Stress response variability can cause up to 3-fold increase in the thermal resistance of Salmonella strains

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Stress response variability can cause up to 3-fold increase in the thermal resistance of *Salmonella* strains☆

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ABSTRACT

The variability in the bacterial stress response has received plenty of attention during the last years, partly due to its relevance to microbial risk assessment. Although the microbial response is affected by numerous variability sources, previous studies focused mostly on strain variability (inherent differences between strains of the same bacterial species) under optimal growth conditions. Here, we analyze a variability source relatively unexplored within microbial risk assessment: stress response variability. This refers to physiological differences due to prior exposure to stressful environments. Namely, we studied the impact of sub-optimal pre-culture conditions or the application of an acid shock on the thermal resistance of two strains of *Salmonella* (a reference strain and a highly heat resistant one). We observed that stress response variability is strain dependent. The heat resistance of the reference strain had a significant increase in heat resistance (up to 3-fold increase), whereas the conditions tested resulted in a reduction of thermal resistance with respect to control conditions (up to 2-fold reduction). Considering that magnitude of these changes are comparable to strain variability, and that stress response variability might be common throughout the food supply chain, this study evidences the need to study this phenomenon further in order to incorporate it into quantitative microbial risk assessments.

1. Introduction

Microbial Risk assessment (MRA) entails analyzing scientific data to assess the risks related to certain hazards (WHO, 2021). It is a useful method to inform risk management of microbiological hazards, with most food safety standards being established with the assistance of MRA (Cassini et al., 2016). Currently, Quantitative Microbial Risk Assessment (QMRA) is often preferred. QMRA uses mathematical models to quantitatively describe the changes in microbial populations along the food supply chain, finally estimating the probability of consumers being exposed to bacterial pathogens, as well as the probability of contracting an illness (EFSA, 2012). Microbial inactivation models from predictive microbiology are a keystone of QMRA, due to their ability to predict the reduction in the microbial concentration for a particular time/temperature combination (Valdramidis, 2016).

Uncertainty and variability are keystones of modern QMRA (EFSA, 2018). The term "variability" reflects sources of variation that are inherent to the microorganism, the food supply chain or the environment (e.g. differences in the response of single cells or in the composition of the food media). It is different from uncertainty, which includes sources of variation that can be tracked down to the use of partial or imperfect information (e.g. measurement errors or model misspecifications). Therefore, while it is possible to minimize uncertainty by amassing more data from experiments of a higher quality, variability is an inherent element of the process and cannot be eliminated merely by gathering more and better data (Nauta, 2000).

Previous studies on microbial variability from the perspective of QMRA addressed the influence of genetic factors, especially on the differences between strains of the same species (den Besten et al., 2018, 2017; Guillén et al., 2020; Lianou et al., 2017). This source of variability is of high relevance for risk assessment, as the phenotypes of bacterial populations within the food supply chain likely vary with respect to those used in microbiology laboratories. However, the variability in the bacterial response is also ruled by other factors. Bacterial cells along the

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food supply chain are exposed to conditions that differ from those used in laboratory settings (e.g., incubation at 37 °C and pH 7). Although the impact of these factors on inactivation kinetics has not been studied in depth, previous studies have shown that their impact on inactivation rates can be of a similar order of magnitude as the one of strain variability (Aryani et al., 2015; Clemente-Carazo et al., 2020; O'Driscoll et al., 1996; Ferreira et al., 2003, 2001).

Consequently, this study puts the focus on quantifying the effect of those variability sources in the microbial response. To underline this conceptual difference, we use the term "strain variability" to refer to genetic differences between strains and "stress response variability" for variability related to the history of the cell (incubation conditions or the application of a sub-lethal stress), in line with Wagner and Altenberg (1996). Using *Salmonella* spp. as case study, we focused on two stress response variability sources: the incubation under suboptimal pH conditions (herein as acid-adaptation) and the application of an acid shock before the thermal treatment. In the context of risk assessment, the first one would represent bacterial populations thriving on ingredients with acidic pH, whereas the second one would stand for bacteria surviving an acidic wash.

The experiments were performed with two *Salmonella* strains: a reference strain (*S.* Enteritidis CECT 4300) and one with extremely high heat resistance (*S.* Senftenberg CECT 4565). Although *S.* Senftenberg is not one of the most prevalent serotypes associated with foodborne illness (e.g. liquid whole egg; *S.* Enteritidis and *S.* Typhimurium) it may present a considerable food safety challenge due to its inherent heat resistance (Mañas et al., 2003). Several authors frequently use this strain for assessment of heat treatment efficacy (Lianou and Koutsoumanis, 2013; Alvarez-Ordonez et al., 2009) and to compare it with that of emerging food decontamination technologies (Mañas et al., 2003). It is thus relevant for inclusion in this study, as this approach enables the study of the most likely response of this species, as well as the analysis of whether stress response variability is strain-dependent.

2. Materials and methods

2.1. Bacterial culture and media

Experiments were performed using *Salmonella enterica* serovar Enteritidis CECT 4300 and *Salmonella enterica* serovar Senftenberg CECT 4565. Both strains were provided by the Spanish Type Culture Collection (CECT, Valencia, Spain). They were selected because *S.* Enteritidis is a reference strain and the most prominent serovar associated with salmonellosis outbreaks (EFSA and ECDC, 2024; Álvarez-Ordóñez et al., 2012), while *S.* Senftenberg is extremely heat-resistant (Guillén et al., 2020). Both bacterial strains were stored at −80 ± 2 °C (20 % glycerol) until use. To perform experiments, the stock cultures were streaked on trypticase soy agar (TSA, Scharlau Chemie, Barcelona, Spain). The fresh cultures were incubated for 24 h at 37 ± 1 °C in an incubator (Memmert GmbH, Nuremberg, Germany), stored at 5 °C and renewed weekly.

2.2. Acid shock

To analyze the effect of an acid-shock on the thermal inactivation kinetics of both *Salmonella* strains, the inoculum was prepared by transferring a single colony from the fresh culture plate (section 2.1) to 10 mL trypticase soy broth (TSB; Scharlau Chemie). The cells were incubated at 37 ± 1 °C for 24 h till stationary phase (validated both by OD measures and by plating). A volume of 1 mL of bacterial suspensions were centrifuged at 3600 rpm for 10 min at 4 ± 1 °C. Pellets were washed with TSB (pH 7.0) and resuspended in acidified TSB (pH 4.5, 5.0, 5.5, 6.0) adjusted with HCl (min. 37 %, Sigma-Aldrich, Seelze, Germany). Then, the cell suspensions were incubated at 37 ± 1 °C for 60 min. Finally, pellets were obtained as previously, washed once with peptone water (pH 7.2) and immediately heat treated (section 2.4). The pH of the cultures prior to the centrifugation step was measured with a pH meter (Basic20, Crison; Alella, Cataluña, Spain) under strict aseptic conditions.

2.3. Growth under acid adaptation conditions

The term acid-adapted will be used herein for cultures exposed to sublethal acidic conditions during growth. To study its effect on thermal inactivation kinetics, a single colony from the fresh culture plate (section 2.1) was transferred to 10 mL of trypticase soy broth (TSB; Scharlau Chemie) with suboptimal pH values (5.0, 5.5 and 6.0) adjusted with HCl (min. 37 %, Sigma-Aldrich, Seelze, Germany). Cultures were also prepared at pH 7.0 as control. The cells were incubated at 37 ± 1 °C until stationary phase (validated both by OD measures and by plating) and immediately centrifuged at 3600 rpm for 10 min at 4 ± 1 °C. Pellets were washed once with peptone water (pH 7.2) and immediately heat treated. Samples incubated at pH 7.0 were used as control. The pH was measured with a pH meter (Basic20, Crison; Alella, Cataluña, Spain) during the incubation under suboptimal pH conditions (supp. Table 3).

2.4. Thermal treatments

A Mastia thermoresistometer was used to conduct thermal treatments (Conesa et al., 2009). A volume of 400 mL of sterile peptone water (10 g/L peptone from casein, Scharlau Chemie) and 5 g/L NaCl (Scharlau Chemie's normal heating medium) were added to the vessel before the treatment. The thermoresistometer's vessel was continuously stirred throughout the procedure to provide a uniform temperature distribution. Once the liquid had stabilized at the target temperature, a volume of 0.2 mL of the respective bacterial suspension (acid-shocked; section 2.2, acid-adapted; section 2.3, or control) was inoculated into the thermoresistometer as inoculum, reaching an initial concentration of approximately 6 $\log_{10}$ CFU/mL.

S. Enteritidis heat resistance experiments were conducted at 55 and 60 °C. Due to its higher resistance, *S.* Senftenberg underwent thermal treatment at 60 and 65 °C. The bacterial suspension was inoculated once the vessel's temperature had stabilized. The duration of the experiment was adapted for each condition to reach approx. 4 log reductions with respect to the initial bacterial concentration. For both strains, the pH of the heating medium was set to 7.0.

To determine the viable cell counts at each data point, sterile test tubes were used to collect a sample of 3 mL at pre-set time intervals (at least 8 time points per treatment) and, after appropriate serial dilutions in sterile 0.1 % peptone water, they were plated in TSA and incubated at 37 °C for 48 h. A minimum of three experiments were performed per condition.

2.5. Mathematical modelling

Depending on the conditions, survivor curves with linear or non-linear shapes were observed (Supp. Figs. 1 & 2). Survivor curves for *S. Enteritidis* showed either log-linearity or a clear shoulder followed by a log-linear phase (Supp. Fig. 1). For the former case, we used the log-linear inactivation model (often called "Bigelow model" for his pioneering work (Bigelow, 1921)) to explain the variation in the microbial concentration (N) in time (t) with respect to the initial one (N_0). In this model, represented in Eq. (1), the inactivation rate is described by the D-value (D), which represents the treatment time required to reduce the microbial concentration by a ten-fold.

$$\log N = \log N_0 - \frac{t}{D} \quad (1)$$

For cases with a shoulder followed by log-linear decay, we used the Geeraerd model without tail (Geeraerd et al., 2000), as shown in Eq. (2) where SL stands for the duration of the shoulder length and k is the inactivation rate, related to the D-value by the identity $k = \ln(10)/D$.

$$N = -N_0 \cdot e^{-k \cdot t} \cdot \frac{e^{k \cdot SL}}{1 + (e^{SL} - 1) \cdot e^{-k \cdot t}} \quad (2)$$

The survivor curves for *S. Senftenberg* had a smooth nonlinear shape, without a clear tail or shoulder (Supp. Fig. 2). Hence, they were described by the Mafart inactivation model (Mafart et al., 2002), shown in Eq. (3). In this model, the parameter δ (δ-value) represents the time for the first log reduction. The nonlinearity is defined by parameter β (β-value) with values of $\beta < 1$ indicating an upwards curvature in the survivor curve and $\beta > 1$ indicating a downwards one.

$$logN = logN_0 - \left(\frac{t}{\delta}\right)^{\beta} \quad (3)$$

We estimated different values of β depending on the condition tested, making it impossible to compare between conditions only based on the δ-value. Hence, we compared the time to reach four log-reductions (t_{4D}) calculated using Eq. (4). Calculations were also done for 3 and 5 log-reductions, reaching the same qualitative results (not shown).

$$t_{4D} = \delta \cdot 4^{1/\beta} \quad (4)$$

The models were fitted by nonlinear regression using the web version of *bioinactivation* (Garre et al., 2017, 2018), currently available at https://foodlab-upct.shinyapps.io/bioinactivation4/. The goodness of the fit was evaluated qualitatively by visually comparing the fitted curves against the observations, and quantitatively using the Root Mean Squared Error ($RMSE = \sqrt{\frac{1}{n}\sum_{i=1}^{n} e_i^2}$; with n the number of data points and $e = \log N_{obs} - \log N_{fit}$ the residuals).

2.6. Statistical analysis

The kinetic parameters of the microbial inactivation models were used as estimates of the thermal resistance of the microbial population for each condition tested. Welch's *t*-test was used to conclude whether the application of different stresses affected the thermal resistance with respect to control conditions. Accordingly, the *t* statistic was calculated as shown in Eq. (5), where θ_i and θ_c are the parameter estimates for some condition *i* and for control conditions. Parameter uncertainty estimated by non-linear regression were used as variance estimates.

$$t = \frac{\theta_i - \theta_c}{\sqrt{\sigma_i^2 + \sigma_c^2}} \quad (5)$$

The *t* statistics obtained were analyzed using the typical hypotheses testing framework, with a significance level $\boldsymbol{\alpha = 0.05}$.

3. Results and discussion

3.1. Relevance of stress response variability in the heat resistance of *Salmonella* Enteritidis

Figs. 1 shows the *D*-values (of the Bigelow or Geeraerd models) estimated for *S. Enteritidis* on each condition (numeric values are included in supp. Table 1). The results show that acid stress response variability is very relevant for the thermal resistance of this strain. The results under control conditions (incubation at optimal pH at pH 7.0) are similar those reported previously in a previous study by our group for this same strain (Georgalis et al., 2022). However, cells grown under suboptimal acidic conditions or that withstood an acid shock after growth under optimal conditions had a higher thermal resistance, in agreement with previous studies on *Salmonella* (Alvarez-Ordóñez et al., 2010; Clemente-Carazo et al., 2021; Leyer and Johnson, 1993; Sharma et al., 2005).

The increase in thermal resistance was larger for cells grown under suboptimal growth conditions. The effect was largest for the lowest pH tested (pH 5), where the *D*-value increased ~3-fold at both temperatures tested. At pH 6, the effect was more moderate, although the *D*-value was still ~50 % higher than the one observed under control conditions. The effect of an acidic shock was more moderate than the one of suboptimal acidic growth. Only the lowest pH tested (4.5) resulted in statistically significant increase in thermal resistance, with a ~ 50 % increase in the *D*-value, similar in magnitude to the increase observed for cells grown at pH 6. This underlines the differences between suboptimal acidic growth and acid shock: although the agent causing the stress is the same, they

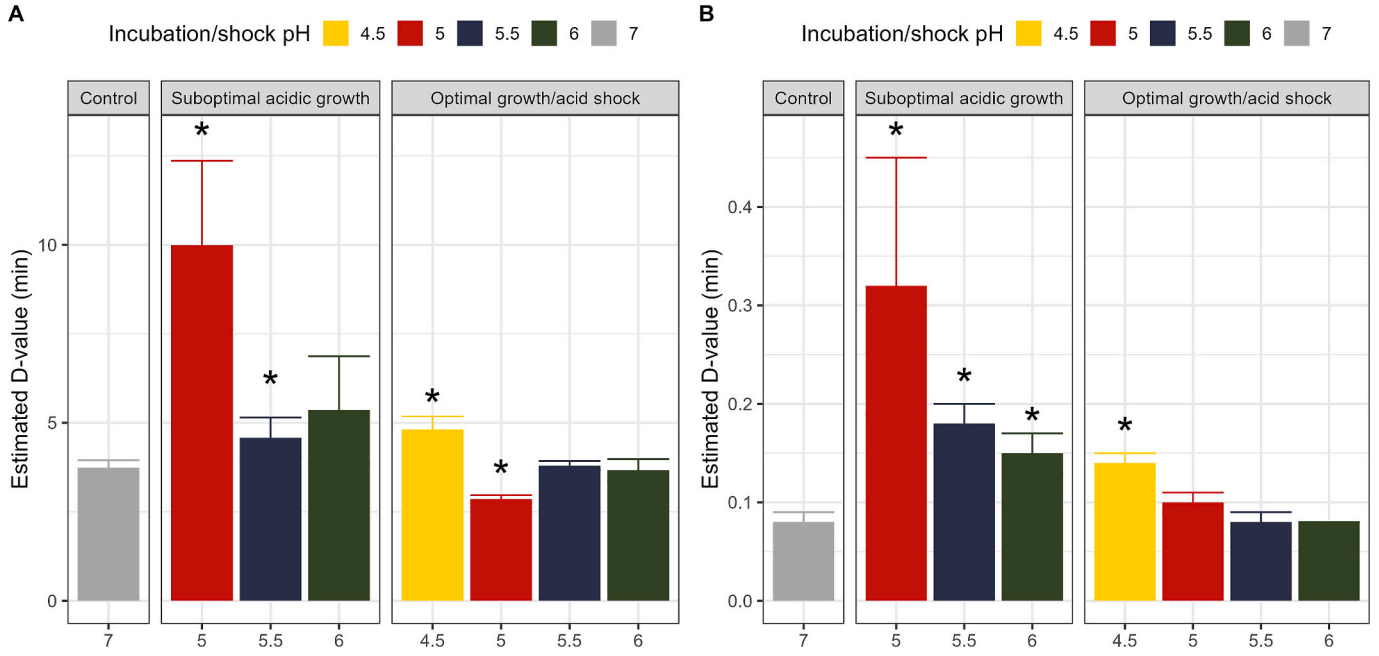

Fig. 1. *D*-values of *Salmonella Enteritidis* (either from the Bigelow or Geeraerd models) for isothermal heat treatments at 55 °C (A) and 60 °C (B). Stress response variability was studied performing experiment for control cells (stationary phase cells incubated at pH 7.0), and cells grown at suboptimal pH (5.0, 5.5 and 6.0) or subject to an acid shock (1 h at pH 4.5, 5.0, 5.5 or 6.0) after optimal growth conditions. Error bars illustrate the standard error of the regression. Conditions that are significantly different from the control (Walsh's test; $\alpha = 0.05$) are indicated by an asterisk (*).

result in different bacterial responses, ultimately having a different impact on thermal resistance.

Our results are generally in accordance with previous studies (even if there are differences in experimental design), underlining the relevance of stress response variability for the heat resistance of *Salmonella* spp. Koutsoumanis and Sofos (2004) reported that acid-adaptation resulted in increased acid resistance in a pH range of 4.0–5.0 in glucose-free media. In addition, previous studies have highlighted the emergence of cross-resistance to thermal stress after the application of acidic conditions within this pH range (Alvarez-Ordóñez et al., 2008, 2009; Spector and Kenyon, 2012). More specifically, Malheiros et al. (2009), demonstrated the acid adaptation capacity of *S.* Enteritidis SE86 grown at pH 4.39, which induced a cross-protection at 52, 56 and 60 °C. The D_{56} values of acid-adapted cells (7.79 min) obtained by Malheiros et al. (2009) were comparable to the ones obtained in this study.

Previous studies have justified the emergence of cross-resistances through class I stress proteins GroEL and DnaK acting as protection/repair mechanisms. Wilde et al. (2000) observed enhanced survival of acid-adapted *S.* Enteritidis PT4 when incubated at lower pH, in line with our study (Fig. 1). The increased heat resistance in that study was RpoS-independent in the presence of glucose as evidenced by the inclusion of *rpoS* mutants.

Rather than RpoS, OmpR was found to be induced upon acid-adaptation at pH 4.5 of *S.* Enteritidis SE86 conferring cross-protection to heat stress at 52 and 60 °C (Ritter et al., 2014). OmpR is a member of OmpR-EnvZ two-component intracellular signal transduction and regulatory system important for osmoregulation and related to high temperature exposure. This acid-shock protein (ASP) is stationary phase-dependent, part of the general stress response system (GSR; pH-independent) and its induction is RpoS-independent (Ritter et al., 2014; Bang et al., 2000). Studies on *S.* Typhimurium (Alvarez-Ordóñez et al., 2008) and *S.* Senftenberg (Alvarez-Ordonez et al., 2009) reported decreased membrane fluidity (lower USFA/SFA ratio) during acid-adaptation as the cause for increased thermotolerance. Interestingly, induction of the two-component signal transduction system PhoP/PhoQ after acid-adaptation at pH 5.4 for 90 min protected *S.* Typhimurium to 55 °C (Gao et al., 2024).

A major advantage of our experimental design is that the inclusion of several time points per experiment, enabling a detailed analysis of the linear and non-linear parts of the survivor curves (Supp. Fig. 1). Fig. 2 illustrates the shoulder length estimated for each condition. Although survivor curves obtained under control conditions were log-linear, the application of stress (either suboptimal acidic growth or an acid shock) introduced a shoulder in the survivor curve of the microorganism in almost every situation tested. This implies that the increase in thermal resistance is not limited to an overall increase in the *D*-value (Fig. 1). It also results in the microbial population being able to withstand the lethal stress for some time without reducing its size.

The shoulder length depended both on the nature of the stress (suboptimal acidic growth or acid shock) and the pH level. In every case tested, suboptimal acidic growth introduced a shoulder whose length was not significantly different at each temperature between pH values tested. On the other hand, the shoulder introduced by the application of an acid shock depended on the pH. An acid shock of pH 4.5 did not introduce any shoulder, whereas an acid shock at pH 6 introduced the longest shoulder at both temperatures tested. This could be due to sub-lethal damage on the cells caused by the acid shock treatment at the lowest pH, whereas treatment at pH 6.0 may have caused acid adaptation and cross-resistance. Interestingly, the acid shock at pH 5.5 did not induce a shoulder for the samples treated at 55 °C. This is probably an artefact of our experimental design, which did not provide enough resolution to identify a shoulder in this experiment.

These findings emphasize the critical role of acid adaptation in enhancing thermal resistance of *Salmonella* strains across various serovars and experimental conditions. The mechanisms underlying this increased thermotolerance appear to involve complex regulatory networks and stress response systems, including the induction of specific stress proteins, signal transduction systems and membrane modifications. These mechanisms seem to differ between suboptimal acidic growth and an acid shoc, as evidenced by our results (Figs. 1 and 2).

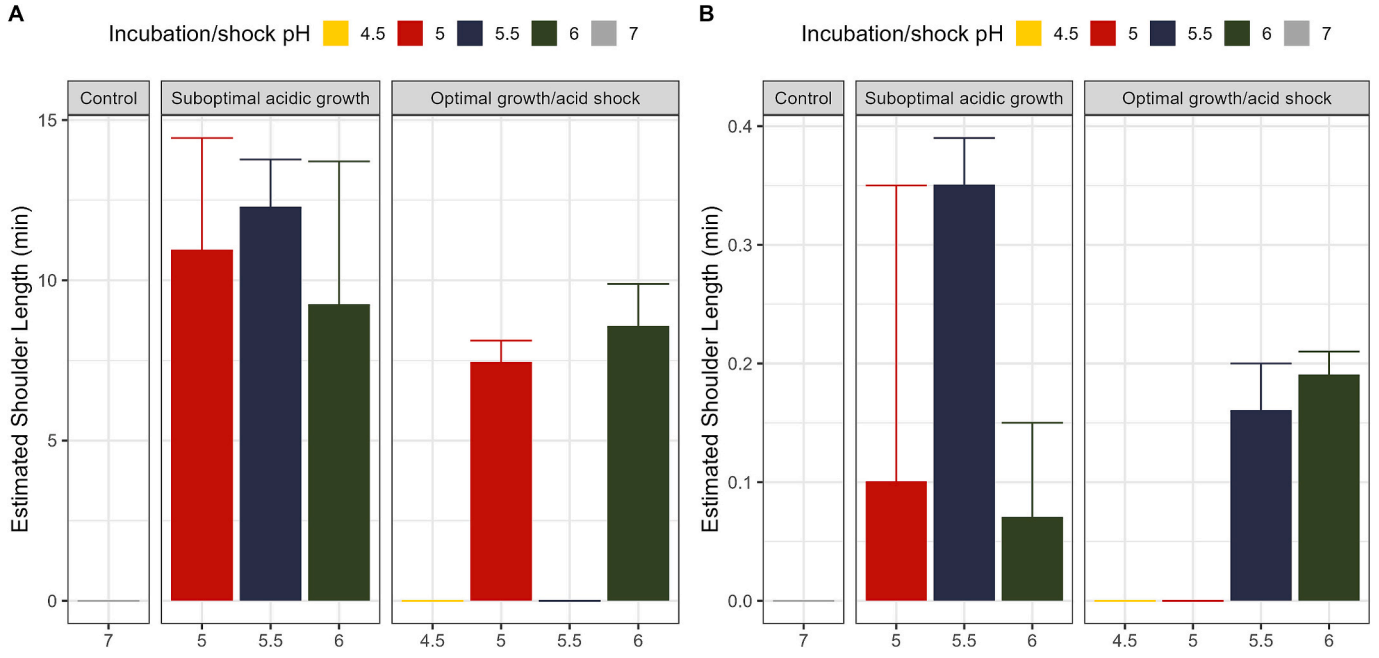

Fig. 2. Shoulder length (*SL* of the Geeraerd model) of *Salmonella Enteritidis* for isothermal heat treatments at 55 °C (A) and 60 °C (B). Stress response variability was studied performing experiment for control cells (stationary phase cells incubated at pH 7.0), and cells grown at suboptimal pH (5.0, 5.5 and 6.0) or subject to an acid shock (1 h at pH 4.5, 5.0, 5.5 or 6.0) after optimal growth conditions. Conditions without a shoulder (i.e., linear inactivation) are shown as a horizontal line on the x-axis. Error bars illustrate the standard error of the regression.

3.2. Relevance of stress response variability in the heat resistance of *Salmonella* Senftenberg

Survivor curves for *S.* Senftenberg showed a smooth non-linearity, without a clear shoulder and/or tail (Supp. Fig. 2). Fig. 3 depicts the treatment time required for 4 log-reductions according to the Mafart model fitted to each condition (parameter estimates in supp. Table 2). As expected, this strain was more heat resistant than the *S.* Enteritidis strain, with a *D*-value at 60 °C ~ 20 times larger ($\delta_{60} = 2.62 \pm 1.85$ min for *S.* Senftenberg; $D_{60} = 0.08 \pm 0.01$ min for *S.* Enteritidis). Furthermore, whereas the survivor curves for *S.* Enteritidis under control conditions were linear, the ones for *S.* Senftenberg had a significant curvature ($\beta = 0.63 \pm 0.25$), in agreement with our previous results for the same strain (Georgalis et al., 2022).

Stress response variability had a relevant impact on the thermal resistance of the microbial population. However, the effect on this strain was opposite to the one observed for the *S.* Enteritidis strain. For cells treated at 60 °C, the reduction in thermal resistance was independent of the pH level and the type of stress (suboptimal acidic growth or acid shock). In every case, the time for 4 log-reductions was reduced ~50 % with respect to control cells. These results are in-line with those previously reported for the same strain, where exposure to an acid-shock lowered the heat resistance of this same strain, an observation attributed to sublethal membrane injury (Clemente-Carazo et al., 2020).

Interestingly, Alvarez-Ordonez et al., 2009 reported induced thermotolerance in late stationary phase (36 h) *S.* Senftenberg CECT 4384 grown in acidified BHI broth (pH 4.5, 5.4, 6.4) and treated at 63 °C, which was attributed to alterations in membrane fluidity. The authors also observed a similar trend for increased thermotolerance in juices due to acid-adaptation, that was even more profound compared to that of *S.* Typhimurium (Alvarez-Ordonez et al. (2009).

Although differences in experimental designs (e.g. 36 h incubation, different media etc.) may explain discrepancies between studies, it is most likely due to differences in the phenotypes of strains of the same serovar. Our study uses *S.* Senftenberg CECT 4565, a strain of an extremely high thermal resistance that is not displayed by strain *S.* Senftenberg CECT 4384. Nguyen et al. (2017) recently analyzed the complete genome of *S.* Senftenberg ATCC 43845 and identified two loci on a conjugative plasmid exhibiting ≥98 % identity to loci previously associated with thermotolerance in Enterobacteriaceae. These loci were proposed to be responsible for the microorganism's high heat resistance. Accordingly, our population-level results indicate that both acid adaptation or the application of an acid-shock might alter the regulation of this system, compromising the innate thermotolerance of this strain.

It is well documented that stress resistance is tightly regulated in bacteria due to high energy costs mainly incurred from the implementation of multiple protective mechanisms (e.g. ASPs & HSPs) (Alvarez-Ordóñez et al., 2015). Our results would then point out a trade-off between the "baseline" stress resistance (the one observed under standard incubation conditions) of a bacterial strain and its ability to adapt and increase that resistance, a concept that has not yet been explored in depth. Hence, it is possible that relatively weak bacterial strains under optimal incubation conditions become relatively resistant when exposed to conditions that enable bacterial adaptation. This underlines the need to analyze thermal resistance at the strain-level, rather than at the serovar-level because the baseline thermal resistance and adaptation capacity of bacterial species is likely strain-dependent.

4. Conclusions

Variability sources in the bacterial response to thermal treatments can take many forms. This study analyzes stress response variability (differences among cells of the same strain due to a different history prior to a treatment) of *Salmonella* spp. We focused on how suboptimal acidic growth (acid-adapted cells) or the application of an acid shock influence the thermal resistance of two *Salmonella* strains. For *S.* Enteritidis CECT 4300 (reference strain), we observed that acid-adapted cells could have a 3-fold increase in their *D*-value with respect to control conditions, as well as shoulders in the survivor curves. On the other hand, the thermal resistance of *S.* Senftenberg CECT 4565 (a highly resistant variant) was reduced when acid-adapted or after an acid shock. Therefore, the magnitude (and direction) of stress response variability would be dependent on the strain and the type of stress. This variability source could be of great relevance for risk assessment, as its magnitude is

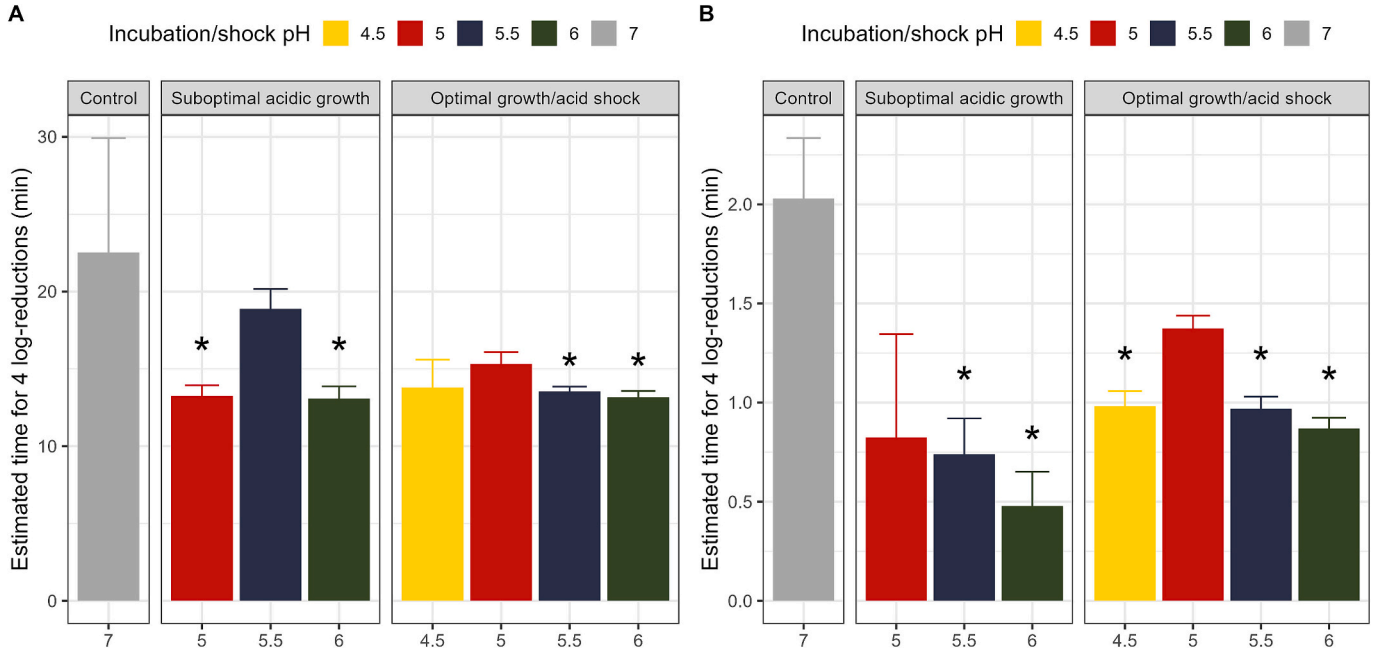

Fig. 3. Time to reach 4 log-reductions in the concentration of *Salmonella* Senftenberg according to the parameters of the Mafart model fitted for an isothermal treatment at 60 °C (A) and 65 °C (B). Stress response variability was studied performing experiment for control cells (stationary phase cells incubated at pH 7.0), and cells grown at suboptimal pH (5.0, 5.5 and 6.0) or subject to an acid shock (1 h at pH 4.5, 5.0, 5.5 or 6.0) after optimal growth conditions. Conditions that are significantly different from the control (Walsh's test; $\alpha = 0.05$) are indicated by an asterisk (*).

comparable to the one previously reported for strain variability. We discussed that adaptive reponses include a complex interplay between inducible acid-adaptation mechanisms and cross-protection phenomena with potential involvement of repair and regulatory mechanisms. This underlines that studying the impact of pre-culturing *Salmonella* in various pH conditions is crucial for understanding its survival mechanisms towards heat.

Supplementary data to this article can be found online at https://doi.org/10.1016/j.ijfoodmicro.2025.111347.

CRediT authorship contribution statement

Leonidas Georgalis: Writing – original draft, Validation, Data curation, Conceptualization. **Theocharia Tsagkaropoulou:** Writing – original draft, Methodology, Investigation, Data curation, Conceptualization. **Kimon Andreas G. Karatzas:** Writing – review & editing, Supervision, Investigation, Formal analysis, Conceptualization. **Pablo S. Fernandez:** Writing – review & editing, Supervision, Investigation, Funding acquisition, Conceptualization. **Alberto Garre:** Writing – review & editing, Supervision, Software, Investigation, Formal analysis, Data curation, Conceptualization.

Declaration of competing interest

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests: Pablo S Fernandez reports financial support was provided by the National Research Council. Pablo S Fernandez reports financial support was provided by EuAGROALNEXT programme and was supported by MCIN with funding from European Union NextGenerationEU and by Fundación Séneca Comunidad Autónoma Región de Murcia Spain. If there are other authors, they declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Data availability

Data will be made available on request.

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