetic background. While *hral/hek* was originally

643 considered absent from DEC but restricted to UPEC, NMEC and sepsis *E. coli*
644 (Dobrindt *et al.*, 2002, Cooke *et al.*, 2010), it later became clear that *hra1* and *tia* are
645 common among DEC, especially EAEC but also EPEC (Fleckenstein *et al.*, 1996,
646 Mancini *et al.*, 2011). In the EAEC strain 60A, Hra2 it is not involved in
647 autoaggregation or invasion, but only in adherence to epithelial cells (Mancini *et al.*,
648 2011); its involvement in bacterial adhesion to abiotic supports and biofilm formation
649 remains to be elucidated. The prevalence of *hra2*, however, seems to be very low
650 among DEC.

651 More recently, a novel member of the Hra family has been identified in STEC,
652 namely Hes (Hemagglutinin from shigatoxin-encoding *E. coli*) (Montero *et al.*, 2017).
653 Hes was shown to promote autoaggregation and biofilm formation as well as
654 erythrocyte agglutination and adherence to epithelial cells, but not invasion. The gene
655 was observed to be present in LEE-negative STEC but not LEE-positive STEC
656 (Montero *et al.*, 2017).

657 1.3.1.3.3. Iron-regulated protein A homologue adhesin (Iha)

658 Iha is an adherent-conferring protein homologous to IrgA (iron-regulated
659 protein A) found in *Vibrio cholerae* (Tarr *et al.*, 2000). As well as a β -barrel structure
660 enabling membrane anchoring as in any OMP, Iha has externally exposed domains.
661 Rather than localised adherence, Iha confers a diffuse adherence pattern in *E. coli*
662 O157:H7. Besides STEC, *iha* has been identified in EPEC and UPEC (Szalo *et al.*,
663 2002, Kanamaru *et al.*, 2003, Gomes *et al.*, 2011). In UPEC, Iha was shown to further
664 act as a catecholate siderophore receptor (Herold *et al.*, 2009) and a virulence factor
665 (Johnson *et al.*, 2005) but these roles in DEC remain to be established. In EHEC, Iha
666 has been clearly demonstrated to be involved in intestinal colonisation and contribute
667 to pathogenesis by promoting adherence to the intestinal epithelium (Yin *et al.*, 2009).

668 *1.3.1.4. Secreted and surface-associated lipoprotein of E. coli*

669 *(SslE)*

670 SslE, formerly known as YghJ (Yang *et al.*, 2007, Iguchi *et al.*, 2009), was
671 recently described as a novel *E. coli* mucinase thanks to its zinc metallopeptidase motif
672 (Luo *et al.*, 2014, Nesta *et al.*, 2014). This protein is secreted by a Type II, subtype a,
673 secretion system (T2aSS) but the molecular mechanisms of its maturation as a surface
674 lipoprotein remains unclear. The gene encoding SslE is present in different DEC
675 pathotypes such as EPEC, ETEC and EHEC (Decanio *et al.*, 2013). In EPEC, SslE was
676 shown to mediate biofilm formation and intestinal colonisation (Baldi *et al.*, 2012,
677 Vermassen *et al.*, 2019). This protein can be divided into two main variants and
678 antibodies raised against variant I (from ExPEC strain IHE3034) are able to inhibit
679 translocation of *E. coli* strains through a mucin-based matrix. In addition, immunisation
680 of animals with SslE I significantly reduces gut colonisation by strains of different
681 pathotypes expressing SslE II (Nesta *et al.*, 2014). These observations make SslE a key
682 factor in *E. coli* colonisation of the mucosal surface in humans and could serve as a
683 component for a protective vaccine against DEC (Naili *et al.*, 2016, Naili *et al.*, 2017,
684 Rojas-Lopez *et al.*, 2018, Rojas-Lopez *et al.*, 2019).

685 *1.3.1.5. E. coli factor adherence 1 (Efa-1)*

686 Efa-1, also known as Lifa (lymphostatin A), present in EPEC and some non-
687 O157 EHEC strains, is known to inhibit the proliferation of mitogen-activated
688 lymphocytes and the synthesis of proinflammatory cytokines, and gamma interferon
689 (Klapproth *et al.*, 2000, Abu-Median *et al.*, 2006). Efa-1 has been shown to mediate
690 colonisation of the calf intestine independently of glycotransferase and cysteine
691 protease motifs (Deacon *et al.*, 2010). In EHEC O157 strains, ToxB is homologous to

692 Efa-1 and appears to contribute to adherence to cultured epithelial intestinal cells
693 (Tatsuno *et al.*, 2001). However, no lymphostatin-like activity has been associated with
694 this protein and it is not involved in intestinal colonisation in animal models (Stevens
695 *et al.*, 2004, Abu-Median *et al.*, 2006). While Efa-1 has an extracytoplasmic domain
696 and is presumably cell-surface exposed (Nicholls *et al.*, 2002), the molecular
697 mechanisms at play for its secretion and cell-surface display remain unknown.

698 *1.3.1.6. Dispersin*

699 Dispersin is an anti-aggregation protein (Aap) involved in the spreading of
700 bacterial cells along the host intestinal mucosa (Sheikh *et al.*, 2002). This protein
701 contributes to adherence and colonisation of EAEC by preventing hyper-aggregation
702 and collapse of AAF (aggregative adherence fimbriae). Dispersin is present at the
703 bacterial cell-surface *via* binding to LPS in a non-covalent manner after secretion
704 through a Type I secretion system (T1SS) (Velarde *et al.*, 2007). This secretion system
705 and cognate secreted protein are encoded in the *aat* (aggregative ABC transporter) locus
706 located in the pAA plasmid of some EAEC (Nishi *et al.*, 2003). Dispersin is also present
707 in some STEC strains (Monteiro *et al.*, 2009, Muniesa *et al.*, 2012).

708 *1.3.1.7. Moonlighting proteins*

709 At the bacterial cell surface of *E. coli*, some unexpected proteins primarily
710 known to be localised in the cytoplasm have been reported. Among these unexpected
711 cell surface proteins, glycolytic enzymes are frequently uncovered (Henderson &
712 Martin, 2011). These so-called moonlighting proteins have been demonstrated to
713 exhibit a secondary function at the bacterial cell-surface, completely unrelated to their
714 primary function in the cytoplasm (Khan *et al.*, 2014). As a common glycolytic enzyme
715 frequently found at the bacterial cell surface, GAPDH (glyceraldehyde 3-phosphate

716 dehydrogenase) has been demonstrated to bind plasminogen and fibrinogen in EHEC
717 and EPEC (Egea *et al.*, 2007); although there is no evidence of GAPDH acting directly
718 as a plasminogen activator (Coleman & Benach, 1999, Seidler, 2013). In addition,
719 GAPDH is clearly involved in adhesion to intestinal epithelial cells upon infection. A
720 common theme for moonlighting proteins present at the bacterial cell surface is that
721 these proteins lack a N-terminal signal peptide for translocation across the CM and the
722 protein secretion systems enabling their translocation across the OM are often
723 unknown, which is covered by the generic term of non-classical protein secretion
724 (Bendtsen & Wooldridge, 2009, Desvaux *et al.*, 2009). For GAPDH, though, it has been
725 strongly suggested to occur *via* piggybacking through the Type III, subtype a, secretion
726 system (T3aSS) (Aguilera *et al.*, 2012). While it is also known that enolase can also be
727 extracellularly located in *E. coli* (Boel *et al.*, 2004), its contribution to bacterial
728 adhesion remains to be determined. The elongation factor Tu (EF-Tu) is also found at
729 the bacterial cell surface and has been reported to be involved in bacterial aggregation
730 (Amimanan *et al.*, 2017). In DEC, the contribution of putative moonlighting glycolytic
731 enzymes and other moonlighting proteins to the colonisation process deserves more
732 thorough investigation.

733 **1.3.2. Multimeric proteinaceous surface colonisation factors**

734 Multimeric protein complexes acting as SCFs can be classified as (i)
735 homooligomeric proteins, namely the trimeric autotransporter adhesins (TAAs), and
736 (ii) cell-surface supramolecular structures, including flagella, and numerous pili.

737 *1.3.2.1. Trimeric autotransporter adhesins (TAAs)*

738 TAAs are characterised by the presence of a short translocator domain, which
739 is functional upon homotrimeric assembly and corresponds to the Type V, subtype c,

740 secretion system (T5cSS) (Cotter *et al.*, 2005, Leo *et al.*, 2012). In DEC, TAAs include
741 UpaG (UPEC autotransporter G), Eib (*E. coli* immunoglobulin-binding protein), Sab
742 (STEC-autotransporter mediating biofilm formation) and Saa (STEC autoagglutinating
743 adhesin).

744 1.3.2.1.1. UPEC autotransporter G (UpaG)

745 While UpaG was originally identified in UPEC, it was also found in the EAEC
746 042 strain (Zude *et al.*, 2014). UpaG is involved in autoaggregation, biofilm formation,
747 adhesion to fibronectin, and laminin, as well as human epithelial cells (Valle *et al.*,
748 2008). In EHEC, EhaG (EHEC autotransporter G) is a positional orthologue of UpaG,
749 which is also involved in autoaggregation, biofilm formation, adhesion to laminin,
750 fibronectin and collagens I, II, II and IV as well as some epithelial cells (Valle *et al.*,
751 2008, Totsika *et al.*, 2012, Zude *et al.*, 2014). The gene encoding EhaG has been also
752 identified in a wide range of DEC including EPEC, EIEC, ETEC and EAEC (Zude *et*
753 *al.*, 2014).

754 1.3.2.1.2. *E. coli* immunoglobulin-binding protein (Eib)

755 Eibs were originally characterised for their ability to bind immunoglobulin
756 fractions, especially to the Fc (fragment crystallisable) region of IgA and IgG (Sandt &
757 Hill, 2000, Sandt & Hill, 2001, Leo & Goldman, 2009); up to 7 different Eibs have
758 been identified to date, namely EibA, B, C, D, E, F and G. In LEE-negative STEC O91,
759 it further appeared that EibG is involved in adherence to epithelial cells in a chain-like
760 adhesion (CLA) pattern (Lu *et al.*, 2006). CLA corresponds to the formation of a long
761 chain cell aggregate, which EibG induces on both human and bovine intestinal
762 epithelial cells. The gene encoding EibG is distributed into 21 different alleles clustered
763 into three *eibG* subtypes, namely *eibG*- α , - β , and - γ (Merkel *et al.*, 2010). While EibG-
764 α and EibG- β are responsible for the typical CLA phenotype, EibG- γ induces adherence

765 in much shorter cell chains and smaller cell aggregates, corresponding to an atypical
766 CLA. EibD has been further shown to promote autoaggregation and biofilm formation
767 (Leo *et al.*, 2011). Considering their structural similarity, other Eibs have been
768 suggested to have similar biological functions but experimental confirmation is still
769 required to ascertain this. Eib genes are found in some STEC strains, as well as some
770 *E. coli* commensal strains (Lu *et al.*, 2006).

771 1.3.2.1.3. STEC-autotransporter mediating biofilm formation (Sab)

772 Sab contributes to the diffusive adherence of STEC to human epithelial cells
773 and biofilm formation to abiotic surfaces (Herold *et al.*, 2009, Farfan & Torres, 2012).
774 Genes encoding Sab are especially present in LEE-negative STEC.

775 1.3.2.1.4. STEC autoagglutinating adhesin (Saa)

776 Saa promotes adhesion to HEp-2 cells in a semilocalised adherence pattern
777 (Paton *et al.*, 2001). So far, the *saa* gene has only been reported in some STEC,
778 including some LEE-negative EHEC strains (Paton & Paton, 2002, Jenkins *et al.*, 2003,
779 Monaghan *et al.*, 2011).

780 *1.3.2.2. Cell-surface supramolecular structures*

781 Flagella and pili are organelles resulting from the supramolecular assembly of
782 different protein subunits to form heteromultimeric protein complexes on the bacterial
783 cell-surface.

784 1.3.2.2.1. Flagella

785 Flagellar components are secreted and assembled *via* the Type III, subtype b,
786 secretion system (T3bSS) and more than fifty genes divided in three hierarchical classes
787 are involved in the flagellar apparatus formation (Young *et al.*, 1999, Chilcott &
788 Hughes, 2000). The main component of the flagellum filament is the flagellin, which

789 has considerable diversity in ultrastructure and is responsible for the H-antigen
790 variability (H1 to H56) (Zhou *et al.*, 2015). In *E. coli*, the flagellation is peritrichous
791 but the sites of cell surface localisation and the number of flagella (typically around 6-
792 10) are considered random (Macnab, 1987a, 1987b). Nonetheless, it must be stressed
793 that when swimming, the flagella in motion coalesce into an undulating bundle, forming
794 one rigid helical ponytail about 14 nm in diameter and 10 µm long that appears as
795 polarly localised in *E. coli* (Bray, 2001). A swimming bacterial cell has a run-and-
796 tumble behaviour, where it progresses linearly (run) and then changing abruptly in
797 direction (tumble), but also slow-random-walk behaviour, where it moves at a relatively
798 low speed (Qu *et al.*, 2018). Upon chemotaxis, the rotational direction of the flagella
799 motor can be switched to control motility, a factor that might help approaching the
800 intestinal mucosa in a more coordinated movement (Kitao & Hata, 2018, Rossi *et al.*,
801 2018). The approach to the surface is an important step towards initial bacterial
802 adhesion and subsequent sessile development. Active motility involving the flagella
803 allows the bacterial cells to overcome repulsive electrostatic and hydrodynamic forces
804 at the adhesion site (Donlan, 2002).

805 Besides swimming, flagella can participate in an alternative type of motility
806 called swarming where bacterial cells move and spread on a surface (Kaiser, 2007).
807 Swarming directly contributes to the surface colonisation process and is associated with
808 the expression of an alternative system, the lateral flagella (Merino *et al.*, 2006). In
809 EAEC O42, the Flag-2 locus encodes such a system (Ren *et al.*, 2005), although, a
810 mutation frameshift has likely inactivated this system in this strain. Nonetheless, the
811 Flag-2 cluster appeared to be present in about 20 % of *E. coli* strains from the ECOR
812 collection. In the environmental strain *E. coli* SMS-3-5, although the Flag-2 gene
813 cluster is complete and intact, swarming motility could not be observed (Fricke *et al.*,

814 2008); to date, the functionality of this system in *E. coli* remains to be elucidated. In
815 the absence of polar flagella, *E. coli* is not as efficient at surface colonisation but is still
816 considered a temperate swarmer, enabling it to swarm over surfaces with rheology
817 corresponding to 0.5 %-0.8 % agar (in comparison to ≥ 1.5 % agar for robust swarmers)
818 (Partridge & Harshey, 2013).

819 Besides motility, flagella can directly act as adhesins, as shown in EPEC, where
820 they are involved in adhesion to epithelial cells (Giron *et al.*, 2002, Cleary *et al.*, 2004).
821 In EAEC, flagella contribute to adhesion to plant leaves (Berger *et al.*, 2009). In EHEC,
822 the flagellin FliC favours initial attachment, adhesion to epithelial cells and biofilm
823 formation on abiotic surfaces as well as spinach leaves (McNeilly *et al.*, 2008, Mahajan
824 *et al.*, 2009, Xicohtencatl-Cortes *et al.*, 2009, Vikram *et al.*, 2013, Nagy *et al.*, 2015).
825 In ETEC, flagella contribute to bacterial adhesion to salad leaves and intestinal
826 epithelial cells, as well as biofilm formation (Shaw *et al.*, 2011, Duan *et al.*, 2012, Zhou
827 *et al.*, 2013, Zhou *et al.*, 2014). Interestingly, in this pathotype, flagella can also mediate
828 indirect adhesion through EtpA (ETEC two-partner secretion protein A), a protein
829 secreted by a T5bSS (two-partner secretion system), which bridges the flagella with
830 host cell receptors, thus allowing bacterial cell attachment to some epithelial cells and
831 mucin-expressing regions in mouse small intestine (Fleckenstein *et al.*, 2006, Roy *et*
832 *al.*, 2009). In EHEC and EPEC, the adhesion of H6 and H7 flagella to the intestinal
833 epithelium and epithelial cells has been suggested to occur through mucins (Giron *et al.*,
834 2002, Mahajan *et al.*, 2009) as reported for H1 flagella from the probiotic *E. coli* Nissle
835 1917 (Troge *et al.*, 2012). In some EHEC/STEC strains, namely LEE-negative EHEC
836 O113:H21 and STEC O139:H1:F18ab strains, flagella can also contribute to bacterial
837 invasion of intestinal epithelial cells but the molecular mechanisms at work remains to

838 be clarified (Luck *et al.*, 2006, Rogers *et al.*, 2012, Duan *et al.*, 2013). These latter
839 aspects would undoubtedly deserve further in-depth investigation.

840 While different flagellin variants have been shown to be involved in direct
841 binding to host cells, such as H1 and H19 flagella in ETEC (Duan *et al.*, 2012, Duan *et*
842 *al.*, 2013), systematic analysis of the colonisation properties of all of the different H-
843 antigens in *E. coli* has not been investigated as yet. Except for EIEC which are generally
844 considered as nonmotile (Nataro & Kaper, 1998), the contribution of flagella as a
845 motility factor over an adhesion factor in the colonisation processes has not been clearly
846 resolved as of yet in DEC, particularly regarding bacterial adhesion and biofilm
847 formation to biotic and abiotic surfaces (Wood *et al.*, 2006, Servin, 2014).

848 1.3.2.2.2. Pili

849 Pili, also referred to in *E. coli* literature as fimbriae, are key actors during the
850 initial attachment of bacteria to surfaces, which is characterised by a stronger and longer
851 interaction coupled with a decrease of bacterial motility (Pruss *et al.*, 2006). While
852 binding can be considered reversible as evidenced for the chaperon-usher fimbriae to
853 lectin (Hultgren *et al.*, 1989, Lin *et al.*, 2002), bacterial binding can also be very strong
854 due to the numerous pili expressed simultaneously by a single cell creating an avidity
855 effect, as well as the flexibility of the stalk itself (Andersson *et al.*, 2006). These pili
856 can be secreted and assembled by different protein secretion systems, namely the Type
857 II, subtype c (T2cSS), Type III, subtype a (T3aSS), Type IV, subtype b (T4bSS), Type
858 VII (T7SS) or Type VIII (T8SS) secretion systems (Figure 2). It should be stressed that
859 this numerical protein secretion nomenclature was intended and restricted to the LPS-
860 diderm bacteria in the first place (Desvaux *et al.*, 2009). In mycolate diderm bacteria
861 (archetypical acid-fast bacteria, namely mycobacteria) and some parietal monoderm
862 bacteria, the ESX (ESAT-6) system involved in protein export across the IM (or

863 cytoplasmic membrane) was also termed T7SS, which is (i) misleading when
864 considering that no ESX component enabling protein translocation across the mycolic
865 outer membrane has yet been identified (Converse & Cox, 2005, Bitter *et al.*, 2009,
866 Groschel *et al.*, 2016, Bosserman & Champion, 2017, Unnikrishnan *et al.*, 2017, Vaziri
867 & Brosch, 2019), and (ii) a misnomer with respect to both the bacterial export systems
868 (and especially parietal monoderm bacteria), which do not follow the numerical
869 nomenclature (e.g. Sec or Tat), and the numerical nomenclature for protein secretion
870 systems in LPS-diderm, which is primarily based on the presence of a translocon at the
871 OM (Desvaux *et al.*, 2004, Desvaux *et al.*, 2009, Desvaux *et al.*, 2009, Sutcliffe, 2011).
872 In diderm bacteria, the ESX is truly an export system in the same line than the Sec or
873 Tat systems (van der Woude *et al.*, 2013) but not a secretion system *per se*. In the
874 present review, the T7SS refers exclusively to the chaperone-usher pathway in LPS-
875 diderm bacteria (Desvaux *et al.*, 2009, Desvaux *et al.*, 2009, Chagnot *et al.*, 2013, Abby
876 *et al.*, 2016, Gagic *et al.*, 2016, Monteiro *et al.*, 2016), which is the main pathway
877 responsible for the secretion of a wealth of pili in *E. coli* (Wurpel *et al.*, 2013). Of note,
878 P pili have been well investigated in UPEC infection (Kuehn *et al.*, 1992, Lillington *et*
879 *al.*, 2014, Behzadi, 2020) but their prevalence in DEC and potential contribution (or
880 not) in diarrhoeic infection is much less documented although they contribute to
881 intestinal colonisation of commensal *E. coli* (Nowrouzian *et al.*, 2001) and have been
882 detected in some strains causing bovine diarrhoea (Dozois *et al.*, 1997).

883 1.3.2.2.1. The injectisome

884 The injectisome is a bacterial molecular syringe assembled and secreted by the
885 T3aSS (Desvaux *et al.*, 2006, Galan & Waksman, 2018). The injectisome forms a
886 needle which is functionally closer to the Hrp (hypersensitive response and
887 pathogenicity) pilus in *Pseudomonas syringae* than to a flagellum (He & Jin, 2003,

888 Tampakaki *et al.*, 2004, Cornelis, 2006). This cell-surface appendage can vary in size
889 depending on the bacterial species and even bacterial strains (Cornelis, 2006); in a
890 controlled process, the pilus length can further adapt for cell surface contact. In DEC,
891 this peculiar pilus is encoded by genes located in the LEE pathogenicity island
892 (McDaniel TK, 1995), a landmark for all EPEC but is also present in some EHEC
893 strains (namely the LEE-positive strains), such as *E. coli* O157:H7, and EIEC
894 (including *Shigella* spp.) (Hueck, 1998, Galan & Wolf-Watz, 2006, Coburn *et al.*,
895 2007). Tir (translocated intimin receptor) is encoded by the *tir* gene located in the LEE
896 and is injected in the host cell by the injectisome (Hueck, 1998). This protein is then
897 exposed at the host cell surface and serves as the receptor for the intimin, enabling
898 intimate bacterial interaction with the intestinal epithelia (Donnenberg *et al.*, 1993, Liu
899 *et al.*, 1999). In EPEC, the injectisome is involved in cell adhesion and pedestal
900 formation that occurs during the formation of attaching and effacing lesions upon actin
901 rearrangement in the infected eukaryotic cell (A/E) (Wong *et al.*, 2011). Of note, while
902 A/E lesions are observed *in vitro* from infected epithelial cell cultures or colonic
903 epithelium with LEE-positive EHEC (Lewis *et al.*, 2015), these kinds of lesions are
904 never observed from clinical samples of EHEC infections (Nataro & Kaper, 1998); a
905 clear explanation of why this is the case is unclear but would undoubtedly deserve
906 further investigation to match up lab experiments with clinical observations (Lewis *et*
907 *al.*, 2015). In addition to the infection of mammalian cells, the injectisome is involved
908 in adhesion to plants with a marked tropism for the stomata (Schroeder & Hilbi, 2008,
909 Shaw *et al.*, 2008, Berger CN, 2010, Croxen *et al.*, 2013). EspA, the main component
910 of the filament in the injectisome is directly involved in adhesion, as well as in biofilm
911 formation, in EPEC (Knutton *et al.*, 1998, Moreira *et al.*, 2006). In EIEC, the
912 injectisome contributes to the invasion capabilities (Hueck, 1998).

913 1.3.2.2.2.2. Type 4 pili (T4P)

914 T4P are assembled and secreted by the T2cSS (Ramer *et al.*, 2002, Chagnot *et*
915 *al.*, 2013). T4P have been demonstrated to play a role in several *E. coli* pathotypes,
916 including host cell adherence and bacterial aggregation (Craig *et al.*, 2004). Some of
917 these pili can exhibit a unique feature in their ability to extend and retract, which results
918 in twitching motility further contributing to biofilm formation (Mattick, 2002, Craig *et*
919 *al.*, 2019). In EPEC, T4P are also known as BFP (bundle-forming pili) and their
920 subunits assemble in a helical manner to form polymeric fibres and can further interact
921 to create higher-order bundles or tangled aggregates (Giltner *et al.*, 2012, Melville &
922 Craig, 2013). These T4P are involved in the colonisation of the GIT and contribute to
923 bacterial virulence (Bieber *et al.*, 1998, Tacket *et al.*, 1998). BFP are encoded by the
924 *bfp* operon comprising of 14 genes, including *bfpA*, which encodes the major repeating
925 subunit of the pilus fibre (Ramer *et al.*, 1996, Sohel *et al.*, 1996). In EHEC strains, the
926 T4P are called HCP (haemorrhagic *E. coli* pili) (Xicohtencatl-Cortes *et al.*, 2009).
927 Inactivation of the *hcpA* gene in EHEC O157:H7 reduces adherence to human and
928 bovine epithelial cells. HCP is also able to bind to fibronectin and laminin, to
929 agglutinate rabbit red blood cells, to mediate biofilm formation and to promote
930 twitching motility (Xicohtencatl-Cortes *et al.*, 2009). HCP are also encoded in some
931 STEC strains (Farfan & Torres, 2012). Because of their size, peculiar T4P called longus
932 pili have been reported in ETEC (Giron *et al.*, 1994). The N-terminal part of the major
933 subunit LngA is homologous with Bfp of EPEC, CofA subunit of CFA/III (colonisation
934 factor antigen) of ETEC and TCP (the toxin-coregulated pilin) of *V. cholerae* (Giron *et*
935 *al.*, 1995, Gomez-Duarte & Kaper, 1995). Longus pili are involved in colonisation of
936 the human gut (Clavijo *et al.*, 2010, Mazariego-Espinosa *et al.*, 2010), in bacterium-

937 bacterium interaction and resistance to antimicrobial agents as a result of biofilm
938 formation (Clavijo *et al.*, 2010).

939 1.3.2.2.3. Conjugative pili (CP)

940 CP are assembled and secreted through T4bSS (Lawley *et al.*, 2003).
941 Classically, the genes encoding for F-plasmid transfer are encoded on the *tra* operon
942 located in the conjugative F plasmid (Manwaring *et al.*, 1999). CP are responsible for
943 nucleoprotein transfer between a donor bacterial cell (harbouring the F plasmid) and a
944 recipient bacterial cell *via* the T4bSS (Lawley *et al.*, 2003). Bacterial conjugation is a
945 well-known process enabling horizontal transfer of genes including virulence or
946 colonisation factors (Manwaring *et al.*, 1999, Mazel & Davies, 1999, Llosa *et al.*, 2002,
947 Sorensen & Mortensen, 2005). Gene transfer is especially promoted in biofilm where
948 physical contact between sessile donor and recipient cells is favoured (Lebaron *et al.*,
949 1997, Hausner & Wuertz, 1999, Dionisio *et al.*, 2002, Molin & Tolker-Nielsen, 2003,
950 Maeda *et al.*, 2006). Besides the transfer of genetic material, CP can be directly
951 involved in bacterial adhesion (Beloïn *et al.*, 2008, May & Okabe, 2008, May *et al.*,
952 2011). In biofilm, this can be further amplified as cells carrying a conjugative F plasmid
953 promote the establishment of F pili mating pairs and consequently induce adhesion and
954 biofilm formation between abiotic surfaces and poor biofilm former cells. EAEC strains
955 expressing F pili have been demonstrated to improve mixed biofilm formation (Pereira
956 *et al.*, 2010). In EAEC C1096, pili encoded on the conjugative plasmid Inc11 further
957 contributed to adherence to abiotic surfaces and epithelial cells (Dudley *et al.*, 2006).
958 In EHEC O157:H7 Xuzhou, a novel conjugative plasmid called pO157-Sal encoding a
959 complete set of genes for the T4bSS was identified, but its involvement in the
960 colonisation process has not been investigated as yet (Wang *et al.*, 2011, Zhao *et al.*,
961 2013).

962 1.3.2.2.4. Type 1 pili (T1P)

963 T1P (also called Type 1 fimbriae) are the most investigated pili secreted and
964 assembled *via* a T7SS (Capitani *et al.*, 2006). The expression of T1P is induced during
965 the initial bacterial adhesion step (Harris *et al.*, 1990, Pratt & Kolter, 1998, Cookson *et*
966 *al.*, 2002, Orndorff *et al.*, 2004, Reisner *et al.*, 2014) and they are involved in the early
967 and late stages of biofilm formation (Schembri *et al.*, 2003, Beloin *et al.*, 2004, Reisner
968 *et al.*, 2014). T1P also have a role in the formation of SIgA (secretory IgA) mediated
969 biofilm of the normal flora within the gut (Bollinger *et al.*, 2003, Orndorff *et al.*, 2004,
970 Bollinger *et al.*, 2006). T1P are composed of FimA (fimbrillin A), which constitutes
971 the pilus rod, and FimH at the apex of the pilus tip. FimH is the key adhesin component
972 in T1P as it can link to mannose residues of some receptors on eukaryotic cells (Kaper
973 *et al.*, 2004, Duncan *et al.*, 2005) but also has nonspecific binding activity to abiotic
974 surfaces (Pratt & Kolter, 1998, Beloin *et al.*, 2008). The absence of the FimH adhesin
975 has been shown to hinder biofilm formation by preventing cell-to-surface and cell-to-
976 cell contacts (Danese *et al.*, 2000). In *E. coli*, different *fimH* alleles have been reported
977 as conferring distinct colonisation abilities and thus playing different roles in biofilm
978 formation (Martinez *et al.*, 2000, Weissman *et al.*, 2006). It was shown that contact
979 between T1P and abiotic surfaces alters the composition of the OM and changes some
980 physicochemical properties of the bacterial surface, which in turn influences adhesion
981 (Otto *et al.*, 2001, Orndorff *et al.*, 2004). While the laboratory *E. coli* K12 strain and
982 UPEC NU14 strain are the focus of the majority of the investigations about T1P, their
983 involvement in bacterial adhesion and/or biofilm formation has been further
984 demonstrated in EPEC, EAEC, ETEC and STEC strains (Elliott & Kaper, 1997,
985 Cookson *et al.*, 2002, Moreira *et al.*, 2003, Sheikh *et al.*, 2017). T1P are encoded in the
986 *fimBEAICDGHF* gene cluster, which is quite widespread in *E. coli* in both commensal

987 and pathogenic isolates (Sauer *et al.*, 2000, Kaper *et al.*, 2004, Wurpel *et al.*, 2013).
988 While present in EHEC O157:H7 (Abraham *et al.*, 1988, Li *et al.*, 1997, Roe *et al.*,
989 2001, McWilliams & Torres, 2014), their contribution to the colonisation process has
990 yet to be demonstrated.

991 Genes encoding the F1C pili are present in approximately 7 % of *E. coli* faecal
992 isolates (Werneburg & Thanassi, 2018). F1C pili have been characterised in UPEC
993 strains where they are encoded in the *foc* (fimbriae of serotype 1C) operon homologous
994 to the *fim* locus (Klemm *et al.*, 1994). In UPEC, F1C pili are involved in adherence to
995 the bladder and kidney cells, as well as in biofilm formation (Werneburg & Thanassi,
996 2018). Their prevalence and contribution to the colonisation process in DEC remains
997 to be investigated.

998 1.3.2.2.2.5. CS31A pili

999 The CS31A (coli surface associated 31a antigen) plays a key role in the
1000 virulence of septicemic *E. coli* and ETEC, as well as some EPEC and DAEC (Girardeau
1001 *et al.*, 1988, Contrepolis *et al.*, 1989, Jallat *et al.*, 1994, Adams *et al.*, 1997). Because of
1002 their thin structure, as well as their close and packed association to the bacterial cell
1003 surface, CS31A was initially described as capsule-like or even nonfimbrial antigens
1004 (Bertin *et al.*, 1993, Mechin *et al.*, 1996) before being clearly identified as thin capsular
1005 pili secreted and assembled by a chaperone-usheer pathway (T7SS) (Thanassi *et al.*,
1006 1998). These pili are synthesised from the *clp* operon located on a high-molecular-
1007 weight self-transmissible R plasmid, called p31A (Martin *et al.*, 1991, Jallat *et al.*, 1994,
1008 Martin, 1996). CS31A are considered homologous to the K88/F4 (*fae* operon) and F41
1009 pili but with some functional dissimilarities, such as that CS31A does not exhibit
1010 haemagglutinin activity (Girardeau *et al.*, 1991). In ETEC, F4 pili allow bacterial
1011 adherence to F4-specific receptors present on the brush borders of villous enterocytes

1012 thus promoting the colonisation of the small intestine (Snoeck *et al.*, 2008). The locus
1013 for diffuse adherence (*ldaCDEFGHI*) (Scaletsky *et al.*, 2005) from EPEC is
1014 homologous to the K88 *fae* and ETEC CS31A *clp* operons. LdaH mediates diffuse
1015 adherence to Hep-2 cells. The LdaH encoding gene has also been found in STEC strains
1016 but no functional characterisation has been reported as yet (Scaletsky *et al.*, 2005).

1017 1.3.2.2.2.6. Aggregative adherence fimbriae (AAF)

1018 AAF belongs to the Afa/Dr (afimbrial adhesin/decay-accelerating factor
1019 receptor) haemagglutinin family together with F1845 pili (Nowicki *et al.*, 1990, Le
1020 Bouguenec & Servin, 2006). In DAEC and EIEC, Afa and Dr hemagglutinins recognise
1021 the Dr blood group antigen (Nowicki *et al.*, 1990). Among the five genes encoded in
1022 the *afa* cluster, *afaB*, *afaC* and *afaE* are required for mannose-resistant
1023 hemagglutination (MRHA) (Servin, 2005). The Dr hemagglutinin is encoded by the
1024 *draABCDE* operon, where *draA*, *draB*, *draC*, and *draD* encode accessory proteins and
1025 *draE* encodes the adhesin part (Nowicki *et al.*, 1987, Servin, 2005). In addition, it
1026 specifically binds collagen IV (Nowicki *et al.*, 1988). Afa and Dr haemagglutinins can
1027 link to decay-accelerating factor (DAF) and to carcinoembryonic antigen-related
1028 cellular adhesion molecules (CEACAMs) (Nowicki *et al.*, 1988, Westerlund *et al.*,
1029 1989, Berger *et al.*, 2004). While some members of the Afa/Dr family were believed
1030 not to form pili as they could not be observed by electron microscopy examination, it
1031 is now clear they are secreted as AAF and F1845 by T7SS, to form pili of various
1032 architecture depending on the pilin subunits (Anderson *et al.*, 2004, Pettigrew *et al.*,
1033 2004).

1034 In EAEC, the colonisation of the gut occurs through aggregative adherence
1035 (AA) due to AAF, which binds to ECM proteins such as fibronectin, laminin and
1036 collagen IV (Farfan *et al.*, 2008, Berry *et al.*, 2014) and then promotes biofilm

1037 formation (Hicks *et al.*, 1996, Wakimoto *et al.*, 2004). To date, five AAFs (AAF/I to
1038 AAF/V) have been identified, all encoded by virulence plasmids of EAEC (pAA) and
1039 the main subunits of which are AggA, AafA, Agg3A, Agg4a and Agg5a respectively
1040 (Nataro *et al.*, 1992, Czeczulin *et al.*, 1997, Boisen *et al.*, 2008, Jonsson *et al.*, 2015).
1041 Another hypothetical Dr-related pilin called HdaA (HUS-associated diffuse adherence)
1042 also appears to confer the capacity to cause the AA phenotype in EAEC (Boisen *et al.*,
1043 2008). In DAEC and EIEC, F1845 pili are involved in gut colonisation (Servin, 2005).
1044 F1845 pili are responsible for diffuse adherence to epithelial cells of the gut and are
1045 encoded by the *daaABCDE* operon (Bilge *et al.*, 1989, Bilge *et al.*, 1993).

1046 1.3.2.2.7. Colonisation factor antigens (CFA)

1047 In ETEC, colonisation factor antigens (CFA), also called coli surface antigens
1048 (CS), form pili that take part in adhesion to the small intestine and are critical for
1049 virulence (Gaastra & Svennerholm, 1996). CFA/I, CFA/II (CS1, 2 and 3) and CFA/IV
1050 (CS4, 5 and 6) are the most virulent (Sjoberg *et al.*, 1988, Knutton *et al.*, 1989,
1051 Taniguchi *et al.*, 1995, Gaastra & Svennerholm, 1996, Svennerholm & Lundgren,
1052 2012) but CS12, 14, 17, 18, 19, 20 and 31 can also adhere to intestinal cells (Werneburg
1053 & Thanassi, 2018). CFA/CS are encoded in operons; taking CFA/I as an example, it is
1054 encoded by the *cfaABCE* operon, where *cfaB* encodes the main subunit, *cfaE* the distal
1055 subunit, *cfaA* a chaperone and *cfaC* the usher involved in pilin transport across the OM
1056 (Jordi *et al.*, 1992). Cell adhesion is enabled by CfaB through its ability to bind
1057 glycosphingolipid (Jansson *et al.*, 2006).

1058 1.3.2.2.8. F9 pili

1059 In EHEC O157:H7, F9 pili are involved in the colonisation of epithelial bovine
1060 cells, bovine gastrointestinal tissue explants and can also bind to fibronectin (Low *et*
1061 *al.*, 2006). Mutants of the main subunit of F9 pili are still able to colonise the terminal

1062 rectum, indicating that the adhesin is not solely responsible for the rectal tropism
1063 observed but may contribute to colonisation at other sites, especially in young animals
1064 (Low *et al.*, 2006). These pili are short but are able to form longer bundles (Low *et al.*,
1065 2006). They are encoded in the F9 gene cluster, a six genes operon located on the
1066 pathogenicity island O161 (Low *et al.*, 2006, Wurpel *et al.*, 2013). This operon has also
1067 been identified in EPEC, as well as EAEC (Wurpel *et al.*, 2013). F9 pili are secreted
1068 and assembled by a T7SS (Wurpel *et al.*, 2013).

1069 1.3.2.2.9. *E. coli* YcbQ laminin-binding fimbriae (ELF)

1070 In EHEC O157:H7, it has been shown that *E. coli* YcbQ laminin-binding
1071 fimbriae (ELF) bind laminin and are involved in adherence to epithelial cells in humans,
1072 cows and pigs (Samadder *et al.*, 2009). ELF form peritrichous flexible fine fibres and
1073 are encoded by the *elfADCG* operon, originally called the *ycbQRST* operon, which was
1074 previously identified in UPEC and some commensal *E. coli* strains (Spurbeck *et al.*,
1075 2011). This operon is homologous to the F17 pili biogenesis genes found in ETEC,
1076 which are assembled and secreted by a T7SS (Lintermans *et al.*, 1988, Lintermans *et*
1077 *al.*, 1991, Bertin *et al.*, 1996, Bertin *et al.*, 2000). More generally, ELF are also
1078 homologous to 20K, K99 and G pili found in various pathogenic *E. coli* (Guinee *et al.*,
1079 1976, Contrepolis *et al.*, 1983). These pili have been shown to mediate binding to
1080 intestinal mucosal cells, especially to N-acetyl-D-glucosamine-containing receptors
1081 (Bertin *et al.*, 1996). The composition of the pili and the sequence of the tip-adhesin
1082 differ between the strains and could explain the phenotypic divergence associated with
1083 the expression of this family of pili in different *E. coli* strains (Korea *et al.*, 2010).

1084 1.3.2.2.10. Long polar fimbriae (LPF)

1085 LPF are encoded by two operons *lpf1* and *lpf2* located on the pathogenicity
1086 islands O141 and O154 in EHEC O157:H7, respectively (Perna *et al.*, 2001). LPF are

1087 also present in other DEC, e.g. LEE-negative EHEC, EPEC, rabbit-specific EPEC,
1088 EAEC and ETEC, as well as in several commensal strains (Doughty *et al.*, 2002,
1089 Wurpel *et al.*, 2013). They share homology with the LPF of *Salmonella enterica* serovar
1090 Typhimurium which are involved in adherence to Peyer's patches and M cells in the
1091 human gut (Baumler & Heffron, 1995, Baumler *et al.*, 1996). The *lpf1* operon is
1092 composed of five genes, with *lpfA* encoding the main pilus subunit, *lpfD* and *lpfE*
1093 encoding minor subunits, and *lpfB* and *lpfC* encoding the chaperone and usher
1094 respectively (Doughty *et al.*, 2002, Torres *et al.*, 2004). The *lpf2* operon also contains
1095 five genes with a duplication of *lpfD* called *lpfD'* but with no *lpfE* paralogue (Torres *et*
1096 *al.*, 2004). In *E. coli* O157:H7, it has been proposed that LPF2 is expressed in early
1097 stages whereas LPF1 is expressed in late stages of growth (Torres *et al.*, 2004). LPF
1098 are secreted and assembled by a T7SS and can bind fibronectin, laminin and collagen
1099 IV, as well as the follicle-associated epithelium (FAE) of Peyer's patches in humans
1100 (Fitzhenry *et al.*, 2006, Farfan & Torres, 2012, McWilliams & Torres, 2014).
1101 Expression of *lpf2* is increased under conditions similar to those for biofilm formation
1102 (Torres *et al.*, 2007). Recently, it has been demonstrated that STEC isolates positive for
1103 *lpf2* formed significantly more biofilm than *lpf2*-negatives isolates (Vogeleer *et al.*,
1104 2015). In EPEC, LPF have been shown to contribute to the early stages of colonisation
1105 of rabbits and the severity of diarrhoea (Newton *et al.*, 2004).

1106 1.3.2.2.2.11. *E. coli* common pilus (ECP)

1107 In EHEC, ECP (previously called Mat for meningitis-associated temperature
1108 dependent pilus) provides adherence to HEP-2, HeLa and HT-29 cells and allows
1109 interaction between bacterial cells (Rendon *et al.*, 2007). Secreted and assembled by a
1110 T7SS, ECP expression is increased under environmental conditions that are
1111 experienced in the GIT, e.g. low oxygen and high CO₂ concentrations (Rendon *et al.*,

1112 2007). However, its role seems to be secondary in the colonisation of the human or
1113 bovine gut (Tatsuno *et al.*, 2000, Dziva *et al.*, 2004). The *ecp* operon has been identified
1114 in numerous commensal and pathogenic *E. coli*, including DEC (Rendon *et al.*, 2007).

1115 1.3.2.2.2.12. Sorbitol-fermenting fimbriae protein (SFP)

1116 In EHEC, the expression of sorbitol-fermenting fimbriae protein (SFP) pili is
1117 induced in anaerobic conditions and leads to an increased adherence to Caco-2 and
1118 HCT-8 cells, with a mannose-resistance hemagglutination phenotype (Brunner *et al.*,
1119 2001, Musken *et al.*, 2008, Bielaszewska *et al.*, 2009). These pili are encoded on the
1120 *sfpABDCDJG* operon harboured in the virulence plasmid pSFO157 (Brunner *et al.*,
1121 2006). SFP pili are secreted and assembled by a T7SS (Brunner *et al.*, 2001). Besides
1122 *E. coli* O157, *sfp* has been identified in other EHEC serotypes, such as O165
1123 (Bielaszewska *et al.*, 2009), but its prevalence among STEC in general is thought to be
1124 quite low (Toma *et al.*, 2004). Distribution of the *sfp* operon in other DEC has not been
1125 investigated in detail as of yet.

1126 1.3.2.2.2.13. Curli

1127 Curli are thin aggregative pili generally considered as one of the major
1128 proteinaceous components of the *E. coli* biofilm matrix (Smyth *et al.*, 1996,
1129 Stathopoulos *et al.*, 2000, Kostakioti *et al.*, 2005, Evans & Chapman, 2014). These
1130 peculiar pili are secreted and assembled by the T8SS through the extracellular-
1131 nucleation-pathway (ENP). Curli are helical filamentous amyloid fibres that facilitate
1132 cell-surface and cell-cell interactions and promote biofilm formation (Olsen *et al.*,
1133 1993, Cookson *et al.*, 2002, Szabo *et al.*, 2005, Beloin *et al.*, 2008, McCrate *et al.*,
1134 2013). In EHEC O157:H7, curli are associated with cellulose production, adherence to
1135 spinach leaves and Hep-2 cells as well as abiotic surfaces (Kim & Kim, 2004, Pawar *et*
1136 *al.*, 2005, Macarasin *et al.*, 2012). In ETEC, curli facilitate adherence to plastic surfaces

1137 (Szabo *et al.*, 2005). Although curli were originally thought not be expressed by EPEC
1138 (Ben Nasr *et al.*, 1996), some strains were later reported to synthesise curli, playing a
1139 role in bacterial adhesion and biofilm formation in condition mimicking human or
1140 bovine hosts (Saldana *et al.*, 2009). However, curli do not seem to be required for
1141 biofilm formation and/or adhesion of EAEC strains (Sheikh *et al.*, 2001, Berger *et al.*,
1142 2009, Pereira *et al.*, 2010). In *Shigella spp.* and EIEC, CsgD and curli expression is
1143 often inactivated (Sakellaris *et al.*, 2000). Two operons are involved in curli production,
1144 (i) the *csgBAC* operon, encoding the structural components of curli (CsgA and CsgB)
1145 and an accessory protein (CsgC), and (ii) the *csgDEFG* operon, encoding a
1146 transcriptional regulator (CsgD) and the secretion machinery for transport across the
1147 OM (CsgE-G) (Arnqvist *et al.*, 1994, Hammar *et al.*, 1995, Beloin *et al.*, 2008). In the
1148 current model, CsgB is proposed as embedded in the OM where it acts as a nucleator
1149 for the polymerisation of the major CsgA curlin (Van Gerven *et al.*, 2015, Jain &
1150 Chapman, 2019). While the exact structure of curli fibres has not yet been elucidated
1151 with molecular resolution (Van Gerven *et al.*, 2015, Jain & Chapman, 2019), the fibres
1152 have been reported to display irregular thin branches, which would result from minor
1153 incorporation of CsgB along the curli and promoting the formation of branched fibres
1154 (Bian & Normark, 1997, Soto & Hultgren, 1999, Shu *et al.*, 2012, DeBenedictis *et al.*,
1155 2017). Recently, CsgC and CsgE were demonstrated to highly inhibit CsgA aggregation
1156 and CsgE was shown to prevent pellicle biofilm formation when added exogenously
1157 (Andersson *et al.*, 2013, Evans *et al.*, 2015).

1158 1.3.2.2.2.14. Haemolysin-coregulated protein (Hcp)

1159 In EAEC, the haemolysin-coregulated protein (Hcp) tube formed by the Type
1160 VI secretion system (T6SS) was suggested to be of importance for biofilm formation
1161 (Aschtgen *et al.*, 2008). More than ten orthologues of the T6SS components have been

1162 identified in EHEC and EPEC strains. This system can also contribute to bacterial
1163 aggregation at the host cell surface (Dudley *et al.*, 2006, Shrivastava & Mande, 2008,
1164 Lloyd *et al.*, 2009, Aschtgen *et al.*, 2010, Moriel *et al.*, 2010). Further investigations
1165 are required in DEC to determine the exact role and molecular mechanisms involved in
1166 the colonisation processes by the Hcp and T6SS.

1167 **2. The different regulation levels involved in the expression** 1168 **of colonisation factors**

1169 In general, the expression of genes encoded on genomes into proteins can be
1170 regulated at pre-transcriptional, transcriptional, post-transcriptional, translational
1171 and/or post-translational levels, as well as at translocational and post-translocational
1172 levels, the latter of which are especially relevant and important for molecular
1173 determinants expressed at the bacterial cell surface (Figure 3). With the rise of omic
1174 approaches, however, some basic bacterial physiology concepts may sometimes be
1175 overlooked and gene/protein expression is very often considered as being limited to
1176 regulatory networks involving transcriptional repressors or activators. However, when
1177 it comes to functions and activities, it is primarily proteins that can help to comprehend
1178 bacterial physiology. It must also be kept in mind that the relationship between mRNA
1179 and protein abundances only very partially correlates; mRNA levels are just a proxy for
1180 the presence of a protein but is not directly proportionate with the increase or decrease
1181 folds of protein expression and even less with its activity when we consider an enzyme
1182 for instance (Vogel & Marcotte, 2012). Here, the different regulatory levels involved
1183 in bacterial adhesion and biofilm formation are highlighted using key examples of
1184 different SCFs.

1185 **2.1. Regulation at the pre-transcriptional level: phase variation**

1186 Prior to transcription, some regulatory mechanisms can already be at work at
1187 the DNA level, through phase variation. There are four main mechanisms of phase
1188 variation (i) DNA inversion, (ii) slipped-strand mispairing, (iii) DNA methylation, and
1189 (iv) DNA deletion (Henderson *et al.*, 1999). As a commonality, all these regulatory
1190 mechanisms primarily occur at the stage of DNA replication and a large majority of
1191 genes regulated by phase variation are bacterial cell surface molecular determinants
1192 (Owen *et al.*, 1996, Holden & Gally, 2004).

1193 In *E. coli* K12, T1P are well-known to be subjected to phase variation following
1194 DNA inversion (Blomfield, 2001). The expression of the *fim* operon is under the control
1195 of the *fim* promoter, which is located within the *fimS*-invertible element (Abraham *et al.*,
1196 1985, Wright *et al.*, 2007). The orientation of the promoter determines the ON or
1197 OFF phase and then induces the expression of upstream genes or not. Two tyrosine
1198 recombinases, FimB and FimE, are known to control the orientation of the *fimS*-
1199 invertible region. FimB predominantly switches the *fim* operon transcription from OFF
1200 to ON, while FimE mediates ON to OFF phase switching (Klemm, 1986, Gally *et al.*,
1201 1996, Hannan *et al.*, 2008). Of note, two DNA topological effectors participate in this
1202 regulation, namely H-NS (histone-like nucleoid-structuring protein) and IHF
1203 (integration host factor); these histones play complementary role, as the DNA inversion
1204 is absolutely dependent upon IHF, whereas the inversion rate is slowed down with high
1205 levels of H-NS and *vice versa* (Dorman & Ni Bhriain, 1993). The existence of this
1206 regulation in DEC has not been examined as of yet.

1207 Slipped-strand mispairing occurs in the course of DNA replication in repetitive
1208 DNA regions, which can be positioned either upstream of a coding DNA sequence

1209 (CDS) and then influences the transcription, such as the promoter efficiency, or within
1210 a CDS and can affect the translational reading frame resulting in a mutation frameshift
1211 (Henderson *et al.*, 1999). In *E. coli*, phase variation resulting from strand-slippage has
1212 not been reported as yet, nonetheless, there is no molecular mechanistic constraint for
1213 it not to occur (Torres-Cruz & van der Woude, 2003).

1214 Phase variation resulting from DNA methylation corresponds to a bacterial
1215 epigenetic mechanism (Henderson *et al.*, 1999). Ag43 is probably one of most
1216 investigated surface proteins subjected to such a regulatory mechanisms (van der
1217 Woude & Henderson, 2008). This epigenetic regulation involves two proteins, the DNA
1218 adenine methylase (Dam) and the OxyR transcriptional regulator (van der Woude &
1219 Henderson, 2008). When Dam has methylated the GATC sites present in the operator
1220 region in the course of DNA replication, the repressor OxyR cannot bind and
1221 transcription by the RNA polymerase occurs and Ag43 is expressed (ON phase);
1222 however, if OxyR binds the GATC sites before they are methylated by Dam, there is
1223 no transcription and no Ag43 expression (OFF phase). Besides Ag43, several pili
1224 secreted and assembled by the T7SS have been reported to be subjected to such an
1225 epigenetic regulation in *E. coli* (Henderson *et al.*, 1999, Blomfield, 2001). The *pap*
1226 (pyelonephritis-associated pilus) operon in UPEC is considered as a paradigm where
1227 the Dam methylation of a GATC-II site in the operator region prevents binding of the
1228 repressor Lrp (leucine-responsive regulatory protein), and consequently the *papBA*
1229 operon is transcribed and the pili are expressed (ON phase). In the absence of
1230 methylation at GATC-II, Lrp can bind to the operator, repress the transcription and
1231 ultimately prevent pili formation (OFF phase). Additionally, this repression can be
1232 lifted when Lrp binds to another site called GATC-I. Among DEC, CS31A pili are
1233 subjected to this same regulatory mechanism (Crost *et al.*, 2003, Graveline *et al.*, 2014).

1234 As a general trend, phase variation due to DNA deletion is irreversible due to
1235 the loss of the genetic element bearing the gene of interest. In *E. coli*, DNA deletion is
1236 responsible for unilateral flagellar phase variation as reported in the H3, H47 and H17
1237 strains (Zhou *et al.*, 2015). While most flagellins are encoded by *fliC* in *E. coli*, H3 and
1238 H47 are encoded by *flkA* and H17 is encoded by *flnA*. For H3 and H47, their production
1239 results from the expression of *flkAB* operon, where the transcriptional regulator FlkB
1240 represses *fliC* (Feng *et al.*, 2008). Upon excision of the *flk* region from the chromosome,
1241 *flkAB* is irreversibly deleted, the repression of *fliC* is released and the FliC flagellin is
1242 produced. Similarly, the H17 strain can irreversibly switch flagellar antigens to H4
1243 (Ratiner, 1967). It appears this flagellar phase variation can be caused by excision of
1244 *flnA* (Liu *et al.*, 2012). When *flnA* is present in the chromosome, the translation of FliC
1245 H4 is inhibited and only FlnA H17 is produced; once *flnA* is excised, the repression of
1246 the *fliC* is released and only the FliC H4 is produced. The ~35 kb DNA deletion region
1247 containing the *flnA* gene is excised as a covalently closed extrachromosomal circular
1248 form. While some DNA deletion can occur through homologous recombination
1249 (Henderson *et al.*, 1999), flagellar phase variation is mediated by non-homologous
1250 recombination *via* an integrase of the tyrosine recombinase family (Feng *et al.*, 2008).
1251 The flagellar phase variation mechanisms in some other *E. coli* H variants and
1252 especially in DEC remain to be defined.

1253 **2.2. Regulation at the transcriptional level: regulators and** 1254 **effectors**

1255 Regulation at the transcriptional level is the most well-known level of gene
1256 regulation and quite often the only one really considered as a proxy for protein
1257 expression levels. Transcriptional regulators can either be repressors or activators but

1258 it is wrong to assume a repressor will systematically repress transcription or an activator
1259 will activate transcription. A second crucial partner to the process must also be
1260 considered, that is the effector, which can be of two types, either an inducer or a co-
1261 repressor. Four possibilities for regulation at the transcriptional level can be
1262 discriminated: (i) positive control of an inducible gene, where an activator is activated
1263 by an inducer, (ii) positive control of a repressible gene, where an activator is
1264 inactivated by an inhibitor, (iii) negative control of an inducible gene, where a repressor
1265 is inactivated by an inducer, or (iv) negative control of a repressible gene, where a
1266 repressor is activated by a co-repressor. Additionally, a so-called repressor can act as
1267 an activator for some genes and vice versa. In other words, the up-expression or down-
1268 expression of a regulator is not sufficient to know what kind of transcriptional
1269 regulation is taking place without knowing the nature and level of the inducer.

1270 Bacteria can sense and respond to environmental cues thanks to a large range of
1271 two-component signal transduction systems where a sensor activates a transcriptional
1272 regulator, which further represses or activates gene expression (Hoch, 2000,
1273 Zschiedrich *et al.*, 2016). Some of these systems participate in cell-to-cell
1274 communication (CTCC) *via* a signal molecule called auto-inducer (AI) (Bassler, 2002).
1275 Quorum sensing (QS) is only one of the different functions of CTCC, which specifically
1276 refers to the sensing of the cell density (quorum); QS should not be considered
1277 synonymous with CTCC because some sensing can be unrelated to QS *sensu stricto*
1278 but to diffusion sensing, confinement or efficiency sensing for instance (Redfield, 2002,
1279 Platt & Fuqua, 2010, West *et al.*, 2012). This semantic issue is of particular importance
1280 in biofilm formation, since by definition, bacteria cells are at a high density following
1281 sessile development and therefore the notion of QS makes little sense. Transcriptional
1282 regulators of virulence and SCFs have been the subject of intense and extensive

1283 research and scientific literature in DEC (Beloin *et al.*, 2008, Tobe, 2008, Pruss, 2017,
1284 Rossi *et al.*, 2018). For these reasons only some key examples will be provided to
1285 illustrate the relevance of differentiating the regulation at different levels.

1286 At the transcriptional level, PNAG production is regulated by NhaR, a
1287 transcriptional regulator of the LysR family, which activates the transcription of the
1288 *pgaABCD* operon by binding to two sites near the -35 region of the promoter (Goller *et*
1289 *al.*, 2006). In EPS, the production of colanic acid is consistently upregulated within
1290 biofilms by the RcsA transcriptional activator (Matthysse *et al.*, 2008, May & Okabe,
1291 2008). The transcription of the *wca* operon is regulated by the *rcaABCF* locus that
1292 encodes a two-component system (Gervais & Drapeau, 1992, Ebel & Trempey, 1999,
1293 Beloin *et al.*, 2008). However, the signal sensed by the RcsC sensor kinase remains
1294 unknown (Whitfield & Roberts, 1999, Oropeza *et al.*, 2015). H-NS is known to act as
1295 a transcriptional repressor in bacteria, a so-called bacterial transcriptional silencing,
1296 analogous to eukaryotic silencing by histones (Landick *et al.*, 2015, Grainger, 2016).
1297 While RcsA is present at a low amount in the cell, this was found to be partially due to
1298 transcriptional silencing by H-NS (Sledjeski & Gottesman, 1995). Cellulose synthesis
1299 is under the control of the CsgD transcriptional regulator (Romling *et al.*, 2000,
1300 Zorraquino *et al.*, 2013). Interestingly in EIEC, *csgD* expression is often inactivated
1301 (Sakellaris *et al.*, 2000), suggesting that biofilm formation can interfere with
1302 pathogenesis, making these strains poor biofilm formers.

1303 While no specific transcriptional regulator has been identified for the expression
1304 of AIDA-I, it was shown that transcription was enhanced in the absence of H-NS and
1305 RfaH transcriptional regulators (Benz *et al.*, 2010). Similarly, the transcription of *ehaG*
1306 and *fdeC* is regulated by H-NS (Totsika *et al.*, 2012, Easton *et al.*, 2014).

1307 CS31A synthesis is dramatically reduced in media containing alanine or
1308 leucine, suggesting that these amino acids can play a role as effectors (Crost *et al.*,
1309 2003). The ON/OFF switch is locked in the OFF phase by alanine, whilst leucine
1310 repressed transcription but without affecting the switch frequency. Analysis of *clp*
1311 expression indicated that alanine and leucine could repress *clp* transcription by a
1312 methylation-independent mechanism but also by either promoting methylation or
1313 methylation protection of GATC-II and GATC-I respectively, which increased the
1314 methylation pattern characteristic of repressed cells. Furthermore, alanine prevented
1315 the AfaF-dependent methylation protection and thus the appearance of cells in the ON
1316 phase. Additional regulatory proteins, including ClpB, cAMP, receptor protein (CRP)
1317 and H-NS, also play important roles in the transcriptional expression of the operons of
1318 the *pap* family combined with regulation at a pre-transcriptional level by phase
1319 variation (Blomfield & van der Woude, 2007).

1320 For the T4P in EPEC, the expression of the *bfp* operon is controlled by the BfpT
1321 (also called PerA) transcriptional regulator, a member of the AraC family, encoded on
1322 the enteroadherence factor plasmid (Tobe *et al.*, 1992, Gomez-Duarte & Kaper, 1995).
1323 The expression of CFA/I is positively regulated by CfaR, whereas for the expression of
1324 CFA/II, CS1 and CS2 is positively regulated by the *rns* gene product (a homologue to
1325 *cfaR* with 96 % identity) (Caron & Meyer, 1989, Caron & Scott, 1990, Savelkoul *et al.*,
1326 1990). The expression of AAF is induced by the transcriptional activator AggR (an
1327 homologue of AraC) also located on pAA (Nataro *et al.*, 1994); YafK and Fis (factor
1328 for inversion stimulation) have also been reported to regulate AAF/II transcription
1329 (Sheikh *et al.*, 2001). From a transcriptional regulation point of view, *lpf1* is repressed
1330 by H-NS and activated by Ler in response to different environmental conditions (Torres
1331 *et al.*, 2007, Rojas-Lopez *et al.*, 2011), whereas *lpf2* transcription appears to be

1332 activated by Fur (Torres *et al.*, 2007). Regulation of curli biogenesis is complex and
1333 involves several two-component systems, such as EnvZ/OmpR, CpxA/CpxR or
1334 CpxR/H-NS/RstA/IHF/OmpR (Vidal *et al.*, 1998, Prigent-Combaret *et al.*, 2000,
1335 Prigent-Combaret *et al.*, 2001, Beloin *et al.*, 2008, Ogasawara *et al.*, 2010, Lavery *et*
1336 *al.*, 2014). In EPEC, Fis has been identified as a negative transcriptional regulator of
1337 *csgA* expression (Saldana *et al.*, 2009). Curli expression can be triggered by a large
1338 range of environmental signals such as the temperature, osmolarity or redox potential
1339 (Olsen *et al.*, 1993, Prigent-Combaret *et al.*, 1999, Gerstel & Romling, 2001, Evans &
1340 Chapman, 2014).

1341 The transcriptional regulatory control of the locus of enterocyte effacement
1342 (LEE) encoding the injectisome is undoubtedly one of the most extensively investigated
1343 in DEC, and in particular in EPEC and EHEC (Schmidt, 2010, Stevens & Frankel,
1344 2014, Franzin & Sircili, 2015). For additional information about the complex regulation
1345 networks of specific, global and phage encoded regulators, as well as environmental
1346 signals such as nutrient sources or metabolic products from the host or microbiota that
1347 can affect the transcription of the LEE-encoded genes, readers are referred to recent,
1348 specific reviews on the topic (Connolly *et al.*, 2015, Furniss & Clements, 2018,
1349 Platenkamp & Mellies, 2018, Turner *et al.*, 2018).

1350 **2.3. Regulation at a post-transcriptional level**

1351 At least three main regulation mechanisms can occur post-transcriptionally, (i)
1352 the stability of mRNA, which can be quantified by determining its half-life, (ii) a
1353 riboswitch, where a molecule such as a metabolite can change the folding of an mRNA
1354 with the formation of a termination hairpin that stops the on-going transcription by the
1355 RNA polymerase, or (iii) attenuation based on the formation of terminator/anti-

1356 terminator loops, which couple or uncouple the transcription by the RNA polymerase
1357 with the translation of the mRNA. Such post-transcriptional regulations are important
1358 regulatory mechanisms that are generally overlooked and underestimated, most likely
1359 because they cannot be easily investigated and estimated by transcriptomic analysis on
1360 its own (Vogel & Marcotte, 2012).

1361 Recently, it was shown that the expression level of *agn43* can be controlled by
1362 antitermination of transcription and translation initiation in the leader mRNA
1363 (Wallecha *et al.*, 2014). Among EPS determinants, PNAG production is regulated by
1364 the RNA-binding protein CsrA (carbon storage regulatory protein A) post-
1365 transcriptionally (Boles & Horswill, 2011, Wang *et al.*, 2017), where CsrA binds
1366 cooperatively to the *pgaA* mRNA and competes for recognition with the 30S ribosomal
1367 subunit. By binding to sites located in the mRNA leader, CsrA can further destabilise
1368 the *pgaA* transcript. The transcription of *yeeJ* is increased in absence of the mRNA
1369 regulator PNPase, an exoribonuclease polynucleotide phosphorylase component of the
1370 degradosome (Martinez-Gil *et al.*, 2017).

1371 Pili produced by the *pap* operon appears to be regulated post-transcriptionally
1372 as a result of differential mRNA stability (Baga *et al.*, 1988). The study demonstrated
1373 that the *papBA* transcript is processed and the resulting mRNA encoding the major pilin
1374 subunit accumulated. The difference in abundance of the two mRNA species could be
1375 readily explained by differences in their half-life. In *E. coli*, RNA degradation occurs
1376 *via* the degradosome thanks to the combination of endoribonuclease and
1377 exoribonuclease activities (Burger *et al.*, 2011, Bandyra *et al.*, 2013).

1378 **2.4. Regulation at the translational level**

1379 While attenuation collaterally affects the translation, three main mechanisms
1380 are directly involved in the regulation of translation, (i) anti-sense RNAs (including the
1381 small RNAs), which hybridise with mRNA and thus block the binding of the ribosome,
1382 (ii) riboregulation, where a ligand changes the mRNA folding, which consequently
1383 prevents the binding of the ribosome, and (iii) translational efficiency depending on the
1384 codon usage.

1385 In addition to CsrA, PNAG synthesis is regulated by two small RNAs, CsrB
1386 and CsrC, which actually sequester CsrA and thus activate the translation of the
1387 *pgaABCD* transcript (Liu *et al.*, 1997, Weilbacher *et al.*, 2003). For colanic acid
1388 production, the low level of expression from the *rcaA* promoter by H-NS transcriptional
1389 silencing is alleviated by the DsrA small RNA (Sledjeski & Gottesman, 1995).

1390 In *E. coli*, the OmpA protein is expressed to very high levels, is growth rate
1391 dependent and is a paradigm for riboregulation (Lugtenberg *et al.*, 1976, Koebnik *et*
1392 *al.*, 2000). Actually, the *ompA* mRNA half-life increases proportionally with the
1393 bacterial growth rate (Nilsson *et al.*, 1984). While a specific region of the transcript is
1394 targeted by the RNaseE (endoribonuclease E), binding of the ribosome induces
1395 conformational changes that mitigate the mRNA degradation (Emory & Belasco, 1990,
1396 Emory *et al.*, 1992, Hansen *et al.*, 1994). As an antagonist, Hfq can bind the transcript
1397 to decrease its stability, thus inducing RNA decay (Nilsson *et al.*, 1984, Vytvytska *et*
1398 *al.*, 2000). Hfq facilitates the binding of a small RNA called MicA in the vicinity of the
1399 ribosome-binding site, thus preventing ribosomal recruitment (Udekwu *et al.*, 2005).

2.5. Regulation at the post-translational level

1400
1401 Regulations at the post-translational level comprises the most diverse range of
1402 molecular mechanisms and is hierarchically the most important (Figure 3). In metabolic
1403 pathways, regulation at the post-translational levels is a key mechanism, particularle in
1404 relation to the modulation of the enzymatic activity, which can be influenced by
1405 physical parameters (pH, temperature, ionic force, redox, etc...), inducers and
1406 inhibitors (irreversible or reversible: competitive, non-competitive, uncompetitive or
1407 mixed inhibition) (Guedon *et al.*, 2000, Desvaux & Petitdemange, 2002, Desvaux,
1408 2004); retro-inhibition and pro-activation can also occur and may also involve allosteric
1409 enzymes. Protein activity can be further altered by numerous post-translational
1410 modifications, namely (i) proteolytic cleavage, and (ii) chemical modifications such as
1411 disulphide bonds, phosphorylation, acetylation, methylation, adenylation or
1412 uridylation. Post-translational regulation also includes the protein folding,
1413 association/dissociation of homo- and heteromers, the degradation of proteins
1414 following the N-terminal rule by the ClpAP proteolytic complex, which can all
1415 influence the protein half-life, as well as the protein translocation to a final subcellular
1416 location. Indeed, the maturation of a protein can also occur at translocational and post-
1417 translocational levels.

1418 As an example of post-translational regulation, the decreased production of
1419 colanic acid at 37°C results from the degradation of the RcsA transcriptional activator
1420 by the Lon protease (Ebel & Trempy, 1999). This post-translational regulation
1421 alleviates the *wca* transcription and explain the low amount of RcsA in cell (Sailer *et*
1422 *al.*, 2003). As a two-component system, the RcsA regulator is activated by the transfer
1423 of a phosphate group from the RcsC sensor, which is *per se* another post-translational
1424 regulation level (Desai & Kenney, 2017). For cellulose biosynthesis, the catalytic

1425 activity of the BcsA-B complex using UDP-glucose as a substrate is allosterically
1426 controlled by cyclic-di-GMP (c-di-GMP) on the PilZ domain of the cellulose synthetase
1427 BcsA (Omadjela *et al.*, 2013). Actually, the PilZ domain was the first effector identified
1428 that is activated upon binding of c-di-GMP (Ryan *et al.*, 2012). Furthermore, the
1429 diguanylate cyclase AdrA exhibiting a GGDEF domain regulates c-di-GMP production
1430 (Romling *et al.*, 2000, Zorraquino *et al.*, 2013). C-di-GMP is a ubiquitous second
1431 messenger produced by the diguanylate cyclase exhibiting GGDEF domain, which is
1432 antagonistically degraded by the phosphodiesterases exhibiting EAL domain (Romling
1433 & Amikam, 2006). This molecule controls the motility and virulence of planktonic
1434 cells, as well as cell adhesion and persistence of multicellular communities (Jenal &
1435 Malone, 2006, Romling & Amikam, 2006, Beloin *et al.*, 2008).

1436 As an autotransporter, Ag43 exhibits a signal peptide, which drives the
1437 preprotein to the Sec export system for translocation across the CM before being
1438 cleaved off after translocation into the periplasm. In the periplasm, several chaperones
1439 participate in the folding prior to the translocation across the OM through a cooperative
1440 mechanism involving the translocation assembly (TAM) and β -barrel assembly (BAM)
1441 machineries (Selkrig *et al.*, 2014). Additionally, the passenger of Ag43 is glycosylated,
1442 which stabilises its conformation (Sherlock *et al.*, 2006). These different post-
1443 translational, translocational and post-translocational levels all contribute to the
1444 regulation of the expression of this surface protein. While glycosylation is not that
1445 important for the functions of Ag43 (Reidl *et al.*, 2009), in TibA it is necessary for
1446 autoaggregation, adhesion to epithelial cells and biofilm formation (Cote *et al.*, 2013).

1447 **Conclusion and perspectives**

1448 Reviewing the different cell-surface molecular determinants that can participate
1449 in the surface colonisation process in DEC, from bacterial adhesion to biofilm
1450 formation, the wealth of SCFs at play is clearly highlighted. While some of these
1451 molecular determinants still remain to be fully characterised, their interplay in surface
1452 colonisation must also be carefully considered and kept in mind. The flagella, as force-
1453 generating cell-surface organelles, have been demonstrated to be important for biofilm
1454 formation (Hobley *et al.*, 2015), but expression of strong adherence factors could
1455 replace motility in the early stages of biofilm formation (Pratt & Kolter, 1998, Donlan,
1456 2002). Although flagella expression is repressed during the switch from the planktonic
1457 to sessile lifestyle to reduce the motility capacity of the bacteria, these surface
1458 organelles have a structural and architectural role in the EPM (Hung *et al.*, 2013, Serra
1459 *et al.*, 2013). While the expression of flagellar genes are repressed, genes involved in
1460 the biosynthesis of the EPM components are generally activated during the biofilm
1461 maturation step (Guttenplan & Kearns, 2013). In *E. coli* K12, capsule polysaccharide
1462 and T1P appear to block the autoaggregation mediated by Ag43 by physically shielding
1463 intercellular Ag43-Ag43 interaction (Hasman *et al.*, 1999, Schembri *et al.*, 2004),
1464 whilst, in turn, the autoaggregation overrides bacterial motility (Ulett *et al.*, 2006). In
1465 some ExPEC, T1P expression appears to be further modulated and influenced by
1466 OmpA or OmpX, together with an increase of exopolysaccharide production, as well
1467 as a decrease in bacterial motility (Otto & Hermansson, 2004, Teng *et al.*, 2006). In
1468 NMEC, OmpA would act together with Hek in the invasion of epithelial cells (Smith
1469 *et al.*, 2007, Fagan *et al.*, 2008). All-in-all, this suggests the OMPs' composition of the
1470 OM may act as a signal in physiological adaptation of bacteria for surface adhesion and
1471 colonisation; this research direction is one of the next frontiers to be explored in DEC.

1472 As a general trend, the average number of pili types appears lower in commensal
1473 compared to pathogenic *E. coli* (Spurbeck *et al.*, 2011). For instance, curli or
1474 conjugative pili can compensate for motility during initial adhesion and biofilm
1475 development (Prigent-Combaret *et al.*, 2000, Ghigo, 2001, Reisner *et al.*, 2003, Beloin
1476 *et al.*, 2008). Plasmids in general can encode numerous SCFs as shown in ETEC and
1477 EAEC (Amabile-Cuevas & Chicurel, 1996, Mainil *et al.*, 1998, Ghigo, 2001, Molin &
1478 Tolker-Nielsen, 2003, Kaper *et al.*, 2004, Wuertz *et al.*, 2004, Beloin *et al.*, 2008, Ong
1479 *et al.*, 2009). While conjugative plasmids can confer initial adhesion capacity and
1480 modulate the biofilm architecture (Ghigo, 2001, Wuertz *et al.*, 2004), the genetic
1481 mobility of this extrachromosomal gene pool and its contribution to biofilm formation
1482 remain poorly investigated in DEC (Dudley *et al.*, 2006). In *Pseudomonas aeruginosa*,
1483 T4P have been primarily regarded as involved in the attachment of epithelial cells in
1484 the course of an infection but later were demonstrated to also bind to abiotic surfaces
1485 such as polyvinyl chloride, polystyrene and stainless steel (Giltner *et al.*, 2006) and it
1486 even appeared to exhibit a much higher affinity towards steel than the mucosal
1487 epithelial surface, which emphasises the relevance of examining T4P in both
1488 environmental and clinical conditions (Yu *et al.*, 2007, Burgess *et al.*, 2014). In the
1489 human and animal cutaneous pathogens *Erysipelothrix rhusiopathiae*, the RspA
1490 (rhusiopathiae surface protein A) and RspB surface proteins have been shown to
1491 specifically bind several ECM components, namely fibronectin, collagens I and IV, but
1492 also polystyrene shedding light on the ecophysiology of this microorganism through its
1493 binding ability to adhere to both biotic and abiotic surfaces (Shimoji *et al.*, 2003). These
1494 aspects have not been reported or examined as yet in DEC but are particularly relevant
1495 considering the presence of T4P and ECM-binding proteins, especially some ATs, in
1496 the various *E. coli* enteropathotypes.

1497 The regulatory network for the production of colonisation factors is often
1498 depicted as being restricted to the transcriptional level. However, this review clearly
1499 demonstrates that the range of regulation levels is much broader and even more
1500 complex (Figure 3). As a general trend, it is important to stress and keep in mind that
1501 the primary functional and regulation level is post-translational and not transcriptional,
1502 as is sometimes assumed. Whenever DNA replication, RNA polymerisation or protein
1503 synthesis occur, enzymes are essential and required for these physiological processes
1504 at pre-transcriptional, transcriptional and translational regulation levels, respectively;
1505 any abrupt changes in the environmental conditions, such as some physicochemical
1506 parameters (e.g. pH, temperature, redox potential), will have a first and direct effect on
1507 the enzyme activity before the cell can even change its transcription profile. For the
1508 SCFs, the interplay taking place at the other regulation levels is extremely complex and
1509 their hierarchy is extremely difficult to establish at a global scale. As well as this, some
1510 regulatory mechanisms in the expression of SCFs in DEC have not been fully
1511 investigated, such as attenuation, riboswitches or translational efficiency, but their
1512 involvement cannot be excluded. As molecular cell-surface determinants, the SCFs in
1513 DEC need to be translocated across a LPS-derm bacterial cell envelope to be
1514 functional and active, which involves further translocational and post-translocational
1515 regulation levels that should not be overlooked in a regulatory network. To this end,
1516 our view of the regulatory network for the production of SCFs in *E. coli* remains
1517 incomplete and there is far from an integrated view of all regulation mechanisms. In
1518 addition, findings from investigations using domesticated laboratory strains of *E. coli*
1519 must be interpreted with caution and reinvestigation in DEC genetic backgrounds
1520 would be wise (Hobman *et al.*, 2007). This will undoubtedly lead to new discoveries in

1521 the field in the years to come and contribute to our understanding of DEC colonisation
1522 mechanisms.

1523 In DEC, SCFs have often been examined for their contribution to bacterial
1524 virulence and thus investigated in conditions related to human infection (Nataro &
1525 Kaper, 1998, Kaper *et al.*, 2004, Rossi *et al.*, 2018). In addition to humans, the GIT of
1526 a wide range of animals also harbours *E. coli* strains, both commensal and pathogenic
1527 (Escobar-Paramo *et al.*, 2006, Croxen *et al.*, 2013, Smati *et al.*, 2015, Torres, 2017).
1528 Following shedding from these animal reservoirs, *E. coli* is also found in the
1529 environment. Outside the host, the range of extraintestinal environmental conditions
1530 that can be encountered by this species is wide, ranging from soil, water to plants, as
1531 well as food matrices and food processing facilities (van Elsas *et al.*, 2011, Giaouris *et*
1532 *al.*, 2014, Jang *et al.*, 2017). As foodborne zoonotic pathogens, understanding the
1533 ecophysiology of DEC necessitates considering its lifestyle outside the human host. In
1534 fact, the role of SCFs should be placed in a context much broader than the colonisation
1535 of the GIT, as they can also play an important role in the colonisation of other
1536 environmental niches. A focus solely on the physiopathology and GIT environment
1537 may bias and limit a full understanding of the wide diversity of SCFs in *E. coli*. While
1538 the notion of virulence factors is a major contribution to the field of microbial
1539 pathogenesis (Falkow, 1988, Finlay & Falkow, 1989), a change of paradigm with the
1540 concept of coincidental by-products of commensalism (Le Gall *et al.*, 2007, Diard *et*
1541 *al.*, 2010, Leimbach *et al.*, 2013) or niche factors (Hill, 2012) is necessary to more
1542 accurately apprehend and understand the ecophysiology of pathogenic species in the
1543 food chain and in one-health approach.

1544 Taking a one-health approach considering the whole food chain, the physiology
1545 of DEC should not only be considered with respect to human infection only, but also in

1546 conditions representative of upstream, *i.e.* from the natural environments,
1547 animal/human reservoirs, agri-food environments and foodstuffs (Burgess *et al.*, 2014).
1548 Investigating the ecophysiology of the DEC with respect to the various biotopes and
1549 biocoenoses encountered in different ecosystems from natural environments, animal
1550 reservoirs, food matrices, food-processing environments, to human ingestion should
1551 shed new light on the relevance and contribution of the SCFs for this species and inform
1552 the design of strategic, targeted interventions to improve public health.

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1553 **Acknowledgments**

1554 This work was supported in part by INRAE ("Institut National de Recherche
1555 pour l'Agriculture, l'Alimentation et l'Environnement", previously called INRA
1556 ("Institut National de la Recherche Agronomique"), by EU FP7 (European Union 7th
1557 Framework Programme) Marie Curie Actions ITN (Initiale Training Network) EID
1558 (European Industrial Doctorate) DISCo (A multidisciplinary doctoral industrial school
1559 on novel preventive strategies against *E. coli* infections) project (n°FP7-PEOPLE-
1560 607611), by the RA-FEDER-Bpifrance ("Région Auvergne-Fonds Européen de
1561 Développement Régional-Banque Publique d'Investissement des entrepreneurs
1562 français") FRI ("Fonds Régional Innovation") Cluster IRP ("Institut de Recherche
1563 Pharmabiotique") CoMBa ("Colonisation de la matrice par les bactéries") project
1564 (n°AV0003483 and n°DOS0019690/00), by ANR (Agence National de la Recherche
1565 Agronomique) PathoFood (Deciphering the cell heterogeneity and the spatial
1566 distribution of foodborne bacterial pathogens in food matrices in interaction with the
1567 microbial communities) project (n°ANR-17-CE21-0002) and by France-Ireland PHC
1568 ("Programme Hubert Curien") ULYSSES 2016 Campus France-Irish Research Council
1569 ARBiC project (n°36044YD). RM was a Marie Curie PhD research fellow granted by
1570 the ITN EID DISCo. VA was a PhD research fellow granted by the RA-FEDER-
1571 Bpifrance FRI IRP CoMBa. All authors declare that the work was conducted in the
1572 absence of any commercial or financial relationships that could be construed as a
1573 potential conflict of interest.

1574 **Authors contribution statement**

1575 VA and MD wrote the first overall draft of the manuscript and draw the original
1576 pictures; RM, SL, MP, CMB, and FCD wrote sections of the manuscript. MD

1577 contributed to conceptualise the overarching aims and had management as well as
1578 coordination responsibility for the execution of the work. MD, MP, CMB and FCD
1579 contributed to the acquisition of the financial supports and resources leading to this
1580 publication. All authors contributed to the critical revision of the manuscript, read and
1581 approved the submitted version.

1582 **Conflict of interest statement**

1583 MP is permanent employee of GSK. FCD is permanent employee of Lallemand.
1584 These authors declare that their respective company provided support in the form of
1585 salaries. All other authors declare no competing interests.

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3366

3367 **Figure legends**

3368 **Figure 1: Schematic representation of the exopolymeric matrix (EPM) in *E. coli***
3369 **biofilm.** By analogy with the extracellular matrix (ECM) in mammalian tissue, the
3370 EPM in bacterial biofilm can be further discriminated between (i) the EPM closely
3371 associated with the bacterial cells, i.e. the cell-associated EPM (caEPM) (purple shade
3372 background), and (ii) the interstitial EPM (iEPM) (white background). Molecular
3373 determinants of the caEPM are attached, anchored or linked to the bacterial cell surface.
3374 Besides cell-surface proteinaceous determinants including monomeric proteins (not
3375 depicted in the picture) and supramolecular protein structures, such the flagella and pili,
3376 molecular components of caEPM further comprise extracellular polysaccharides (EPS),
3377 namely some lipopolysaccharides (LPS) as well as poly- β -1,6-N-acetyl-D-glucosamine
3378 (PNAG) and colanic acid, which both form a capsule. Together with colanic acid that
3379 can be released from the bacterial cell surface, cellulose can compose the EPS part of
3380 the iEPM. Besides extracellular DNA (eDNA), some exoproteins (not depicted in the
3381 picture) and outer membrane vesicles (OMV) may also constitute the iEPM in *E. coli*
3382 biofilm.

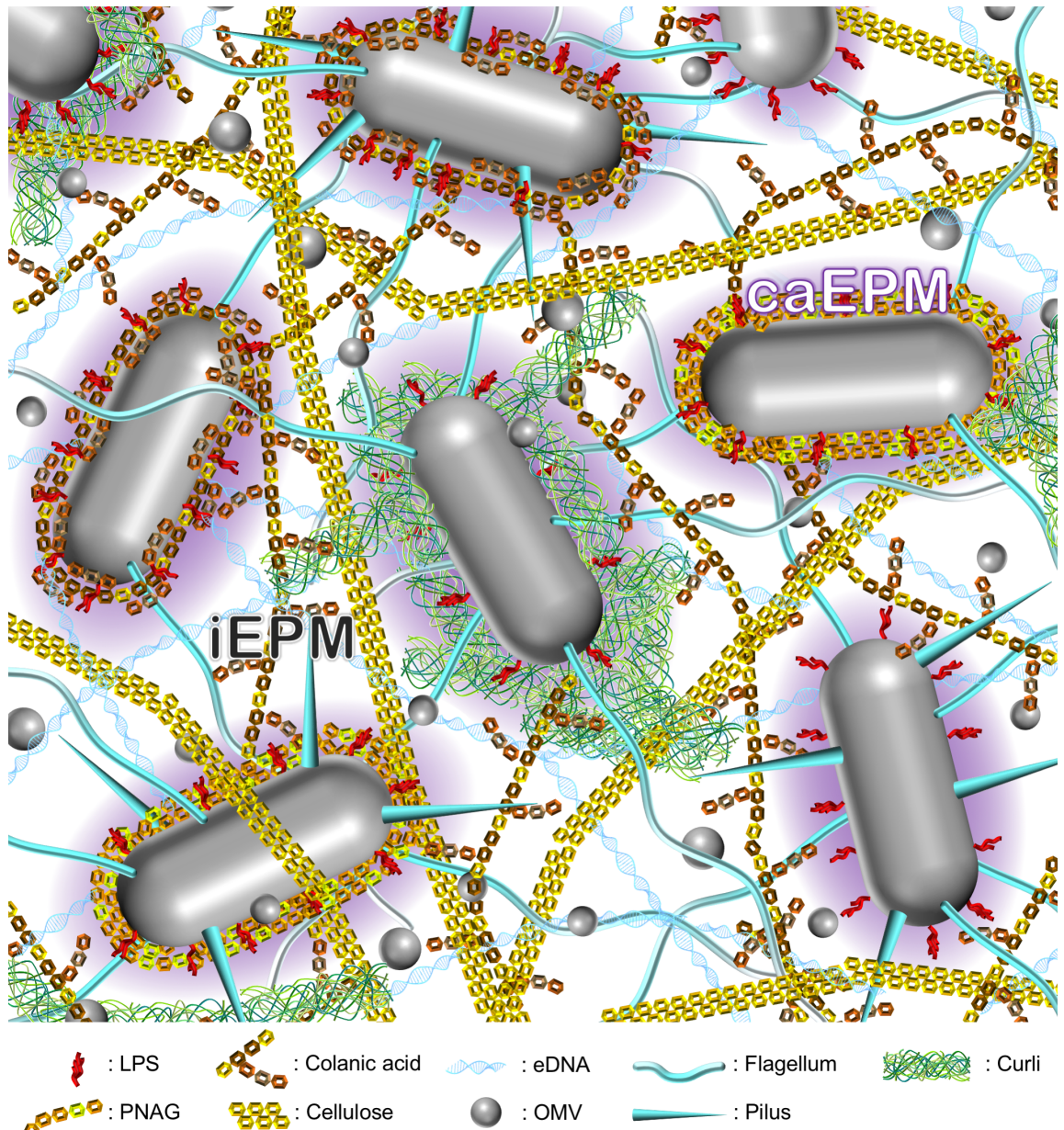
3383 **Figure 2: Schematic representation of the cell-surface proteinaceous determinants**
3384 **acting as CFs in DEC.** Monomeric proteins are depicted in shades of red, whereas
3385 multimeric protein complexes are depicted in shades of blue. Whenever possible,
3386 molecular structures were obtained from the protein databank (PDB) (Berman *et al.*,
3387 2002, Rose *et al.*, 2017) or the electron microscopy databank (EMBD) (Lawson, 2010).
3388 Regarding ATs, no structure for ATAs is currently available but Ag43 (PDB: 4KH3) is
3389 provided as a representative of a SAAT and EspP (PDB: 3SLI, 3SZE) as a
3390 representative of SPATE. Intimin (PDB: 3NCW, 4E1S) is given as a representative of

3391 an IAT. Proteins secreted across the OM by the T5SS are first exported *via* the Sec
3392 translocase (SecYEG-DF/SecA) (PDB: 2AKH, 3AQO, 5XAM) across the inner
3393 membrane (IM). Dispersin (PDB 2JVU) is secreted *via* T1SS (PDB: 5066). Besides
3394 ATs, all OMPs including the Hra, OmpA (PDB: 2GE4) and Iha are first exported *via*
3395 Sec before being integrated into the OM *via* the β -barrel assembly machinery (Bam)
3396 complex (BamABCDE) (PDB: 5LJO). The surface-associated lipoprotein of *E. coli*
3397 (SslE) is secreted by a T2aSS (EMDB: 1763, PDB: 3CIO, 3OSS, 4KSR, 2W7V, 2BH1)
3398 after Sec export. Like the moonlighting proteins represented here by GAPDH
3399 (PDB: 5ZA0), the secretion mechanisms of Efa-1 remain unknown. EibD
3400 (PDB: 2XQH) is provided as a representative of TAAs. The injectisome is secreted and
3401 assembled by the T3aSS (EMDB: 1875). The flagellum (EMDB: 1132, 1873;
3402 PDB: 1IO1) is secreted and assembled by the T3bSS (EMDB: 1887). The T4P
3403 (EMDB: 0070) is secreted and assembled by the T2bSS. The conjugative pilus (CP)
3404 (PDB: 5LEG) is secreted and assembled by the T4bSS (EMDB: 2567). The T1P
3405 (EMDB: 3222), CS31A, AAF (PDB: IUT2, 2XQ), CFA (EMDB: 1952), F9 pilus, ELF,
3406 LPF (PDB: 5AFO), ECP (PDB: 3QS3) and SFP are all secreted and assembled by T7SS
3407 (PDB: 4J3O) after Sec export. The curli are secreted and assembled by the T8SS
3408 (EMDB: 2750). Hcp form a tube, which is displayed extracellularly upon triggering of
3409 the T6SS (EMDB: 2524; PDB: 4HKH, 3RX9, 4JIV).

3410 **Figure 3: Regulation levels and control mechanisms for the expression of genes**
3411 **encoding colonisation factors in DEC.** Respective to biochemical process, the
3412 sequential steps and events for gene/protein expression flow from pre-transcriptional,
3413 transcriptional, post-transcriptional, translational to post-translational regulation levels
3414 (as depicted by blue arrows). Thus, at least five regulation levels can be considered in
3415 bacteria and at each level, different control mechanisms can be at play. Besides, for a

3416 same protein encoded gene different regulation levels and regulatory mechanisms can
3417 intervene, *e.g.* the expression of Ag43 is regulated at pre-transcriptional level by DNA
3418 methylation, at transcriptional level by OxyR, at post-transcriptional level by
3419 antitermination of transcription and translation initiation in the leader mRNA, and also
3420 at post-translational levels with its autoaggregative activity modulated by pH, its native
3421 folding requiring chaperones and final subcellular localisation by translocation across
3422 the OM. Besides rRNA, tRNA and sRNA, biological functions and activities are
3423 essentially represented by proteins and the hierarchy of regulations levels and control
3424 mechanisms (as depicted by shades of red) is opposite to the gene/protein expression
3425 flow; *e.g.* whatever the pre-transcriptional (with DNA replication), transcriptional (with
3426 mRNA synthesis), post-transcriptional (with the modulation of transcripts) or
3427 translational (with the protein synthesis) levels, they are all strictly depend on enzyme
3428 activities which can be regulated at post-translational levels in the first place with direct
3429 and immediate effect due to modulation of their catalytic activity by temperature or pH
3430 for instance.

3431

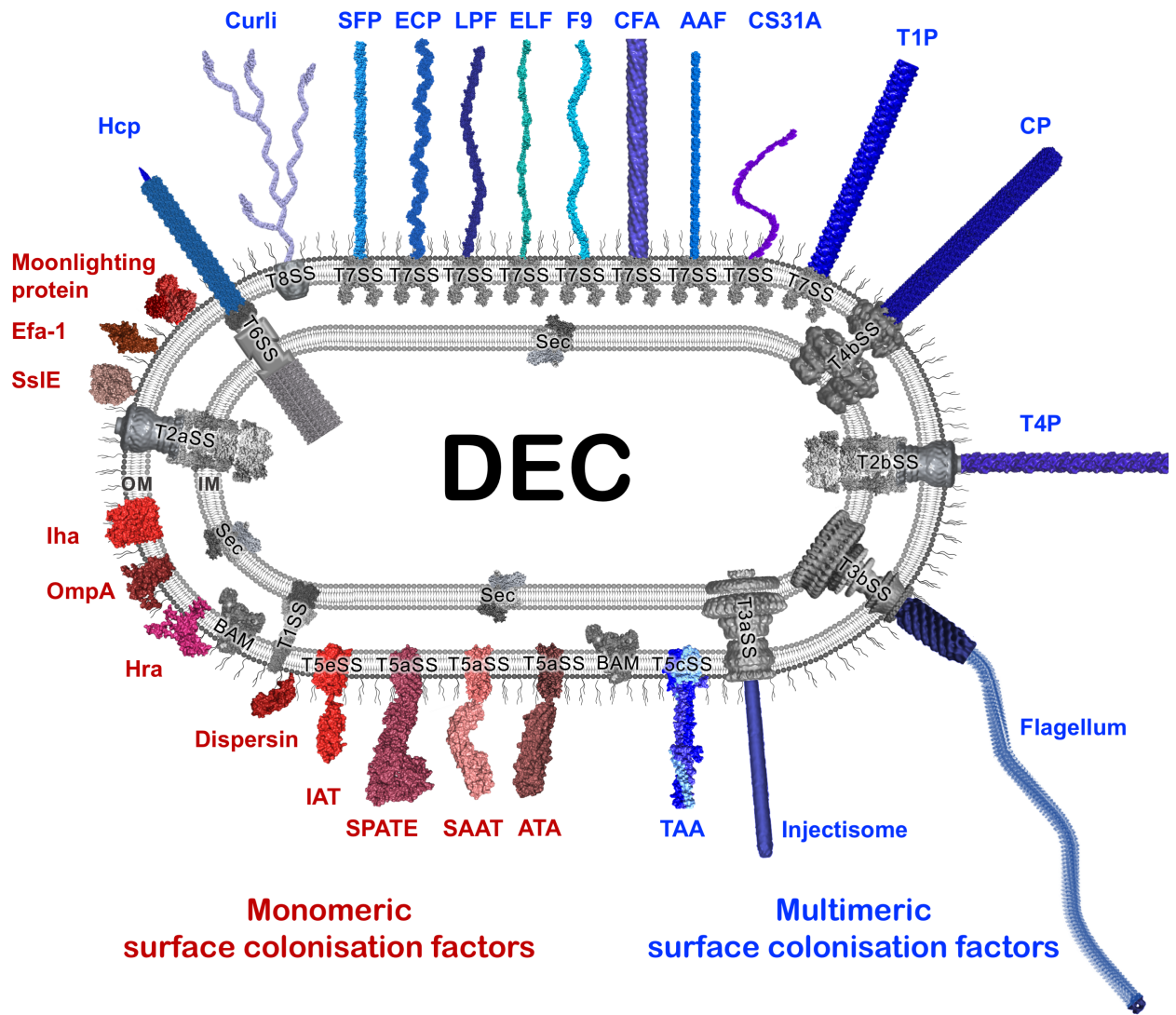


3432

3433

Figure 1

PA



3434

3435 Figure 2

PRE

Regulation levels	Control mechanisms	Examples
Pre-transcriptional	DNA replication (phase variation): -DNA inversion -Slipped-strand mispairing -DNA methylation -DNA deletion	<i>fim</i> operon (T1P) <i>agn43</i> (Ag43), <i>clp</i> operon (CS31A) <i>fliC</i> (flagellin)
Transcriptional	Rate of mRNA synthesis (regulators / effectors): -Positive control of an inducible gene -Positive control of a repressible gene -Negative control of an inducible gene -Negative control of a repressible gene	<i>pga</i> operon (PNAG), <i>wca</i> operon (colanic acid) <i>cfa</i> operon (CFA), LEE (injectisome) <i>agn43</i> (Ag43) <i>clp</i> operon (CS31A)
Post-transcriptional	Modulate transcripts for translation initiation: -Stability of the mRNA (half-life) -Riboswitch -Attenuation	<i>agn43</i> (Ag43), <i>pga</i> operon (PNAG) <i>pap</i> (CU pili)
Translational	Rate of protein synthesis: -Anti-sense RNA (including small RNA) -Riboregulation -Codon usage (translation rate)	CsrB and CsrC (PNAG), DsrA (colonic acid) OmpA
Post-translational	Modulate protein activity: -Physical parameters (pH, etc...) -Inducers and inhibitors (allostery) -Proteolytic cleavage -Chemical modifications (glycosylation, etc..) -Protein folding (chaperones) -Association/dissociation multimers -Stability of the protein (half-life) -Translocation and final subcellular localisation	Ag43 BcsA (cellulose) TibA, Ag43 Ag43 RcsA (colanic acid) Ag43

3436

3437 Figure 3

PREPRINT