INTRODUCTION

Most bacteria live in association with a wide variety of abiotic and biological surfaces in their environments (Costerton, 1999; O'Toole, Kaplan, & Kolter, 2000). Surface attachment plays a key role during infection when bacteria grow and colonise or invade a tissue (Klemm & Schembri, 2004). It is also typically the first step in formation of biofilms, the sessile bacterial communities resistant to the host defences and to antimicrobial agents (Abdallah, Benoliel, Drider, Dhulster, & Chihib, 2014; Monroe, 2007; Shirtliff & Leid, 2009; Tuson & Weibel, 2013).

Overall, bacterial surface attachment is a complex and highly regulated multistep process that is still only partly understood (Berne, Ellison, Ducret, & Brun, 2018). Like most other bacteria,
Escherichia coli possesses several surface adhesins (Korea, Ghigo, & Beloin, 2011), with different roles in attachment dependent on the surface and the stage of biofilm formation (Beloin, Roux, & Ghigo, 2008; Monteiro et al., 2012; Wang et al., 2018). Type 1 fimbriae are crucial for the attachment to eukaryotic cells, because of their capacity to specifically bind mannosylated proteins. This binding is mediated by FimH at the fimbrial tip that interacts with mannosides in a force-dependent catch bond fashion, which allows bacteria to remain attached even when exposed to high shear flow experienced by E. coli during urinary tract infection (Aprikian et al., 2011; Nilsson, Thomas, Sokurenko, & Vogel, 2006). Type 1 fimbriae also bind non-specifically to a variety of abiotic surfaces (Beloin et al., 2008; Chao & Zhang, 2011; Pratt & Kolter, 1998) being important at the early stages of biofilm formation (Monteiro et al., 2012; Wang et al., 2018). Fimbriae-mediated attachment further contributes to the catheter-associated infections (Reisner et al., 2014).

Besides possessing adhesive structures, planktonic bacteria need to establish contact with the surface in order to attach. Flagella-driven motility could contribute to attachment, and thus to biofilm formation (Guttenplan & Kearns, 2013) and structure (Wood, Gonzalez Barrios, Herzberg, & Lee, 2006) or invasion (Misselwitz et al., 2012), by helping bacteria to overcome repulsive forces at the surface (Berke, Turner, Berg, & Lauga, 2008; Drescher, Dunkel, Cisneros, Ganguly, & Goldstein, 2011; Frymier, Ford, Berg, & Cummings, 1995; Pratt & Kolter, 1998, 1999; Vigeant, Ford, Wagner, & Tamm, 2002). Indeed, E. coli strains that either lack flagella or are flagellated but non-motile have severely reduced ability to form biofilms (Genevaux, Muller, & Bauda, 1996; Niba, Naka, Nagase, Mori, & Kitakawa, 2007; Pratt & Kolter, 1998, 1999). Additionally, flagella may also function as adhesins (Friedlander et al., 2013; Friedlander, Vogel, & Aizenberg, 2015; Haiko & Westerlund-Wikstrom, 2013).

Motility in E. coli and other bacteria is controlled by the chemotaxis pathway (Sourjik & Wingreen, 2012; Wadhams & Armitage, 2004) as well as by the biofilm-associated second messenger c-di-GMP (Guttenplan & Kearns, 2013; Hengge, 2009; Jenal & Malone, 2006; Romling, Maciag, Antoniani, & Landini, 2010), and c-di-GMP is thus generally viewed as a biofilm-promoting second messenger (Guttenplan & Kearns, 2013; Hengge, 2009; Jenal & Malone, 2006; Romling, Galperin, & Gomelsky, 2013).

The goal of our study was to analyse the importance of motility and its regulation by the chemotaxis pathway and c-di-GMP during attachment of E. coli to both abiotic and biomimetic mannosylated surfaces. We demonstrate that fimbriae-mediated surface attachment of E. coli is enhanced by the chemotactant-induced smooth swimming and by higher swimming velocity in absence of c-di-GMP signalling. These findings suggest that c-di-GMP can act as a negative rather than positive regulator of E. coli surface attachment, and that low c-di-GMP levels and low activity of the chemotaxis pathway promote surface attachment of E. coli in a concerted way.

2 | RESULTS

2.1 | Smooth swimming promotes surface attachment of E. coli

To investigate possible functions of motility and its regulation in surface attachment of E. coli W3110 (Hayashi et al., 2006), we first quantified biomass of cells attached on microtiter plates after 24 hr incubation using crystal violet (CV) staining (Figure 1a). In line with previous studies that studied effects of flagella and motility on biofilm formation (Genevaux et al., 1996; Niba et al., 2007; Pratt & Kolter, 1999), ΔfliC strain that lacks flagella and ΔmotA strain that has paralysed flagella showed strongly reduced surface colonisation under these conditions. Biomass of surface-attached cells was also reduced for ΔcheZ strain that has an increased level of tumbling, but not for the smooth-swimming ΔcheY and ΔcheA strains. Defects in motility apparently impair surface colonisation by reducing initial attachment, as time-resolved microscopy showed a steady increase in the number of attached wild-type cells during the first 12 hr of incubation (Boehm et al., 1995; Pratt & Kolter, 1999). 

Binding of YcgR to flagellar motor in the presence of c-di-GMP affects motility, either by decreasing cell swimming speed (Boehm et al., 2010) or by suppressing CW rotation of flagellar motor (Fang & Gomelsky, 2010; Paul et al., 2010). High levels of c-di-GMP also stimulate synthesis of biofilm matrix factors, such as curli in E. coli (Pesavento et al., 2008; Sommerfeldt et al., 2009; Tagliabue, Maciag, Antoniani, & Landini, 2010), and c-di-GMP is thus generally viewed as a biofilm-promoting second messenger (Guttenplan & Kearns, 2013; Hengge, 2009; Jenal & Malone, 2006; Romling, Galperin, & Gomelsky, 2013).

Interestingly, in contrast to both our (Figure 1a) and previous (Pratt & Kolter, 1998) results based on CV staining of cell biomass at the late stages of attachment and biofilm formation, we observed a much better attachment of ΔcheA and ΔcheY strains at the early time points of incubation (Figure 1b,c, and Figure S2a,b in the Supporting Information).
Motility and chemotaxis regulate *E. coli* surface attachment. (a) Relative surface colonisation by motility and chemotaxis mutants. *E. coli* cultures were incubated on polystyrene Corning Costar tissue culture TC-treated plates for 24 h in M9 medium at 30°C. Colonisation was quantified using staining with crystal violet (CV) and normalised to the CV value of the wild type (wt). Shown are mean and standard error of three to eight replicates. (b, c) Time course of the relative surface attachment of indicated motility and chemotaxis mutants. Wild-type cells labelled with cyan fluorescent protein (CFP) or with mCherry were mixed 1:1 with wild-type or mutant cells labelled with yellow or green fluorescent protein (YFP or GFP) and incubated for indicated time in M9 medium at 30°C on polystyrene BD Falcon TC-treated plates. (b) Exemplary images of mixed cultures of wild-type cells labelled in magenta and wild type or mutants in green. Scale bar: 25 µm. (c) Relative numbers of attached cells at different time points. The number of attached cells in each image was normalised to the number of wild-type cells, and the values were normalised again to the wild type/wild type ratio in the same experimental series, to compensate for any growth effects of fluorescent protein expression. Inset shows absolute numbers of attached wild-type and ∆fliC cells. Shown are mean and standard error of three replicates. (d, e) Swimming behaviour at the surface of a glass slide. Cells were grown in planktonic cultures to post-exponential phase and their swimming was analysed in motility buffer. (d) Exemplary images of tracks of wild-type and ∆cheY cells. (e) Quantification of trajectory durations and lengths. Shown are mean and standard error of three replicates. (f) Relative surface attachment in the presence of chemoattractants. Attachment of wild-type and ∆cheY cells in motility buffer with and without 10 mM of methyl aspartate and serine to ibidi uncoated imaging plates was quantified after 20 min incubation (see Experimental procedures), and normalised to the number of wild-type cells that were attached without chemoattractants. Shown are mean and standard error of six replicates. Statistical analyses were performed here and throughout using a two-sample t-test with unequal sample size and unequal variance, with \( p < .05 \) (*), \( p < .005 \) (**), \( p < .0005 \) (**), NS, not significant.
information). These results indicate that cell attachment may be directly related to the ability of bacteria to make smooth runs, which is strongly reduced in ∆cheZ but is increased in ∆cheA and ∆cheY strains. Confirming that the enhanced attachment of ∆cheY is motility dependent, no increase in attachment was observed upon deletion of ∆cheY in ΔfliC background (Figure 1c). Moreover, adhesiveness of ∆cheY cells seemed to be even lower than that of the wild type, judging by their poorer attachment in the centrifugation assay (Figure S2c in the Supporting information). Smooth swimming may lead to trapping of cells in the proximity of the surface (Berke et al., 2008; Drescher et al., 2011; Frymier et al., 1995; Vigent et al., 2002), and indeed ∆cheY cells spend more time swimming at the surface than wild-type cells (Figure 1e) and their trajectories are longer (Figure 1d,e). Both measures confirm entrapment of ∆cheY cells at the surface, which means that the smooth swimming mutants sample a larger surface area than wild-type cells and therefore have an increased probability of attachment.

Since stimulation with chemotranscients transiently inhibits CheA activity and therefore reduces cell tumbling, we expected it to enhance attachment similar to the effects of cheA and cheY knockouts. Indeed, we observed that stimulation with 10 mM of α-methyl-DL-aspartate and L-serine, strong chemotranscients sensed by two major E. coli chemoreceptors, elevated attachment of the wild-type cells to the levels similar to, and even slightly higher than that of ∆cheY strain (Figure 1f). The origins of such better attachment of the attractant-stimulated wild type compared to ∆cheY mutant need further investigation. The generally reduced attachment of stimulated cells, apparent for ∆cheY but also presumably true for the wild-type cells, is likely to be non-specifically caused by high millimolar levels of amino acids.

### 2.2 Low levels of c-di-GMP enhance surface attachment

We next observed that colonisation of microtiter plate surface by E. coli is also affected by c-di-GMP signalling. Deletion of the gene encoding DgcE (YegE), the dominant c-di-GMP cyclase under our growth conditions (Sarenko et al., 2017) and the major cyclase controlling motility via c-di-GMP in E. coli (Pesavento et al., 2008), led to increased biomass of surface-associated cells (Figure 2a). Inversely, the deletion of the major diesterase PdeH (YjhH) markedly reduced colonisation (Figure 2a). These effects were mediated by the motility control via YcgR, because ∆ycgR showed similar increase in the colonisation as ∆dgcE, and no additional increase was observed for ∆ycgR∆dgcE strain (Figure 2a). Consistently, deletion of ycgR also abolished the effect of the pdeH deletion (Figure 2a). Deletion of other cyclases with lesser contributions to the global pool of cellular c-di-GMP (Sarenko et al., 2017) showed weaker or no increase in attachment (Figure S3a in the Supporting information). Furthermore, overexpression of DgcE, which is expected to globally elevate the cellular level of c-di-GMP, has reduced attachment in the YcgR-dependent manner (Figure S3b in the Supporting information).

Although ∆dgcE and ∆pdeH strains showed similar surface attachment after 1 hr of incubation in the microscopy assay, which might be due to inherently low c-di-GMP levels at the early phase of culture growth, the effects of the deletions become visible between 3 and 6 hr and further increased up to 12 hr of attachment (Figure 2b,c). At this time point, the results of microscopy and CV staining became comparable, and no further enhancement in the differences between strains was observed until 24 hr (Figure S4 in the Supporting information). Interestingly, the attachment of ∆ycgR cells could be further strongly enhanced during the early stage of incubation by deleting cheY in this background (Figure 2c), suggesting that effects of ycgR and cheY mutations are multiplicative.

To rule out that the effect of ycgR deletion on attachment is due to altered adhesiveness, we again performed the centrifugation assay. Indeed, no differences in adhesion were observed between wild-type and ∆ycgR cells (Figure S5 in the Supporting information). Relative attachment of ∆dgcE cells was even mildly reduced, whereas that of ∆pdeH cells was increased (Figure S5 in the Supporting information), which could be explained by involvement of c-di-GMP in the production of extracellular matrix (Hengge, 2009; Romling et al., 2013; Serra & Hengge, 2019). Since these effects are opposite to the differences in attachment observed for the swimming cells, they do not appear to play major role in attachment under our conditions.

Finally, we observed that when compared to the wild type, not only the number of attached ∆ycgR cells but also the strength of attachment apparently increased (Figure S6 in the Supporting information).

### 2.3 C-di-GMP controls swimming speed via YcgR

To get insights into the mechanism of YcgR-mediated reduction of surface attachment by c-di-GMP, we directly compared surface attachment and motility of wild-type and ∆ycgR cells. Even when pre-grown in a shaking culture to OD600 of 0.5 and subsequently imaged in the motility buffer (i.e., in absence of further growth), ∆ycgR cells showed pronouncedly enhanced surface attachment compared to the wild type (Figure 3a). Thus, the enhanced attachment of ∆ycgR cells does not require growth or differentiation on the surface. Among the analysed motility parameters, differences in swimming speed between mutants were consistent with the previous report (Boehm et al., 2010), with cells swimming faster at low c-di-GMP levels (Figure 3b). In contrast, no differences in cell tumbling rates could be detected under our conditions (Figure 3c). These results indicate the increased swimming speed of ∆ycgR and ∆dgcE cells as the cause of their more efficient attachment to surfaces. Although swimming speed could in principle also influence surface trapping of cells, we could not observe significant differences between trajectory lengths on the surface for ∆ycgR, ∆dgcE or ∆pdeH cells (Figure 3d). This invariance of trajectory length in spite of increased swimming speed could be explained by the compensating shortening of trajectory durations at low levels of c-di-GMP (Figure S7 in the Supporting information). Thus, swimming speed rather than the trajectory length seems to correlate with enhanced attachment.
Type 1 fimbriae mediate swimming-speed dependence of surface attachment

Since flagella apparently have only a minor role in attachment under our conditions, we tested the potential involvement of type 1 fimbriae, one of the major and best characterised adhesins of E. coli (Korea et al., 2011). Indeed, ∆fimA strain that lacks a major subunit of fimbria showed clearly reduced attachment (Figure 4a,b). In contrast, deletion of csgA encoding the subunit of curli fibres, major component of E. coli biofilm matrix (Vidal et al., 1998), had no significant effect on attachment under our conditions (Figure 4a,b). Same results were observed when the surface colonisation was assessed for these strains after 24 hr (Figure S8 in the Supporting information), which is consistent with the previously observed higher importance of type 1 fimbriae for early biofilm formation (Monteiro et al., 2012).

Strikingly, lack of fimbriae also abolished the effect of the YcgR deletion, with attachment of ∆fimA and ∆fimA∆ycgR being identical. This indicates that fimbriae are responsible for the increased number of attached cells seen for the ∆ycgR mutant, which was confirmed by similar attachment of fimbria-less strains with and without ycgR. In contrast, ∆cheY cells attached better even in absence of fimbriae (Figure 4b), although this enhancement was weaker than in the wild-type background. This suggests that prolonged swimming enhances attachment independently of the adhesin involved. Comparable results were obtained when the experiment was performed at higher temperature, 30°C (Figure S9 in the Supporting information).

Specificity of ∆fimA effects on attachment could be confirmed by complementation with FimA expression from a plasmid (Figure S10 in the Supporting information). Moreover, the activity of type 1 fimbriae fimD promoter was not affected by ycgR deletion (Figure S11a in the Supporting information), favouring the hypothesis that attachment mediated by type 1 fimbriae is directly promoted by cell swimming speed. Similarly, no differences in fimD promoter activity were observed in ∆dgcE cells (Figure S11b in the Supporting information).
In contrast, we observed that \textit{fimD} promoter activity was decreased in \textit{\Delta pdeH} and elevated in \textit{\Delta cheY} cells. Nevertheless, these modest changes were opposite to the observed adhesiveness of \textit{\Delta pdeH} and \textit{\Delta cheY} strains in centrifugation experiments (Figures S2c and S5 in the Supporting information) and are thus unlikely to explain differences in their attachment.

The effect of c-di-GMP was also observed for the specific attachment on mannosylated surface, which is strictly dependent on type 1 fimbriae (Figure 4c,d and Figure S12 in the Supporting information). For a relatively brief (20 min) incubation, the attachment to this surface was strongly promoted by motility (Figure 4c,d), although flagella-less \textit{\Delta fliC} cells attached somewhat better than \textit{\Delta motA} cells, possibly because immobile flagella partially hinder the attachment. Importantly, the attachment of \textit{\Delta ycgR} cells under these conditions was almost twice that observed for the wild type. In contrast, attachment of \textit{\Delta ycgR\Delta fliC} and \textit{\Delta fliC} cells was indistinguishable, confirming that the effect of YcgR on attachment is motility-dependent. Strongly increased attachment was also observed for the smooth-swimming \textit{\Delta cheY} cells. However, motility became less important for attachment at later time points, with attachment of \textit{\Delta motA} and \textit{\Delta ycgR} cells becoming similar to that of the wild type after 1h, and the advantage of \textit{\Delta cheY} cells being reduced (Figure S12 in the Supporting information). At this later time point, the lack of flagella reduces the number of attached cells, as \textit{\Delta fliC} cells attached less efficiently than \textit{\Delta motA} cells, pointing to a possible role of flagella as (non-specific) secondary adhesins that can stabilise the fimbriae-mediated primary adhesion.

3 DISCUSSION

Although multiple studies have demonstrated that flagella and motility play an important role for bacterial attachment to biotic and abiotic surfaces (Duan et al., 2013; Friedlander et al., 2015; McClaine & Ford, 2002; Pratt & Kolter, 1999; Zhou et al., 2013), the importance of motility control through cell signalling in this transition from planktonic to the sessile lifestyle remains unclear. Here, we could show that both established networks that post-translationally regulate swimming of \textit{E. coli}, the chemotaxis pathway and the c-di-GMP signalling pathway, also control initial stages of cell adhesion.
First, we confirmed previous observations (Pratt & Kolter, 1998, 1999) that flagella-driven motility strongly promotes initial colonisation of the abiotic as well as of the mannosylated surface by E. coli. For both types of studied surfaces, the cell adhesion is primarily mediated by type 1 fimbriae, whereas flagella might function as auxiliary adhesins. This does not exclude that flagella are more important as adhesins on other types of surfaces (Friedlander et al., 2013; Haiko & Westerlund-Wikstrom, 2013).

Second, although mutations abolishing the motility control by chemotaxis pathway in E. coli had little effect on the long-term surface colonisation as shown already in previous work (Pratt & Kolter, 1998), we observed that smooth-swimming cheA and cheY mutants show largely enhanced initial surface attachment. This was particularly pronounced during the first several hours of incubation and it required swimming, since attachment of ΔcheY strain was rather reduced in the swimming-independent centrifugation strain with the effect of genetic inactivation of the chemotaxis pathway, we observed that E. coli attachment is also promoted by the chemotaxin stimulation that lowers the pathway activity. The chemotaxis could thus control attachment of E. coli (and other bacteria) to surfaces that secrete chemoeffectors, with attractant secretion promoting attachment and repellent secretion inhibiting it. This might affect surface colonisation during formation of submerged biofilms, but also attachment to the epithelial cells that are known to secrete chemoeffectors (Keilberg & Ottemann, 2016; Lopes & Sourjik, 2018). Nevertheless, effects of motility and chemotaxis on attachment and biofilm formation are likely to be species-specific. For example, although motility was similarly important for biofilm formation by Agrobacterium tumefaciens, in that case deletion of cheA had no apparent effect on cell attachment under static conditions and resulted in reduced biofilm formation under flow (Merritt et al., 2007).

Lastly, our results also show that c-di-GMP signalling mediated by the flagellar brake YcgR promotes surface adhesion via type 1 fimbriae (Berke et al., 2008; Drescher et al., 2011; Frymier et al., 1995; Vigeant et al., 2002). Such hydrodynamic entrapment may be a general mechanism promoting bacterial attachment at abiotic and biotic surfaces (Berke et al., 2008; Drescher et al., 2011; Frymier et al., 1995; Misselwitz et al., 2012; Vigeant et al., 2002). Consistent with the effect of genetic inactivation of the chemotaxis pathway, we observed that E. coli attachment is also promoted by the chemoeffectant stimulation that lowers the pathway activity. The chemotaxis could thus control attachment of E. coli (and other bacteria) to surfaces that secrete chemoeffectors, with attractant secretion promoting attachment and repellent secretion inhibiting it. This might affect surface colonisation during formation of submerged biofilms, but also attachment to the epithelial cells that are known to secrete chemoeffectors (Keilberg & Ottemann, 2016; Lopes & Sourjik, 2018). Nevertheless, effects of motility and chemotaxis on attachment and biofilm formation are likely to be species-specific. For example, although motility was similarly important for biofilm formation by Agrobacterium tumefaciens, in that case deletion of cheA had no apparent effect on cell attachment under static conditions and resulted in reduced biofilm formation under flow (Merritt et al., 2007).
fimbiae. This YcgR-dependent regulation of attachment apparently correlates with perturbations of global cellular c-di-GMP levels, primarily controlled by the major diguanylate cyclase/ phosphodiesterase pair DgcE/ PdeH. Although this contrasts with a previous study that instead reported an increased attachment of E. coli at high levels of c-di-GMP (Fang & Gomelsky, 2010), this discrepancy could be explained by the observed YcgR-independent effects of c-di-GMP on cell surface adhesiveness, which might have had stronger effect on attachment in the study of Fang and Gomelsky. Notably, it has been previously reported in Salmonella that the effect of dgcE knockout could be different from deletions of other cyclases (Ahmad et al., 2011).

Our results suggest that the most likely cause of better attachment of ΔycgR cells is their faster swimming. In agreement with a previous report (Boehm et al., 2010), we indeed observed that YcgR negatively regulates swimming speed in presence of high levels of c-di-GMP. In contrast, we observed no evidence for the YcgR-mediated control of cell tumbling rate, as could be expected given previously reported influence of c-di-GMP on the CW rotation bias of flagellar motor (Girgis, Liu, Ryu, & Tavazoie, 2007; Paul et al., 2010). One possible explanation of this discrepancy is that the effect of YcgR on flagellar motor rotation is load-dependent, so that the CW bias is only sensitive to YcgR binding at high load such as present in tethered cells, but not in cells swimming in a liquid with low viscosity. We further observed that the effects of chemotaxis activity and c-di-GMP levels on attachment were multiplicative. The negative regulation of surface attachment by c-di-GMP suggests that this second messenger might have dual function during biofilm formation dependent on its stage (Romling, 2012), with low levels of c-di-GMP being important for efficient early attachment, while high levels being required for matrix production in mature biofilms (Hengge, 2009; Romling et al., 2013).

At least in the case of mannosylated surface, enhanced attachment of faster bacteria could be explained by the known force-dependence of mannose binding by FimH (Aprikian et al., 2011; Sauer et al., 2016). This catch-bond mechanism is normally assumed to promote attachment under high shear force, for example, in the urinary tract, but the same effect could also strengthen attachment of faster cells. Indeed, the effects of high fluid flow and fast swimming on cell interaction with the surface may be similar, increasing tensile force on adhesins interacting with the surface. And while the mechanism of type 1 fimbiae interaction with abiotic surfaces has not being established, it is at least possible that the same large force-induced conformational change in FimH is also responsible for better attachment of faster cells to these surfaces. In contrast, the residual attachment to abiotic surface that was observed in ΔfimA cells and is apparently mediated by one or several of other E. coli adhesins that can attach to plastic (Korea et al., 2011), was not regulated by YcgR. Thus, faster swimming bacteria with low c-di-GMP levels could have an advantage when attaching to biological surfaces, such as bladder epithelial cells, as well as to abiotic surfaces such as catheters via type 1 fimbiae.

4 | EXPERIMENTAL PROCEDURES

4.1 | Bacterial strains and plasmids

All strains and plasmids used in this study are listed in Table S1. E. coli strains used in this work were derived from W3110 (Hayashi et al., 2006). Genes were deleted using either the one-step method for inactivation of genes (Datsenko & Wanner, 2000) or by P1 transduction using strains of the Keio collection (Baba et al., 2006) as donors. Kanamycin resistance was excised using FLP recombination expressed from the plasmid pCP20 (Cherepanov & Wackernagel, 1995). Plasmids pVS130 and pVS147 were made by cloning ecpf and eyfp genes into pTrc99a vector with SacI/XbaI (Amann, Ochs, & Abel, 1988). For the construction of pME7, fimA gene was cloned into pBAD33 vector using XbaI/HindIII.

4.2 | Growth conditions for surface colonisation

Cells were grown in 96-well polystyrene tissue culture (TC)-treated plates from Corning® Costar® (Corning Inc.) or where indicated polystyrene TC-treated plates from BD Falcon™ (Becton, Dickinson and Co.) or ibidi® uncoated imaging plates (ibidi GmbH) in M9 minimal medium (47.7 mM Na2HPO4·12H2O, 22 mM KH2PO4, 8.55 mM NaCl, 18.7 mM NH4Cl, 2 mM MgSO4, 0.1 mM CaCl2) supplied with 0.4% ribose. Pre-cultures were grown in 24-well plates (Corning® Costar®) in 1 ml of Tryptone broth (TB: 1% tryptone, 0.5% NaCl) for 48 hr at 30°C. For growth in M9 medium, cells were first diluted to an OD600 of 1.0 in TB and then to 0.1 in M9. For mixed culture experiments, cells with different fluorescent labels were mixed as indicated. About 125 µl of cell suspension was seeded into the wells. On plates used for imaging, additional 175 µl of medium was added to the wells. Media were supplied with 100 µg/ml of ampicillin (Amp), 50 µg/ml of kanamycin (Kan), 0.005% arabinose and/or 50 µM isoprpyl-β-D-thiogalactopyranosid (IPTG) when applicable. Cells were grown at 30°C without shaking for the indicated time.

4.3 | Crystal violet staining of attached cell biomass

Wells were washed 3 times with 200 µl of sterile water and stained with 200 µl of 1% crystal violet (CV) for 20 min. CV was removed and plates were washed with water until no further destaining was visible. Plates were dried overnight and subsequently incubated with 200 µl of 96% ethanol for 1 hr at 150 rpm on a rotary shaker to solubilise CV. CV absorption was then measured in a plate reader at 600 nm. A blank control with an empty medium was included during surface colonisation and this control was treated in the same way as the samples. Blank values were subtracted from the raw values of the samples. Values obtained for each strain were further normalised to the wild-type control. For normalisation of CV to the OD of the culture, OD600 was measured in a plate reader before washing and staining the cells.
4.4 | Imaging of attachment of planktonic cells on abiotic surfaces

Cells were grown in 10 ml of TB supplemented with 100 µg/ml of Amp overnight at 30°C and 200 rpm, diluted 1:100 in 10 ml of fresh TB supplemented with 100 µg/ml of Amp, 34 µg/ml chloramphenicol (Cm), 0.005% arabinose and/or 50 µM IPTG and grown at 30°C and 200 rpm to the indicated OD<sub>600</sub>. Cells were harvested by centrifugation (5 min, 3,200 g), washed once with motility buffer (10 mM KPO<sub>4</sub>, 0.1 mM EDTA, 67 mM NaCl, 0.5% glucose), resuspended in motility buffer and incubated at 4°C for 20 min. For attachment experiments, cells were diluted to an OD<sub>600</sub> = 0.4 in motility buffer, and cells expressing different fluorescent proteins were mixed as indicated. About 200 µl of cells was seeded in wells of 96-well imaging uncoated plates from ibidi®. After 20 min or 1 hr incubation at room temperature as indicated, plates were washed three times with 200 µl of motility buffer. Attached cells were covered with 200 µl of motility buffer and imaged immediately.

For attachment in the presence of chemoattractants, cells were grown and harvested as described above. After incubation in motility buffer for 20 min at 4°C, cells were diluted to an OD<sub>600</sub> = 0.04 in motility buffer with 10 mM α-methyl-DL-aspartic acid (Sigma Aldrich, USA) and 10 mM L-serine (Acros Organics—Thermo Fisher Scientific, Niddereau, Germany). About 200 µl of cells was seeded in wells of 96-well imaging uncoated plates from ibidi® and imaged without washing after 20 min.

4.5 | Surface mannosylation

In order to create surfaces containing mannose residues, 1% BSA was added to 96-well imaging uncoated plates from ibidi® and incubated for 30 min at room temperature. Surfaces were then washed to remove the unattached BSA and 0.1 mg/ml of 4-Methylumbelliferyl α-D-mannopyranoside (Sigma Aldrich, USA) was added. Plates were incubated for 15 min under UV light using an Ebox VX5 system (Vilber Lourmat, France) in order to activate the fluorophore as previously described (Belisle, Correia, Wiseman, Kennedy, & Costantino, 2008). Wells were then washed and cells were added as explained for attachment of planktonic cells.

4.6 | Fluorescence microscopy

Wide-field fluorescence imaging was performed on a Zeiss Observer Z1 microscope equipped with an ORCA CCD camera (Hamamatsu), 40x NA 0.75 and 100x NA 1.46 objectives, and mCherry (Ex572/25, Em645/90), GFP (Ex470/40, Em525/50), YFP (Ex500/25, Em535/30) and CFP (Ex434/25, Em479/40) filter sets using the AxiVision software, or on an Olympus IX81 microscope equipped with a C9100 EM-CCD camera (Hamamatsu) or an ORCA-R2 camera (Hamamatsu), 20x (UPLSAPO NA 0.75) or 40x (UPLSAPO NA 0.95) objectives, and mCherry (Ex562/40, Em641/75), GFP (Ex474/23, Em525/45), YFP (Ex504/12, Em535/22) and CFP (Ex434/17, Em479/40) filters using Xcelflource rt software (Olympus).

Colonised surfaces for imaging were grown as described above (growth conditions) and imaged in tethering buffer (10 mM KPO<sub>4</sub>, 0.1 mM EDTA, 1 µM methionine, 10 mM lactic acid, 67 mM NaCl, pH 7) after washing with buffer for 3 times. Cells for fluorescent imaging were labelled with eYFP, eCFP, eGFP or mCherry expressed from plasmids pVS147, pVS130, pVM42 or pOB2. Images from wide-field microscopy were analysed using ImageJ (http://imagej.nih.gov/ij/).

4.7 | Centrifugation-enforced cell attachment

Samples of mixed cultures labelled with different fluorophores were prepared as for surface colonisation experiments on TC-treated BD Falcon™ imaging plates and the plates were centrifuged for 2 min at 650 g. After centrifugation, cells were washed with tethering buffer and imaged in motility buffer.

4.8 | Quantification of attached cells by microscopy

For quantification of the area covered by attached cells, a stack of five images acquired within two second time intervals was acquired for each fluorescence channel. Each image in the stack was corrected for background fluorescence with Fiji (https://fiji.sc/) using rolling ball background subtraction (https://imagej.net/plugins/rolling-ball.html). To eliminate swimming cells, the minimal image per stack was calculated. Minimal images were converted to binary images by automated local thresholding using Phansalkar’s method. Cells and cell clumps were identified in binary images using segmentation with Fiji. The fraction of the area covered by identified cells and cell clumps in the area of the whole image was calculated (=area fraction). Ratios of area fractions of mutant cells were determined and finally normalised to the ratio of wild-type cells.

4.9 | Tracking experiments

Cells were grown to OD<sub>600</sub> ~ 0.6 or OD<sub>600</sub> ~ 1.05, as indicated, following the same protocol as for attachment experiments, harvested and washed once by centrifugation at 4,000 rpm (1,300 rcf) for 5 min, resuspended in motility buffer, cooled at 4°C for about 20 min and then diluted to the final OD<sub>600</sub> of ~ 0.02 and ~ 0.005. A small droplet (4 µl) of the suspension was trapped between a slide and a coverslip along with a large volume of air in a grease-sealed compartment. The bacterial motion was observed and recorded at the bottom hydrophilic surface of the sample using phase contrast microscopy at 10x magnification (NA = 0.3) and a Mikrotron Eosens camera (1 px = 0.7 µm) running at 100 frames per seconds (fps) for...
30 s. The bacteria were tracked using the Mosaic analysis program (Sbalzarini & Koumoutsakos, 2005) running as an ImageJ plugin, and extracted trajectories were analysed using custom-made algorithms running as ImageJ plugins, to evaluate tumbling rate, run speeds and trajectory durations and lengths.

4.10 | Tracking analysis

The trajectories were analysed as follows. For trajectories longer than 0.5 s, discrimination between Brownian and stuck particles on the one hand and swimmers on the other hand was performed using a radius of gyration criterion: the measure of diffusivity $max_{i,j} \rho(r_j-r_i)/t_{raw}$ must be larger than 0.2 px²/μsr for the particle to be considered a swimmer, where $t_{raw}$ is the trajectory duration and $r_j$ and $r_i$ are any couple of times within the trajectory. The tumbles were identified using the following criteria. The velocity of the cells is computed as $v = 1/\Delta t \sum_{\Delta t/2}^{\Delta t/2} \Delta v$, with $\Delta t = 0.2$ s. The average displacement $d = 1/\Delta t \sum_{\Delta t/2}^{\Delta t/2} \Delta \vec{r}$ is computed over the same duration, and the ratio $r = |\vec{v}|/d$ is then evaluated. This quantity is close to 1 during the runs and low (typically less than 0.6–0.7) during the tumbles. A threshold $r_{th}$ is computed which maximises Shannon’s entropy, following Huang’s fuzzy thresholding method (Huang & Wang, 1995), using all the tracks of swimmers in one movie as the sample. This threshold is used to separate each trajectory into putative run and tumble segments. To reduce the noise, the variance $v_{run}$ of $r$ during the run segments is computed, and each putative tumble is accepted as a tumble if $\sum (r-r_{th})^2 > 3v_{run}t^2/2$ (i.e., if the magnitude of the tumble excursion is larger than what a random fluctuation would be). The criteria we used are thus based on how directed the motion is and not on changes of orientations, because a tumble can happen without a change of direction. The tumbling rate is then the overall number of transitions from run to tumble in all trajectories divided by the sum of the durations of the trajectories. The average swimming speed was determined as the average of the speed during the runs of all swimmers in the film. The average trajectory durations and lengths were computed using only the trajectories identified as swimmers.

4.11 | Swimming speed measurements

The same culture growth and cell harvesting protocols were used as for tracking experiments, except that the cells were resuspended to a final $OD_{500}$ of 0.5. The sample preparation was the same. It was observed under the same microscope. The motion was then recorded for 100 s at 100 fps. The movie was then analysed using the Differential Dynamic Microscopy algorithm (Wilson et al., 2011), which evaluates the average swimming speed of the population of cells, as well as the standard deviation of swimming speeds within the population and the fraction of swimming cells. The fraction of swimming cells was above 80% in any case, and the standard deviation about 30% of the mean.

4.12 | Flow cytometry

Activity of $fimD$ promoter was assessed using a plasmid-based eGFP reporter from E. coli promoter library (Zaslaver et al., 2006). Samples were grown and prepared as described for measurements of planktonic attachment. Cells were diluted to an $OD_{600}$ of 0.4 in motility buffer and their fluorescence was measured. Alternatively, to investigate promoter activity in swimming and attached cells, 500 μl of this cell suspension was seeded in wells of 8-well ibidi® imaging plates. After 1 hr incubation at room temperature, supernatants were taken and attached cells were scraped in 200 μl of fresh motility buffer. Fluorescence levels of both fractions were measured with BD LSRFortessa SORP cell analyzer (BD Biosciences, Germany).

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CONFLICT OF INTEREST

The authors declare no conflict of interest.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available from the corresponding author upon request.

AUTHOR CONTRIBUTIONS

VMS, MEL, RC, OB and VS designed the experiments and wrote the manuscript. VMS, MEL and RC performed the experiments. VMS, MEL, RC and KF analysed the data.

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