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Using growth kinetics to evaluate the stress factors faced by *Salmonella* Typhimurium during survival on meat

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Abstract

The poultry sector, a major source of animal protein worldwide, confronts serious food safety and contamination issues. Where *Salmonella* is the most common pathogen. This study looks at the growth kinetics of different genetic variants of *Salmonella enterica* serovar Typhimurium to see whether stressors affect their survival in chicken meat. Nine strains, including the wild-type ST4/74, and eight isogenic deletion mutants (Δ*fur*, Δ*rpoE*, Δ*dam*, Δ*hilD*, Δ*ssrB*, Δ*fnr*, Δ*slyA*, and Δ*entC*), were investigated for their growth kinetics in chicken meat during a 16-hour period. A Complete Randomized Design was used in this study, where the control was ST4/74. A Student's t-test was performed to determine statistical significance ($P < 0.05$). Except for the Δ*fur* mutant, all other strains displayed identical growth kinetics to the wild-type. This suggests that the assessed genes, excluding fur, are not required for *Salmonella*'s survival in chicken meat under this study conditions. The Δ*fur* mutant exhibited substantial inhibition due to intracellular iron toxicity. Treatment with the iron chelator bipyridine alleviated this effect. These findings highlight the fur gene's critical function in regulating iron homeostasis, which helps *Salmonella* survive in chicken meat. Insights into the amount of iron as a stress factor in chicken meat were established when ST4/74 was treated with bipyridyl. Understanding these genetic underpinnings provides a better insight into the molecular biological behavior in this food matrix.

Keywords: chicken meat, fur gene, genetic mutants, growth kinetics, iron homeostasis.

Resumen

El sector avícola, una fuente principal de proteína animal a nivel mundial, enfrenta serios problemas de seguridad alimentaria y contaminación, donde *Salmonella* es el patógeno más común. Este estudio examina la cinética de crecimiento de diferentes variantes genéticas de *Salmonella enterica* serovar Typhimurium para ver si los factores de estrés afectan su supervivencia en la carne de pollo. Se investigaron nueve cepas, incluyendo el tipo salvaje ST4/74, y ocho mutantes isogénicos de eliminación (Δ*fur*, Δ*rpoE*, Δ*dam*, Δ*hilD*, Δ*ssrB*, Δ*fnr*, Δ*slyA*, and Δ*entC*), para ver su cinetica de crecimiento en la carne de pollo durante un período de 16 horas. Se uso un Diseño Completamente al Azar (DCA), donde el control fue ST4/74 y para el análisis de los resultados se usó una prueba t Student para determinar la significancia estadística ($P < 0.05$). Excepto por el mutante Δ*fur*, todas las demás cepas mostraron cinéticas de crecimiento idénticas al tipo salvaje. Esto sugiere que los genes evaluados, excluyendo *fur*, no son necesarios para la supervivencia de *Salmonella* en la carne de pollo bajo las condiciones de este estudio. El mutante Δ*fur* exhibió una inhibición sustancial debido a la toxicidad del hierro intracelular. El tratamiento con el quelante de hierro bipiridina alivió este efecto. Estos hallazgos destacan la función crítica del gen *fur* en la regulación de la homeostasis del hierro, lo que ayuda a la supervivencia de *Salmonella* en la carne de pollo. Se establecieron conocimientos sobre la cantidad de hierro como un factor de estrés en la carne de pollo cuando ST4/74 fue tratado con bipiridina. Comprender estos fundamentos genéticos proporciona una mejor comprensión del comportamiento biológico molecular en esta matriz alimentaria.

Palabras clave: carne de pollo, cinética de crecimiento, gen fur, homeostasis del hierro, mutantes genéticos.

Introduction

Over recent decades, the food industry has undergone remarkable transformations, enhancing its ability to produce, process, and market food products on an unprecedented scale, all while prioritizing consumer safety (Ene, 2020). This rapid growth has ushered in complex challenges related to ensuring the ongoing safety and integrity of food products. Food safety, encompassing both the maintenance of food quality and the prevention of contamination, has become a critical issue for consumers worldwide (Ene, 2020). Within this evolving landscape, the poultry sector stands out as a global leader in providing high-quality, efficient sources of animal protein (Korver, 2023). With chicken meat being the most widely consumed meat, boasting an annual per capita consumption of 314.3 kg, its role in the global food supply chain is unparalleled (Baldi et al., 2020). However, this prominence comes with significant challenges, particularly concerning public health.

The increased demand and consumption of chicken meat can exponentially raise the risk of contamination during its production, processing, and marketing (Kumar et al., 2020). In 2015, the World Health Organization (WHO) estimated that foodborne diseases caused by 31 hazards transmitted through food resulted in 600 million illnesses annually, affecting 1 in 10 people worldwide (Kumar et al., 2020). Many of these foodborne diseases originate from poultry products. *Campylobacter jejuni*, *Salmonella* Typhimurium, *Escherichia coli* O157:H7, and *Listeria* are among the most common emerging pathogens in poultry meat (Mor-Mur & Yuste, 2010). From 1998 to 2012, the United States Foodborne Disease Outbreak Surveillance System (DFOSS) reported 1,114 outbreaks, with 279 (25%) associated with poultry. The main pathogenic bacteria involved were *Salmonella* and *Campylobacter* (Chai et al., 2017).

Poultry is a major conduit for *Salmonella* transmission to humans. In 2014, a total of 88,175 confirmed cases of human salmonellosis were reported across the European Union. Among these cases, *Salmonella enterica* serovar Typhimurium was responsible for 17 % of the infections (Teklemariam et al., 2023). This strain has a great adaptability to new environments and food matrices, often causing diarrhea, sepsis, abdominal pain, gastroenteritis, and even death (Chen et al., 2023).

The adaptability of *Salmonella* Typhimurium is due to its ability to engage the innate immune system and modulate numerous cellular processes (Hurley et al., 2014). Host cells are invaded via a type-three secretion system (T3SS-1) encoded by *Salmonella* pathogenicity island I (SPI-1), forming pores that allow invasive proteins to enter the cell cytoplasm (dos Santos et al., 2020). This leads to the formation of ruffles, enabling the engulfment and stabilization of the *Salmonella*-containing vacuole (SCV). Intracellularly, SPI-2 encodes a second T3SS-2 that operates within the phagosome, delivering effectors into the vacuolar space (Figueira & Holden, 2012). Both SPI-1 and SPI-2 play crucial roles in the invasion and persistence phases within host cells, contributing to the pathogen's survival and adaptability in adverse environments (Kröger et al., 2013).

Salmonella Typhimurium counts with 4,941 different genes (Moore et al., 2024), each with a different function. The genes selected for this study are part of the 3,846 core genes defined in *Salmonella* Typhimurium (Fu et al., 2015). This means they are critical for survival; they are classified under general crucial functions such as housekeeping, virulence, stress response, and those involved in nutrient acquisition. Functions such as the homeostasis or regulation of iron uptake by *fur* (ferric uptake regulator) gene, by encoding the Fe^{2+}-binding protein (Garcia-del Portillo et al., 1993). Iron is required by the bacteria to survive, but having this element in high concentrations can be toxic. *Fur* also regulates genes for siderophore biosynthesis and iron transport systems (Kaushik et al., 2016). The *rpoE* gene principal function is encoding an extracytoplasmatically stress response (Miticka et al., 2003). The product of *rpoE*, sigma factor σ^E, regulates the expression of several virulence factors (Chaudhuri et al., 2013). While *Dam*, is a gene that codes for DNA adenine methyltransferase (*Dam*), which methylates adenine into specific GATC sequences (Giacomodonato et al., 2014; Giacomodonato et al., 2009). *HilD* is a homologous transcription factor that activates the transcription of *hilA* which is the master regulator of SPI-1 (Petrone et al., 2014). On the other hand, *ssrB* is a gene required by SPI-2 since it promotes the transcription of multiple genes there (Choi et al., 2010). At the same time, *fnr* is one of the main proteins in charge of regulating the anaerobic metabolisms in bacteria (Fink et al.,

2007). *SlyA* is a gene that is required for virulence, and survival in macrophages (Shi et al., 2004), resists oxidative stress, and activates de SPI-2 gene expression (Bharathan et al., 2023). The function *entC* plays in the biosynthesis of enterobactin, in addition to its high affinity siderophore that is essential for iron acquisition (Mohakud et al., 2022).

Understanding the genetic underpinnings of *Salmonella*'s survival in chicken meat, a nutrient-rich and commonly contaminated medium, is paramount. Current research has primarily focused on the rapid detection of *Salmonella*, leaving a gap in understanding the complex biological behaviors of these bacteria. Initial findings into ST4/74's behavior in milk environments have begun to map out the growth kinetics of various mutants, revealing critical genes necessary for survival (Bharathan et al., 2023). Chicken meat and *Salmonella* are well-known for having several encounters and causing many foodborne diseases. Many studies focus on the occurrence, genetic characterization, and antimicrobial resistance of this pathogen and practices on how to avoid it in chicken meat (Abd-Elghany et al., 2015). However, the essential factors for *Salmonella*'s adaptation and survival in this food matrix remain unclear.

This study aims to evaluate the growth kinetics of various *Salmonella* Typhimurium genetic mutants in chicken meat over a 16-hour period. As well as understand and stress factors affecting the survival of *Salmonella enterica* serovar Typhimurium in chicken meat. Determine the role of the fur gene in regulating iron homeostasis and its impact on *Salmonella* Typhimurium survival in iron-rich environments. For this study, the model organism used was *Salmonella enterica* serovar Typhimurium (ST4/74), due to its transcriptional landscapes being well characterized (Bharathan et al., 2023). This strain, along with other eight isogenic deletion mutants of ST4/74 (Δ*fur*, Δ*rpoE*, Δ*dam*, Δ*hilD*, Δ*ssrB*, Δ*fnr*, Δ*slyA*, and Δ*entC*) were analyzed to determine if their absence affected the pathogen's survival in chicken meat. Identifying the significance of these genes develops data that will futuristically aid the development of novel technologies aimed at making food safer.

Materials and Methods

Study Location

This research was made at the Department of Poultry Science at Auburn University in the state of Alabama, United States of America. All the microbiological studies were handled in Dr. Srikumar's Food Safety Laboratory.

Bacterial Strains and Growth Conditions

To determine the role of some genes of ST4/74 genome, in the survival of *Salmonella*, growth curves were made. For this study, a total of nine strains were used mentioned in Table 1. *Salmonella enterica* serovar Typhimurium (ST4/74) and other eight isogenic deletion mutants from ST4/74. All these strains were obtained from Dr. Srikumar´s Laboratory.

The inoculation was made in 1 g chicken meat samples prepared from "chicken breast tenders" commercially sold in a supermarket. Samples were cut and weighed for its for subsequent inoculation.

From the glycerol stock at -80 °C where all strains mentioned in Table 1 were found, the necessary inoculums were made. When required, they were streaked out into Lennox broth (LB) agar plates. From the streaked plate with the strain, a colony was picked, inoculated in 5 mL LB broth, and then incubated overnight at 37 °C. Then a 0.5 McFarland solution was made. This solution was done using a spectrophotometer at a wavelength of 625 nm until an optical density reading of 0.08 -0.1 was obtained. Under this methodology an approximate cell counts of 1-5×10^8 CFU/mL was obtained, to subsequently be inoculated.

Table 1

List of bacterial strains used in this study

Strains/Complements		Role	Source
ST4/74	Wild-type *S.* Typhimurium 4/74		Gifted by Dr. Srikumar´s laboratory
ST4/74Δ*fur*	*S.* Typhimurium 4/74 Δ*fur*	Regulates iron uptake or homeostasis	

Strains/Complements		Role	Source
ST4/74 Δ*rpoE*	*S.* Typhimurium 4/74 Δ*rpoE*	Envelope stress response	
ST4/74 Δ*dam*	*S.* Typhimurium 4/74 Δ*dam*	Attenuate the virulence of several pathogens	
ST4/74 Δ*hilD*	*S.* Typhimurium 4/74 Δ*hilD*	Activates transcription of *hilA*, a master regulator of SPI-1	
ST4/74 Δ*ssrB*	*S.* Typhimurium 4/74 Δ*ssrB*	Required for the SPI-2 expression	
ST4/74 Δ*fnr*	*S*. Typhimurium 4/74 Δ*fnr*	Regulate the anaerobic metabolism	
ST4/74 Δ*slyA*	*S*. Typhimurium 4/74 Δ*slyA*	Required to activate SPI-2 gene expression, resist oxidative stress, virulence, and survival in macrophages	
ST4/74 Δ*entC*	*S.* Typhimurium 4/74 Δ*entC*	Encodes a protein involved in the biosynthesis of enterobactin	

The 1 g chicken meat samples were UV-treated for 20 minutes. Afterward, 10 µL of 0.5 McFarland solution, representing an approximate cell count of 1-5×10^6 CFU/mL was inoculated to the chicken samples. After inoculation samples were incubated at 37 °C. An inoculated 1 g piece was periodically obtained from the incubator at 37 °C at 0, 2, 4, 6, 8, 10, 12, 14, and 16 h post-inoculation, placed in 5mL 1X phosphate-buffered saline (1X- PBS) and well vortex. Serial dilutions were made as required by timepoint. The counts were determined by spread plating in Xylose Lysine Decarboxylase agar (XLD). Colonies were counted using the colony counter IncuCount Automatic Colony Counter 150 (Revolutionary Science, USA) and the growth curve was plotted. All the experiments were conducted in three repetitions.

ST4/74 and ST4/74 Δ*fur* + Bipyridyl

To prove the hypothesis that intracellular iron intoxication is inhibiting ST4/74Δ*fur*. A confirmatory study was handled where the use of bipyridine was required. Bipyridyl is a chelator that can cross cell membranes and is recognized for its ability to bind and remove Fe2+ from the internal iron stores of cells (Thompson et al., 2017). This confirmatory experiment was handled using 1M bipyridyl (Sigma-Aldrich, USA). The 1 g chicken meat samples before inoculation were treated with

50mL 1X-PBS + 10 µL of 1M bipyridyl. All the growth conditions were the same as in non-treated samples.

Experimental Design and Statistical Analysis

For the statistical analysis of this study, a Completely Randomized Design (CRD) for the evaluation of 8 isogenic deletion mutants and a control. Three repetitions were performed for each treatment, resulting in 27 experimental units. The wild-type strain, ST4/74, served as the control, and all eight mutant strains were compared to this control. Data were collected at nine different time points: 0, 2, 4, 6, 8, 10, 12, 14, and 16 hours. Each growth curve was conducted in three repetitions, and the resulting growth kinetics were based on average data with standard deviations. Statistical analyses were performed using the online version of SAS (SAS Studio). A series of Student's t-tests ($P < 0.05$) were used to determine the statistical differences in bacterial survival between the wild-type strain and the eight mutant strains at various time points. Descriptive statistics, including mean and standard deviation, were calculated for each treatment and time point. Statistical significance was determined using Student's t-test, with $P< 0.05$ indicating a significant difference among treatments. Based on this criterion, significant differences were observed at various time points, providing insights into the effects of the mutations on bacterial growth kinetics.

Results and Discussion

Growth Curves

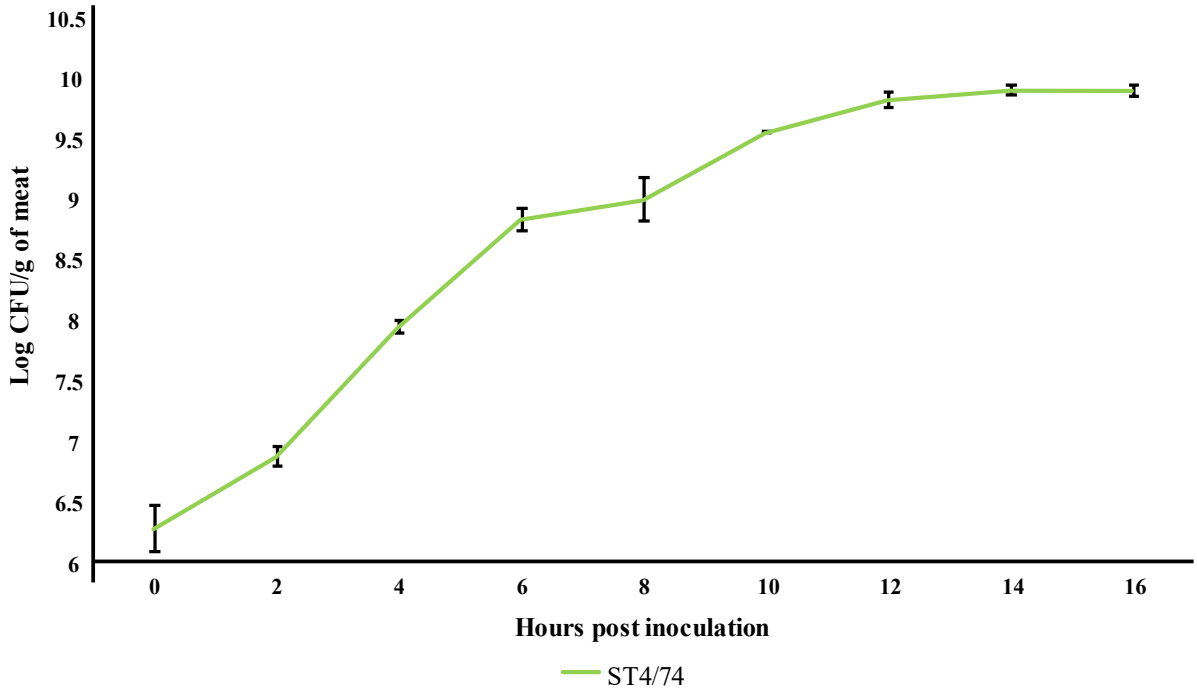
Wild-type S. Typhimurium 4/74

The growth kinetics of the Wild-type *S*. Typhimurium 4/74 (ST4/74) strain in chicken meat, is illustrated in Figure 1, where its survival over a 16-hour period at 37 °C is shown. The x-axis represents the hours post-inoculation of the chicken meat with the strain, while the y-axis indicates the bacterial concentration (Log CFU/g of meat). This growth kinetics serves as the control for the study, facilitating a comparison between ST4/74 and the other strains listed in Table 1. The error bars on the graph represent the standard deviation among three repetitions of data taken at each time point. The graph demonstrates that ST4/74 was able to proliferate in chicken meat, exhibiting a notable logarithmic growth phase from hour 2 through hour 10, after which the growth rate plateaus, indicating the onset of a stationary phase. In Appendix 2 the means of each microbial count is displayed, providing more specific proliferation data.

Chicken meat is known as an ideal medium for bacterial proliferation. Where *Salmonella* is a common infector of this food matrix. Temperature is a determinant factor for the proliferation of microorganisms. For this study, the incubation of ST4/74 and the eight isogenic deletion mutant, mentioned in Table 1, was of 37 °C. As mentioned by (Joubert & Britz, 1987), the optimum temperatures for this facultative anaerobe organism proliferation are 32 °C through 37 °C. Under the optimum temperature a stress factor was deleted. These results can be enforced by the ones obtained from (McKay et al., 1997), where similar growth kinetics can be observed in chicken meat. Where at 30°C *Salmonella* had a log phase from 4 to 25 hours. Another study by Juneja et al. (2007), evaluated for a 6 hour-period the growth kinetics of different serovars of *Salmonella enterica*, in which similar results to those of this study were obtained. These findings suggest that chicken meat is a conducive medium for the proliferation of ST4/74.

Figure 1

Growth kinetics of ST4/74 in chicken meat for 16 hours post-inoculation



S. Typhimurium 4/74 ΔrpoE

The x-axis represents the hours post-inoculation, while the y-axis indicates the bacterial concentration (Log CFU/g of meat). Error bars denote the standard deviation from three repetitions at each time point. The data reveals that ST4/74Δ*rpoE* exhibits a slower proliferation compared to the control strain ST4/74. Specifically, ST4/74Δ*rpoE* shows a logarithmic growth phase until approximately hour 8, followed by a stationary phase. A Student's t-test ($P < 0.05$) was conducted for each time point to compare the growth of ST4/74Δ*rpoE* with the control strain ST4/74, with the significance levels marked on the graph. The results indicate that there is no significant difference in growth between ST4/74Δ*rpoE* and the control strain for most time points, except for significant differences observed at hours 2, 8, and 16.

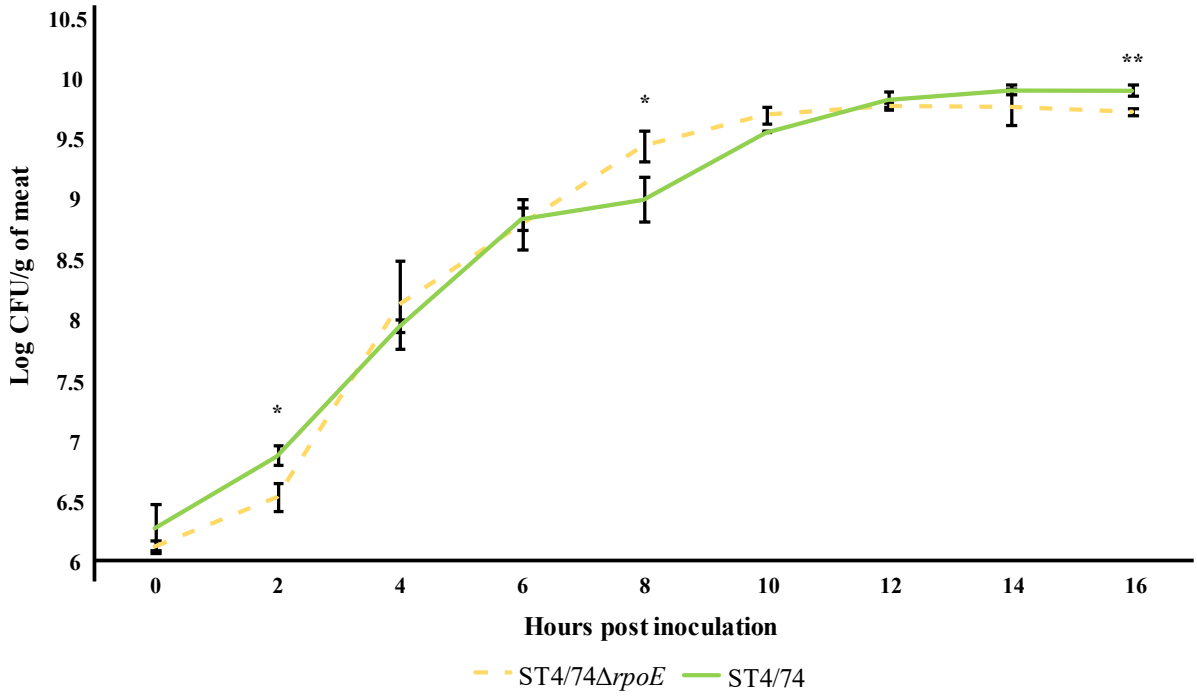
Complementary data is presented in Appendix B, which provides detailed quantitative information on the bacterial counts. Including the means and standard deviation of each microbial

count for both strains at each time point. As well as the p-values from the statistical comparisons. This tabular data supports graphical representation, offering a comprehensive view of the bacterial growth dynamics and the statistical significance of the observed differences.

To help the bacterium control and repair damage to its cell envelope, *rpoE* controls the expression of genes involved in the bacterial envelope stress response (Birhanu et al., 2021). This reaction is crucial when the bacteria encounter harmful conditions inside the host, such as an acidic pH, antimicrobial peptides, and other stressors found in the gut environment (Appia-Ayme et al., 2011). ST4/74Δ*rpoE* was inhibited in both cow and camel milk (Bharathan et al., 2023), which are typically rich environments for *Salmonella* proliferation. Due to the function of *rpoE* mentioned in Table 1, it can be inferred that bovine and camel milk were environments that caused extracytoplasmatic stress on the cell. On the other hand, the study by (Karash et al., 2022), shows that *rpoE* is required for *S.* Typhimurium fitness under iron-restricted conditions. Iron in chicken was not a limiting factor; however, this may have been the case in the study by (Bharathan et al., 2023), on cow and camel milk. In contrast, to the growth kinetics observed in chicken meat, this food matrix is recognized as ideal for *Salmonella´*s growth. The availability of nutrients is greater here than in milk even though milk is more humid (Tan et al., 2022). These characteristics present in chicken meat for the bacterium growth can be a key point for survival. As well as an environment with non-acidic conditions or any antimicrobial peptide known, that will require the expression of the gene. So, the absence of the *rpoE* gene didn't show a significant effect on the survival of ST4/74 in chicken meat under the conditions managed in this study.

Figure 2

Growth kinetics of ST4/74ΔrpoE and ST4/74 in chicken meat for 16 hours post-inoculation



Note. The asterisk indicates statistical significance based on Student's t-test P< 0.05 (*), P < 0.01 (**), and P < 0.001 (***).

S. Typhimurium 4/74 Δdam

The growth kinetics of S. Typhimurium 4/74 Δ*dam* and ST4/74 strains in chicken meat over a 16-hour period at 37 °C is illustrated in Figure 3. The x-axis represents the hours post-inoculation, while the y-axis indicates the bacterial concentration (Log CFU/g of meat). Error bars denote the standard deviation from three repetitions at each time point. The data reveals that, overall, there is no significant difference in the growth between ST4/74Δ*dam* and the control strain ST4/74. However, significant differences were observed at specific time points. ST4/74Δ*dam* shows a logarithmic growth phase until approximately hour 8, followed by a stationary phase. A Student's t-test ($P < 0.05$) was conducted for each time point to compare the growth of ST4/74Δ*dam* with the control strain ST4/74, with the significance levels marked on the graph. Significant differences in growth were observed at hours 2, 12, and 14.

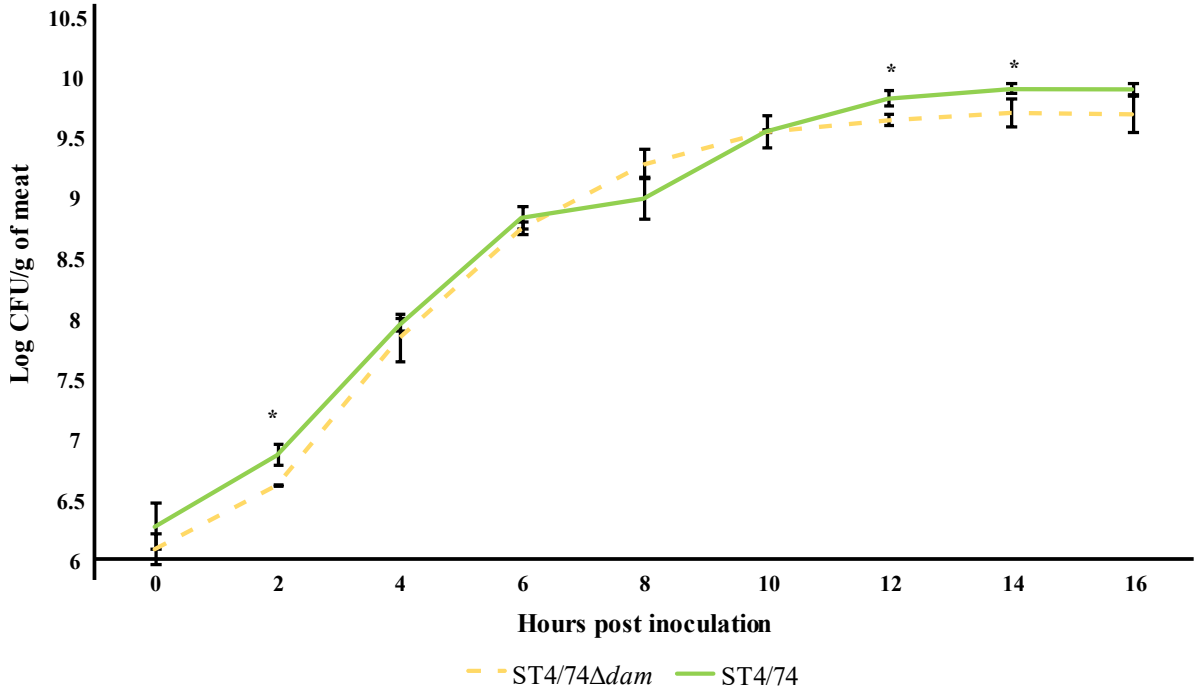
Complementary data is presented in Appendix C, which provides detailed quantitative information on the bacterial counts. Including the means and standard deviation of each microbial count for both strains at each time point. As well as the p-values from the statistical comparisons. This tabular data supports graphical representation, offering a comprehensive view of the bacterial growth dynamics and the statistical significance of the observed differences.

DNA adenine methylation (*dam*) regulates genes involved in the intestinal stage of infection (Giacomodonato et al., 2014). As well as, attenuating the virulence of several pathogens. Genes involved in virulence are considered important due to their work enabling *Salmonella* to invade host cells and immune response (Birhanu et al., 2021). *Dam* has an influence on the expression of virulence factors involved in the T3SS and it also contributes to the bacterium responses to several environmental stress (Liu et al., 2020). In the study presented by (Chaudhuri et al., 2009), the relevance of *dam* in ST4/74 for virulence was shown when grown in vitro. Other study by (Guo et al., 2020) demonstrated that homeostasis of *dam* expression was essential for Salmonella virulence and the ability to induce inflammasome activation. Considering the relevance of this gene in establishing on its host. It was considered relevant to evaluate if its absence in ST4/74 is relevant for its survival in this food matrix. After running the three repetitions for ST4/74Δ*dam* kinetics on chicken meat. No inhibition was present from the mutant.

This could have occurred due to the absence of an immune system in the chicken meat. As well as the functions mentioned, they were not necessary to survive. Additionally, the conditions were such that the bacteria were not exposed to severe stress, allowing them to proliferate without the need to activate invasion and survival mechanisms, which would require significant energy expenditure (Hamilton et al., 2009). Therefore, the absence of the *dam* gene didn't show a significant effect on the survival of ST4/74 in chicken meat under the conditions managed in this study.

Figure 3

Growth kinetics of ST4/74Δdam and ST4/74 in chicken meat for 16 hours post-inoculation



Note. The asterisk indicates statistical significance based on Student's t-test P< 0.05 (*), P < 0.01 (**), and P < 0.001 (***).

S. Typhimurium 4/74 ΔhilD

The growth kinetics of S. Typhimurium 4/74 Δ*hilD* and ST4/74 strains in chicken meat over a 16-hour period at 37°C is illustrated in Figure 4. The x-axis represents the hours post-inoculation, while the y-axis indicates the bacterial concentration (Log CFU/g of meat). Error bars denote the standard deviation from three repetitions at each time point. The data reveals that, overall, there is no significant difference in the growth between ST4/74Δ*hilD* and the control strain ST4/74. However, significant differences were observed at specific time points. ST4/74Δ*hilD* shows a logarithmic growth phase until hour 10, followed by a stationary phase. A Student's t-test (P < 0.05) was conducted for each time point to compare the growth of ST4/74Δ*hilD* with the control strain ST4/74, with the significance levels marked on the graph. Significant differences in growth were observed at hours 4, 6, 8 and 10.

Complementary data is presented in Appendix D, which provides detailed quantitative information on the bacterial counts. Including the means and standard deviation of each microbial count for both strains at each time point. As well as the p-values from the statistical comparisons. This tabular data supports the graphical representation, offering a comprehensive view of the bacterial growth dynamics and the statistical significance of the observed differences.

For ST4/74 to infect host cells it requires the function of the two pathogenic islands SPI-1 and SPI-2. These pathogenic islands are crucial for the bacterium's ability to invade host cells and survive within them (Oladapo et al., 2022). The genes located in these islands are required to encode the two types of three secretion systems T3SS-1 and T3SS-2 (Gaviria-Cantin et al., 2017). *HilD* is a master transcriptional regulator important in the activation of the SPI-1. This pathogenic island includes the genes that are necessary for the T3SS. One of these genes is *HilA* which is the master regulator of the SPI-1 (Guerra et al., 2020). Which transcription is activated by *HilD.*

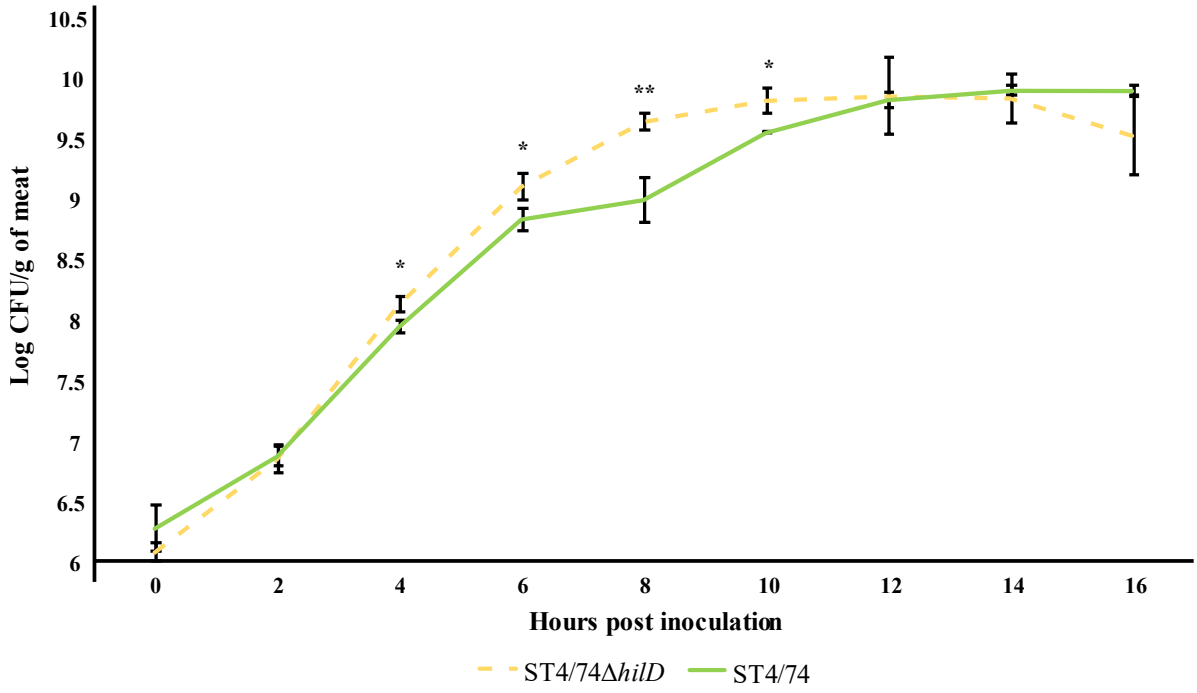
On the other hand, functions are also extended to help the bacteria adapt to the environment. The bacterium can face stress in the environment when trying to infect a new host. Acidic conditions can be critical for ST4/74 in some food matrixes. *HilD* regulates genes able to face these conditions as well as oxidative stress and limited nutrient availability (Colgan et al., 2016).

Although *HilD* function is important, in this study, it was observed that there was no inhibition of ST4/74Δ*hilD*. Chicken meat doesn’t have an active immune system as well as macrophages, which are the targets of the bacteria to invade. And even if it did, most likely other master regulators such as *HilA*, *HilC*, *HilD*, and *RtsA*, which are part of ST4/74 genome, enabled survival in chicken meat (Guerra et al., 2020). Likewise, chicken is a rich medium for ST4/74 survival, nutrient availability can be discarded as a stress factor, as well as having an acidic condition (Bhawana et al., 2023). Similarly, considering the incubation temperature under which the study was conducted, it can't be considered a stress factor that could have limited its proliferation. Therefore, the absence of the *hilD* gene didn’t

show a significant effect on the survival of ST4/74 in chicken meat under the conditions managed in this study.

Figure 4

Growth kinetics of ST4/74ΔhilD and ST 4/74 in chicken meat for 16 hours post-inoculation



Note. The asterisk indicates statistical significance based on Student's t-test P< 0.05 (*), P < 0.01 (**), and P < 0.001 (***).

S. Typhimurium 4/74 ΔssrB

The growth kinetics of S. Typhimurium 4/74 Δ*ssrB* and ST4/74 strains in chicken meat over a 16-hour period at 37 °C is illustrated in Figure 5. The x-axis represents the hours post-inoculation, while the y-axis indicates the bacterial concentration (Log CFU/g of meat). Error bars denote the standard deviation from three repetitions at each time point. The data reveals that, overall, there is no significant difference in the growth between ST4/74Δ*ssrB* and the control strain ST4/74. However, significant differences were observed at specific time points. ST4/74Δ*ssrB* shows a logarithmic growth phase until approximately hour 8, followed by a stationary phase. A Student's t-test ($P < 0.05$) was conducted for each time point to compare the growth of ST4/74Δ*ssrB* with the control strain ST4/74,

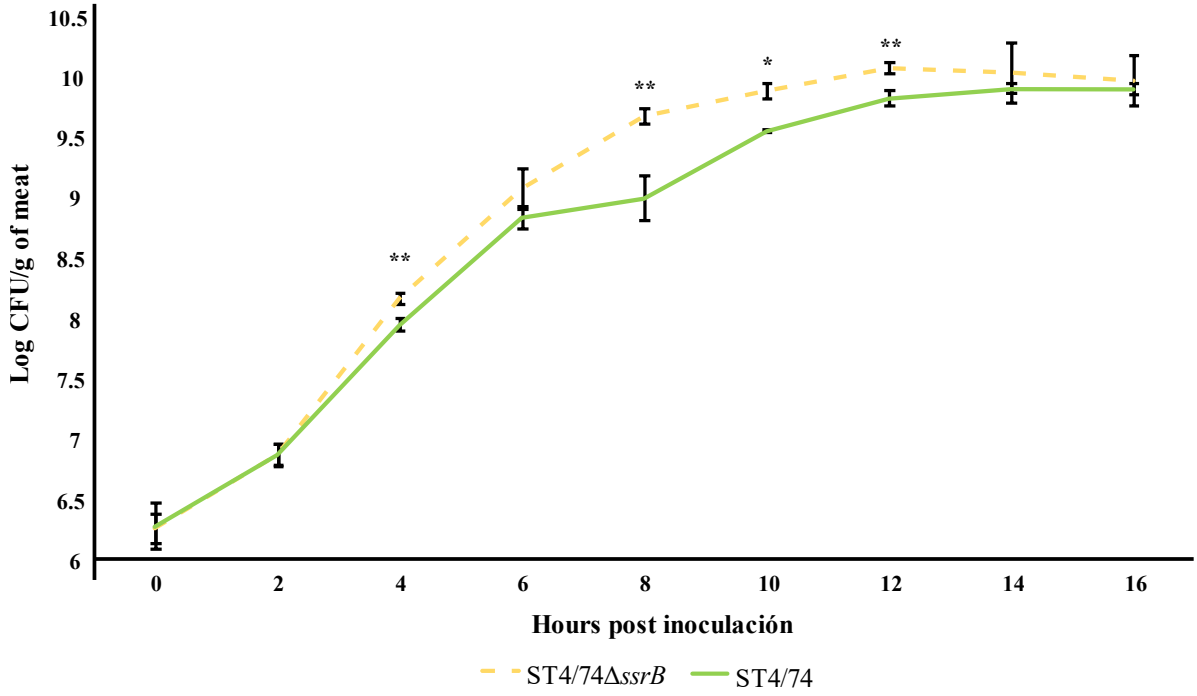
with the significance levels marked on the graph. Significant differences in growth were observed at hours 4, 8, 10 and 12.

Complementary data is presented in Appendix E, which provides detailed quantitative information on the bacterial counts. Including the means and standard deviation of each microbial count for both strains at each time point. As well as the p-values from the statistical comparisons. This tabular data supports the graphical representation, offering a comprehensive view of the bacterial growth dynamics and the statistical significance of the observed differences.

SrrB is a core gene in ST4/74 that together with *ssrA* form the two-component regulatory system. These two genes together are required for the expression of genes located in the SPI-2 (Lee et al., 2000). The genes activated in the SPI-2 by *ssrB*, are necessary for the formation of the SCV (Shetty & Kenney, 2023), which is a specific area inside host cells where the bacteria can multiply. SCV provides an advantage for ST4/74 cells to replicate under protection avoiding a response from the immune system (Röder et al., 2021). Because of this, *Salmonella* can withstand the host's immune system and survive inside the host (Pérez-Morales et al., 2017). Additionally, *ssrB* facilitates the transition of *Salmonella* between its several virulence regimes. It does this by activating SPI-2 genes and suppressing the expression of SPI-1 genes involved in the initial invasion of host cells (Osborne & Coombes, 2009). Even though *ssrB* is a gene with several important functions in ST4/74 such as the invasion and survival within the host in an adverse environment (Kröger et al., 2013). In this study, no inhibition was observed for ST4/74Δ*ssrB*. Leading to the hypothesis that, due to the absence of an active immune system in the inoculated chicken meat, genes encoded by *ssrB* in the SPI-2 required for the SCV formation, doesn't need to be activated. Therefore, the absence of the *ssrB* gene didn't show a significant effect on the survival of ST4/74 in chicken meat under the conditions managed in this study.

Figure 5

Growth kinetics of ST4/74ΔssrB and ST4/74 in chicken meat for 16 hours post-inoculation



Note. The asterisk indicates statistical significance based on Student's t-test P< 0.05 (*), P < 0.01 (**), and P < 0.001 (***).

S. Typhimurium 4/74 ΔslyA

The growth kinetics of *S*. Typhimurium 4/74 Δ*slyA* and ST4/74 strains in chicken meat over a 16-hour period at 37 °C is illustrated in Figure 6. The x-axis represents the hours post-inoculation, while the y-axis indicates the bacterial concentration (Log CFU/g of meat). Error bars denote the standard deviation from three repetitions at each time point. The data reveals that, overall, there is no significant difference in the growth between ST4/74Δ*slyA* and the control strain ST4/74. However, significant differences were observed at specific time points. ST4/74Δ*slyA* shows a logarithmic growth phase until approximately hour 8, followed by a stationary phase. A Student's t-test (P < 0.05) was conducted for each time point to compare the growth of ST4/74Δ*slyA* with the control strain ST4/74, with the significance levels marked on the graph. Significant differences in growth were observed at hours 2, 6 and 16.

Complementary data is presented in Appendix F, which provides detailed quantitative information on the bacterial counts. Including the means and standard deviation of each microbial count for both strains at each time point. As well as the p-values from the statistical comparisons. This tabular data supports the graphical representation, offering a comprehensive view of the bacterial growth dynamics and the statistical significance of the observed differences.

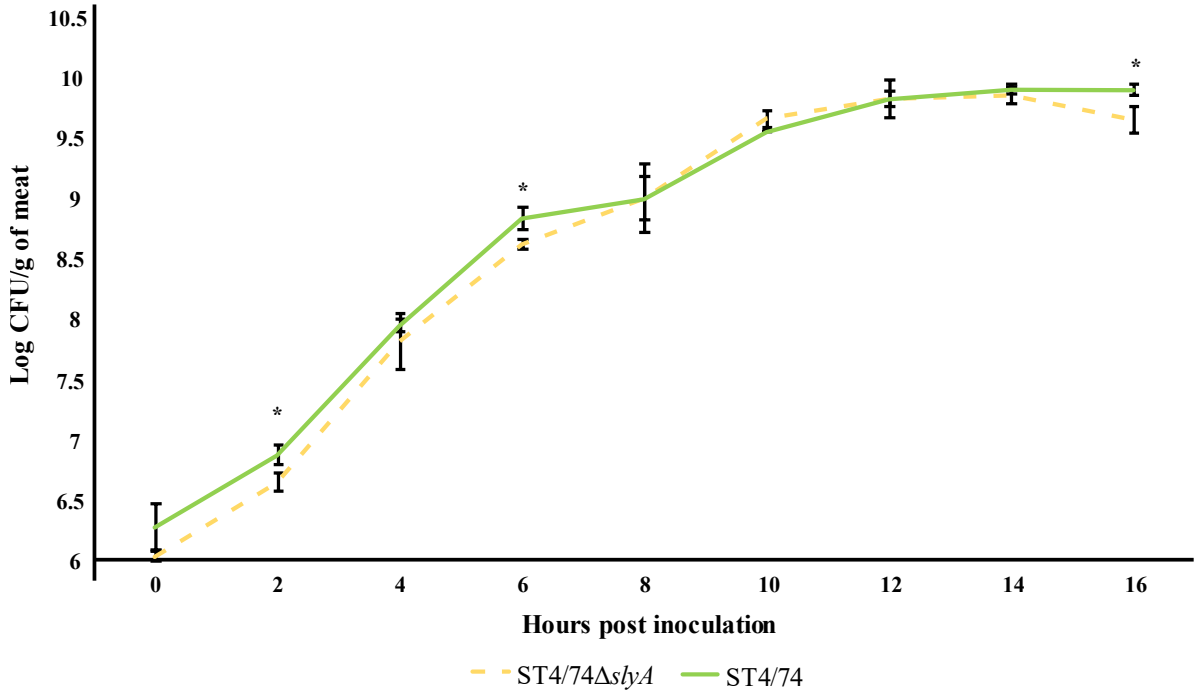
Salmonella Typhimurium's virulence and intracellular survival rely heavily on the *slyA* transcriptional regulator. First, the expression of the SPI-2 genes, which codes for elements of the T3SS essential to the bacterium's survival and reproduction in host cells, must be activated by *slyA* (Linehan et al., 2005). For *Salmonella* to flourish in the intracellular milieu of macrophages, this activation is essential (Navarre et al., 2005). Moreover, *slyA* gives the bacteria the ability to withstand oxidative stress, which is a defense mechanism frequently used by host macrophages to eliminate invasive pathogens (Zhang et al., 2018). *Fur*thermore, by controlling several genes involved in ST4/74 pathogenic potential, *slyA* considerably raises the bacteria's overall virulence (Bartoli et al., 2020). Finally, *slyA* regulates the production of genes that mitigate the harsh environment these immune cells encounter, which is essential for the bacterium's survival within macrophages. Because of these combined roles, *slyA* is necessary for *Salmonella* to be able to infect and live inside the host. Due to the complex array of functions these genes exert on the bacteria, inhibition was anticipated. However, ST4/74 was able to proliferate.

In the study by (Kim et al., 2024), which aimed to determine a specific function of the gene, a comparison was made between the wild-type and ST4/74Δ*slyA*. It was found that there was no significant difference in growth between them. Only when the bacteria faced challenges to survive in macrophages did the expression of the *slyA* gene become necessary. Another study by (Cabezas et al., 2018) suggests that *hilA* is downregulated by *slyA*. By a RNA-seq analysis that showed a −2.8 $\log_2$ fold-change in *hilA* expression levels in the WT compared to Δ*slyA*. *HilA* is the master regulator of the SPI-1 (Guerra et al., 2020). Based on these results, it can be inferred that due to the absence of

macrophages in chicken meat. Since macrophages are cells part of their immune system, which isn't active in meat. As well as none stressing conditions to activate invasion and survival mechanisms, which would require significant energy expenditure (Hamilton et al., 2009). Therefore, the absence of the *slyA* gene didn't show a significant effect on the survival of ST4/74 in chicken meat under the conditions managed in this study.

Figure 6

Growth kinetics of ST4/74ΔslyA and ST4/74 in chicken meat for 16 hours post-inoculation



Note. The asterisk indicates statistical significance based on Student's t-test P< 0.05 (*), P < 0.01 (**), and P < 0.001 (***).

S. Typhimurium 4/74 Δfnr

The growth kinetics of S. Typhimurium 4/74 Δ*fnr* and ST4/74 strains in chicken meat over a 16-hour period at 37 °C is illustrated in Figure 7. The x-axis represents the hours post-inoculation, while the y-axis indicates the bacterial concentration (Log CFU/g of meat). Error bars denote the standard deviation from three repetitions at each time point. The data reveals that, overall, there is no significant difference in the growth between ST4/74Δ*fnr* and the control strain ST4/74. However,

significant differences were observed at specific time points. ST4/74Δ*fnr* shows a logarithmic growth phase until approximately hour 8, followed by a stationary phase. A Student's t-test ($P < 0.05$) was conducted for each time point to compare the growth of ST4/74Δ*fnr* with the control strain ST4/74, with the significance levels marked on the graph. Significant differences in growth were observed at hours 2 and 8.

Complementary data is presented in Appendix G, which provides detailed quantitative information on the bacterial counts. Including the means and standard deviation of each microbial count for both strains at each time point. As well as the p-values from the statistical comparisons. This tabular data supports the graphical representation, offering a comprehensive view of the bacterial growth dynamics and the statistical significance of the observed differences.

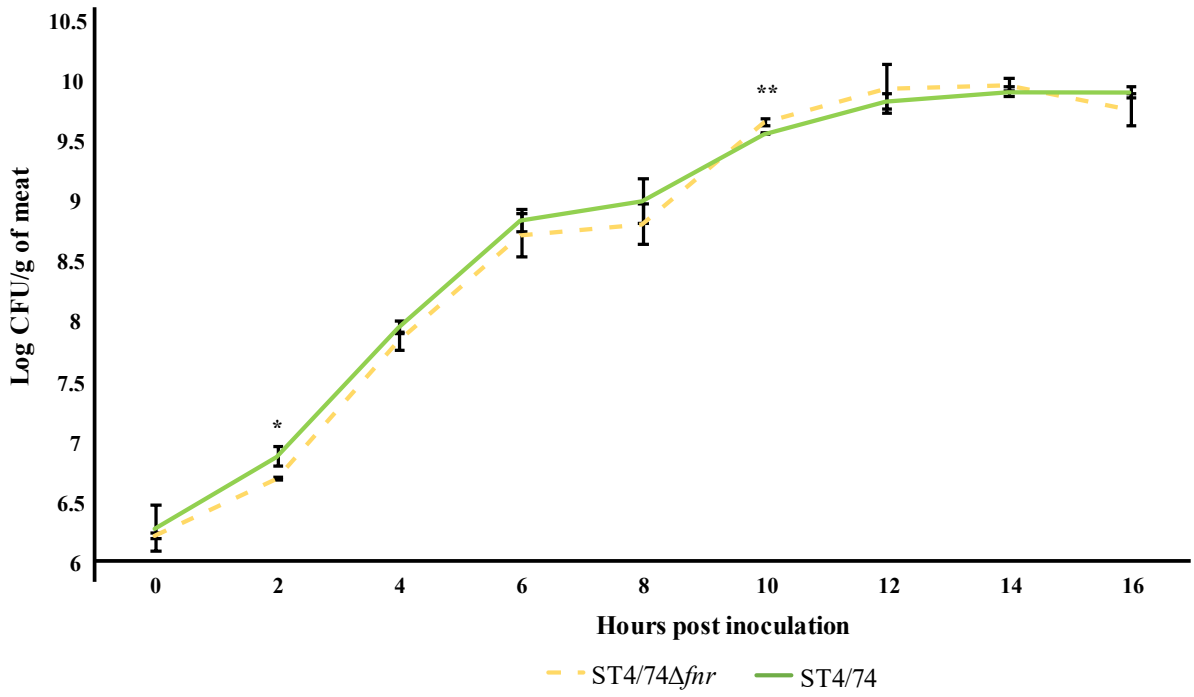
Fumarate nitrite reduction regulator, or *fnr,* is a key gene for ST4/74. This indicates a crucial role in bacteria's survival and pathogenicity under anaerobic conditions. Anaerobic metabolism and virulence genes are among the more than 300 genes whose expression is regulated by the global regulator *fnr* (Nikhil et al., 2022). For the host to adapt to low-oxygen conditions, such the gastrointestinal tract, this gene is necessary (Kiley & Beinert, 1998). As well the study (Fernández et al., 2018) showed that the deletion of *fnr* in the genome can cause significant changes to this membrane. Because *fnr* plays an important role in the modification of the lipid A membrane in *S. enteriditis*. This study showed that by this the ability of bacteria to survive under different oxygen levels is also affected. Other studies as (Chaudhuri et al., 2013), demonstrated that mutations in the *fnr* gene significantly reduce *Salmonella* Typhimurium's overall pathogenicity by limiting its capacity to live and multiply within host tissues.

Despite having different functions relevant to bacterial survival, ST4/74Δ*fnr* proliferated in chicken meat similarly to ST4/74. It can be hypothesized that this is due to the uncontrolled and therefore variable oxygen conditions in the environment. Studies have shown that the FNR protein switches between active and inactive forms depending on oxygen availability, directly impacting the

metabolic pathways (Kiley & Beinert, 1998). The interplay between *FNR* and other global regulators like *arcA* could explain the observed growth of the ST4/74Δ*fnr* mutant under aerobic conditions (Spiro & Guest, 1990). Results in the study by (Bharathan et al., 2023), ST4/74Δ*fnr* showed to be significantly inhibited for survival in both camel and bovine milk, this study highlighted microaerophilic conditions, during our incubation conditions, which could´ve required the gene expression. However, the absence of the *fnr* gene didn't show a significant effect on the survival of ST4/74 in chicken meat under the conditions managed in this study.

Figure 7

Growth kinetics of ST4/74 Δfnr and ST4/74 in chicken meat for 16 hours post-inoculation



Note. The asterisk indicates statistical significance based on Student's t-test P< 0.05 (*), P < 0.01 (**), and P < 0.001 (***).

S. Typhimurium 4/74 ΔentC

The growth kinetics of S. Typhimurium 4/74 Δ*entC* and ST4/74 strains in chicken meat over a 16-hour period at 37 °C is illustrated in Figure 8. The x-axis represents the hours post-inoculation, while the y-axis indicates the bacterial concentration (Log CFU/g of meat). Error bars denote the standard deviation from three repetitions at each time point. The data reveals that, overall, there is no

significant difference in the growth between ST4/74Δ*entC* and the control strain ST4/74. However, significant differences were observed at specific time points. ST4/74Δ*entC* shows a logarithmic growth phase until approximately hour 8, followed by a stationary phase. A Student's t-test ($P < 0.05$) was conducted for each time point to compare the growth of ST4/74Δ*entC* with the control strain ST4/74, with the significance levels marked on the graph. Significant differences in growth were observed at hours 8 and 16.

Complementary data is presented in Appendix H, which provides detailed quantitative information on the bacterial counts. Including the means and standard deviation of each microbial count for both strains at each time point. As well as the p-values from the statistical comparisons. This tabular data supports the graphical representation, offering a comprehensive view of the bacterial growth dynamics and the statistical significance of the observed differences.

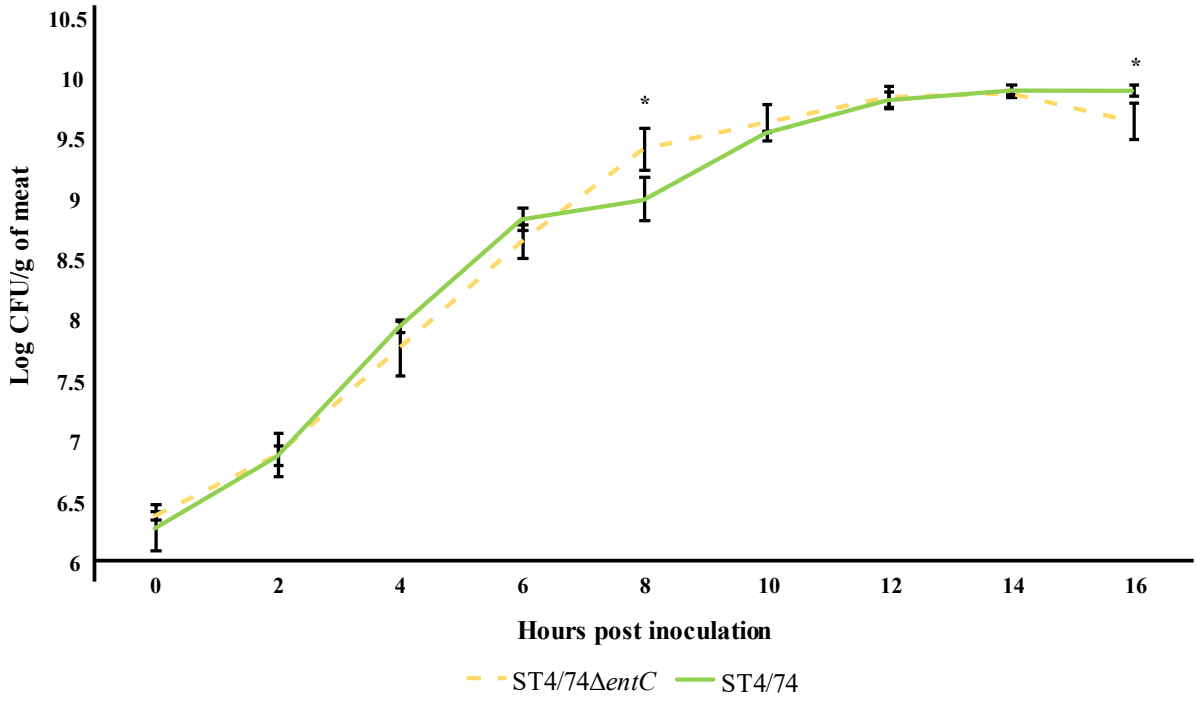
In ST4/74 *entC* is a core gene that has the function of encoding an enzyme that's involved in the biosynthesis of enterobactin, which is a high affinity siderophore. These iron-chelating molecules produced in response to low iron levels in bacteria, are also known as essential virulence factors for the bacterium (Khasheii et al., 2021). For many bacterial functions, such as respiration and DNA synthesis, iron is an essential nutrient. As a defense mechanism during infection, the host sequesters iron, making it more difficult for bacteria like *Salmonella* to get this essential mineral (Tan et al., 2021).

Salmonella can scavenge iron from the host by producing enterobactin, which is controlled by the *entC* gene (Mey et al., 2021). This aids in the pathogen's proliferation and pathogenicity. Studies have demonstrated that when exposed to iron restrictions, *entC* mutants grow significantly less than wild-type strains, because these mutants can grow quite normally in media rich in iron, but when iron is scarce, their growth is severely inhibited (Karash et al., 2022). In addition, research employing genome-wide methods, like transposon sequencing or Tn-seq, has revealed *entC* to be one of the conditionally required genes for growth in iron-restricted environments (Karash & Kwon, 2018). All these studies indicate a relation between survival and growth of ST4/74Δ*entC* in rich iron media.

Due to the fact, that chicken meat is considered a rich font of iron, containing 8.8 μgrams of iron per gram (Hazell, 1982), this can be an insight to its survival. Nevertheless, other siderophores in *Salmonella* serve the function of acquiring iron necessary for bacterial proliferation. Salmochelin is one such siderophore. The synthesis, excretion, and uptake of salmochelin require five genes: *iroB*, *iroC*, *iroD*, *iroE*, and *iroN* (Müller et al., 2009). Then, the similar growth observed compared to the wild-type cannot be attributed solely to the iron-rich nature of the food, as other mechanisms for iron acquisition may have aided bacterial survival. However, the inhibition of ST4/74Δ*fur* under the same study conditions suggests iron toxicity within the cytoplasm, supporting the hypothesis that the iron content in the food matrix is high, as mentioned by (Hazell, 1982). This is corroborated by the treatment 0.2 mM bipyridyl in ST4/74Δ*fur*, illustrated in Figure 10 and Appendix K. Therefore, it can be inferred that the *entC* gene did not need to be expressed due to the availability of iron. Additionally, since chicken meat lacks an active immune system to limit bacterial iron access, *entC* likely did not require expression. Consequently, under the conditions of this study, *entC* did not affect the survival of ST4/74 in chicken meat.

Figure 8

Growth kinetics of ST4/74 ΔentC and ST4/74 in chicken meat for 16 hours post-inoculation



Note. The asterisk indicates statistical significance based on Student's t-test P< 0.05 (*), P < 0.01 (**), and P < 0.001 (***).

S. Typhimurium 4/74 Δfur

The growth kinetics of S. Typhimurium 4/74 Δ*fur* and ST4/74 strains in chicken meat over a 16-hour period at 37°C is illustrated in Figure 9. The x-axis represents the hours post-inoculation, while the y-axis indicates the bacterial concentration (Log CFU/g of meat). Error bars denote the standard deviation from three repetitions at each time point. The data reveals that ST4/74Δ*fur* exhibits a 2 to 3-fold lower proliferation compared to the control strain ST4/74. Statistical analysis using Student's t-test ($P < 0.05$) at each time point underscores significant differences in growth, evidencing an inhibition of the Δ*fur* mutant in chicken meat. The corresponding significance levels are marked on the graph.

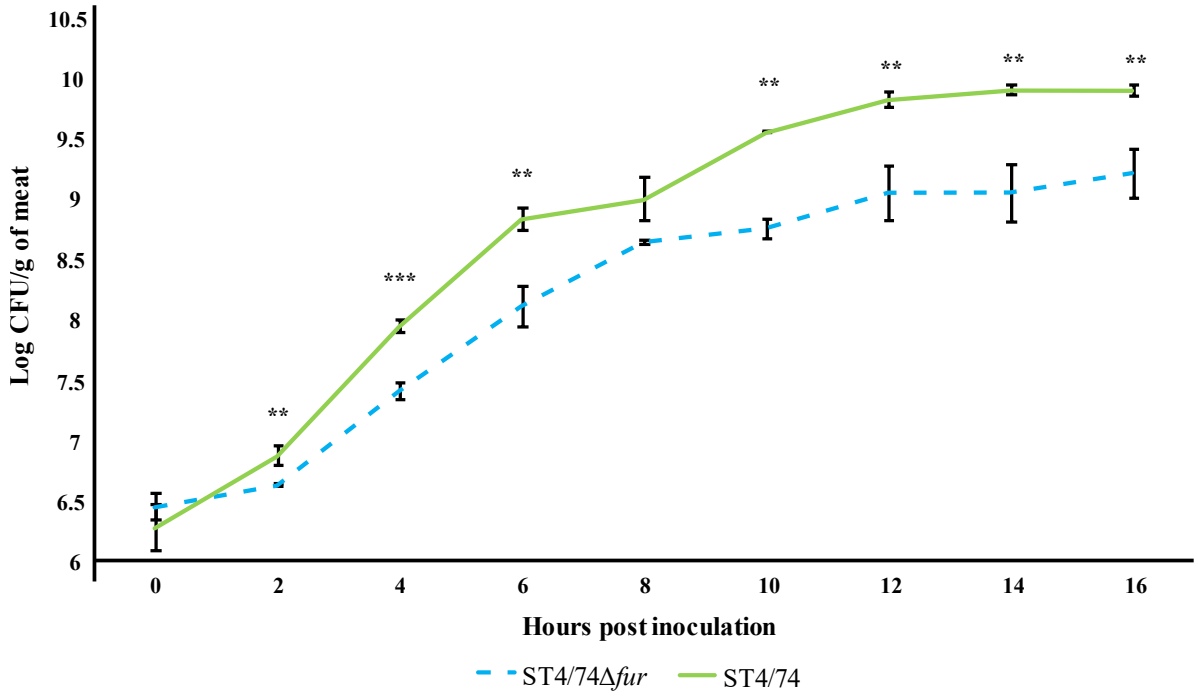
Complementary data is presented in Appendix I, which provides detailed quantitative information on the bacterial counts. Including the means and standard deviation of each microbial count for both strains at each time point. As well as the p-values from the statistical comparisons. This

tabular data supports the graphical representation, offering a comprehensive view of the bacterial growth dynamics and the statistical significance of the observed differences.

Fur function is regulating the amount of iron inside *Salmonella´*s cytoplasm. *Fur* binds to iron and inhibits the transcription of iron acquisition genes when iron levels are high enough to keep excess iron from having harmful effects on cells (Troxell et al., 2011). The presence of iron is important for the microorganism, due to its requirement in different redox reactions. Hence, in large amounts, it becomes toxic to the bacteria (Domínguez-Acuña & Garcia-Del Portillo, 2022). Host organisms, activate several methods, such as nutritional immunity to avoid the proliferation of pathogenic bacteria (Marchetti et al., 2020). This makes iron unavailable for bacteria. However, these can´t be proven because chicken meat doesn´t have an active immune system. Therefore, the results obtained led to the hypothesis that the bacteria were inhibited due to iron intoxication in the cytoplasm. From the literature review, it was observed that the strain *S*T4/74Δ*fur* was inhibited in both cow and camel milk (Bharathan et al., 2023), which are typically rich environments for *Salmonella* proliferation. Although, considering this study proves iron intoxication was inhibiting ST4/74Δ*fur* in bovine milk, this food matrix is not considered a font of iron having 0.40-0.59 µg/ml (Fransson & Lönnerdal, 1983). Meaning, that even though the medium isn´t iron-rich the lack of *fur* causes the bacterium inhibition. Then, chicken meat is a richer iron font with 8.8 µg/g of chicken meat (Hazell, 1982). Inhibition of ST4/74Δ*fur* can be expected and said due to iron toxicity inside the cytoplasm. Having stated this hypothesis, it was necessary to perform confirmatory experiments to prove that the excessive entry of iron, due to the lack of *fur*, into the cytoplasm is inhibiting *Salmonella.* The results of this confirmatory experiment are illustrated in Figure 10 and Appendix K.

Figure 9

Growth kinetics of ST4/74Δfur and ST4/74 in chicken meat for 16 hours post-inoculation



Note. The asterisk indicates statistical significance based on Student's t-test P< 0.05 (*), P < 0.01 (**), and P < 0.001 (***).

Confirmatory Experiment

Bipyridyl

The growth kinetics of *S*. Typhimurium 4/74, ST4/74Δ*fur*, and their counterparts treated with 0.2 mM Bipyridyl in chicken meat over a 16-hour period at 37°C is illustrated in Figure 10. The x-axis represents the hours post-inoculation, while the y-axis indicates the bacterial concentration (Log CFU/g of meat). Error bars denote the standard deviation from three repetitions at each time point. The data reveal significant differences in the growth patterns among the strains and their treatments.

ST4/74 shows a clear logarithmic growth phase until approximately hour 8, after which the growth rate slows down, indicating the onset of the stationary phase. The addition of 0.2 mM Bipyridyl to ST4/74 results in significantly enhanced growth compared to the control ST4/74, particularly notable from hours 4 through 16, as shown in Figure 10 and Appendix J. In Appendix J, the data show

that from hour 4 onward, the bacterial counts for ST4/74 treated with Bipyridyl are significantly higher, with p-values less than 0.05 indicating statistical significance at most time points.

ST4/74Δ*fur* demonstrates a slower growth rate compared to ST4/74, indicating an inhibition due to the Δ*fur* mutation. When 0.2 mM Bipyridyl is added to the chicken inoculated with ST4/74Δ*fur*, the growth is enhanced compared to ST4/74Δ*fur* alone, indicating that the Bipyridyl mitigates some of the inhibitory effects of the Δ*fur* mutation. This is reflected in the higher bacterial counts observed in Appendix K for the strain treated with Bipyridyl compared to the untreated Δ*fur* strain. Significant differences in growth are observed at multiple time points, with the Bipyridyl treatment leading to higher counts at several points.

A Student's t-test ($P < 0.05$) was conducted for each time point to compare the growth of each strain with its respective control, with the significance levels marked on the graph. Significant differences in growth were observed at several time points for each comparison. From the results obtained from this study for the ST4/74Δ*fur* strain. Where a significant inhabitation of this strain was observed compared to ST4/74. A complementary experiment was required.

The confirmation of findings was essential for ST4/74Δ*fur*; however, the kinetics of the wild-type (WT) strain were also evaluated to gain new insights into the composition of the food matrix. When treated with bipyridyl, the WT strain showed a significant difference of 1 to 3-fold compared to untreated ST4/74, suggesting that, as (Hazell, 1982) noted, the food matrix, such as chicken meat, is a rich iron source with 8.8 µg/g. This significant inhibition in growth after bipyridyl treatment indicates that the iron content in the food matrix plays a crucial role in bacterial survival. Further experiments treating chicken meat with varying concentrations of bipyridyl could determine whether the surface iron content is toxic to the bacteria or if reducing the iron content substantially allows faster bacterial growth.

Although iron is necessary for bacterial growth, in large amounts, it becomes toxic to the bacteria (Domínguez-Acuña & Garcia-Del Portillo, 2022). From this study, it was insight, an

environment with limited iron can be favorable under optimal conditions without external stress factors. As mentioned in the study by (Varghese et al., 2020), bacteria often encounter various environmental stresses and may respond by forming multicellular structures, such as biofilms. This study, showed the formation of novel macroscopic and multicellular structures by *Salmonella* Typhimurium, resembling small strings approximately 1 cm in length. These structures are induced under specific stress conditions, including iron deprivation caused by 2,2-bipyridyl and exposure to low concentrations of antibiotics or ethanol in minimal media. Notably, these string-like structures revert to planktonic growth when returned to nutrient-rich media and exhibit greater resistance to antibiotics and oxidative stress compared to planktonic cells.

The formation of these strings in most bacteria represents a defense mechanism. Additionally, understanding the strategies used by *Salmonella* Typhimurium for host adaptation, as outlined by (Anderson & Kendall, 2017), can further elucidate how these bacteria manage to survive under diverse environmental conditions.

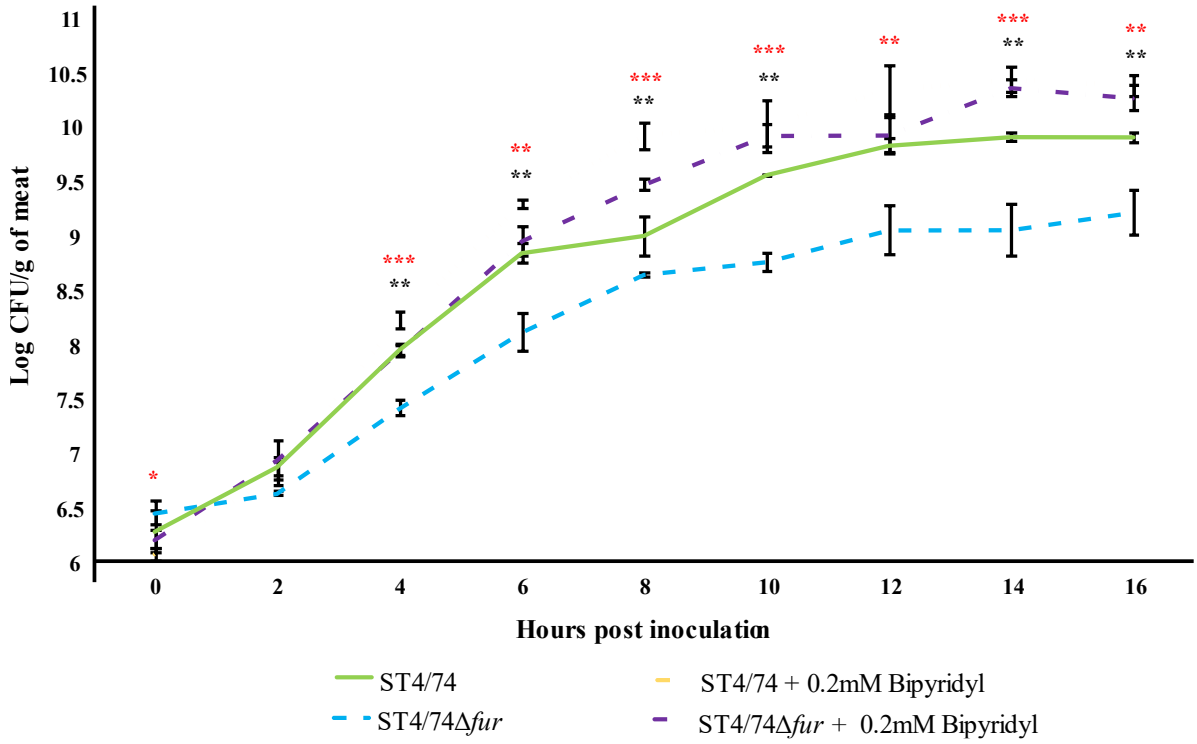
As mentioned, *fur* is important for regulating iron that comes to the bacterium's cytoplasm (Marcoleta et al., 2018). Iron in large amounts can be toxic for the cell. As the strain lacks *fur* in charge of regulating the amount of iron coming into the cell. It was hypothesized that large amounts of iron were coming inside the cell causing toxicity and inhibiting the mutant. To prove or disapprove that ST4/74Δ*fur* was being inhibited due to iron toxicity. The samples were treated with 0.2mM Bipyridyl. This chemical compound 2, 2'-bipyridine (bipyridyl, 0.2 mM), a membrane-permeable Fe^{2+} chelator (Tan et al., 2019). By chelating the Fe^{2+} molecules present in the chicken meat. Even if the *fur* regulator is not present in the mutant ST4/74Δ*fur*, bacteria should grow similarly to the wild type and be significantly different from ST4/74Δ*fur*. Studies show that by reducing iron restriction, bipyridine can promote the proliferation of *fur* mutants in *Salmonella* Typhimurium (Karash et al., 2022). Bipyridine treatment causes *fur* mutants of *S.* Typhimurium, which has poor iron management, to proliferate more rapidly, according to a study by (Troxell et al., 2011). Compared to wild-type strains, which still

depend on *Fur*-regulated iron uptake systems, the mutants are better able to adapt to situations with reduced iron availability because of this compound's iron chelating effects. Which was proven in this study, by chelating the iron. Giving a better view of what is happening to the bacteria to understand its biological behavior in the food matrix. As well as, relating the composition of this to the factors that inhibited bacteria.

In this study, as shown in Figure 10, bipyridine chelated the iron present in the chicken meat. This way a significant growth of ST4/74Δ*fur* + 0.2mM Bipyridyl can be shown in contrast with ST4/74Δ*fur*. Therefore, what was hypothesized can be proved, that iron toxicity was inhibiting ST4/74Δ*fur*.

Figure 10

Growth kinetics of ST4/74, ST4/74 Δfur, ST4/74 + 0.2mM Bipyridyl and ST4/74 Δfur + 0.2mM Bipyridyl in chicken meat for 16 hours post-inoculation



Note. The asterisk indicates statistical significance based on Student's t-test P< 0.05 (*), P < 0.01 (**), and P < 0.001 (***). * Indicates statistical significance between ST4/74Δ*fur* and ST4/74Δ*fur* + 0.2mM Bipyridyl based on Student's t-test P < 0.05.

Conclusions

This investigation analyzed *Salmonella* Typhimurium's ability to proliferate in chicken meat and discovered that seven of the eight tested mutations of this *Salmonella´*s serovar were able to proliferate. This implies that under the conditions handled in this study, the evaluated genes did not significantly affect the growth of the bacteria in the tested food matrix.

The assessment of different stress factors inherent to the chicken meat matrix on the survival of *Salmonella* Typhimurium revealed that the Δ*fur* mutant's survival was significantly impacted. This highlights the critical role of iron homeostasis in enabling *Salmonella* to withstand the inherent challenges present in the food matrix.

It was determined, nevertheless, that the *fur* gene is in fact essential for bacterial development in this setting. Moreover, the study verified that iron overload in the bacterial cytoplasm is the source of the inhibition seen in the ST4/74Δ*fur* mutant. This suggests that the *fur* gene is important for controlling iron levels and maintaining *Salmonella* Typhimurium viability in chicken flesh.

Recommendations

Similar studies should be conducted on other frequently infected food matrices, such as dairy, pork, and beef, to learn more about how *Salmonella* Typhimurium and its mutations behave in various settings.

To replicate more accurate food processing and storage settings, research the impact of various environmental stress factors, such as temperature, pH, and oxygen levels, on the growth and survival of *Salmonella* strains.

Conduct an experiment focused on the *fur* gene, using different iron concentrations to determine bacterial growth at various levels.

Evaluate the bacterial growth kinetics under conditions that necessitate the deleted gene for survival.

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Appendices

Appendix A

Growth kinetics data of ST4/74 in chicken meat for 16 hours post-inoculation

Hours post inoculation	Log CFU/g of meat (Control ST4/74) $\pm$ S.D
0	6.271 ± 0.19
2	6.865 ± 0.08
4	7.941 ± 0.05
6	8.832 ± 0.09
8	8.991 ± 0.17
10	9.548 ± 0.01
12	9.820 ± 0.06
14	9.898 ± 0.03
16	9.896 ± 0.44

Note. S.D = Standard Deviation. Log CFU/g of meat: logarithms of concentration of Colony – Forming Units per gram in meat. Each microbiological count of hours post inoculation represents the mean of three repetitions.

Appendix B

Comparative growth kinetics data of ST4/74ΔrpoE and control (ST4/74) in chicken meat for 16 hours post-inoculation

Hours post inoculation	Log CFU/g of meat (ST4/74Δ*rpoE*) ± S.D	Log CFU/g of meat (Control ST4/74) ± S.D	P-value
0	6.111 ± 0.05	6.271 ± 0.19	0.236
2	6.521 ± 0.11	6.865 ± 0.08	0.013
4	8.119 ± 0.36	7.941 ± 0.05	0.492
6	8.783 ± 0.20	8.832 ± 0.09	0.722
8	9.435 ± 0.12	8.991 ± 0.17	0.025
10	9.692 ± 0.06	9.548 ± 0.01	0.068
12	9.764 ± 0.02	9.820 ± 0.06	0.232
14	9.754 ± 0.15	9.898 ± 0.03	0.188
16	9.713 ± 0.02	9.896 ± 0.44	0.003

Note. S.D = Standard Deviation. Log CFU/g of meat: logarithms of concentration of Colony – Forming Units per gram in meat. P- value = P< 0.05 is considered statistically significant. Each microbiological count of hours post inoculation represents the mean of three repetitions.

Appendix C

Comparative growth kinetics data of ST4/74Δdam and control (ST4/74) in chicken meat for 16 hours post-inoculation

Hours post inoculation	Log CFU/g of meat (ST4/74Δ*dam*) $\pm$ S.D	Log CFU/g of meat (Control ST4/74) $\pm$ S.D	P-value
0	6.080 ± 0.13	6.271 ± 0.19	0.228
2	6.611 ± 0.00	6.865 ± 0.08	0.033
4	7.831 ± 0.19	7.941 ± 0.05	0.387
6	8.740 ± 0.05	8.832 ± 0.09	0.208
8	9.274 ± 0.12	8.991 ± 0.17	0.088
10	9.543 ± 0.13	9.548 ± 0.01	0.952
12	9.641 ± 0.04	9.820 ± 0.06	0.016
14	9.701 ± 0.11	9.898 ± 0.03	0.050
16	9.691 ± 0.15	9.896 ± 0.44	0.090

Note. S.D = Standard Deviation. Log CFU/g of meat: logarithms of concentration of Colony – Forming Units per gram in meat. P- value = P< 0.05 is considered statistically significant. Each microbiological count of hours post inoculation represents the mean of three repetitions.

Appendix D

Comparative growth kinetics data of ST4/74ΔhilD and control (ST4/74) in chicken meat for 16 hours post-inoculation

Hours post inoculation	Log CFU/g of meat (ST4/74Δ*hilD*) ± S.D	Log CFU/g of meat (Control ST4/74) ± S.D	P-value
0	6.071 ± 0.07	6.271 ± 0.19	0.168
2	6.846 ± 0.11	6.865 ± 0.08	0.827
4	8.128 ± 0.06	7.941 ± 0.05	0.019
6	9.103 ± 0.11	8.832 ± 0.09	0.031
8	9.640 ± 0.07	8.991 ± 0.17	0.004
10	9.817 ± 0.10	9.548 ± 0.01	0.046
12	9.854 ± 0.31	9.820 ± 0.06	0.862
14	9.836 ± 0.20	9.898 ± 0.03	0.633
16	9.528 ± 0.32	9.896 ± 0.44	0.189

Note. S.D = Standard Deviation. Log CFU/g of meat: logarithms of concentration of Colony – Forming Units per gram in meat. P- value = P< 0.05 is considered statistically significant. Each microbiological count of hours post inoculation represents the mean of three repetitions.

Appendix E

Comparative growth kinetics data of ST4/74ΔssrB and control (ST4/74) in chicken meat for 16 hours post-inoculation

Hours post inoculation	Log CFU/g of meat (ST4/74Δ*ssrB*) ± S.D	Log CFU/g of meat (Control ST4/74) ± S.D	P-value
0	6.251 ± 1.93	6.271 ± 0.19	0.888
2	6.860 ± 1.35	6.865 ± 0.08	0.944
4	8.159 ± 0.55	7.941 ± 0.05	0.005
6	9.069 ± 1.89	8.832 ± 0.09	0.103
8	9.670 ± 0.63	8.991 ± 0.17	0.003
10	9.879 ± 0.65	9.548 ± 0.01	0.011
12	10.066 ± 0.44	9.820 ± 0.06	0.005
14	10.029 ± 2.48	9.898 ± 0.03	0.418
16	9.963 ± 2.08	9.896 ± 0.44	0.613

Note. S.D = Standard Deviation. Log CFU/g of meat: logarithms of concentration of Colony – Forming Units per gram in meat. P- value = P< 0.05 is considered statistically significant. Each microbiological count of hours post inoculation represents the mean of three repetitions.

Appendix F

Comparative growth kinetics data of ST4/74ΔslyA and control (ST4/74) in chicken meat for 16 hours post-inoculation

Hours post inoculation	Log CFU/g of meat (ST4/74Δ*slyA*) ± S.D	Log CFU/g of meat (Control ST4/74) ± S.D	P-value
0	6.025 ± 0.03	6.271 ± 0.19	0.095
2	6.641 ± 0.07	6.865 ± 0.08	0.026
4	7.809 ± 0.22	7.941 ± 0.05	0.383
6	8.613 ± 0.04	8.832 ± 0.09	0.020
8	8.994 ± 0.28	8.991 ± 0.17	0.991
10	9.656 ± 0.06	9.548 ± 0.01	0.112
12	9.819 ± 0.15	9.820 ± 0.06	0.987
14	9.847 ± 0.06	9.898 ± 0.03	0.327
16	9.644 ± 0.11	9.896 ± 0.44	0.021

Note. S.D = Standard Deviation. Log CFU/g of meat: logarithms of concentration of Colony – Forming Units per gram in meat. P- value = P< 0.05 is considered statistically significant. Each microbiological count of hours post inoculation represents the mean of three repetitions.

Appendix G

Comparative growth kinetics data of ST4/74Δfnr and control (ST4/74) in chicken meat for 16 hours post-inoculation

Hours post inoculation	Log CFU/g of meat (ST4/74Δ*fnr*) ± S.D	Log CFU/g of meat (Control ST4/74) ± S.D	P-value
0	6.206 ± 0.02	6.271 ± 0.19	0.620
2	6.682 ± 0.01	6.865 ± 0.08	0.019
4	7.827 ± 0.07	7.941 ± 0.05	0.099
6	8.707 ± 0.17	8.832 ± 0.09	0.336
8	8.798 ± 0.16	8.991 ± 0.17	0.242
10	9.645 ± 0.02	9.548 ± 0.01	0.005
12	9.925 ± 0.19	9.820 ± 0.06	0.437
14	9.954 ± 0.05	9.898 ± 0.03	0.216
16	9.751 ± 0.13	9.896 ± 0.44	0.157

Note. S.D = Standard Deviation. Log CFU/g of meat: logarithms of concentration of Colony – Forming Units per gram in meat. P- value = P< 0.05 is considered statistically significant. Each microbiological count of hours post inoculation represents the mean of three repetitions.

Appendix H

Comparative growth kinetics data of ST4/74ΔentC and control (ST4/74) in chicken meat for 16 hours post-inoculation

Hours post inoculation	Log CFU/g of meat (ST4/74Δ*entC*) $\pm$ S.D	Log CFU/g of meat (Control ST4/74) $\pm$ S.D	P-value
0	6.371 ± 0.03	6.271 ± 0.19	0.423
2	6.875 ± 0.18	6.865 ± 0.08	0.933
4	7.759 ± 0.22	7.941 ± 0.05	0.245
6	8.642 ± 0.13	8.832 ± 0.09	0.114
8	9.411 ± 0.17	8.991 ± 0.17	0.043
10	9.630 ± 0.15	9.548 ± 0.01	0.455
12	9.840 ± 0.09	9.820 ± 0.06	0.768
14	9.871 ± 0.02	9.898 ± 0.03	0.397
16	9.639 ± 0.15	9.896 ± 0.44	0.046

Note. S.D = Standard Deviation. Log CFU/g of meat: logarithms of concentration of Colony – Forming Units per gram in meat. P- value = P< 0.05 is considered statistically significant. Each microbiological count of hours post inoculation represents the mean of three repetitions.

Appendix I

Comparative growth kinetics data of ST4/74Δfur and control (ST4/74) in chicken meat for 16 hours post-inoculation

Hours post inoculation	Log CFU/g of meat (ST4/74Δ*fur*) ± S.D	Log CFU/g of meat (Control ST4/74) ± S.D	P-value
0	6.444 ± 0.11	6.271 ± 0.19	0.246
2	6.624 ± 0.01	6.865 ± 0.08	0.007
4	7.405 ± 0.07	7.941 ± 0.05	<0.001
6	8.106 ± 0.17	8.832 ± 0.09	0.002
8	8.636 ± 0.02	8.991 ± 0.17	0.07
10	8.750 ± 0.08	9.548 ± 0.01	0.003
12	9.043 ± 0.22	9.820 ± 0.06	0.004
14	9.045 ± 0.23	9.898 ± 0.03	0.003
16	9.206 ± 0.20	9.896 ± 0.44	0.004

Note. S.D = Standard Deviation. Log CFU/g of meat: logarithms of concentration of Colony – Forming Units per gram in meat. P- value = P< 0.05 is considered statistically significant. Each microbiological count of hours post inoculation represents the mean of three repetitions.

Appendix J

Comparative growth kinetics data of ST4/74 + 0.2 mM Bipyridyl and control (ST4/74) in chicken meat for 16 hours post-inoculation

Hours post inoculation	Log CFU/g of meat (ST4/74 + Bipyridine) $\pm$ S.D	Log CFU/g of meat (Control ST4/74) $\pm$ S.D	P-value
0	6.061 ± 0.05	6.271 ± 0.19	0.142
2	6.808 ± 0.10	6.865 ± 0.08	0.510
4	8.215 ± 0.07	7.941 ± 0.05	0.007
6	9.283 ± 0.03	8.832 ± 0.09	0.001
8	9.914 ± 0.12	8.991 ± 0.17	0.001
10	10.003 ± 0.23	9.548 ± 0.01	0.080
12	10.333 ± 0.22	9.820 ± 0.06	0.018
14	10.427 ± 0.11	9.898 ± 0.03	0.001
16	10.371 ± 0.09	9.896 ± 0.44	0.001

Note. S.D = Standard Deviation. Log CFU/g of meat: logarithms of concentration of Colony – Forming Units per gram in meat. P- value = P< 0.05 is considered statistically significant. Each microbiological count of hours post inoculation represents the mean of three repetitions.

Appendix K

Comparative growth kinetics data of ST4/74Δfur + 0.2 mM Bipyridyl and control (ST4/74 Δfur) in chicken meat for 16 hours post-inoculation

Hours post inoculation	Log CFU/g of meat (ST4/74Δ*fur* + Bipyridine) ± S.D	Log CFU/g of meat (ST4/74Δ*fur*) ± S.D	P-value
0	6.197 ± 0.08	6.444 ± 0.11	0.036
2	6.928 ± 0.18	6.624 ± 0.01	0.099
4	7.931 ± 0.05	7.405 ± 0.07	<0.001
6	8.944 ± 0.13	8.106 ± 0.17	0.002
8	9.464 ± 0.04	8.636 ± 0.02	<0.001
10	9.913 ± 0.10	8.750 ± 0.08	<0.001
12	9.917 ± 0.16	9.043 ± 0.22	0.005
14	10.355 ± 0.07	9.045 ± 0.23	<0.001
16	10.260 ± 0.11	9.206 ± 0.20	0.001

Note. S.D = Standard Deviation. Log CFU/g of meat: logarithms of concentration of Colony – Forming Units per gram in meat. P- value = P< 0.05 is considered statistically significant. Each microbiological count of hours post inoculation represents the mean of three repetitions.