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HbiF Regulates Type 1 Fimbriation Independently of FimB and FimE

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Type 1 fimbriae have been suggested to play a role in the pathogenesis of extraintestinal *Escherichia coli* infection. Type 1 fimbriation in *E. coli* is phase variable and known to be dependent upon FimB and FimE, the two recombinases that invert the molecular switch *fimS* and control the expression of the downstream *fim* operon. Here we showed that HbiF, a novel site-specific recombinase, inverted *fimS* independently of FimB and FimE. HbiF-mediated *fimS* inversion appeared to be predominantly switching from "off" (termed OFF) to "on" (termed ON) orientation. This is different from the *fimS* inversion mediated by either FimB (bidirectional ON to OFF and OFF to ON) or FimE (unidirectional ON to OFF). Constitutive expression of the *hbiF* gene in *E. coli* resulted in a *fimS*-locked-ON phenotype, which resulted in the pathogenic *E. coli* K1 strain being incapable of inducing a high degree of bacteremia in neonatal rats. Discovery of HbiF-mediated OFF-to-ON *fimS* switching provides a new opportunity to develop a strategy for the prevention and therapy of extraintestinal *E. coli* infection including bacteremia and meningitis.

Type 1 fimbriae are filamentous surface organelles produced by many members of the *Enterobacteriaceae* including *Escherichia coli* and mediate mannose-sensitive adhesion to various eukaryotic cells. The importance of type 1 fimbriae was implicated in *E. coli* colonization of the gastrointestinal tract and also in the pathogenesis of extraintestinal *E. coli* infections (20, 41, 45). For example, we have demonstrated that more than 80% of *E. coli* K1 bacteria bound onto the surface of human brain microvascular endothelial cells are type 1 fimbriated, suggesting that type 1 fimbriae are important in the pathogenesis of *E. coli* meningitis (41).

Type 1 fimbriae are encoded by the *fimAICDFGH* gene cluster and are primarily composed of the major subunit FimA and a small tip structure containing FimF, FimG, and the adhesin FimH (18). FimH protein has a lectin-like activity with high affinity to α-D-mannose or mannosylated glycoproteins/glycolipids (13, 39, 41). Type 1 fimbriae are assembled via a chaperone-usher pathway, where two specialized accessory proteins are required, the periplasmic chaperone FimC and the outer membrane usher FimD (30).

The expression of the *fim* operon is phase variable. The phase variation of type 1 fimbriae is mediated by an invertible 414-bp *cis* element, *fimS*, that is located upstream of *fimA* and contains a vegetative promoter for the *fim* operon when *fimS* is in the "on" orientation (1). Flipping of this molecular switch alternates *E. coli* between type 1 fimbriated and nonfimbriated states, termed ON and OFF, respectively (1, 19). The switch of *fimS* is a site-specific recombination process and dependent on either FimB (ON-to-OFF or OFF-to-ON switching) or FimE (ON-to-OFF switching only) (19, 22).

In this report, we examined a genomic island deletion mutant (designated *E. coli* YX101 in this study) that failed to induce a high degree of bacteremia in neonatal rats. We found that *E. coli* YX101 is a natural switch-locked-ON mutant for type 1 fimbria expression and that type 1 fimbriation was responsible for the failure to induce bacteremia. The *fimS* switch locking is due to the induction of a tyrosine site-specific recombinase, HbiF, whose effect on *fimS* is independent of FimB or FimE invertase.

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MATERIALS AND METHODS

Strains, plasmids, and growth conditions. *E. coli* strains and plasmids used in this study are listed in Table 1. Bacteria were grown in Luria-Bertani (LB) or brain heart infusion (BHI) broth supplemented with appropriate antibiotics at 37°C overnight statically unless otherwise specified. The antibiotics were used at the indicated final concentrations: streptomycin, 100 μg/ml; ampicillin, 100 μg/ml; kanamycin, 50 μg/ml; and chloramphenicol, 40 μg/ml.

Expression profiling of *E. coli* RS218 and its derivatives with DNA microarray. Expression profiling of *E. coli* RS218 and its derivatives was conducted using an *E. coli* DNA microarray (8, 41). This microarray is composed of 8,144 oligonucleotides that covered open reading frames present in *E. coli* K-12 strain MG1655, *E. coli* O157:H7 strains EDL933 and Sakai, uropathogenic *E. coli* strain CFT073, and other uropathogenic or meningitis-related virulence factors and pathogenicity islands (8, 41).

Overnight cultures of *E. coli* were grown in fresh BHI broth for 30 min at 37°C. Subsequently, bacteria were recovered with centrifugation and bacterial total RNA was extracted with the Ribopure Bacteria kit (Ambion, Austin, TX) followed by an RNeasy minicolumn cleanup (QIAGEN, Valencia, CA). Cy3- and Cy5-labeled cDNA probes were generated with an indirect labeling procedure as previously described (8, 41). The purified Cy3- and Cy5-coupled cDNAs were combined and hybridized to an *E. coli* DNA microarray at 42°C for 18 h. After stringent washings, hybridized microarray slides were scanned (GenePix 4000B; Axon Instruments, Foster City, CA). DNA microarray images were analyzed with GenePix 4.0 software (Axon). The signal intensities between two channels were normalized with print-pin group *loess* normalization. The differential expression of genes was also determined with the Limma package from Bioconductor (http://www.bioconductor.org) (37).

Individual gene or gene cluster knockout. A targeted gene or gene cluster knockout was constructed with a "one-step PCR" gene inactivation method (7). The targeted gene or gene cluster was replaced with a chloramphenicol or kanamycin cassette that is PCR synthesized with long primers based on the antibiotic resistance gene in the template plasmid (Table 2). Both primers have 50-nucleotide extensions that are homologous to boundaries of the targeted gene or gene cluster. After electroporation into the host strain, the targeted gene or gene cluster was replaced with a chloramphenicol or kanamycin cassette via

TABLE 1. Strains and plasmids used in the studies

Strain or plasmid	Relevant characteristic(s)	Reference or source
Strains		
RS218^{str}	Spontaneous streptomycin-resistant mutant of *E. coli* RS218 (O18:K1:H7), isolated from the cerebrospinal fluid of a neonate with *E. coli* meningitis	(34)
10G	F^- *mcrA* Δ(*mrr-hsdRMS-mcrBC*) φ80d*lacZ*Δ*M15* Δ*lacX74 endA1 recA1 araD139* Δ(*ara leu*)*7697 galU galK rpsL nupG* λ^- *tonA*	Lucigen
fimS-ON	RS218^{str} *fimS*-locked-ON	(41)
fimS-OFF	RS218^{str} *fimS*-locked-OFF	(41)
YX101	RS218^{str} ΔRDI 21	(41)
YX102	YX101 Δ*fimAICDFGH*::Cm	This study
YX104	YX101 Δ*hbiF*::Cm	This study
YX105	YX104 carrying pSMART-hbiFc	This study
YX106	YX104 carrying pSMART vector	This study
YX107	YX101 Δ*hbiF*::Cm Δ*fimBE*::Kan and *fimS*-locked-OFF	This study
YX108	YX107 carrying pSMART-hbiFc	This study
YX109	YX107 carrying pSMART vector	This study
YX110	YX101 Δ*fimBE*::Kan	This study
Plasmids		
pBAD-Thio	Expression control vector for pBAD promoter carrying ampicillin resistance gene	Invitrogen
pBAD-hbiF	*hbiF* gene from RS218 under the control of pBAD promoter in pBAD/Thio-TOPO vector	This study
pBAD-fimB	*fimB* gene from RS218 under the control of pBAD promoter in pBAD/Thio-TOPO vector	This study
pBAD-fimE	*fimE* gene from RS218 under the control of pBAD promoter in pBAD/Thio-TOPO vector	This study
pSMART vector	Low-copy-number transcription and translation-free cloning vector derived from pSMARTLCAmp carrying ampicillin resistance gene	Lucigen
pSMART-hbiFc	*hbiF* gene under the control of its native promoter in pSMART vector	This study

homologous recombination, which was facilitated by the λ Red system (23). Subsequently, chloramphenicol or kanamycin was used to identify mutants and confirmation of correct gene replacement was conducted by PCR with screening primers and sequencing of PCR products (Table 2).

Construction of plasmids for expression, complementation, and switch evaluation. Plasmids with inducible *hbiF*, *fimB*, and *fimE* expression were constructed using the pBAD/Thio-TOPO cloning kit (Invitrogen, Carlsbad, CA). The full gene was amplified by PCR from *E. coli* RS218 genomic DNA using Elongase (Invitrogen) and cloned into the pBAD/Thio-TOPO vector according to the manufacturer's instructions. The colonies with recombinant pBAD/Thio fusion plasmids were selected using ampicillin, and the clones with an intact invertase insert in the correct orientation were screened using combined primers from both insert and vector. The correctness of each invertase cloning was confirmed by plasmid sequencing.

Gene cloning for complementation was conducted using a pSMARTLCAmp-based low-copy-number cloning vector (Lucigen, Middleton, WI). The individual gene or gene cluster including the upstream promoter region from *E. coli* RS218 was amplified with Phusion high-fidelity DNA polymerase (New England Biolabs, Inc., Beverly, MA), followed by T4 polynucleotide kinase phosphorylation (Table 2). The phosphorylated PCR product was ligated into pSMARTLCAmp using the CloneSMART kit (Lucigen). Successful cloning of the target gene or gene cluster was confirmed by PCR and plasmid sequencing. For complementation, the resultant plasmid was electroporated into the desired gene knockout mutant.

Invertible element *fimS* orientation assay. A qualitative assay of *fimS* orientation was conducted using the asymmetrical digestion of a *fimS*-containing fragment as previously described (41). The 602-bp fragment containing the invertible element *fimS* was PCR amplified using a pair of primers, fimS-F and fimS-R (Table 2), directly from broth culture or colonies. Asymmetrical restriction digestion of this PCR product with SnaBI produced 404- and 198-bp fragments when type 1 fimbriation was in phase-ON or 442- and 160-bp fragments when fimbriation was in phase-OFF. The SnaBI-digested PCR products were separated on a 2.0% agarose gel.

Yeast aggregation assay. The abilities of *E. coli* strains to aggregate yeast cells were measured as previously described (39). *E. coli* strains were washed and resuspended in phosphate-buffered saline at an optical density at 530 nm of 0.4. These normalized *E. coli* suspensions were subjected to twofold serial dilutions in phosphate-buffered saline and subsequently mixed with commercial baker's yeast cells (5 mg/ml) with or without 1% D-mannose. Aggregation was monitored visually, and the titer was recorded as the highest dilution giving a positive aggregation result.

Animal model of *E. coli* bacteremia. *E. coli* bacteremia was induced in 5-day-old rats by subcutaneous injection of *E. coli* as previously described (17). The outbred, pathogen-free pregnant Sprague-Dawley rats with timed conception were purchased from Charles River Breeding Laboratories (Wilmington, MA). The rats usually deliver in our vivarium 5 to 7 days after arrival. At 5 days of age, all members of each litter were randomly divided into designated experimental groups to receive subcutaneously 10^6 CFU of *E. coli* RS218 or its derivatives. At 18 h after bacterial inoculation, blood specimens were obtained for quantitative cultures as previously described (17).

RESULTS

***E. coli* YX101 is a type 1 fimbrial molecular switch *fimS*-locked-ON mutant.** Recently, we showed that *E. coli* YX101, a genomic island mutant derived from meningitis-causing *E. coli* K1 strain RS218, failed to induce any detectable bacteremia in neonatal rats after subcutaneous inoculation of 10^6 CFU of bacteria (44). In contrast, the neonatal rats infected with wild-type *E. coli* RS218 showed bacteremia of 10^8 CFU/ml of blood. To understand why *E. coli* YX101 failed to induce a high degree of bacteremia in the neonatal rats, we compared the expression profiles of this strain with those of *E. coli* RS218 using DNA microarrays. One of the most drastic changes in *E. coli* YX101 was the induction of *fim* operon expression (Fig. 1A). The *fimAICDFGH* genes in the *fim* operon were significantly induced 6-to 17-fold in *E. coli* YX101 compared to the parent strain *E. coli* RS218, suggesting that *E. coli* YX101 is highly type 1 fimbriated.

Since type 1 fimbriation is phase variable, we examined the status of molecular switch *fimS* in *E. coli* YX101 with PCR amplification combined with SnaBI asymmetrical restriction digestion (41). The wild-type *E. coli* RS218 was composed of a mixture of fimbriated and nonfimbriated cells when grown in BHI broth under static conditions as previously shown (Fig. 1B, lane 1). However, it is striking that the *fimS* switch in *E. coli*

TABLE 2. Oligonucleotides used in the studies

Oligonucleotide	Sequence[a]	Usage
fimS-F	AGTAATGCTGCTCGTTTTGC	*fimS* inversion assay
fimS-R	GACAGAGCCGACAGAACAAC	*fimS* inversion assay
hbiF-ATG	ATGACGAGAAAATATCTCACAC	*hbiF* coding region cloning into pBAD/ Thio-TOPO
hbiF_stop2	GGTACTGTTTTAACGAGGCT	*hbiF* coding region cloning into pBAD/ Thio-TOPO and *hbiF* knockout checking
fimB-ATG	ATGAAGAATAAGGCTGATAACAA	*fimB* coding region cloning into pBAD/ Thio-TOPO
fimB-stop	CTATAAAACAGCGTGACG	*fimB* coding region cloning into pBAD/ Thio-TOPO
fimE-ATG	GTGAGTAAACGTCGTTAT	*fimE* coding region cloning into pBAD/ Thio-TOPO
fimE-stop	ATTATCAATTAGTTAAATCAAGC	*fimE* coding region cloning into pBAD/ Thio-TOPO and *fimBE* knockout checking
hbiFc_F	TTCTACTATGAGGACCGAATCCTT	*hbiF* cloning into pSMART
hbiFc_R	TTGAATCAGAAGGTACTGTTTTAACG	*hbiF* cloning into pSMART
fim_KOF	TGGCAGTCAAACTCGTTGACAAAACAAAGTGTACAGAACGACTGC CCATGgtgtaggctggagctgcttc	*fim* knockout
fim_KOR	TAGCTTCAGGTAATATTGCGTACCTGCATTAGCAATGCCCTGTGAT TTCTcatatgaatatcctcct	*fim* knockout
fim_CKF	TCTTTTGGGGGAAAACTGTG	*fim* knockout checking
fim_CKR	TCTGGCCTACAAAGGGCTAA	*fim* knockout checking
hbiF_KOF	TACATCGATAACGTTCTGATTGCAGGCATACTTATCTGGGTGTGCC TCTCgtgtaggctggagctgcttc	*hbiF* knockout
hbiF_KOR	GAAGGTACTGTTTTAACGAGGCTTTTTTTCCACACCCCTTTAAAA CGAGcatatgaatatcctcctta	*hbiF* knockout
hbiF_CKF	TGACTTGTAGAAAAGCTGCACAA	*hbiF* knockout checking
fimBE_KOF	ATAATCAGGATTAAAATGTTGGATTATTGCTAACCCAGCACAGCT AGTGCgtgtaggctggagctgcttc	*fimBE* knockout
fimBE_KOR	CCCATAATCCGGCAAAACGAGCAGCATTACTGGCGGTATAACGCA CAGTAcatatgaatatcctcctta	*fimBE* knockout
fimBE_CKF	TCACTCAGAAGAACTGGTCCAC	*fimBE* knockout checking

[a] Lowercase underlined sequences represent sequences matching the template plasmids pKD3 or pKD4 (23).

YX101 was completely in switched-ON orientation under similar growth conditions (Fig. 1B, lane 2). We also examined growth conditions favorable for ON-to-OFF switching, such as passage on blood agar plates. Without exception, *fimS* in *E. coli* YX101 was orientated in the switched-ON state (Fig. 1B, lane 4). In addition to the *fimS* orientation, we also examined the type 1 fimbriation using a yeast aggregation assay. *E. coli* YX101 exhibited abilities to aggregate yeast cells similar to those of highly type 1 fimbriated *E. coli* strain *fimS*-ON, a *fimS*-locked-ON mutant of *E. coli* RS218 previously constructed through the mutation of inverted repeats in the *fimS* molecular switch (41) (Table 3). Based on these findings, we conclude that *E. coli* YX101 is a *fimS*-locked-ON mutant and highly type 1 fimbriated.

Constitutive induction of *hbiF* is responsible for the *fimS*-locked-ON phenotype. Since *E. coli* YX101 is a *fimS*-locked-ON mutant, we examined and compared the expression profiles of *E. coli* YX101 with those of the genetically constructed *fimS*-locked-ON strain (*fimS*-ON) and the parent strain RS218 using DNA microarrays. We showed that the *hbiF* gene was highly induced in *E. coli* YX101 compared to *E. coli* RS218, while it remained relatively unchanged in the genetically constructed *fimS*-locked-ON mutant (Fig. 2A). In contrast to the strong induction of *hbiF*, the expression levels of *fimB* and *fimE* were not significantly changed in *E. coli* YX101 compared to *E. coli* RS218 (Fig. 2A). In addition, deletion of *fimBE* from YX101 did not affect the *fimS* inversion and the *fimBE* deletion mutant remained in the *fimS*-locked-ON state (Fig. 2B).

The *hbiF* gene (GenBank accession number DQ272538) belongs to a gene cluster designated *hbi* (from *H*BMEC [human brain microvascular endothelial cell] *b*inding *i*nduced) due to their high expression levels in human brain microvascular endothelial cell-associated *E. coli* (Y. Xie, unpublished data). The *hbiF* gene was absent in the *E. coli* K-12 strain MG1655 genome (2) but present in approximately 80% of *E. coli* K1 isolates derived from blood and cerebrospinal fluid including strain RS218 (data not shown). Although this gene was also present in the genomes of *E. coli* O157:H7 strains EDL 933 and Sakai, *hbiF* genes in these two strains were truncated due to a frameshift mutation (14, 27). HbiF protein was homologous to two well-known *fimS* invertases, FimB and FimE, with percentages of amino acid identity of 55% and 52%, respectively, suggesting that it may belong to the family of tyrosine site-specific recombinases and have an effect on the type 1 molecular switch *fimS*.

A marked induction of *hbiF* in *E. coli* YX101 and similarity between HbiF and FimB as well as FimE prompted us to hypothesize that HbiF protein might be involved in the *fimS*-locked-ON phenotype in *E. coli* YX101. We cloned the *hbiF* gene into the pBAD/Thio-TOPO vector (designated pBAD-hbiF) so that the expression of *hbiF* was under the tight control of the pBAD promoter. When *E. coli* RS218 was transformed with pBAD-hbiF, *fimS* was switched from OFF to ON orien-

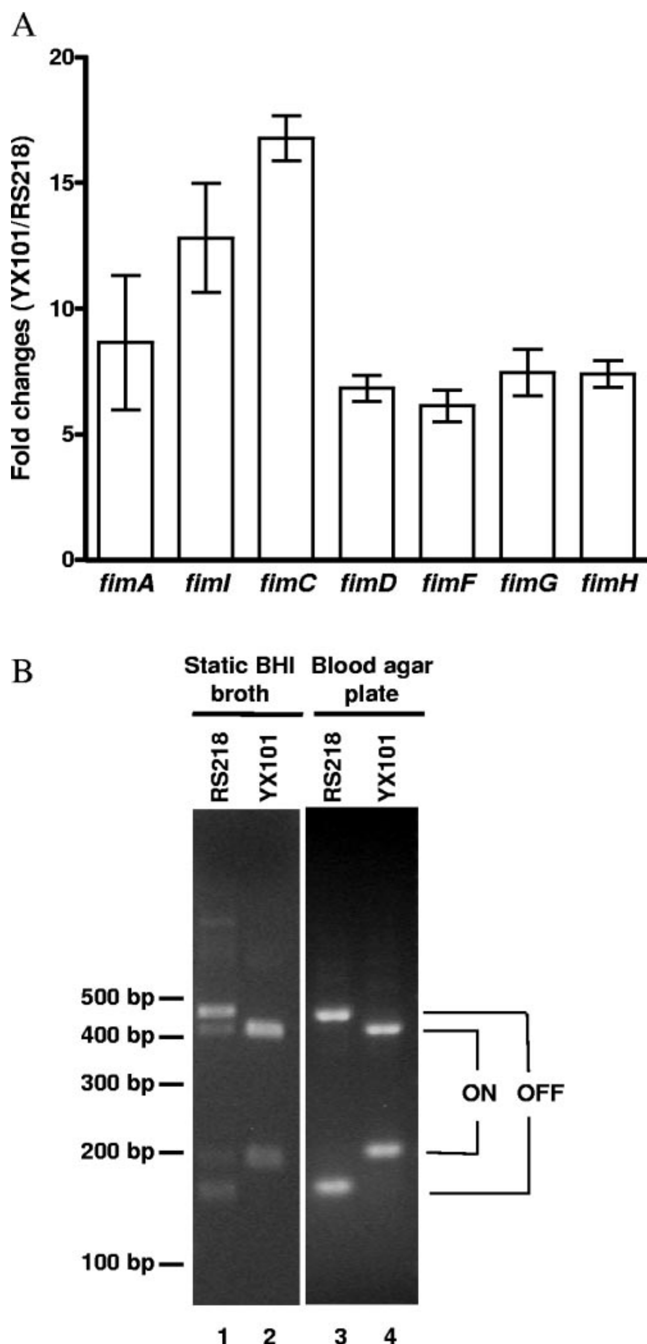

FIG. 1. *E. coli* YX101 is a type 1 molecular switch *fimS*-locked-ON mutant. (A) In *E. coli* YX101 (an RSI 22 deletion derivative of *E. coli* RS218), genes in the *fim* operon were significantly induced compared to *E. coli* RS218 (values are means ± standard errors of the means). The mRNA levels of the *fim* gene cluster were profiled using an *E. coli* DNA microarray. (B) The induction of the *fim* operon in *E. coli* YX101 was due to OFF-to-ON orientation switching of *fimS*. The *fimS* gene was locked in ON orientation in this strain but phase variable in parental strain RS218.

tation with 0.1% arabinose overnight induction compared to that in RS218 with control vector pBAD-Thio (Fig. 2C, lanes 1 and 2). When exogenous FimB and FimE were supplied in a similar fashion, FimB favored OFF-to-ON switching while FimE favored ON-to-OFF switching as expected (Fig. 2C, lanes 3 and 4). These findings suggest that HbiF protein is likely to be responsible for the *fimS*-locked-ON phenotype observed in *E. coli* YX101.

To further confirm the role of *hbiF* in type 1 fimbriation, we constructed an *hbiF* deletion mutant in the background of *E. coli* YX101. Deletion of *hbiF* from *E. coli* YX101 (YX104, the *hbiF*-negative derivative of YX101) restored the *fimS* switch from locked-ON orientation to a mixed population of ON/OFF orientations when grown in BHI broth under static conditions (Fig. 2D, lane 3). Complementation of the *hbiF* gene with its native promoter in the *hbiF* deletion mutant of *E. coli* YX101 (YX105, YX104 plus pSMART-hbiFc) changed the *fimS* switch back to locked-ON orientation (Fig. 2D, lane 4). In contrast, complementation of the *hbiF* deletion mutant of YX101 with the control vector (YX106, YX104 plus pSMART vector) did not affect the mixed ON/OFF *fimS* composition as expected (Fig. 2D, lane 5). These findings indicate that the induction of *hbiF* gene expression is responsible for the *fimS*-locked-ON phenotype in *E. coli* YX101.

HbiF invertase switches molecular switch *fimS* independently of FimB and FimE. Based on the sequence homology and expression profiling results, we hypothesize that HbiF invertase directly affects *fimS* inversion, rather than indirectly through FimB or FimE. In order to distinguish the contributions of FimB and FimE from that of HbiF to *fimS* inversion, we deleted *fimBE* in the *hbiF* mutant strain of YX101. Deletion of both *fimBE* and *hbiF* in YX101 resulted in strain YX107, whose *fimS* switch was locked in OFF orientation. For example, the *fimS* switch in *E. coli* YX107 (*fimBE* negative, *hbiF* negative) was maintained in locked-OFF orientation even after three passages in static BHI broth (Fig. 3A, lanes 5 to 8), while the *fimS* switch in *E. coli* RS218 (*fimBE*$^+$ *hbiF*$^+$) was switched from OFF to ON orientation during these passages (Fig. 3A, lanes 1 to 4). The *fimS* locking in *fimBE*-negative, *hbiF*-negative strain YX107 suggests that there are no other factors capable of switching *fimS* in *E. coli* YX101 except for these three invertases. This is consistent with the findings from genomic sequencing where only three FimB homologues are found in the *E. coli* RS218 genome (i.e., FimB, FimE, and HbiF; http://www.genome.wisc.edu).

To further confirm our hypothesis that HbiF activity in *fimS* inversion is independent of FimB and FimE, we next transformed the invertase-negative mutant YX107 (*fimBE* negative, *hbiF* negative and *fimS*-locked-OFF) with pSMART-hbiFc. Reintroduction of *hbiF* with its native promoter into this in-

TABLE 3. Yeast aggregation abilities of *E. coli* RS218 and its derivatives

Strain[a]	Yeast aggregation titer[b,c]	
	Without D-mannose	With 1% D-mannose
E. coli RS218	1	−
E. coli YX101	16	−
E. coli fimS-ON	16	−
E. coli fimS-OFF	−	−

[a] The strains were grown overnight in static BHI broth at 37°C.
[b] Agglutination titers were recorded as the highest dilution giving a positive aggregation.
[c] Minus sign shows that no aggregation was observed.

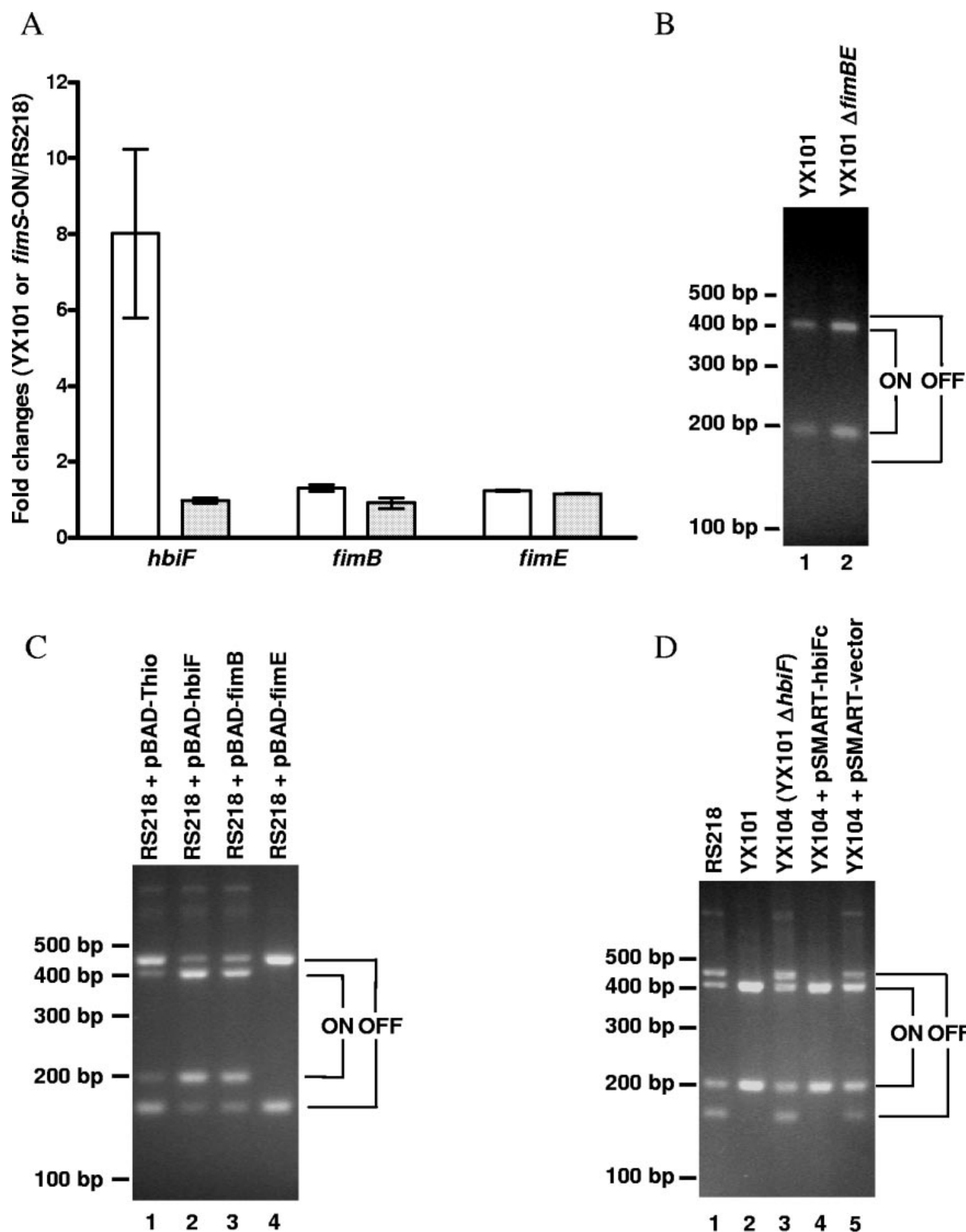

FIG. 2. The *fimS*-locked-ON phenotype in *E. coli* YX101 is due to induction of *hbiF*. (A) DNA microarray results showed that *hbiF* was significantly induced in *E. coli* YX101 compared to RS218 (white bars) but not in the engineered *fimS*-locked-ON mutant (shaded bars) (values are means ± standard errors of the means). Expression of *fimB* and *fimE* genes remained unchanged in both YX101 and *fimS*-ON. (B) Deletion of *fimB* and *fimE* in *E. coli* YX101 did not affect the locked-ON status of *fimS*. (C) Overexpression of *hbiF* in *E. coli* RS218 resulted in *fimS* switching from OFF to ON. (D) *hbiF* was responsible for the *fimS*-locked-ON phenotype in *E. coli* YX101.

vertase-negative mutant changed the *fimS* switch from OFF to ON orientation, while the same strain supplemented with the vector control remained in OFF orientation as in the parent strain when grown on solid medium (Fig. 3B). We also examined the *fimS* status in these transformants growing in static or shaking liquid culture. With no exception, the *fimS* switches in YX107 carrying pSMART-hbiFc were orientated in locked-ON, while those in the same strain carrying vector control were

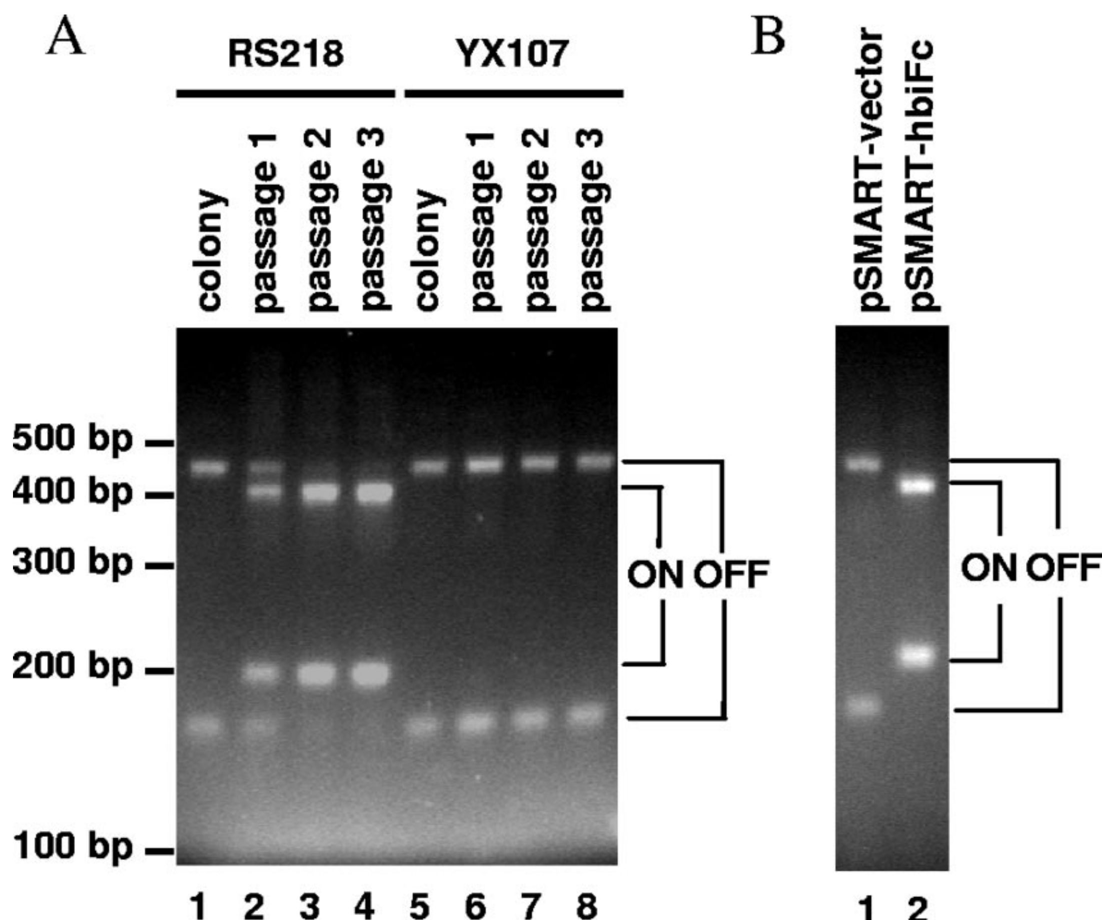

FIG. 3. HbiF inverts the molecular switch *fimS* independently of FimB and FimE. (A) The molecular switch *fimS* was locked OFF in *E. coli* YX107, where both *fimBE* and *hbiF* genes are deleted from the genome of YX101. (B) HbiF was able to switch *fimS* from OFF to ON and remains in locked-ON orientation in the absence of FimB and FimE, the two well-known *fimS* invertases.

orientated in locked-OFF (data not shown). These data illustrate that HbiF invertase is working on the molecular switch *fimS* independently of FimB and FimE recombinases and is responsible for *fimS* changes from OFF to ON orientation in YX101. FimB- and FimE-independent *fimS* inversion is also consistent with the observations where deletion of *fimBE* did not affect the *fimS*-locked-ON phenotype in YX101 (Fig. 2B). In addition, HbiF-mediated *fimS* OFF-to-ON switching is consistent with our demonstrations of *fimS* conversion from locked-OFF in the *fimBE*-negative, *hbiF*-negative strain YX107 (YX101 Δ*fimBE* Δ*hbiF*, Fig. 3A, lanes 5 to 8) to locked-ON in *fimBE*-negative, *hbiF*$^+$ strain YX110 (YX101 Δ*fimBE*, Fig. 2B, lane 2) with the introduction of *hbiF*.

HbiF-mediated type 1 fimbriation results in a marked reduction of bacteremia. We next examined whether type 1 fimbriation in *E. coli* YX101 is related to this strain's failure to induce bacteremia. To evaluate the role of type 1 fimbriation in induction of bacteremia, we first examined genetically engineered type 1 fimbriation mutants *E. coli fimS*-ON and *fimS*-OFF for their abilities to induce bacteremia in neonatal rats. Subcutaneous injection of *E. coli fimS*-ON into neonatal rats did not induce any detectable bacteremia, which was identical

TABLE 4. Magnitude of bacteremia induced by *E. coli* RS218 and its derivatives in neonatal rats

Strain (no. of animals examined)	Bacteremia, log CFU/ml blood (mean ± SD)	Comment(s)
E. coli RS218 (12)	8.59 ± 2.70	Wild type
E. coli YX101 (13)	<1.69[a,b]	Highly type 1 fimbriated, a *fimS*-locked-ON mutant of RS218
E. coli fimS-ON (6)	<1.69[a,b]	Highly type 1 fimbriated, a genetically engineered *fimS*-locked-ON mutant of RS218
E. coli fimS-OFF (6)	8.93 ± 1.20	Non-type 1 fimbriated, a genetically engineered *fimS*-locked-OFF mutant of RS218
E. coli YX102 (5)	8.15 ± 0.60	Non-type 1 fimbriated, *fim* operon deletion mutant of *E. coli* YX101
E. coli YX104 (5)	7.30 ± 2.03	Type 1 fimbriation similar to RS218, *hbiF* deletion mutant of *E. coli* YX101

[a] Bacterial counts in bloodstream of 5-day-old rats were below the detection limits.
[b] The magnitude of bacteremia induced by the mutant was significantly different from that of wild-type *E. coli* RS218 ($P < 0.01$).

to the result observed with *E. coli* YX101 (Table 4). In contrast, the *E. coli fimS*-OFF mutant achieved a magnitude of bacteremia similar to that of wild-type *E. coli* RS218 (Table 4). These results suggest that type 1 fimbriation in *E. coli* YX101 is most likely responsible for its failure to induce bacteremia in neonatal rats.

Further, we constructed a *fim* operon deletion mutant of *E. coli* YX101, where an approximately 7.5-kb chromosomal DNA segment including the molecular switch *fimS* and its downstream structural gene *fimAICDFGH* were deleted from *E. coli* YX101. This type 1 fimbria-negative mutant of *E. coli* YX101 exhibited an ability to induce a high degree of bacteremia in neonatal rats, similarly to the wild-type *E. coli* RS218 (Table 4). We also showed that deletion of *hbiF* from *E. coli* YX101 (which changed *fimS*-locked-ON to a mixed population of *fimS* ON/OFF orientations) restored this bacterial ability to induce a high degree of bacteremia in neonatal rats (Table 4). Taken together, the failure of *E. coli* YX101 to induce bacteremia in neonatal rats is attributed to HbiF-dependent type 1 fimbriation in this strain.

DISCUSSION

In this study, we identified a novel site-specific recombinase, HbiF, that regulates the inversion of *fimS* in *E. coli*. More importantly, HbiF invertase was able to switch *fimS* in the absence of FimB and FimE. The existence of non-FimB- and non-FimE-dependent *fimS* inversion was implied for *E. coli* Nissle 1917. An uncharacterized recombinase was suggested to be responsible for *fimS* OFF-to-ON switching in a *fimBE* double mutant of this strain (40). In addition, HbiF-mediated *fimS* inversion appears to be predominantly unidirectional OFF-to-ON switching. This is different from the *fimS* switching mode mediated by either FimB (bidirectional ON-to-OFF and OFF-to-ON) or FimE (unidirectional ON-to-OFF). The unique switching mode of HbiF also explains why the induction of HbiF resulted in a *fimS*-locked-ON phenotype in *E. coli* YX101. The role of *hbiF* in the *fimS*-locked-ON phenotype was genetically confirmed with deletion and complementation of *hbiF*.

Type 1 fimbrial phase variation is subject to the control of a variety of environmental signals, including physical signals such as temperature, pH, or osmolarity, and nutritional signals such as the availability of amino sugars or branch chain amino acids (10, 11, 26, 32). In addition to environmental signals, alteration of intracellular signals derived from cross-talk among different fimbriae such as P and S fimbriae also affects *fimS* inversion (15). Despite complex regulation mechanisms and the involvement of multiple regulators such as H-NS, Lrp, *leuX*, OmpR, PapB, and SfaB, it is invariable that the type 1 fimbrial phase variations due to those external or internal signals are dependent upon the functions of FimB and FimE directly or indirectly (10, 11, 15, 16, 26, 28, 32, 38, 43). Discovery of a novel regulator coordinating FimB- and FimE-independent type 1 fimbriation in *E. coli* opens a new research opportunity to study the regulation and expression of type 1 fimbriae.

E. coli YX101 is an RDI 21 island deletion derivative of meningitis-causing *E. coli* K1 strain RS218 (44). This island is the largest genomic island in *E. coli* RS218 (~120 kb). Deletion of this island promoted the expression of the *hbiF* gene, suggesting that the factor(s) inside RDI 21 is likely a negative transcriptional regulator for *hbiF* expression. There are at least 11 putative proteins in RDI 21 that potentially function as a transcriptional repressor(s) for the *hbiF* promoter, including PapB, PapI, PapX, a DsdC homologue, and seven other putative transcriptional regulators. It is tempting to speculate that loss of one or more of these transcriptional regulators might be directly or indirectly responsible for *hbiF* induction in YX101. For example, *papB*, *papI*, and *papX* are located inside the *pap* gene cluster, which is responsible for biosynthesis of P fimbriae. It is known that there is cross talk between P fimbriae and type 1 fimbriae in uropathogenic *E. coli* due to the activity of the PapB protein (15, 16, 43). PapB inhibits FimB-mediated *fimS* OFF-to-ON inversion and activates the expression of FimE. On the other hand, there are two highly homologous copies of DsdC in RS218 (98% amino acid residue identity). Transcriptional activation of *dsdXA* via the activity of DsdC is required for D-serine detoxification in *E. coli* (24). Deletion of *dsdA* in uropathogenic *E. coli* CFT073 reduced type 1 fimbriation (29). Since CFT073 harbors only a backbone copy of the *dsd* gene cluster, the effect of deletion of RDI 21-associated *dsd* on type 1 fimbriation is unclear.

Another important finding of this study is that HbiF-dependent type 1 fimbriation drastically reduced the ability of *E. coli* K1 to induce bacteremia in neonatal rats (Table 4). Human neonates, especially premature ones, are at a greater risk for bloodstream infection due to enteric gram-negative bacteria (5). *E. coli* is one of the most common causes of septicemia in infants, which is usually associated with high morbidity and mortality (6, 33). Further, *E. coli* bacteremia is associated with other complications. For example, a high degree of bacteremia is a prerequisite for the induction of *E. coli* meningitis (9). The contribution of type 1 fimbriation to *E. coli* K1 bacteremia has been implied by previous studies (12, 25, 31). For example, type 1 fimbriated *E. coli* K1 strain IH3080 (O18:K1:H7) was found to be less able to induce bacteremia than nonfimbriated bacteria when examined with both neonatal rat and mouse peritonitis models (25, 31). Conversely, disruption of *fimA* in *E. coli* K1 did not affect the ability to induce bacteremia, similar to our results with *E. coli fimS*-OFF (3, 21). The incapability of type 1 fimbriated *E. coli* to induce a high degree of bacteremia is probably related to its susceptibility to opsonin-independent phagocytosis by polymorphonuclear leukocytes (35, 36).

Previously we have demonstrated that type 1 fimbriae are critical for *E. coli* K1 binding to human brain endothelial cells in vitro (41). However, a high level of type 1 fimbriation appears to be detrimental for induction of a high degree of bacteremia as demonstrated with the *fimS*-locked-ON derivatives of *E. coli* K1 strains in neonatal rats (Table 4). Since a high degree of bacteremia is prerequisite for *E. coli* crossing the blood-brain barrier (44), the roles of type 1 fimbriae in *E. coli* meningitis become an apparent paradox. There are several potential explanations that might reconcile the seemingly conflicting roles of type 1 fimbriation in bacteremia and binding to human brain endothelial cells. For example, it is likely that a high level of type 1 fimbriation, i.e., the *fimS*-locked-ON phenotype, is required in the efficient blood clearance of type 1 fimbriated *E. coli*. In contrast, a low level of type 1 fimbriation might be permissible in the host bloodstream, which allows

wild-type *E. coli* K1 strains to cross the blood-brain barrier. Alternatively, it is possible that transient expression of type 1 fimbriation in host blood is sufficient for *E. coli* K1 to bind to brain endothelial cells and cross the blood-brain barrier. Previous studies have demonstrated that type 1 fimbriated *E. coli* was present in infant rat blood at 6 h after intraperitoneal administration of *E. coli* K1 (31). Additional studies are needed to clarify the roles of type 1 fimbriae in *E. coli* bacteremia and meningitis.

In summary, we identified a novel type 1 fimbriation regulator, HbiF, whose function for the type 1 fimbrial molecular switch *fimS* is independent of FimB and FimE invertases. Constitutive induction of *hbiF* in *E. coli* YX101 results in the *fimS*-locked-ON phenotype in this strain. HbiF-dependent type 1 fimbriation is responsible for this strain's failure to induce bacteremia in neonatal rats.

ADDENDUM

During the review of the manuscript, a paper on unlinked FimB and FimE homologues in uropathogenic *E. coli* strain CFT073 was published (4). In CFT073, three non-FimB and non-FimE *fimS* invertases were identified based on genomic sequences, which were designated IpuA, IpuB, and IpbA. IbpA is identical to HbiF and was demonstrated to be capable of inverting *fimS* from OFF to ON orientation. However, the expression of the *ipbA* gene was dormant in wild-type *E. coli* CFT073, because CFT073 derivatives harboring only *ipbA* (i.e., CFT073 Δ*fimBE* Δ*ipuAB*) were locked in either ON or OFF orientation (4). Interestingly, CFT073 lacked a complete RDI 21 genomic island, although it did carry two copies of *pap* gene clusters (responsible for P fimbria biogenesis) (42).

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