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Identification of *fliC* and flagella expression in *Salmonella enterica* subspecies *enterica* serovar *Gallinarum biovar* gallinarum

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***Salmonella enterica* subsp. *enterica* ser. *Gallinarum biovar*. gallinarum (*S. gallinarum*) is the causative agent of fowl Typhoid, a bacterial disease that affects domestic and wild birds. For many years, *S.* gallinarum was defined as an aflagellated bacterium, that is, non-motile. However, the closely related strain *Salmonella pullorum* is capable of expressing a flagellum-like filament. Since these bacteria are practically identical, it was determined if *S.* gallinarum expresses flagellum-like filament under different culture conditions in order to increase the basic knowledge of this bacterium. The motility of *S.* gallinarum was evaluated in different culture media (GI, S and nutrient media), and visualized the bacterial filaments using electron microscopy. *fliC* was identified in some of the studied strains; subsequently, sequencing analysis of *fliC* gene shown 98% of identity with that in *S.* Typhimurium. In the present study, the presence of a flagellum-like structure was demonstrate in different strains of *S.* gallinarum using electron microscopy. In addition, the *fliC* gene was amplified, which allowed us to suggest that this bacterium is capable to shows a flagellum under certain culture conditions, similar to that reported for *S.* pullorum.**

Key words: *fli*C, flagella, *S*. gallinarum, motility, medium-dextrose.

INTRODUCTION

Salmonella enterica subsp. enterica ser. Gallinarum biovar. Gallinarum (Grimont and Weill, 2007) causes fowl Typhoid (Barrow and Freitas, 2011). This disease affects poultry, mainly chickens, turkeys, and pheasants, as well as wild birds, which act as natural reservoirs (Barrow and Freitas, 2011; Foley et al., 2011). The differences in susceptibility and resistance to avian typhoid are related to the virulence of the strain and genetic background of the host, the young birds are most susceptible to the disease, whereas adult birds may have asymptomatic infection (Barrow and Freitas, 2011; Fraser and Hughes, 1999).

Due to the importance of fowl typhoid in Mexico, the government implemented the National Campaign

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against Avian Salmonellosis under the NOM-005-ZOO-1993. This campaign has helped to significantly reduce fowl typhoid cases.

The bacteria of the *Salmonella* genus are short, Gram-negative bacilli; they are aerobes or facultative anaerobes, some display a capsule, do not form spores and are generally motile due the presence of peritrichous flagella (Barrow and Freitas, 2011). The bacteria use different mechanisms to colonize and survive within the host (Monack et al., 1996; Fraser and Hughes, 1999; Marimoto and Minamino, 2014). In this regard, it is suggested that *S*. gallinarum release the outer membrane protein A in the presence of heat-labile serum components, as a mechanism to distract the immune system (Vega-Manriquez et al., 2016). To survive, the bacteria have developed various adaptive mechanisms, including motility (Marimoto and Minamino, 2014). This mechanism makes them more efficient for nutrient acquisition, avoidance of toxic substances, and in the case of pathogenic bacteria allows finding optimal colonization sites to establish a symbiotic relationship with the host (Monack et al., 1996; Fraser and Hughes, 1999).

The flagellum is clinically important because it presents antigenic variability (Marimoto and Minamino, 2014); this structure is critical for chemotaxis because it is responsible for bacterial motility (Fraser and Hughes, 1999). The flagellum is a long helical filament, propelled by a rotary engine embedded in the cell envelope (Marimoto and Minamino, 2014). The basic structure of flagellum consists of the extracellular filament, which is a homopolymer formed by thousands of subunits of FliC or flagellin (Marimoto and Minamino, 2014). The filament ends in a hook that connects it with the cell's engine; this hook is flexible, extracellular and is a homopolymer of 120 to 130 copies of the FlgE protein8 (Marimoto and Minamino, 2014). Finally, the basal body is a multiprotein complex embedded in the cell membrane, wherein the flagella motor is located; it consists of a central axis and four ring-shaped complexes (Marimoto and Minamino, 2014).

There are more than 50 genes involved in the processes of assembly, regulation, rotation and movement of flagella (Marimoto and Minamino, 2014). It is known that expression of flagellar operon is regulated by diverse stimulus such as temperature, grade of DNA supercoiling, phospholipids, OmpR proteins (which act in response to osmolarity changes), DnaJ, K, GrpE (corresponding to thermal shock response), cell division, and the catabolite repression through cAMP levels (Iino, 1969; Wang et al., 2005). For example, if the glucose concentration is high, cAMP levels decrease and the master operon is not expressed (Iino, 1969; Wang et al., 2005; Marimoto and Minamino, 2014).

It has been reported that epinephrine plays an important role in stimulating motility in *S*. Typhimurium, so it seems that it is playing a key role in the mechanism of Quorum Sensing. This would trigger the activation of genes related to motility such as fliC owing to the fact that the presence of this neurotransmitter increases its expression 21 times (Conceição et al., 2015).

In strain z66 of S. Typhi, a significant expression of 187 genes has been observed when the bacterium is in contact with Anti-z66, among these genes *fli*C is highlighted as its expression is reduced more than three times (Zhang et al., 2009). There are some controversial results regarding the participation of the flagellum in the interaction with the host cell, as it depends on the serovar studied, the flagellum changes its participation in the infection phase, as shown in the results found in *S*. Typhimurium *fli*C mutated which is not able to induce an inflammatory response. Opposite to this, *S*. Dublin independently if the gene is present or not, does not to participate in the induction of the inflammatory response, so this structure would not be participating in the systemic phase of the serovar (Olsen et al., 2013). Based on these references, suggests that regulation or expression of the flagellum depends on the serovar or may be different according to the serovar or even according to the environment.

S. enterica subsp. *enterica* ser. Gallinarum biovar. pullorum are considered non-motile bacteria. However, in 1993 Kilger and Grimont conducted a study in *S.* gallinarum and *S.* pullorum where they demonstrated the presence of the *fli*C gene (corresponding to the first part of the flagellum) by PCR (Kilger and Grimont, 1993). They suggested that this gene encodes antigens G and M in these serovars (Kilger and Grimont, 1993). Li et al. (1993) identified and sequenced the complete *fli*C gene in *S.* gallinarum and *S.* pullorum (Li et al., 1993). In addition, Holt and Chaubal (1997) reported the development of flagella-like fibrous filaments in *S.* pullorum grow in special culture media; they also determine that this bacterium belonged to the serogroup D of the Kauffmann-White classification.

Non-mobile strains of Salmonella are known that presents the *fli*C gene, but do not form a functional flagella (Poppof and Le Minor, 1997). However, because the gene has a hypervariable central region, that allows, through the PCR-FRLP technique, to characterize and differentiate strains of these two biovarities (Paiva et al., 2009; Cheraghchi et al., 2014). In studies with a motile mutant of *S*. gallinarum, showed that this strain has the ability to induce an inflammatory response, suggesting that has an increased ability to colonize (de Freitas Neto et al., 2013).

In this study the presence of flagella in *Salmonella* gallinarum were demonstrated and their expression induction when the strain in cultured in specific media. It was confirmed the presence of flagella by electron microscopy and sequencing of *fli*C gene. This study contributes to the knowledge about these presence and expression of flagella in this microorganism.

MATERIALS AND METHODS

Bacterial strains

For this study *Escherichia coli*, ATCC1946, wild type *Salmonella* Typhimurium, eight *Salmonell*a gallinarum strains (ATCC9184, FVB323, FVB347, FVB383, FVB47, FVB41 and SC) were use. *Salmonell*a pullorum ATCC10398 and *S*. pullorum wild type (SP). *Salmonell*a Enteritidis ATCC49214, a wild type *Salmonella* Enteritidis and *Staphylococcus aureus* beta-toxin producer.

Culture media

Culture media were used according to methodology reported by Holt et al. (1997). For motility assays tubes and plates of semisolid medium of beef heart infusion with 0.3% agar (Motility GI Medium, Difco, 38800, France) were used; semisolid medium brain and heart infusion with 0.3% agar (Motility Medium S, Difco, 38800, France) and nutrient medium with 0.5% agar (Nutrient Broth, Merck, 64271, Germany). All culture media were used alone or supplemented with 0.5% dextrose.

Motility assay

Strains were inoculated in isolation in plates with agar A (0.7% nutrient broth, 0.1% yeast extract, 0.2% glycerol, 200 mM K2HPO4 and 9 mM KH2PO4), and were incubated for 24 h at 37 °C. Inoculation in the culture tubes was made using the needle technique; meanwhile, in the agar plates were used the needle technique and by single streak. The cultures were incubated at 37°C; growths were recorded by photographs taken at 24, 48 and 72 h of incubation. After that, 1 to 3 colonies of each strain were inoculated in GI, S, or nutrient medium, alone or supplemented with 0.5% and were incubated at 37 or 42°C for 24, 48 and 72 h. The diameter of the growth halos was measured at 24, 48 and 72 h of incubation. A photographic register also recorded the growth and motility of these cultures.

DNA extraction

All strains were inoculated in 5 ml of broth A and DNA was extracted using the modified guanidine thiocyanate method described by Mozioglu et al. (2014), with minor modifications.

Detection of *fliC* by PCR

The presence of *fliC* gene was determined by PCR using the primers reported by Kilger and Grimont (1993), (forward: 5'-AAGGAAAAGATCATGGCA, reverse: 5'-TTAACGCAGTAAAGAGAG). The PCR was performed in a SelectCycler™ II Thermal Cycler (Select Bio Products, USA) under the following cycling conditions: initial denaturation cycle at 94°C for 5 min, 30 cycles consisting of 94°C for 1 min, 50°C for 1 min, 72°C for 2 min, and final extension at 72°C for 7 min. With these conditions, a 1500 bp fragment was obtained. A DNA concentration of 2-3 ng/µl and 0.5 µM primers were use in this technique.

Restriction assay

To verify if the fragment amplified by PCR correspond to *fli*C, a restriction analysis was performed with *Eco*RV and *Kpn*I according to the manufacturer's recommendations. The reaction was incubated at 37°C for 1.30 h. The restriction profile of wild-type *S.* Typhimurium and in *S.* gallinarum ATCC9184 were used as a reference. The resulting fragments were visualized by electrophoresis on 1% agarose gels stained with ethidium bromide. An *in silico* restriction profile prediction was performed using *fli*C of *Salmonella enterica* subsp. *enterica* serovar Typhimurium str. LT2 (NCBI Reference Sequence: NC_003197.1, 1488 bp) and *Salmonella enterica* subsp. *enterica* serovar Gallinarum str. 287/91 (GenBank: AM933173.1, 1518 bp).

DNA sequencing

To verify the identity of *fli*C of *S.* gallinarum ATCC9184, the amplicon was purified using the QIAquick® Gel Extraction Kit (Qiagen, USA) according to manufacturer recommendations, and the nucleotide sequences were obtained in an automatic sequencer (Sequencing Unit, Institute of Cellular Physiology, UNAM). The sequences obtained were analyzed using Blast® software and compared with the reported sequences of *fliC* in *E. coli* str.K-12 substr. M61655 (NCBI Reference Sequence: NC_000913.2, 1497 pb), *Salmonella enterica* subsp. *enteric* serovar Enteritidis str. SGSC2475 (GenBank: AY649709.1, 1518 pb), *Salmonella enterica* subsp. *enteric* serovar Typhimurium str. LT2 (NCBI Reference Sequence: NC_003197.1, 1488 pb), *Salmonella enterica* subsp. *enteric* serovar Gallinarum str. 287/9 (GenBank: AM933173.1, 1518 pb) and *Salmonella enterica* subsp. enteric serovar Gallinarum str. ST16 incomplete sequence (GenBank: DQ838253.1, 1272 bp).

Electron microscopy

To evidence the presence of flagella by Transmission Electron Microscopy, *S.* Enteritidis ATCC49214, *S.* gallinarum FVB323 and *S.* gallinarum ATCC9184 were growth in GI medium with 0.5% dextrose for 48 h at 37°C. Once the bacterial colonies grew, a swab of each culture was taken, resuspended it in 1ml of sterile distilled water and then stained with 2% uranyl using charcoal grills for visualization. The samples were analyzed in the Transmission Electron Microscopy facility at the Faculty of Veterinary Medicine, UNAM (Mexico City, Mexico), for staining.

RESULTS

Grown in different media

To induce the expression of flagella, all the strains were growth in GI, S, and nutrient media, alone or supplemented with dextrose. It was found that after 48 h of culture in the GI medium supplemented with 0.5% dextrose (GI+D), *Salmonella* gallinarum FVB323 showed wavy expansion and growth effects, characteristic of motile bacteria, compared to the smooth growth of non-motile bacteria. The strongest undulate and irregular growth was observed in plates inoculated by the needle technique while no significant differences were detected in the tubes inoculated by the needle technique (images not showed).

Differences in the growth morphology of the strains cultured in GI medium was observed in comparison with those cultured in GI+D medium. The colonial form of strain growth in GI medium was smooth while in GI+D it was wavy, rhizoidal or lobate (Figures 1 and 2). Following 48 h of incubation in GI medium, an undulated and

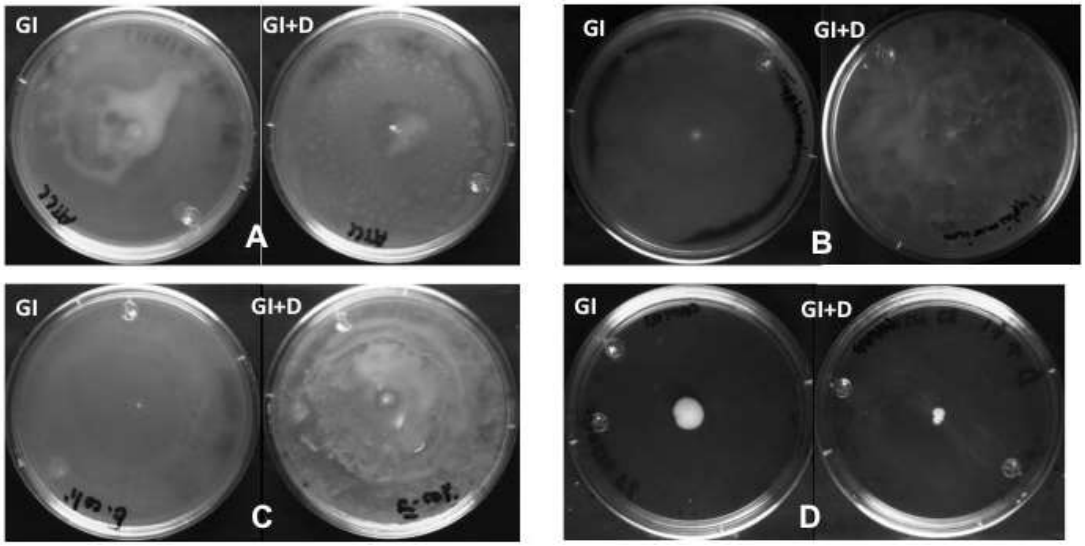

Figure 1. Effect of dextrose on growth and motility of *Salmonella* sp. Strains were cultured in agar plates of GI medium, alone and supplemented with 0.5% dextrose (GI+D) and were incubated for 48 h at 37°C. Panel A shows *Salmonella* Enteritidis ATCC, panel B shows *Salmonella* Typhimurium, panel C shows *Escherichia coli* and panel D shows *Staphylococcus aureus* β-toxin. Strains were inoculated by the needle technique. The effect of wavy growth and expansion of motile bacteria can be better observed, compared with non-motile bacteria in GI medium alone (GI) and supplemented with dextrose (GI+D).

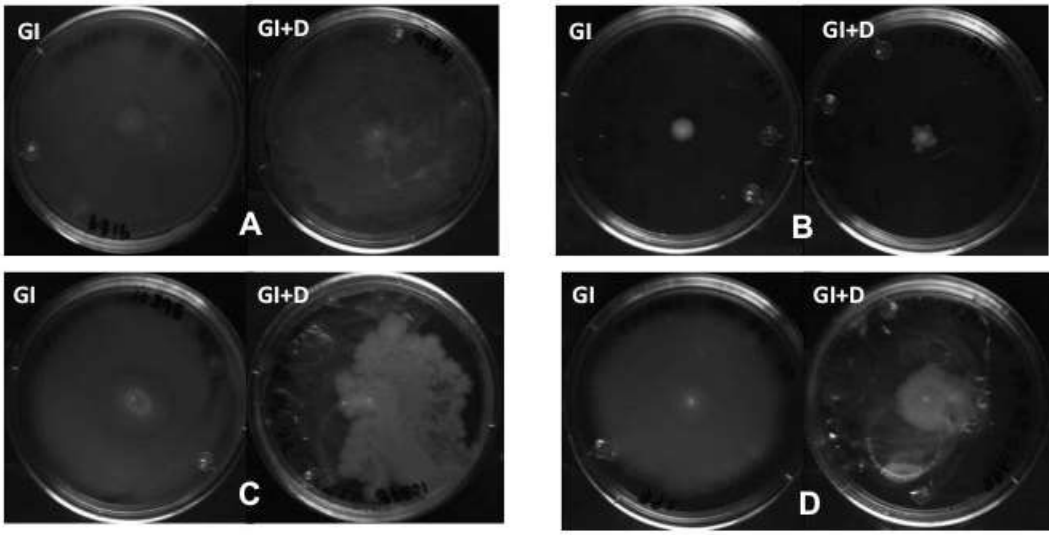

Figure 2. Culture of strains of *Salmonella* gallinarum and *Salmonella* pullorum in GI medium alone and supplemented with 0.5% dextrose in plates at 48 h at 37° C. Panel A shows *Salmonella* gallinarum ATCC9184, panel B *Salmonella* gallinarum FVB323, panel C *Salmonella* pullorum ATCC10398 and panel D *Salmonella* pullorum (SP).

irregular growth pattern at the surface of culture plates in most of the *S*. pullorum and *S*. gallinarum strains was observe, except for the FVB323, FVB383 and FVB47 strains (Figure 3B and D). The temperature had minimal effect on motility since a similar result was observed in the cultures incubated at 37 and 42°C. However, addition of dextrose to GI medium favors the observation of motility (Figures 3 and 4).

The wavy expansion displayed by motile bacteria was evident in the ATCC9184, ATCC10398 and SP strains (Figure 2). The FVB323 strain growth in GI+D

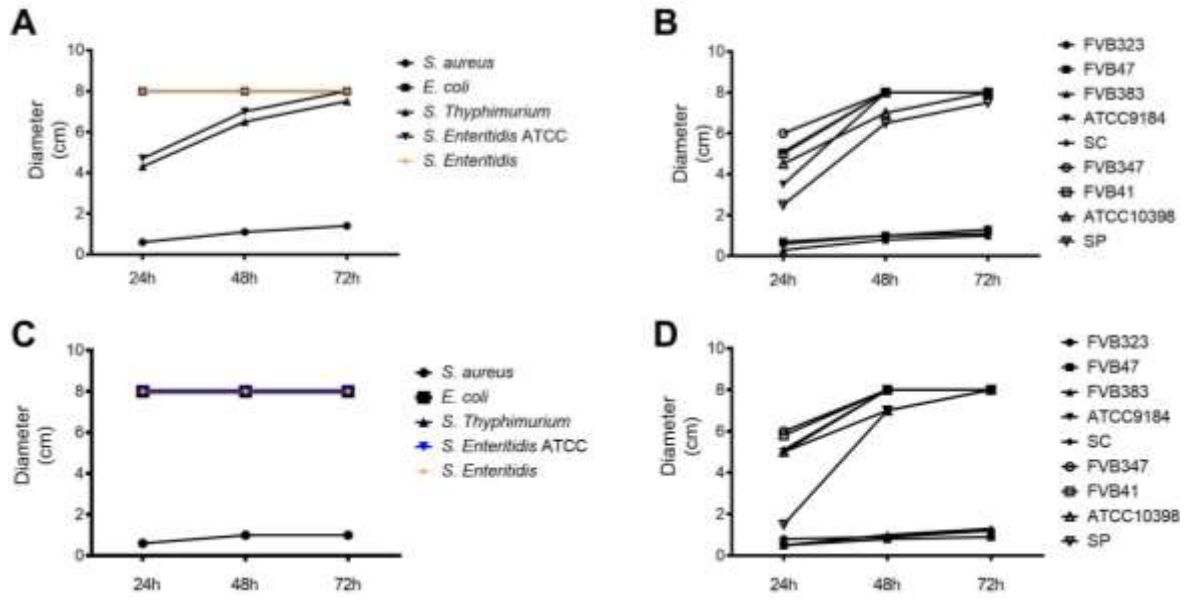

Figure 3. Comparison of halos of growth of *S.* gallinarum and *S.* pullorum. Strains were cultured in GI medium alone (upper panels) or supplemented with 0.5% dextrose (lower panels) at 37°C. Diameter of the halos of growth was measure at 24, 48 and 72 h.

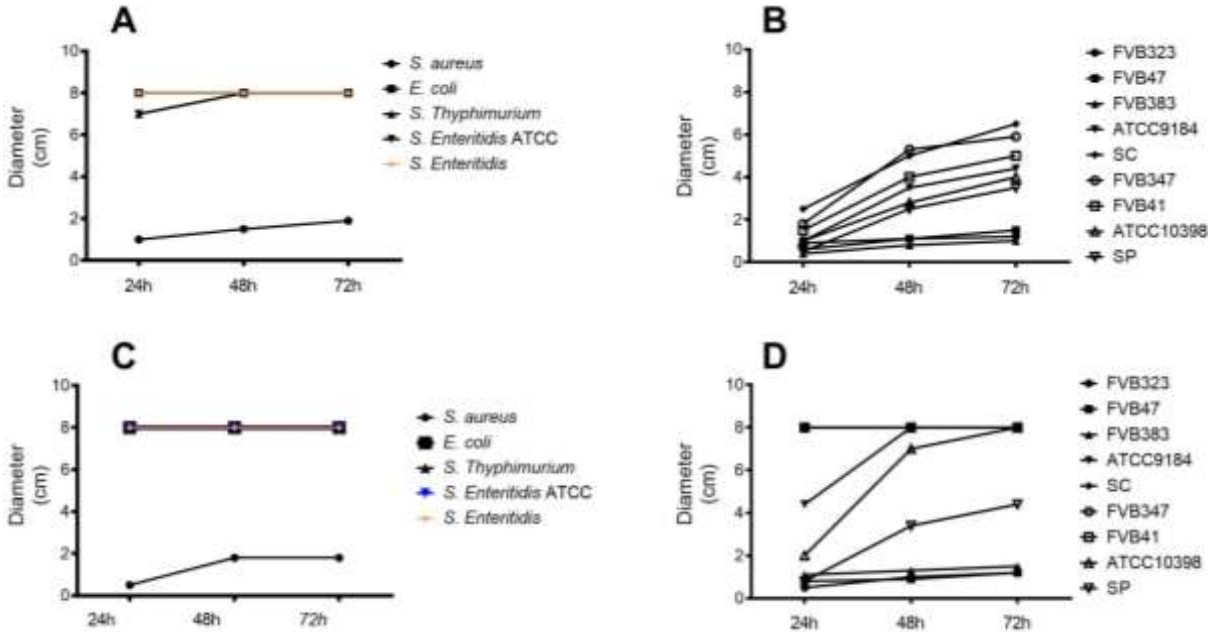

Figure 4. Determination of the diameter of control bacteria and strains of *S.* gallinarum and*S.* pullorum. Strain were cultured in GI medium alone (A and B) and GI medium supplemented with 0.5% dextrose (C and D) at 42°C at 24, 48 and 72 h of incubation.

medium showed the wavy expansion effect observed in motile bacteria (Figure 2B). On the other hand, the growth of FVB323 strain in GI medium was similar to that observed in *S. aureus* (Figure 1D). Additionally, the presence of gas was observed in strains of *S*. pullorum in GI+D medium (Figure 2C and D).

The diameter of halo growth between strains (Figures 3B and 4B) were compared, and observe that almost all *Salmonella* gallinarum strains resemble motile bacteria, except for FVB323, FVB383 and FVB47 strains, which resemble non-motile bacteria (like *S. aureus*). The photographic record clearly illustrates differences in

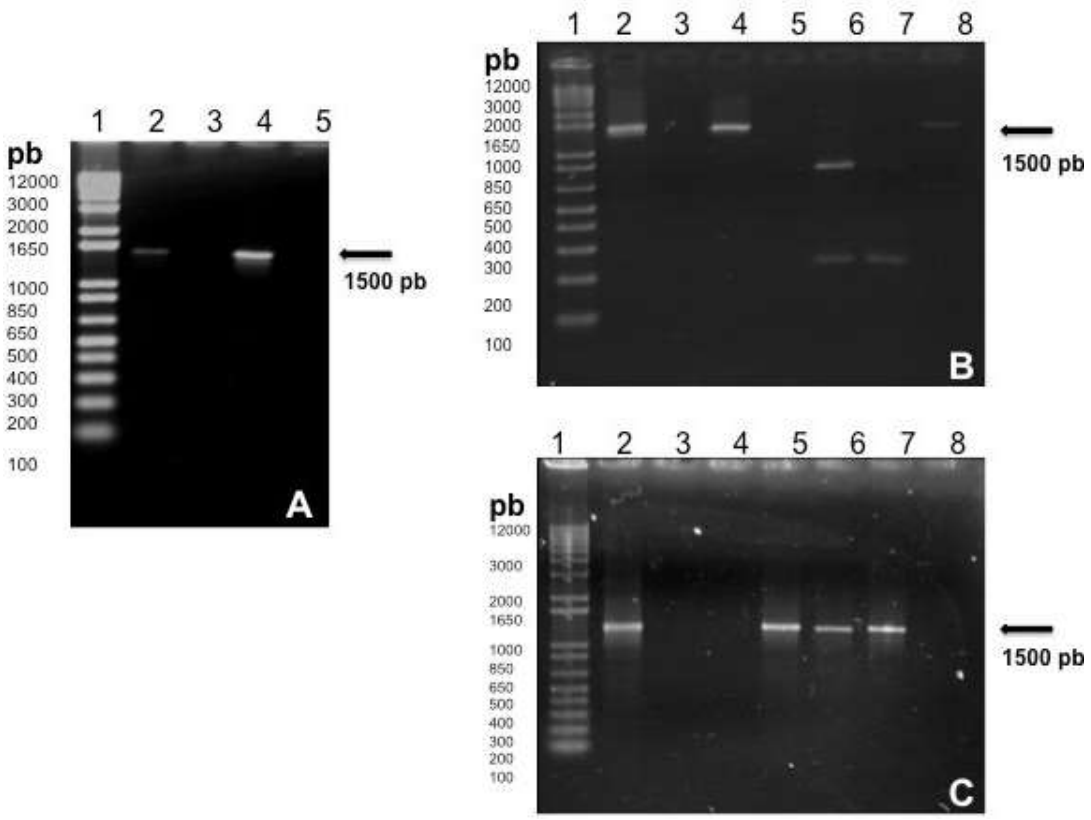

Figure 5. Agarose gels showing the PCR product to amplify *fli*C. Panel A: MWM (lane 1); *S.* Typhimurium (lane 2); *S. aureus* (lane 3); *S*. gallinarum FVB323 (lane 4); and *S*. pullorum ATCC10398 (lane 5). The arrow indicates a 1500 bp amplicon corresponding to *fli*C. Panel B: MWM (lane 1); *S.* Typhimurium (lane 2); *S. aureus* (lane 3); *S*. gallinarum FVB383 (lane 4), *S*. gallinarum FVB347 (lane 5); *E. coli* (lane 6), *S*. Enteritidis wild type (lane 7) and *S*. Enteritidis ATCC (lane 8). Panel C: MWM (lane 1); *S.* Typhimurium (lane 2); *S. aureus* (lane 3); *S*. gallinarum FVB41 (well 4); *S.* gallinarum FVB47 (lane 5); and *S.* pullorum SP (lane 6); *S.* gallinarum ATCC9184 (lane 7); and *S*. gallinarum SC (well 8).

growth morphology between strains cultured in GI medium, with those cultured in medium supplemented with dextrose (Figure 2). The halo of growth of *S.* gallinarum strains was slightly larger in GI+D medium, and the highest growth was observed after 48 h (Figures 2A, B and 3D).

As shown in Figures 3 and 4, the diffusion diameter of the cultures was larger in motile bacteria than in non-motile ones, both in GI and GI+D medium. Motile bacteria showed maximum growth between 24 and 72 h, in contrast with *S. aureus*, which showed the highest growth at 48 h. interestingly, there was no difference between those cultures incubated at 37 or 42°C (Figures 3 and 4A, C). In GI medium, the cultures of *S.* gallinarum FVB323, FVB47 and FVB383 showed a diffusion diameter more like that of *S. aureus* cultures, distinct the other strains, which reassembly that motile bacteria (Figure 3 and 4B, D). The growth morphology of all strains was very similar in GI medium with or without dextrose. The growth of cultures incubated at 42°C was lower to that observed in bacteria incubated at 37°C particularly for SP, ATCC10398, ATCC 9184, FVB41, SC and FVB347 strains (Figure 4B and D). The highest growth was observed in strains cultured for 48 h at 37°C except for the FVB41 strain, which showed maximum growth at 24 h in GI+D medium (Figure 4D).

Identification of *fli*C by PCR and sequencing

Using PCR, a fragment of around 1500 bp was detected in many of the study strains, except for *S. aureus*, *S*. gallinarum strains 347, 41, SC, and *S*. pullorum ATCC10398, in which no amplification was observed. Meanwhile in *E. coli* and *S.* Enteritidis wild type two fragments of approximately 850 and 250 bp were obtained (Figure 5B).

Restriction assay

As was expected, the restriction profile of *fli*C produces

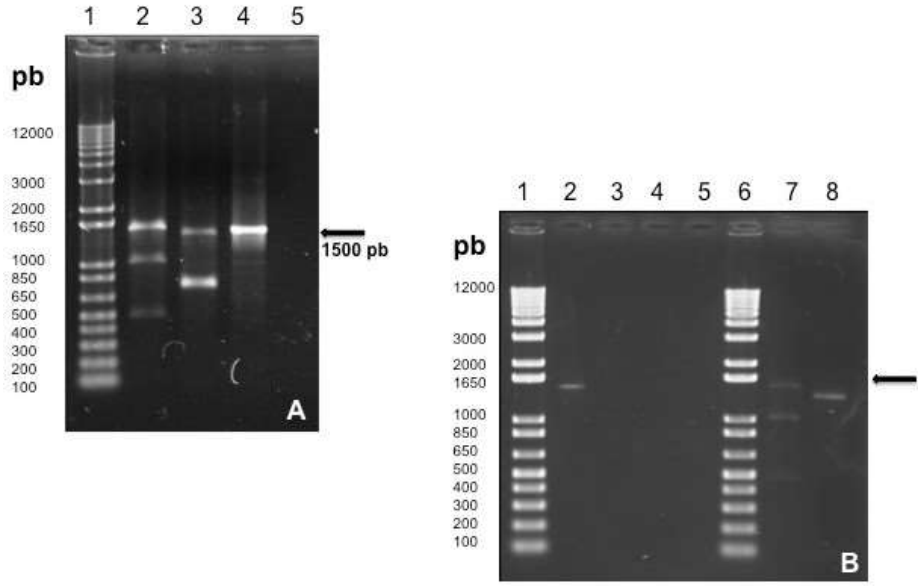

Figure 6. Restriction profile of *fli*C. The *fli*C amplicon of *Salmonella* Typhimurium field strain and *Salmonella* gallinarum ATCC9184 were digested with *Eco*RV or *Kpn*I. Panel A: MWM (lane 1);*S*. Typhimurium wild type (lane 2); *S. Typhimurium* wild type (lane 3); *S*. Typhimurium (lane 4), *fli*C PCR product. Panel B: MWM (lane 1and 6); *S.* gallinarum ATCC9184 (lane 2), the *fli*C PCR product digested with *Eco*RV (lane 7); *fli*C PCR product digested with *Kpn*I (lane 8).

two fragments of 483 and 1017 pb for *Eco*RV and one fragment of 1442 pb for *Kpn*I restriction. The same restriction profile was observed in *S.* gallinarum ATCC9184 and *S.* Typhimurium restricted with *Eco*RV (Figure 6); however, in the case of restriction with *Kpn*I, the expected patrol was only obtained in the *S.* gallinarum ATCC9184 strain (Figure 6B), while in the case of *S.* Typhimurium, restriction assay generates two fragments of approximately 1442 bp and 750 bp (Figure 6A).

Sequencing

A *fli*C PCR product of *Salmonella* gallinarum ATCC9184 (BankIt1674463 SEQ1 KF885733) was sequenced and analyzed using the PSI-Blast® software. The *fli*C genes of *S.* gallinarum ATCC9184 and *S.* Typhimurium showed 99% of identity with *S.* Enteritidis, meanwhile *S.* gallinarum 287/9 and ST showed a 100% identity with *S.* Enteritidis. It is important to note that the sequences of *fli*C of *S.* gallinarum ATCC9184 had a higher identity with *S.* Typhimurium compared with the sequences of *S.* gallinarum (287/9 and ST strains) reported in Genbank, which is identical to *S.* Enteritidis.

Electron microscopy analyzes

To confirm the presence of flagella-like structures in the studied strains, the bacteria cultured in GI+D medium (to stimulate their expression) was analyze by transmission electron microscopy. In *S.* gallinarum ATCC9184 (Figure 7B) filaments similar to the large flagella characteristic of *S.* Enteritis were observed (Figure 7C); interestingly, in *S.* gallinarum FVB323, thick and short filaments were observed, which are different from those observed in *S*. Enteritidis (Figure 7A).

DISCUSSION

This is the first report describing the presence of flagella-like filaments in wild type *Salmonella* gallinarum and ATCC strains. The filaments expression was induced in cultures supplemented with 0.5% dextrose, in which bacterial colony morphology showed a similar growth to that observed in motile bacteria. In addition, the presence of *fliC* gene, involved in flagellar filament formation in *S*. gallinarum FBV323, FBV383, FVB347 and ATCC9184 strains was described.

Motility is an essential property for bacterial colonization; for example, the flagella of *Helicobacter pylori* allow it to move through the gastric mucus to establish itself in the lining of the stomach; in the case of *Salmonella* Enteritidis, adherence to the epithelial surface depends on fimbriae and flagella (Olivares and Gisbert, 2006; de Freitas Neto et al., 2013; Foley et al., 2013; Tomoda et al., 2015). Moreover, flagella cause the activation of proinflammatory cytokines in the host

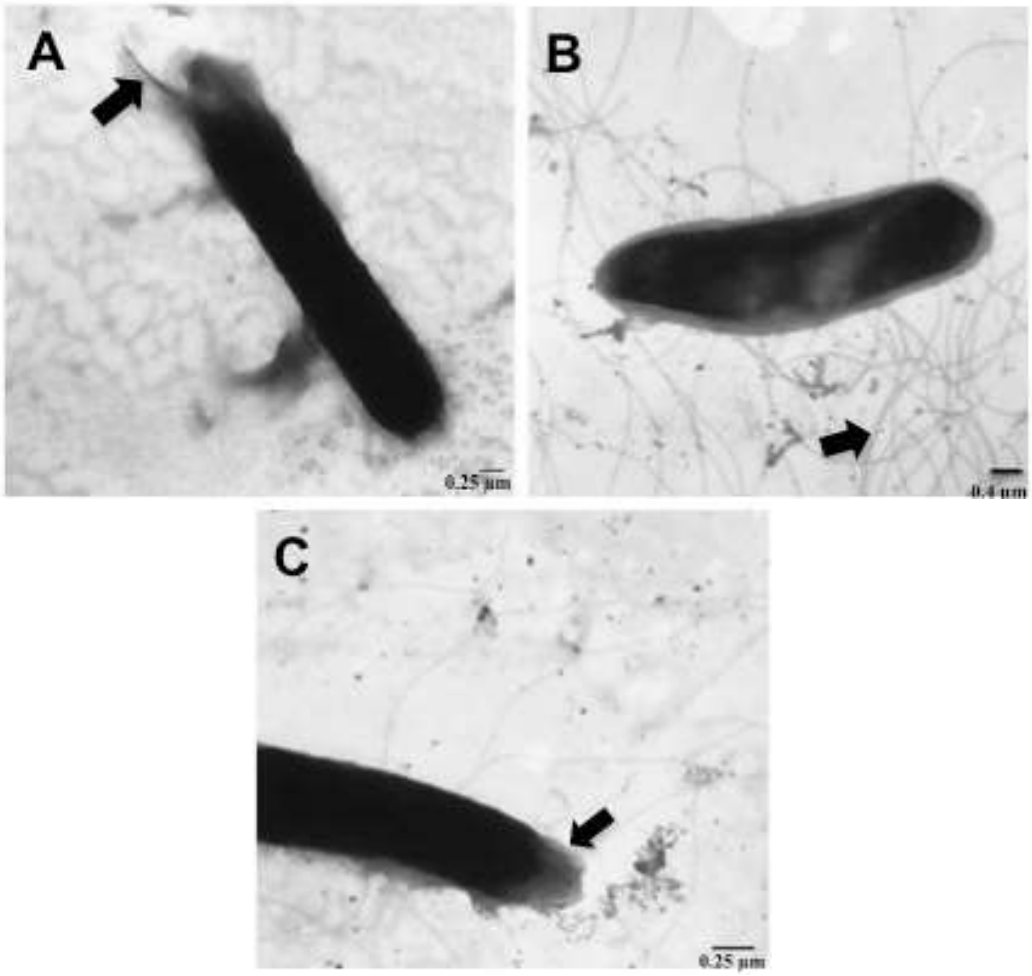

Figure 7. Electron micrograph of *Salmonella* serovars. Strains were stained with negative staining technique contrasted with 2% urany using I30,000X magnification. Panel A: *Salmonella* gallinarum FVB323; panel B: *Salmonella* gallinarum ATCC9184 and panel C: *Salmonella* Enteritidis ATCC49214. The arrows indicate electron-dense filaments emerging from the bacteria (A and C) and loose filaments can be observed in the background (B).

through their recognition by Toll-like receptor 5; however, the bacteria can change their phase and produce a filament with a different protein from that initially recognized by the immune system, and thus evade the immune system, ensuring its establishment in the intestinal epithelium (Santos, 2014; Rogier et al., 2015). In recent studies, participation of the flagellum in the mechanism of respiratory burst in neutrophils was observed in *S*. Typhimurium, because mutated bacteria in *fli*C, *fli*B and *mot*A significantly reduced this event (Westerman et al., 2018). It is also proposed that in order to be unrecognized by the immune system, when under stress signals caused by compounds that act on the cell envelope, such as EDTA or the complement system, it activates *rfl*P transcription and the later, stimulates degradation flagellar proteolytic master regulatory complex $FlhD_4C_2$ by ClpXP protease action. All these events lead to a decrease in the production of the flagellum and loss of motility; this would favor the evasion of the microorganism (Spöring et al., 2018). Study similar to these should be performed in *S*. gallinaurm that presents *fli*C or the complete operon.

In this study, the expression of flagella-like filaments in *Salmonella* gallinarum cultured in a dextrose-rich medium may be related to the regulation of *fli*C. It is known that the regulation of genes by nutrients is an important mechanism of microorganisms to adapt to their nutritional environment, like dextrose, which has been demonstrated, induce the transcription of L-pyruvate kinase genes, fatty acid synthase, acetyl-CoA carboxylase and insulin in humans (Prax and Bestram, 2014). An interesting example of these mechanisms is offered by *V. vulnificus*, who in the presence of glucose, *in vitro*, 87% of the microorganisms do not present flagellum and 13% do develop it but in a short form. In this event, dephosphorylated EIIAGlc is involved and interacts with

FapA, which avoids the biosynthesis of the flagellum and allows the bacteria to remain in the niche rich in glucose; the mechanism is developed through an independent cAMP pathway (Park et al., 2016). On the other hand, it has been reported that in *Saccharomyces cerevisiae*, glucose represses the transcription of a series of genes responsible for respiration, the use of other carbon sources (galactose, maltose, sucrose) and genes encoding enzymes of the gluconeogenic pathway. In contrast, glucose is able to induce genes involved in its own metabolism, especially genes encoding glucose transporters and enzymes of the glycolysis pathway (Meugnier et al., 2007). This work demonstrated that in most strains of *Salmonella* gallinarum, the addition of dextrose to culture medium produces a wavy, rhizoidal or lobate expansion, unlike the media without dextrose in when the bacterial expansion was smooth.

Holt et al. (1997) suggest that the higher growth rate of *S.* gallinarum cultures incubated at 42°C is due to this is the corporal temperature of birds. However, in this study, a decrease in growth rate in cultures incubated at 42°C was observed, contrary to that observed in the motile strains incubated at the same temperature. These discrepancies probably occurred due to the adaptation of the control strains to the *in vitro* culture at this temperature (Foley et al., 2013).

In *S.* gallinarum strain 323, the electron microscopy analyzes confirm the presence of filaments, which appear to be like-fimbriae, since they are small and electron-dense as previously described (Thornley and Horne, 1962). The genome *S.* gallinarum contain 50 genes related to motility and chemotaxis, however, some of them are pseudogenes (de Freitas Neto et al., 2013). By transfecting these genes in wild-type strains, they acquire the ability to produce flagellum and produce a different pathogenesis (Thomson et al., 2008; de Freitas Neto et al., 2013). In this study, the *fliC* gene was analyzed because it is involved in the synthesis of the flagellum, and the protein that encodes forms part of the external structure of flagellar filament. Furthermore, motility was evident in *S*. gallinarum FVB347, FVB41 and SC strains, and in *S.* pullorum ATCC10398 growth in GI and GI+D. However, the *fli*C gene cannot be detected by PCR in these strains; this may be explained by Holt et al. (1997), who argued that the *fli*C gene is not present in all strains of *S*. pullorum; on the other hand Kilger and Grimont (1993) attributed the non-motile phenotype of *S*. Gallinarum-Pullorum to *fliC* gene mutations these could interfere with the detection of the gene by PCR.

Another possibility could be that the *fli*C gene presents phase variation which involves the inversion of a DNA segment from one orientation to another, and a particular gene is expressed when the segment is oriented in one direction, and a different gene is expressed when the fragment is oriented in the opposite direction. Most *Salmonella* strains express both phases while some strains express only one phase (Kilger and Grimont, 1993).

The restriction profile of *fli*C gene of *Salmonella* Typhimurium LT2 digested with *Kpn*I shown an unexpected fragment size, possibly, because the strain analyzed is different to the strain used to predict the restriction profile *in silico*. A previous study reported that the site of the enzymatic cleavage site in might be different according to the bacterial strain (Kilger and Grimont, 1993). Due to the close phylogenetic relationship between different serovars of *Salmonella*, it is not strange that the sequence of *Salmonella* gallinarum ATCC9184 used in this work had greater identity with *Salmonella* Typhimurium. However, it is noteworthy that the sequence of *fli*C obtained in *Salmonella* gallinarum ATCC9184 was incomplete, and represents only half of the sequence reported in *S.* gallinarum 287/9.

Conclusions

This work suggests that *Salmonella* gallinarum develop a motile phenotype under specific culture conditions, and this may be significantly involved in the pathogenesis of the bacteria. In summary, the wavy growth pattern observed in cultures of *Salmonella* gallinarum in media supplemented with dextrose suggests that all *Salmonella* gallinarum strains could present motility under these culture conditions. In addition, the *fli*C gene showed 99% homology with their homolog of *S.* Typhimurium in 4/7 strains of *S.* gallinarum. Finally, our study demonstrated that *S.* gallinarum expressed flagella-like structures after 48h of incubation in media supplemented with dextrose, regards to the temperature of incubation.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

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