Flagellar Motility Is Critical for \textit{Listeria monocytogenes} Biofilm Formation

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The food-borne pathogen \textit{Listeria monocytogenes} attaches to environmental surfaces and forms biofilms that can be a source of food contamination, yet little is known about the molecular mechanisms of its biofilm development. We observed that nonmotile mutants were defective in biofilm formation. To investigate how flagella might function during biofilm formation, we compared the wild type with flagellum-minus and paralyzed-flagellum mutants. Both nonmotile mutants were defective in biofilm development, presumably at an early stage, as they were also defective in attachment to glass during the first few hours of surface exposure. This attachment defect could be significantly overcome by providing exogenous movement toward the surface via centrifugation. However, centrifugation did not restore mature biofilm formation. Our results indicate that it is flagellum-mediated motility that is critical for both initial surface attachment and subsequent biofilm formation. Also, any role for \textit{L. monocytogenes} flagella as adhesins on abiotic surfaces appears to be either minimal or motility dependent under the conditions we examined.

\textit{Listeria monocytogenes} is a gram-positive food-borne pathogen that causes life-threatening infections in fetuses, newborns, and immunocompromised people. It also causes a severe flu-like illness in pregnant women and self-limited gastrointestinal infections in immunocompetent people (8, 15). \textit{L. monocytogenes} successfully contaminates processed foods because it persists on food-processing surfaces in the form of biofilms (16). Unlike most other food-borne pathogens, \textit{L. monocytogenes} grows during refrigeration. Thus, a small inoculum at the time of packaging can lead to a significant burden of organisms by the time it reaches the consumer (16). Biofilm-coated surfaces are particularly difficult to decontaminate, since bacteria in biofilms are more resistant to detergents, biocides, and antibiotics than are their planktonic counterparts (5, 9, 12). Very little is known about the molecular mechanisms of \textit{L. monocytogenes} biofilm formation. Flagellum-mediated motility is important for biofilm formation by several gram-negative bacteria (18). In contrast, flagella are implicated as surface adhesins early in \textit{L. monocytogenes} surface attachment, but a role for motility in biofilm formation has not been examined (29). This prompted us to investigate the role of flagella and flagellum-mediated motility in \textit{L. monocytogenes} biofilm formation.

\textit{L. monocytogenes} has four to six peritrichous flagella per cell, each of which consists of thousands of flagellin monomers which are modified by β-O-linked glycosylation (23). In contrast to the case with many other bacteria, in \textit{L. monocytogenes}, biosynthesis of flagella is temperature dependent and regulated by a distinctly different mechanism than the well-described hierarchical regulation of gram-negative bacteria. Thus, at mammalian host physiologic temperature, 37°C, most \textit{L. monocytogenes} strains do not produce flagella and are nonmotile (21). This is due to MogR repression of flagellar gene transcription at 37°C (11, 24). In contrast, at 30°C and below, \textit{L. monocytogenes} is motile because MogR is inhibited by its antirepressor GmaR, thus permitting flagellar gene transcription (21, 25).

Flagella play important roles in early biofilm formation in several gram-negative bacteria (18). While it has been proposed that flagella might act in biofilm formation both as surface adhesins and as providers of force-generating motility, in at least \textit{Escherichia coli} and \textit{Vibrio cholerae} it is motility itself that is critical (19, 22, 30, 31). To date, the only published report on the role of \textit{L. monocytogenes} flagella in attachment to abiotic surfaces suggests that, in the absence of motility, flagella have a role as adhesins in initial surface attachment to stainless steel (29). In this study, comparing wild-type bacteria to flagellum-minus and paralyzed-flagellum mutants, we demonstrated that flagellum-mediated motility is critical for \textit{L. monocytogenes} biofilm formation on abiotic surfaces, and if there is any role for flagella as surface adhesins, it is either minimal or dependent upon motility.

**MATERIALS AND METHODS**

**Strains and media.** Previous work on biofilm formation by other bacteria suggests that many of the most commonly studied, domesticated strains of bacteria are poor biofilm formers (4, 28). Preliminary experiments suggested that this might also be the case for \textit{L. monocytogenes}. Therefore, we selected an environmental isolate, M35303A (also known as ZK3457), as our wild type because it had been previously identified as a robust biofilm former (3). This strain belongs to serovar 1/2a, a serovar commonly isolated from food preparation sites and one of the two serovars most commonly associated with human listeriosis (15). M35303A is a cytochrome cuxotroph, hence minimal medium was supplemented with 0.1 mg/ml cytochrome (3, 27). The wild-type strain 10403S was grown in minimal medium supplemented with both cysteine and methionine at 0.1 mg/ml each. O’Neil and Marquis constructed and characterized the nonmotile mutant strains, HEL-304 (Δ\textit{motB}) and HEL-742 (\textit{motBD}Δ\textit{A}; paralyzed flagella), and their respective complemented strains, HEL-447 (\textit{Δ\textit{motA}}) and HEL-759 (\textit{motB}Δ\textit{D}), in the 10403S background as previously described (17). Media used were tryptic soy broth yeast extract (TSBYE) containing 3% Bacto tryptic soy broth [Becton, Dickinson, and Co.] and 0.6% Bacto yeast extract [Becton, Dickinson, and Co.] and Hsiang-Ning Tsai medium (HTM) with 3% glucose plus essential amino acid(s), a defined minimal medium (27). Chloramphenicol was used at 10 μg/ml for strain construction via allelic

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exchange in either TSBYE or Bacto brain heart infusion (BHI; Becton, Dickin-
son, and Co.).

Mutant strain construction. Mutants were constructed in the M35303A back-
ground using allelic exchange with plasmid (1) pKS7Δ flaA for the flagellum-
minus mutant (ΔflaA) (26), (2) pKS7ΔmotBD23A for the paralyzed-flagellum
mutant (motBD23A) (17), and (3) pC01N-688* for the nonglycosylated flagel-
lum mutant (gmaRD83N) (25). The ΔflaA mutant (ZK3602) was nonmotile,
independent of temperature, and did not have visible flagella by crystal violet
(CV) staining (13). The motBD23A mutant (ZK3603) was confirmed by colony
PCR of the region with primers K057 (TATATTGTGATGAGGGATG CTC)
and K058 (TTTTTGATACATATGCGGTTCAG) and subsequent sequence-
ning using these same primers. The motBD23N mutant was nonmotile,
independent of temperature, and had visible flagella by CV staining. The
gmaRD83N,D85N mutant (ZK3604) was confirmed by colony PCR of the region
with primers K053 (CACCCTGGCATGACTGCAG) and K054 (GGC
ATTGTTGGTTGTTGTC ACT), followed by sequencing with the same prim-
ers. The gmaRD83N,D85N mutant had motility comparable to that of the
wild-type strain and had wild-type-appearing flagella by CV staining. The lack of
flagellar glycosylation was also confirmed by immunoblottting of surface-ex-
traced protein preparation with anti-β-O-linked N-acetylgalcosamine (Glc-
NAc)-specific monoclonal antibody CTD110.6 (Fierce) as described by
Shen and colleagues (25). All three mutants displayed exponential-phase growth
rates that were indistinguishable from those of wild-type M35303A in HTM
with 3% glucose and cysteine at 30°C in shaking culture and reached the same
total optical density after 24 h of incubation.

Motility assay. Using a sterile toothpick, bacteria were inoculated into 0.3%
BHI agar and incubated at room temperature (−24°C), 30°C, or 37°C. The
diameter of the bacterial swarm in the agar was measured 24 to 48 h later.
The wild type and the nonglycosylated-flagellum mutant were comparably motile
at −24°C and 30°C, and both were nonmotile at 37°C. The flagellum-minus and
paralyzed-flagellum mutants were nonmotile at all temperatures tested.

Biofilm assay. Surface-adhered biofilm formation was assayed in a 96-well format
using a modified version of previously published protocols (6, 20). Over-
night (15 to 20 h) rolling cultures grown in TSBYE at −20°C (optical density
at 600 nm (OD600) = 2.0 to 2.5) were diluted in HB/C medium containing
2.5 to 3.5) were diluted into freshly made HTM with 3%
glucose plus cysteine to an initial OD600 of 0.05 to 0.1. One hundred fifty
wells were inoculated into any empty wells, and a lid was placed and then secured
in the bottom of a well of a 6-well polystyrene plate, inoculated with 2.5
ml of culture, and left at room temperature for 3 h. Following this, DAPI
(4′,6′-diamidino-2-phenylindole) was added to a final concentration of 2
μg/ml for 10 min. Each coupon was washed three times with 2.5 ml of minimal medium
and then removed, and a coverslip was placed on top. Digital images of 5 hpf
per coupon were captured using an Axioskop 2 microscope (Carl Zeiss, Inc.
equipped with a 100× plan-Apochromat oil immersion objective, Ob-100 joy-100 CCD camera (Hamamatsu Photonics), and Openlab software (Improvis).
Images were processed using ImageJ (NIH), and cells/hpf were counted manually.

Centrifugation experiments. Biofilm assays were done as described above,
with the additional step that after inoculation the 96-well plates were centrifuged
at ~1,900 × g for 60 min in a Beckman Coulter Allegra 6KR centrifuge equipped
with a swinging bucket rotor and microplas carriers. To assay the effect of
centrifugation on initial surface attachment, a glass coverslip (22 by 22 by 1 mm;
Fisher) was inoculated into 0.3% TSBYE at −20°C for 11 to 16 h in TSBYE
(OD600 = ~1.8 to 3.2) were inoculated into freshly made HTM with 3%
and cysteine to an initial OD600 of 0.05 and the then 3 ml of each strain was aliquoted into two separate
wells. After centrifugation, coverslips were washed in the well three times
with 2.5 ml of HTM. Microscopy was performed with an Axioskop 2 Plus (Carl Zeiss,
Inc.) upright scope equipped with a 40×-phase-contrast lens and 1.6× optivar.
Images were captured with an Axiocam CCD and AxioVision 4.4 (Carl Zeiss,
Inc.). At least 5 hpf were manually counted for each strain to determine the
average number of cells/hpf.

Statistical analyses were performed using MINTAB soft-
ware. Comparisons were done using one-way analysis of variance (ANOVA),
followed by Tukey’s multiple comparison test (set at 5%).

RESULTS

L. monocytogenes flagellum-minus mutants are defective in
biofilm formation. Compared to the wild-type strain, an iso-
genic flagellum-minus mutant containing an in-frame dele-
tion in the flagellin gene was defective in surfaced-adhered biofilm
formation (Fig. 1). The bacteria were grown in a defined min-
imal medium (HTM with 3% glucose and cysteine) at 30°C in
96-well PVC microtiter plates. After removal of planktonic
cells and washing, surface-adhered biofilms were stained with
CV (Fig. 1A). We observed that the flagellum-minus mutant
was defective in biofilm formation compared to the wild type
over each of five days (Fig. 1B). The difference in biofilm-
forming ability is unlikely to be secondary to a difference in
growth, as both strains grew comparably. We were unable to
assess biofilm formation for more than 5 days, because after 5
to 6 days in standing culture, biofilm levels of the wild type
began to deteriorate. This deterioration might be the result of
either cell death or biofilm dispersion. These data indicate that
flagella play a role in biofilm formation but do not distinguish
whether this role is in motility, adhesion, or both.

Flagellum-mediated motility is critically important for L.
monocytogenes biofilm formation. To determine whether
motility, or simply the presence of flagella, is critical for biofilm
formation, we compared biofilms formed by the wild-type
strain and a flagellum-minus mutant with that of a paralyzed-
flagellum mutant (motBD23A). The paralyzed-flagellum
mutant had a defect in biofilm formation comparable to that of
the flagellum-minus mutant (Fig. 1). MotB is postulated to be part of the flagellar stator (the stationary part of the flagellar motor within which the rotor turns), and the corresponding aspartic acid in E. coli MotB is required for torque generation (32). In the original L. monocytogenes strain background (10403S), the paralyzed-flagellum mutant (motBD23A) is nonmotile, has normal numbers of peritrichous flagella, expresses normal amounts of flagellin, and can be complemented by allelic exchange of wild-type motB back onto the chromosome (17). In our wild-type background, the motBD23A mutant was similarly nonmotile and by CV-based staining had flagella indistinguishable from the wild type. Both the flagellum-minus and paralyzed-flagellum mutants had growth rates indistinguishable from the wild type in shaking culture in HTM with 3% glucose and cysteine at 30°C. The biofilm-defective phenotype of the paralyzed-flagellum mutant indicates that motility plays a critical role in surface-adhered biofilm formation by L. monocytogenes. These data do not, however, exclude some role for flagella as adhesins once cells reach the surface.

M35303A is not a commonly studied strain of L. monocytogenes (it was selected for its robust biofilm forming ability), therefore, we subsequently examined the biofilm phenotype of flagellum-minus and paralyzed-flagellum mutants in the commonly used 10403S background using mutants generated and published by O’Neil and Marquis (17). We observed that, in this background, both the flagellum-minus and paralyzed-flagellum mutants were defective in biofilm formation compared to wild-type 10403S and that complementation of the respective wild-type gene restores wild-type levels of motility. Analysis using one-way ANOVA followed by Tukey’s multiple comparison test (set at 5%) indicated that there was a statistically significant difference between the amounts of biofilm formed by motile and nonmotile strains on each of five days.

tive mutations with a copy of the wild-type gene resulted in strains that formed wild-type levels of biofilm (Fig. 2A). O’Neil and Marquis have previously demonstrated that complementation of both the flaA and motBD23A mutation with a copy of the respective wild-type gene restores wild-type levels of motility. Analysis using one-way ANOVA followed by Tukey’s multiple comparison test (set at 5%) indicated that there was a statistically significant difference between the amounts of biofilm formed by the motile and nonmotile strains (Fig. 2B). In contrast, there was no statistical difference between the amounts of biofilm formed by the two nonmotile mutants. Likewise, there was no statistical difference in biofilm formation by the two complemented strains and the wild-type strain. Based on this, we propose that our findings for M35303A are generalizable to a wide variety of L. monocytogenes strains.

A mutant lacking flagellar glycosylation has no defect in biofilm formation. A nonglycosylated-flagellum mutant (gmaRD83N,D85N) that has wild-type motility formed biofilms generally as well as the wild-type strain (Fig. 1). We had postulated that if flagella have a role as surface adhesins, then there might be a difference in biofilm formation between the wild type and a motile mutant with an altered flagellar surface. L. monocytogenes flagellin is posttranslationally modified with β-O-linked GlcNAc at three to six sites per flagellin monomer (23). A single flagellum contains thousands of monomers, so it is conceivable that removing all glycosylation might drastically change the sur-
face characteristics of flagella. The *L. monocytogenes* glycosyltransferase responsible for flagellin glycosylation, GmaR, is a uniquely bifunctional protein (25). It both glycosylates flagellin and acts as an antirepressor for MogR, which itself represses transcription of flagellar biosynthetic operons (11, 24, 25). GmaR’s antirepression function is independent of its glycosyltransferase function (25). In the original strain background (EGDe), *gmaRD83N,D85N* has wild-type motility with nonglycosylated flagella (25). We also observed wild-type motility when we moved the *gmaRD83N,D85N* mutation into our strain background. Biofilm formation by the wild-type strain and the nonglycosylated-flagellum mutant were statistically indistinguishable, except for the minor difference observed on day 1 (Fig. 1B). Likewise, there was no statistical difference in initial surface adhesion by the two strains (Fig. 3). We do not know what might account for the day 1 observation (Fig. 1B).

The relative biofilm-forming abilities of mutants are similar on various abiotic surfaces. The nonglycosylated-flagellum mutant also formed biofilms comparable to those of the wild type on each of four different abiotic surfaces tested: PVC, polystyrene, polypropylene, and borosilicate glass (data not shown). Likewise, the flagellum-minus and paralyzed-flagellum mutants were both defective in biofilm formation on these four different surfaces (data not shown). The four different surfaces do not constitute an exhaustive search for a surface on which the absence of flagellum glycosylation might effect some difference in biofilm formation. However, the consistency of the biofilm defect that the flagellum-minus and paralyzed-flagellum mutants have on various surfaces further supports our conclusion that the primary role for flagella in biofilm formation by *L. monocytogenes* is in motility.

**Nonmotile mutants are defective in surface attachment during very early biofilm formation.** To further elucidate the role of motility in biofilm formation, we examined bacterial adherence to glass coverslips at the earliest stages of biofilm formation using light microscopy. Both flagellum-minus and paralyzed-flagellum mutants had a statistically significant deficit in the number of cells adhered per hpf between 30 and 200 min after inoculation compared to motile bacteria, both the wild-type strain and the nonglycosylated-flagellum mutant. The numbers of cells/hpf for the strains were compared by one-way ANOVA followed by Tukey’s multiple comparison test (set at 5%) (Fig. 3). While there was a statistically significant difference in adherence between motile and nonmotile strains, there was not a statistically significant difference in adherence between the flagellum-minus and paralyzed-flagellum mutants. Nor was there a statistically significant difference in adherence between the two motile strains (Fig. 3).

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FIG. 5. Centrifugation restored surface attachment of nonmotile mutants. Initial surface attachment of the flagellum-minus (Fla−) and paralyzed-flagellum (Paralyzed) mutants was restored to wild-type levels by exogenously supplying surface-directed motion via centrifugation (−1,900 × g) for 1 h. Also shown are the data for the adherence of the different strains when left in standing culture for 1 h. When the level of adherence by the spun wild-type strain is compared to that of either the spun flagellum-minus mutant or the spun paralyzed-flagellum mutant, it is not significantly different at the 95% confidence interval as determined by one-way ANOVA followed by Tukey’s multiple comparison test (set at 5%), as denoted by the asterisk. In contrast, when the adherence of the spun flagella-minus mutant and the spun paralyzed-flagella mutant are compared using the same statistical analysis, the level of adherence is statistically different at the 95% confidence interval, as denoted by the double dagger. Cells/hpf were counted after glass coverslips were washed. The number of cells attached per hpf to glass coverslips was normalized such that the average number of cells/hpf for the wild type for each experiment was set at 100%. Data are from four independent experiments, each using one coverslip per strain and condition, and 5 hpf were analyzed per coverslip. Error bars represent standard errors of the means.

between the motile and nonmotile strains was statistically significant at the 95% confidence interval as determined by one-way ANOVA followed by Tukey’s multiple comparison test (set at 5%). In contrast, there was no statistical difference between the two motile strains or between the two nonmotile strains. The defect in adhesion to stainless steel by the nonmotile mutants was less pronounced than their defect in adhesion to glass, suggesting the possibility that the role of flagella in adhesion to stainless steel is different from that to glass.

Exogenously supplied surface-directed force restores the initial surface attachment of nonmotile mutants. To test if exogenously supplied movement directed toward the surface might overcome the surface attachment defect of the nonmotile mutants, we compared the numbers of cells adhered to glass coverslips with and without centrifugation (−1,900 × g for 60 min). For both the flagellum-minus and paralyzed-flagellum mutants, centrifugation increased the number of cells adhered to levels comparable to that of the wild-type strain (Fig. 5). When the numbers of cells/hpf for the strains were compared by one-way ANOVA followed by Tukey’s multiple comparison test (set at 5%), the increase in cell adherence after centrifugation was statistically significant for both nonmotile strains, though not for the wild-type strain. While we cannot exclude the possibility that centrifugation for 60 min might have promoted some nonspecific interactions between the bacteria and the surface, we think that this is unlikely given that there was not a statistically significant increase in adherence of the spun wild-type strain compared to the nonspun wild-type strain. At the 95% confidence interval, there was no significant difference between the spun nonmotile strains and the spun wild type; however, when the two nonmotile strains were compared to each other, the higher number of flagellum-minus cells adhered postspin compared to the paralyzed mutant did reach statistical significance (Fig. 5). We speculate that perhaps the presence of paralyzed flagella interferes slightly with contact between putative bacterial surface adhesin(s) and abiotic surfaces when motility is supplied exogenously.

In our experiments, centrifuging the nonmotile mutants in 96-well microtiter plates for up to 60 min at 1,900 × g did not alleviate the biofilm formation defect of either flagellum-minus or paralyzed-flagellum mutants (data not shown). Based on this, we speculate that surface-associated biofilm formation by L. monocytogenes involves both growth of the initially attached cells and ongoing recruitment of planktonic cells into the biofilm. This recruitment appears to be dependent on flagellum-mediated motility, and without it wild-type levels of biofilm development cannot occur.

DISCUSSION

We have found that flagellum-mediated motility is critical for wild-type levels of L. monocytogenes biofilm development. Compared to the wild type, both flagellum-minus and paralyzed-flagellum mutants had comparable defects in initial surface attachment and in subsequent biofilm formation. Supplying surface-directed motility exogenously via centrifugation restored wild-type levels of attachment to both nonmotile mutants. These data indicate that the primary role of flagella in L. monocytogenes biofilm formation is in generating motion, and that if there is any role for L. monocytogenes flagella as surface-adesins in biofilm formation it is either minimal or is dependent upon motility.

Previously published data suggested that flagellated L. monocytogenes cells attach more rapidly to a stainless steel surface in the absence of motility than flagellum-minus cells (29). Based on this report, we initially hypothesized that the paralyzed-flagellum mutant might form a better biofilm than the flagellum-minus mutant, though in the gram-negative bacteria E. coli and V. cholerae, flagellum-minus mutants and paralyzed-flagellum mutants are similarly defective in biofilm formation (22, 30, 31). In the experiments of Vatanyoopaisarn and colleagues, wild-type, flagellated L. monocytogenes cells (NCTC 7973) were rendered nonmotile by washing and resuspending in phosphate-buffered saline (PBS), a condition of complete nutrient deprivation in which cells are unable to move but remain viable (29). In PBS, lacking motility, the flagellated wild type attach 10-fold more to stainless steel in the first 4 h than do the flagellum-minus mutant. By 24 h, however, attachment levels are comparable. This prior study did not examine mature biofilm formation per se, so our observation that there was no statistical difference in biofilm-forming ability between the paralyzed-flagellum mutant and a flagellum-minus mutant at 24 h and beyond is compatible (Fig. 5).
1B). However, when we compared bacterial attachment to
glass coverslips and stainless steel coupons during the first 4 h
of surface exposure, the paralyzed-flagellum and flagellum-
minus mutants also showed comparably defective attachments,
arguing against a role for flagella as surface adhesins. Differ-
ences in experimental protocols might account for the dissimi-
lar results. Our experiments were done in a different strain
background and under nutrient-replete conditions directly
comparing a paralyzed-flagellum mutant with a flagellum-mi-
minus mutant and an isogenic wild-type strain.

We are struck by the parallel between our results for the role
of *L. monocytogenes* flagella in attachment and biofilm forma-
tion on abiotic surfaces and those of O’Neil and Marquis for
the role of flagella in epithelial cell adherence and invasion
(17). *L. monocytogenes* flagella contribute to epithelial host cell
surface adhesion and invasion (2, 7). Recently, O’Neil and
Marquis have shown that the function of flagella in these
processes is in motility and not as adhesins (17). This is in
contrast to the case for other bacteria, such as enteropatho-
genic *E. coli*, for which flagella function as cell-surface ad-
hesins in the absence of motility (10). While in *L. monocytog-
enes* both flagellum-minus and paralyzed-flagellum mutants
are defective in epithelial cell adhesion and invasion, low-
speed centrifugation (40 to 1,000 × g) restores flagellum-minus
mutant adhesion to epithelial cells to nearly wild-type levels (7,
17). In contrast, even after centrifugation the paralyzed flagella
do not adhere to epithelial cells as well as the flagellum-minus
mutant, suggesting that paralyzed flagella might actually inter-
fere with contact between putative bacterial adhesins and the
epithelial cell surface (17). With regard to abiotic surface at-
tachment, we observed that centrifugation fully restored fla-

gellum-minus mutant attachment, suggesting that motility is
the only role for flagella in this process. In comparing spun
wild-type bacteria with either the spun flagellum-minus mutant
or the spun paralyzed-flagellum mutant, there was no statisti-
cally significant difference between their levels of attachment
at the 95% confidence interval (Fig. 5). However, the increased
level of attachment of the spun flagellum-minus mutant com-
pared to the spun paralyzed-flagellum mutant did reach statistical
significance at the 95% confidence interval (Fig. 5), hinting at the
possibility that paralyzed flagella might also interfere with abiotic
surface attachment.

A limited centrifugation of 1 h restored initial surface at-
tachment, yet it did not restore mature biofilm formation to the
nonmotile mutants. This suggests that biofilm development by
*L. monocytogenes* proceeds via both growth of initially surface-
adhered cells and ongoing recruitment of motile cells from the
planktonic phase. In thinking about how flagellum-mediated
motility is critical for *L. monocytogenes* biofilm formation, we
favor a model, previously proposed for other bacteria, that the
primary role of flagellum in surface-associated biofilm forma-
tion is to provide the force necessary to overcome repulsive
forces that might exist between the bacteria and the surface
(22, 30). Incorporated in this idea is also the concept that the
contribution of flagellum-mediated motility is simply to in-
crease the probability of encountering a surface. It appears
that during biofilm development in many other motile bacteria,
motility and extracellular matrix production are inversely reg-
ulated, such that once motile cells contact a surface they switch
to producing matrix (14). We are curious to determine whether

a similar situation exists in *L. monocytogenes* and how it might
be regulated.

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