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Allelic functional variation of FimH among *Salmonella enterica* subspecies

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Abstract

Salmonella enterica has a wide diversity, with numerous serovars belonging to six different subspecies with dynamic animal-host tropism. The FimH protein is the adhesin mediating binding to various cells, and slight amino acid discrepancy significantly affects the adherence capacities. To date, the general function of FimH variability across different subspecies of *Salmonella enterica* has not been addressed. To investigate the biological functions of FimH among the six *Salmonella enterica* subspecies, the present study performed several assays to determine biofilm formation, *Caenorhabditis elegans* killing, and intestinal porcine enterocyte cell IPEC-J2 adhesion by using various FimH allele mutants. In general, allelic mutations in both the lectin and pilin domains of FimH could cause changes in binding affinity, such as the N79S mutation. We also observed that the N79S variation in *Salmonella* Dublin increased the adhesive ability of IPEC-J2 cells. Moreover, a new amino acid substitution, T260M, within the pilin domain in one subspecies IIIb strain beneficial to binding to cells was highlighted in this study, even though the biofilm-forming and *Caenorhabditis elegans*-killing abilities exhibited no significant differences in variants. Combined with point mutations being a natural tendency due to positive selection in harsh environments, we speculate that allelic variation T260M probably contributes to pathoadaptive evolution in *Salmonella enterica* subspecies IIIb.

Keywords *Salmonella* subspecies, FimH, Amino acid substitution, Binding, Allelic variation

Introduction

Salmonella is a widespread foodborne and zoonotic pathogen that causes public health concerns and economic burden worldwide and can successfully infect humans and animals (Knodler and Elfenbein 2019; Tang et al. 2023, 2022; Xu et al. 2020a; Zhou et al. 2023). The genus *Salmonella* is divided into two species: *bongori* and *enterica*, and the species *enterica* is further divided into six subspecies based on antigenic differences and DNA affinity, namely, I (*enterica*), II (*salamae*), IIIa (*arizonae*), IIIb (*diarizonae*), IV (*houtenae*), and VI (*indica*) (Lowther et al. 2011). Among them, subspecies I, isolated from warm-blooded animals and humans, has been extensively studied, including its prevalence, virulence potentials and disease characteristics. The other five subspecies are mostly isolated from cold-blooded animals with a narrower host range and limited pathogenicity (Desai et al. 2013; Elbediwi et al. 2021; Gambi et al. 2022; Li et al. 2022c; Xu et al. 2020b). A review published by Lamas et al. detailed the similarities and differences between five *non-enterica* subspecies, such as biochemical characteristics, ecology, antimicrobial resistance, and colonization (Lamas et al. 2018). However, comparisons among six subspecies remain unaddressed.

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Salmonella contains various virulence factors, including pathogenicity islands, flagella, and fimbriae, which play essential roles in different stages during infection (Chen et al. 2023; Kang et al. 2022; Klasa et al. 2020; Kolenda et al. 2019; Li et al. 2022b). Fimbriae, especially type I fimbriae, enable *Salmonella* to adhere and colonize various host cells and abiotic surfaces (Kolenda et al. 2019; Xu et al. 2021). Type I fimbria is encoded by the *fim* operon (Kolenda et al. 2019), consisting of *fimA*, *fimI*, *fimC*, *fimD*, *fimH* and *fimF,* all of which are controlled by the *fimA* promoter. The other four proteins (FimZ, FimY, FimW and STM0551) are transcriptional regulators involved in fimbrial biosynthesis (Kolenda et al. 2019). Of these, *fimH*, located at the top of the structure, confers *Salmonella* adhesive capability (Kisiela et al. 2011) and binding properties to mannose-containing oligosaccharides. Accumulating evidence shows that FimH has been an excellent paradigm for studying evolution and pathoadaptation since slight amino acid (AA) discrepancies can significantly affect binding capacity (Grzymajło et al. 2010; Kisiela et al. 2006) and biofilm formation (Kisiela et al. 2012). It is known that *Salmonella* Typhimurium (*S.* Typhimurium) strain SL1344 showed greater biofilm-forming capacity on human epithelioma-2 cells (HEp-2) after replacing the 61st glycine and 118th phenylalanine with alanine and serine in *S.* Typhimurium strain LB5010, respectively, which was initially unable to form biofilms (Boddicker et al. 2002). Moreover, the 78th mutation from threonine to isoleucine of the FimH adhesin in *S.* Gallinarum led to the loss of mannose tolerance binding properties (Boddicker et al. 2002).

Based on the above, we speculated that biological functions are highly dependent on the FimH adhesin in different subspecies under direct or indirect selective pressures during evolution and infection. The present study, focusing on the FimH allelic variation in six *S. enterica* subspecies, aims to illuminate the connection between phenotype differences and genetic background.

Results

Identification and comparison of fimH transformants

The *fimH* mutant and complemental strains were identified via polymerase chain reaction (PCR), and the results are shown in Fig. 1A and B, respectively. We constructed the maximum-likelihood phylogenetic tree according to the *fimH* sequences, which showed that the *fimH* sequences from subspecies I formed a clearly distinguishable phylogenetic clade (Fig. 2A). Additionally, the AA differences between SL1344 and other strains from subspecies I-VI are summarized in Fig. 2B. For all strains, an intact open reading frame with a 22-AA long signal peptide, 173 AA lectin domain (N-terminal) and 137 AA pilin domain (C-terminal) was determined, with no truncated sequence. Except for the two strains in subspecies II, which had the same AA sequences, the other subspecies were different, with a maximum of 16 AA differences (one IIIa strain) compared to SL1344. However, these strains, except for SL1344, showed high similarity in position 104 of the lectin domain and position 295 of the pilin domain, which were arginine and asparagine, respectively. Among the six strains in subspecies I, the most diverse was *S.* Typhi, differing by more than 10 AAs.

Growth rate and virulence of fimH transformants

To investigate the roles of *fimH* from different subspecies in growth rate and virulence in *Caenorhabditis elegans* (*C. elegans*), 14 h-growth curve measurements and survival assays of 18 strains were performed. The results suggested that the growth curves of all eighteen strains were highly similar, as shown in Fig. 3A. In addition, the wild-type (WT) and 16 complemental strains did not differ significantly in survival capability when infecting *C. elegans* in contrast with the deletion mutant (Fig. 3B).

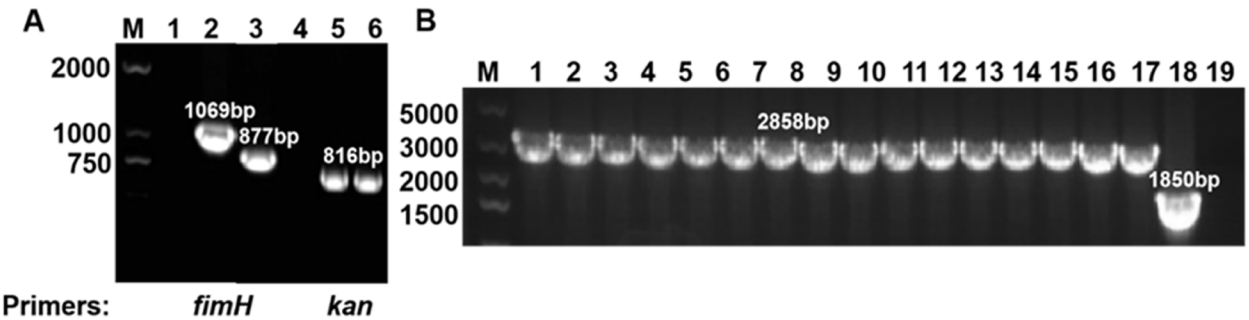
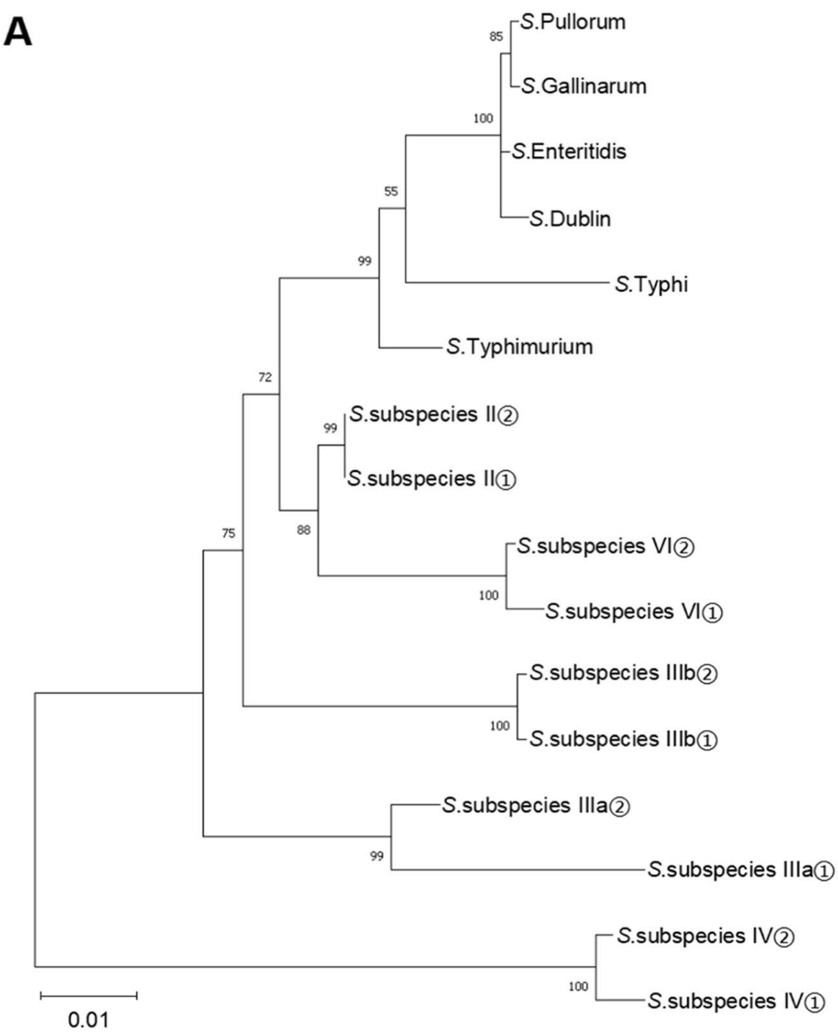


Fig. 1 Identification of *fimH* transformants *via* PCR. **A** The mutant was identified by two pairs of primers, *fimH*-F/R and *kan*-F/R. M: DL 2,000 marker; 1, 4: blank control; 2: SL1344; 3,6: mutant strain; 5: plasmid pET30a. **B** The *fimH* transformants were identified by the primers *Tcr*-F/R. M: DL 5,000 marker; 1–17: *fimH* from different subspecies in the order of I to VI; 18: the plasmid pACYC184-*fimFZ*; 19: blank control

A



B

		SP				Lectin domain/1-173AA																										
Subsp.	Strains	-11	-10	-6	-2	10	27	28	35	36	39	40	41	56	63	64	66	67	79	92	93	102	104	107	109	115	120	137	147	155	160	170
	SL1344	T	A	T	L	A	Q	P	P	E	G	W	V	T	E	L	V	Q	N	V	A	Y	L	V	Y	K	Q	M	T	G	T	K
I	MY0175																						R		S	M						
	MY0193																		S				R		S	M						
	MY0189		T											I									R		S	M						
	MY0187													I									R		S	M						
	SAL01964								L	K	D		C									H	R		S			I				
II	MY0144																I						R			R						
	MY0145																I						R			R						
IIIa	MY0555	A																		Y	S		R			R					A	
	MY0558					P	R					G	G			V				Y	S		R			R						
IIIb	MY0146																	K					R		S	R			S			R
	MY0148																	K					R		S	R			S			R
IV	MY0150				Q	P		S										R		A			R	I		S	L			D		
	MY0155				Q	P		S										R		A			R	I		S	L			D		
VI	MY0153			S																			R			R						
	MY0154			S									A										R			R						

		Pilin domain/177-313AA																		
Subsp.	Strains	183	195	200	202	205	208	213	223	243	250	260	263	274	282	284	290	295	298	No.
	SL1344	Q	S	A	A	R	G	T	V	M	L	T	T	H	R	G	I	I	A	--
I	MY0175																	N		4
	MY0193												I					N		6
	MY0189																	N		6
	MY0187			V														N		6
	SAL01964		P					A										N		11
II	MY0144													R				N		5
	MY0145													R				N		5
IIIa	MY0555						S				V		M	R				N		11
	MY0558						S				V		M	R	H			N	V	16
IIIb	MY0146				M								I	R		S	V	N	T	13
	MY0148				T							M	I	R		S	V	N	T	14
IV	MY0150					W			I	L				R				N		15
	MY0155								I	L				R				N		14
VI	MY0153	K											I	R				N		7
	MY0154												I	R				N		7

Fig. 2 Maximum-likelihood DNA phylograms and FimH AA variations in the different transformants. The *fimH* tree (**A**) was built based on an alignment of *fimH* sequences amplified from sixteen isolates across six subspecies. **B** The signal peptide, lectin domain and pilin domain are filled in gray, blue and pink, respectively. SL1344 (filled in yellow) was used as a reference. Blank cells meant that the AA was the same as that of SL1344. The last column indicates the sum of the number of different AAs (No.)

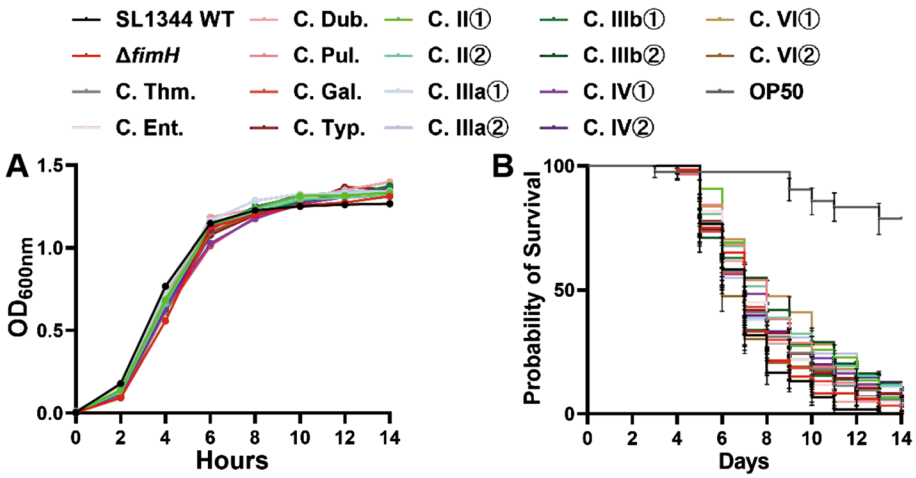


Fig. 3 Different *fimH* transformants had the same growth rate and virulence in *C. elegans*. **A** Growth curves and (**B**) survival curves of WT SL1344 and transformants. The data represent the mean (SEM) of three independent experiments

Biofilm formation of fimH transformants

The biofilm formation ability of the *fimH* transformants of the six subspecies in Luria–Bertani (LB) broth was assessed by crystal violet staining. As depicted in Fig. 4, the biofilm-forming capabilities at 22°C were more potent than those at 37°C, and absorbances at OD_{590nm} of all strains were identical without significant difference.

Adhesive ability of different fimH transformants

FimH, which is responsible for binding to mannose, plays a crucial role in mediating adherence to many kinds of eukaryotic cells. Our study used the IPEC-J2 cell model to evaluate the adhesive ability of different *fimH* transformants. The negative results of fourteen complemental strains suggested a weaker adhesion capacity than the WT, except *fimH* variants cloned from MY0193 and MY0148, separately belonging to subspecies I (*S.* Dublin serovar) and IIIb (Fig. 5). Although no significant differences were observed in the transformants derived from the two strains above, we reasonably presumed that some AAs might play a role in adherence to enterocytes because both strains have unique AA variations (Fig. 2B).

Discussion

Allelic variations have been widely studied as an aspect of pathoadaptive evolution (Sokurenko et al. 1999, 1998). FimH mutations, including A27V and N70S in *Enterobacteriaceae*, were witnessed in global phylogeny and host-adaptation (Hommais et al. 2003). Several studies on subspecies I serovars, such as *S.* Typhimurium, *S.* Pullorum and *S.* Dublin, proved that slight changes in FimH could result in binding preference to various cell types (Dwyer et al. 2011; Guo et al. 2009; Hung et al. 2002; Kisiela et al. 2012). including HEp-2 cells, RAW264.7

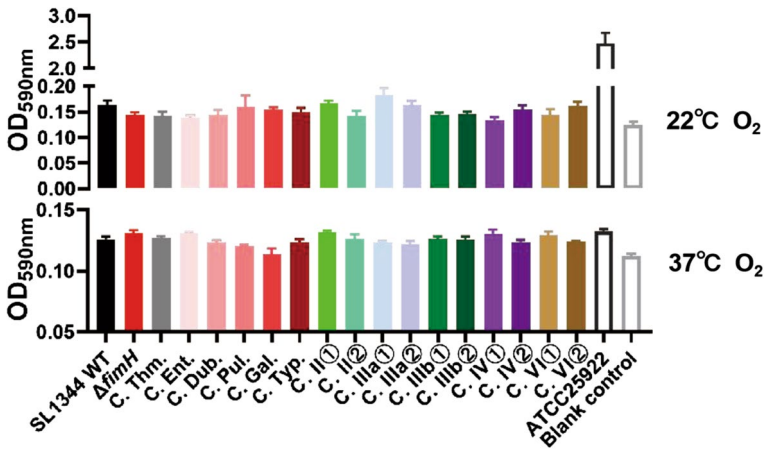


Fig. 4 The measurement of biofilm formation at 22°C and 37°C between WT SL1344 and transformants

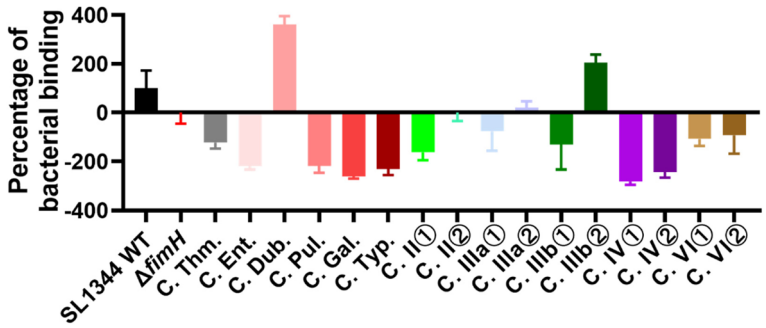


Fig. 5 FimH-mediated bacterial binding to intestinal porcine enterocytes. The data were expressed as a percentage of bacterial binding relative to the difference between WT (100% binding) and the *fimH* mutant (0%)

cells and chicken leukocytes (Guo et al. 2009; Kisiela et al. 2012; Kolenda et al. 2019). The Thm3 strain had a higher adhesive ability to both HEp-2 and RAW264.7 cells, which only differed by the 136th AA compared to SL1344 (Kisiela et al. 2012). However, research on FimH in other subspecies is currently limited to their genetic relationship. The present study, therefore, performed genetic and phenotypic analysis to provide insights into the pattern of FimH variation across different *S. enterica* subspecies, which probably contributes to pathoadaptive evolution in *Salmonella*.

C. elegans has long been a model organism for molecular biology research because of its easy culture, easy observation, and sequenced genome (Léopold Kurz and Ewbank 2003; Peng et al. 2022; Xu et al. 2020b). In this study, given that the other five subspecies except I preferentially infect reptiles and amphibians, *C. elegans* was chosen as the model organism to study the bacterial virulence mediated by different *fimH* transformants from subspecies I-VI. Surprisingly, subspecies II-VI did not show higher lethality to nematodes. We hypothesized that since the FimH adhesin was different from acid tolerance genes such as *fur-1* or *ompR*, which have been confirmed to make a difference during nematode infection, *fimH* variants from various sources did not affect the survival of *C. elegans* (Labrousse et al. 2000).

Biofilm formation, partially attributed to flagella and adhesive fimbriae (Dwyer et al. 2011; Ledeboer et al. 2006), is generally considered an evolutionary advantage for efficient *Salmonella* infection, transmission and colonization, providing a habitat to persist in unfavorable environmentss (Li et al. 2022a; White et al. 2008). Previously, biofilm formation on mannosylated bovine serum albumin (ManBSA) and HEp-2 cells of different *fimH* transformants was evaluated. The result that some transformants producing fimbriae did not grow biofilms on ManBSA or HEp-2 cells illustrated that biofilm formation was independent of fimbriae production (Dwyer et al. 2011). To determine whether *fimH* from different subspecies affects the formation of fimbriae, we conducted a biofilm formation assay under aerobic conditions at 22°C and 37°C. The absorbance of the *fimH* deletion strain after crystal violet staining was similar to that of the WT, consistent with the conclusion above and results elsewhere in *S.* Typhimurium strain CMCC 50115 at 28°C after cultivation for 24 h or 72 h (Xu et al. 2021). It is noteworthy that *fimH* from other strains also did not affect biofilm formation, which may be related to strain differences and culture conditions (Xu et al. 2021).

FimH, as an adhesive factor, was most often assessed for adhesion properties. Mammalian cells, including HEp-2, IPEC-J2 and RAW264.7 cells and the Leghorn Male Hepatoma cell line (LMH), are excellent models to perform the binding assay (Boddicker et al. 2002; Dwyer et al. 2011; Kisiela et al. 2012; Ledeboer et al. 2006; Yue et al. 2015). The IPEC-J2 cell binding assay in the study exhibited the impaired adhesive ability of *S.* Pullorum and *S.* Gallinarum, which was in accordance with previous data (Yue et al. 2015). Importantly, the increased percentage of *fimH* from *S.* Dublin and one IIIb strain manifested possible crucial AA sites. Through alignment of the AA sequences, we concluded that the N79S and T260M substitutions promoted adhesive capacity, the former of which has been demonstrated elsewhere when *S.* Enteritidis underwent the single mutation of N79S, and the binding ability became as strong as that of *S.* Dublin (Kisiela et al. 2012). After performing BLAST analysis, we discovered that the IIIb subspecies exhibited the highest prevalence of the T260M mutation, at approximately 20%, which was significantly higher than that in other subspecies (data not shown). However, since the substitution T260M has not been mentioned previously, some assays, such as allelic mutations in low-binding strains or serovars, should be carried out in the next step to verify the role of the mutation. Additionally, we speculated that the residue substitutions in both the lectin domain and

pilin domain could probably indirectly change the conformation of the binding pocket and thus influence the binding ability since no variable positions were found in the binding pocket (residues 10, 49, 56 and 160, Fig. 6) (Yue et al. 2015), combined with the previous results that strains carrying mutations M137I and R232W in the pilin domain showed a high-binding phenotype (Kisiela et al. 2012).

Within *fim* gene clusters, each gene exerts its own function. Some are involved in the synthesis or expression of fimbriae, and others act as regulators. FimF functions in assembling fimbriae (Dwyer et al. 2011), and FimZ is a sensor DNA-binding protein that can facilitate transcription (Yin et al. 2022). As we constructed the various *fimH* transformants by combining *fimH* from different strains with *fimFZ* from SL1344, it cannot be excluded that their respective FimFZ may not function as expected. In addition, other virulence factors might also play a role in the above assays since pathogens generally utilize several organelles or machinery, such as the type three secretion system or flagella, to facilitate colonization and infection (Yue and Schifferli 2013). Therefore, it was reasonable that no significant differences were observed in biofilm-forming capabilities and *C. elegans* killing. Therefore, we concluded that the new T260M substitution had a great impact on the binding capacity, which may confer the subspecies IIIb advantages to outcompete in harsh environments.

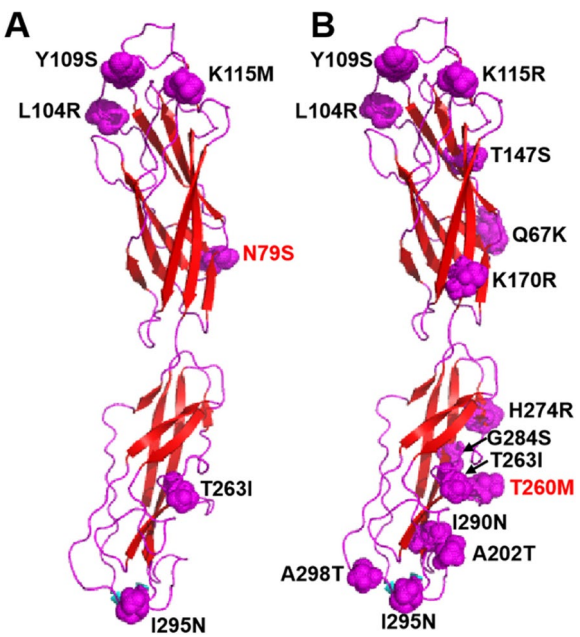


Fig. 6 Substituted sites on FimH alleles visualized on the SL1344 FimH model. The purple balls highlight the substituted residues. The red fonts (N79S and T260M) indicate that the alternative site was unique to this strain. **A** *S.* Dublin strain; **B** subspecies IIIb strain. α-helices are highlighted in blue, β-sheets in red, and loops in purple in the predicted structure of SL1344 FimH

Conclusions

This research investigated the biological functions of FimH among six *S. enterica* subspecies, including *enterica* (I), *salamae* (II), *arizonae* (IIIa), *diarizonae* (IIIb), *houtenae* (IV), and *indica* (VI). First, a SL1344 *fimH* mutant via the λ-red recombination system and several *fimH* complement strains from different subspecies were constructed. By comparing the FimH AA sequences from fifteen strains with SL1344, a maximum of sixteen AA variations (one IIIa strain) was observed. There were few differences in biofilm formation and *Caenorhabditis elegans* killing among the *fimH* transformants. However, two *fimH* variants cloned from subspecies I (*S.* Dublin serovar, MY0193) and IIIb (MY0148) exhibited strong adhesive abilities to IPEC-J2 cells. Combined with the AA comparison results, a novel amino acid substitution within the pilin domain, T260M, was discovered. This mutation might provide subspecies IIIb with a competitive advantage in challenging surroundings.

Methods

Bacterial strains and growth conditions

All bacterial strains, plasmids, and primers used in this study are summarized in Supplementary Tables S1 and S2, respectively. LB medium was most commonly used unless otherwise specified. When necessary, selective antibiotics were added as follows: 25 μg/mL chloramphenicol, 50 μg/mL kanamycin, and 100 μg/mL ampicillin.

Construction of the SL1344 *fimH*::*kan* mutant

The *kan* segment was cloned from the vector pET30a to replace the *fimH* coding region in SL1344 based on the λ-red recombination system (Datsenko and Wanner 2000; Yu et al. 2000). Briefly, the primers *fimH*::*kan*-F/R for *kan* element amplification were flanked by a 60-bp homologous sequence on the chromosome. The 50 ~ 100 ng PCR product was then introduced into SL1344 carrying the plasmid pKD46 by electroporation using a 0.2 cm cuvette at 200 Ω, 2.5 kV, and 25 μF, which expresses recombinase *exo*, *bet* and *gam* in the presence of L-arabinose. The cells recovered at 37°C for one hour were spread onto an LB agar plate with kanamycin and ampicillin. The positive colonies after PCR were finally confirmed by sequencing. Subsequently, the plasmid pKD46 was removed through incubation at 42°C.

Construction of *fimH* complement strains from six *Salmonella* subspecies

A schematic diagram of the plasmid with *fimH* complementation of six *Salmonella* subspecies is shown in Fig. 7. Since FimF, located downstream of FimH (Kolenda et al. 2019),, potentially acts as an adaptor for fimbrial assembly (Dwyer et al. 2011), and FimZ, regulated by leucine-responsive regulatory protein, indirectly affects type I fimbriae synthesis (McFarland et al. 2008), the low copy vector pACYC184, possessing the *fimFZ* segment of SL1344, was created as a complemental plasmid (pACYC184-*fimFZ*) according to the following paper (Hancox et al. 1997). The *fimH* fragments of 16 strains (*S.* Typhimurium, *S.* Enteritidis, *S.* Dublin, *S.* Pullorum, *S.* Gallinarum, and *S.* Typhi, one of each, and subspecies II ~ VI, two of each) (Table S1) were amplified by PCR, ligated into the pACYC184-*fimFZ* plasmid and transformed into the SL1344 *fimH*::*kan* mutant. The colonies were selected by plating on LB plates containing kanamycin (50 μg/mL) and chloramphenicol (25 μg/mL) and verified by PCR.

Construction of the maximum-likelihood phylogenetic tree and comparison of FimH AA sequences

The maximum-likelihood phylogenetic tree was constructed based on a previous paper using MEGA X (Kisiela et al. 2012). The sequenced *fimH* sequences above were then translated into AA in Snapgene 4.1.8. All AA sequences were imported into MEGA X to visualize the differences, of which the AA sequence of strain SL1344 was used as a reference.

Determination of the growth curves

The growth curves of all 18 strains were measured every two hours for 14 h by measuring OD_{600nm}. The overnight bacterial cultures were inoculated into LB broth medium at a 1:100 dilution and incubated at 37°C with shaking at 180 rpm.

Infecting *Caenorhabditis elegans* with *Salmonella*

According to the protocol described previously (Zhang and Jia 2014), *C. elegans* SS104, a temperature-sensitive mutant that does not reproduce at 25°C, was fed *Escherichia coli* (*E. coli*) OP50 coated on nematode growth medium (NGM) plates until the fourth-stage larvae (L4) for infection assays. Meanwhile, infection plates of nineteen strains, including OP50, were prepared by spotting an equal volume of overnight LB culture on NGM plates (Desai et al. 2019). Afterwards, twenty synchronized worms were placed onto the bacterial plates and maintained at 25°C for 14 days. The number of dead nematodes was observed and recorded each day. Three independent experiments were conducted for each strain.

Biofilm formation assay

The fresh LB bacterial broth cultured to OD_{620nm} ≈0.08 was diluted 1:100 with LB before adding 200 μL to a 96 well polystyrene microtiter plate with five replicates.

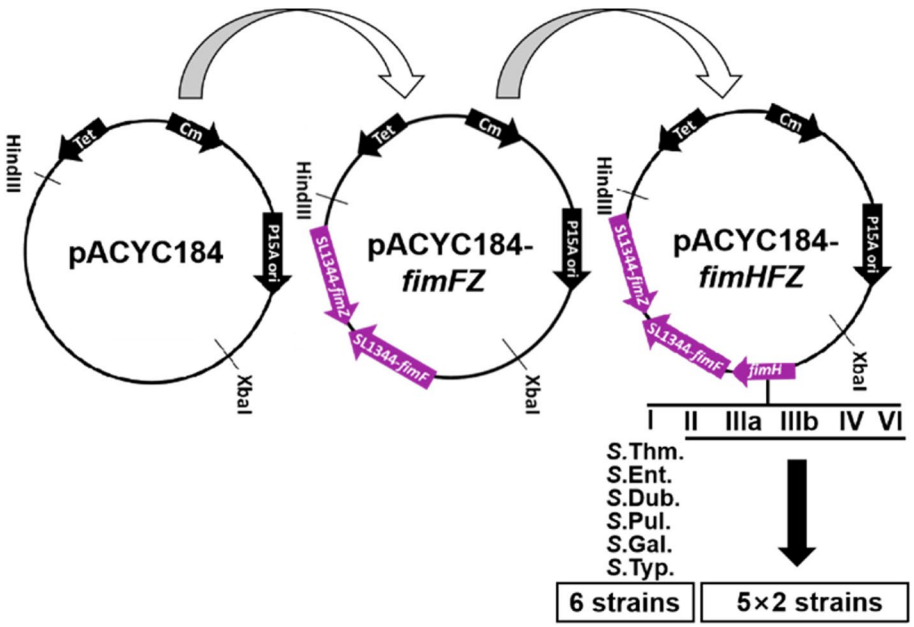


Fig. 7 Construction diagram of the complementation plasmid pACYC184-*fimHFZ*. (Thm.: Typhimurium; Ent.: Enteritidis; Dub.: Dublin; Pul.: Pullorum; Gal.: Gallinarum; Typ.: Typhi)

Marginalization was prevented by adding 200 µL ddH_2O to the surrounding blank wells. The plates were incubated at 22°C and 37°C for three days. The following steps were the same as reported previously (Chen et al. 2023) except for measuring the absorbance value at OD_{590nm}. Three independent experiments were conducted for each strain.

IPEC-J2 cell adhesion assay

IPEC-J2 cells cultured in Dulbecco's modified Eagle's medium/nutrient mixture F-12 (DMEM/F-12) (Thermo Scientific, Shanghai, China) were seeded in 24-well plates at a density of 1×10^5 cells per well. Following washing with PBS three times, the bacterial cultures grown under static conditions were resuspended in DMEM/F-12 and adjusted to 1.25×10^7 colony-forming units (CFU) per milliliter (400 µL per well). After incubation at 37°C for 1 h, the cells were washed thrice with PBS to remove the nonadherent organisms and then lysed with PBS containing 0.1% Triton X-100 (Sigma–Aldrich, Shanghai, China). The CFU in every well was calculated by serial dilutions. The eventual results were presented as percentages of bacterial binding shown previously (Yue et al. 2015). Three independent experiments were conducted for each strain.

Modeling of the FimH structure

First, the SL1344 FimH AA sequence, which included 313 AAs, not counting the signal peptide (22 AAs), was uploaded to the i-TASSER server, a platform to predict protein structure and function (http://zhanglab.ccmb.med.umich.edu/I-TASSER/) (Roy et al. 2010). The predicted three-dimensional structure of FimH was used as a model to mark substitution sites in other strains. The PDB format was viewed, modified, and edited using the PyMOL molecular graphics system (Seeliger and De Groot 2010).

Statistical analysis

All values are presented as the means from three separate experiments ± standard error of the mean (SEM). Statistical differences between the results were computed using one-way analysis of variance (ANOVA) using GraphPad Prism 9.0. A $P<0.05$ was considered statistically significant.

Abbreviations

S. Typhimurium	*Salmonella* Typhimurium
AA	Amino acid
PCR	Polymerase chain reaction
C. Elegans	*Caenorhabditis elegans*
WT	Wild-type
LB	Luria–Bertani
OD_{590nm}	Optical density at 590 nm
E. coli	*Escherichia coli*
NGM	Nematode growth medium
L4	Fourth-stage larvae
DMEM/F-12	Dulbecco's Modified Eagle Medium/Nutrient Mixture F-12
CFU	Colony-forming units
SEM	Standard error of mean
ANOVA	One-way analysis of variance

Supplementary Information

The online version contains supplementary material available at https://doi.org/10.1186/s44149-023-00101-y.

Additional file 1: Table S1. Bacterial strains and plasmids used in this study. **Table S2.** Primers used in this study. All *fim*H nucleotide sequences. All FimH amino acid sequences.

Authors' contributions

X.K.: Methodology, Investigation, Writing – Original Draft, Visualization; J.C.: Methodology, Data curation; X.Z.: Investigation; AE-D.: Revision; Y.M.: Conceptualization, Methodology, Writing – Review and Editing.

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Availability of data and materials

All data generated during this study are included in this article and the Supplementary material file. Supplementary Table 1: Bacterial strains and plasmids used in this study; Supplementary Table 2: Primers used in this study; All *fimH* nucleotide sequences and all FimH amino acid sequences.

Declarations

Ethics approval and consent to participate

Not applicable.

Consent for publication

Not applicable.

Competing interests

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper. Author Min Yue was not involved in the journal's review or decisions related to this manuscript.

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