Differences in resistance to different environmental stresses and non-thermal food preservation technologies among *Salmonella enterica* subsp. *enterica* strains

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Running title: Variability in *Salmonella* stress resistance

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ABSTRACT

In this work the resistance of 15 strains belonging to 11 serovars of *Salmonella enterica* subsp. *enterica* to several different environmental stresses (acid, hydrogen peroxide, NaCl and heat) and non-thermal food preservation technologies (HHP, PEF, UV) was determined and compared. Results obtained showed that differences in resistance among strains, quantified as *2D*-values, varied less than 2.4-fold for all agents, including heat if *S.* Senftenberg 775W is excluded from the analysis. These results also indicate that variability in resistance among strains of the same serovar was comparable to inter-serovar variability. *Salmonella* strains that were the most resistant to a given stress were not more resistant to other types of stress. Nevertheless, a positive correlation was observed between the resistance of *Salmonella* strains to oxidative and osmotic stress, as well as between UV and PEF resistance. These results would be especially helpful in defining safe food preservation processes and might be very useful for improving quantitative microbiological risk assessments of *Salmonella* in food products.

Keywords: cross-resistance; variability; risk-assessments, non-thermal technologies, foodborne pathogen

1. **INTRODUCTION**

The relevance of *Salmonella* as an agent responsible for food-borne toxiinfections is well known. Currently, the microorganisms of the genus *Salmonella* constitute the second most frequent cause of foodborne disease in Europe and the United States (European Food Safety Authority (EFSA), 2018; Scallan et al., 2011), only surpassed by *Campylobacter*. The main reservoir of *Salmonella* is the intestinal tract of animals; this microorganism can thus contaminate food products of animal and plant origin, directly or indirectly. Food products most frequently identified as responsible for foodborne *Salmonella* infections in the European Union in 2017 were eggs and egg products (36.8 % of outbreaks), bakery products (16.7 %), and meat and meat products (8.2 %). However, the range of products that can vehicle *Salmonella* is much broader, including other products of animal origin, vegetables, crustaceans, or milk (EFSA, 2018).

The microorganisms of the genus *Salmonell*a have evolved to survive in naturally stressful conditions such as high osmolarity, extreme temperatures, and low pHs (Fang, Frawley, Tapscott, & Vazquez-Torres, 2016; Spector & Kenyon, 2012). However, inherent genetic differences among serovars and/or strains can lead to substantial changes in their stress tolerance. Whereas the stress resistance of *S.* Enteritidis and *S.* Typhimurium – the most common serovars associated with human infection worldwide – has been studied in detail, much less information is available regarding most of the other 2,500 existing *Salmonella* serovars (Grimont & Weill, 2007).

Previous studies dealing with variability in resistance within the *Salmonella* genus have often been limited, either because they included a low number of serovars/strains or because they only dealt with a small number of stressing agents and/or food preservation technologies (Doyle & Mazzotta, 2000; Gayán, Serrano, Raso, Álvarez, &

Condón, 2012; Lianou & Koutsoumanis, 2013; Saldaña et al., 2009; Sherry, Patterson, & Madden, 2004). In addition, since experimental conditions (culture conditions, strains, etc.) were not the same in most cases, subsequent comparison becomes difficult and/or meaningless. The lack of studies dealing with the stress resistance and adaptive stress responses of *Salmonella* strains and serovars is particularly alarming because such studies are not only necessary to understand their physiology, but also to help designing more efficient inactivation processes and/or action plans throughout the food chain with the purpose of preventing the health risk they pose. Such studies would help to improve the accuracy of quantitative microbial risk assessments.

Thus, this study's aim was to determine and compare the resistance of 15 strains belonging to 11 serovars of *Salmonella enterica* subsp. *enterica* to different environmental stresses and non-thermal food preservation technologies.

2. MATERIAL AND METHODS

2.1. Bacterial strains

15 strains belonging to 11 serovars of *Salmonella enterica* subsp. *enterica* were selected to carry out this investigation: 5 of them corresponded to *S.* Typhimurium. The strains of *S.* Typhimurium (STCC 443, STCC 722, STCC 7162 and STCC 4594), *S.* Enteritidis STCC 4300, *S.* Derby STCC 4397, *S.* Infantis STCC 4373, *S.* Virchow STCC 4154, *S.* Gallinarum STCC 4883, *S.* Senftenberg 775W STCC 4565, *S.* Saintpaul STCC 4153, and *S.* Stanley STCC 4141 were supplied by the Spanish Type Culture Collection. The strains of *S.* Hadar NCTC 13033 and *S.* Newport NCTC 129 were supplied by Public Health England, and the strain of *S.* Typhimurium SL1344 was kindly provided by Tim Brocklehurst from the Institute of Food Research, Norwich. All strains were maintained frozen at -80 °C in cryovials for long-term preservation.

2.2 Growth conditions

Cultures were grown in 96 wells microtiter plates (Thermo Scientific, Roskilde, Denmark). They were prepared by inoculating 100 µl of tryptic soy broth (Oxoid, Basingstoke, UK) supplemented with 0.6 % w/v yeast extract (Oxoid; TSB-YE) with a single colony previously isolated on a plate of tryptone soy agar supplemented with 0.6% w/v yeast extract (Oxoid; TSA-YE). Microtiter plates were sealed with a polyester impermeable film (VWR International, Leuven, Belgium) and incubated overnight at 37 °C under static conditions. One (1) µl of these pre-cultures was inoculated into 100 µl of fresh TSB-YE and incubated for 24 h under the same conditions to obtain the stationary growth phase cultures that were used for stress resistance determinations.

2.3 Acid, hydrogen peroxide, and sodium chloride resistance determinations

The treatment medium for acid-resistance determinations was citrate-phosphate McIlvaine buffer adjusted to different pHs (2.0-3.0) (Dawson, Elliott, Elliott, & Jones, 1974). Hydrogen peroxide resistance was evaluated in 100 mM Tris–HCl buffer (pH 7.0) with hydrogen peroxide added at final concentrations of 10, 30, and 100 mM (Sigma, St Louis, USA). Resistance to osmotic medium was evaluated in TBS-YE supplemented with 25, 30, and 33 % w/v of sodium chloride (VWR International; NaCl). In all cases, treatments were performed on microtiter plate, and cells were added to the treatment medium to an initial concentration of 10^7 cells/ml. After inoculation, the suspensions were incubated at a constant temperature of 25 ºC throughout the treatment, except for the NaCl determinations, which were carried out at 37 ºC due to the low lethality of this agent at room temperature (25 ºC). After the selected contact time (up to 50 minutes, 100 minutes and 32 hours for acid, hydrogen and sodium chloride determinations, respectively) 20 µl samples were withdrawn at preset intervals

and transferred into 180 µl of buffered peptone water (Oxoid; BPW). Subsequent serial dilutions were prepared and pour-plated for survival counts as described below.

2.4 Heat treatments

Heat treatments were carried out in a specially designed resistometer (Condón, Arrizubieta, & Sala, 1993). Briefly, this instrument consists in a 400 mL vessel provided with an electrical heater for thermostation, an agitation device to ensure inoculum distribution and temperature homogeneity, and ports for the injection of microbial suspension and for the extraction of samples. Once treatment temperature had attained stability (55, 58, 61, or 64 ± 0.1 °C), 0.1 mL of the microbial cell suspension was injected into the main chamber containing the treatment media, tryptic soy broth. After inoculation, samples were collected at different heating times (up to 16 minutes) and immediately pour plated and incubated for survival counting.

2.5 High hydrostatic pressure (HHP) treatments

HHP treatments were carried out in a Stansted Fluid Power S-FL-085-09-W (Harlow, London, England) apparatus (Ramos, Chiquirrín, García, Condón, & Pérez, 2015). The pressure-transmitting fluid was a mixture of propylene glycol and distilled water (50/50, v/v). An automatic device was employed to set and/or record pressure and time during the pressurization cycle. Cell suspensions were diluted to a cell concentration of 10^7 cells/ml in citrate-phosphate McIlvaine buffer of pH 7.0, approximately. Samples were packed in plastic bags, which were sealed without headspace and introduced in the treatment chamber. Treatments were applied at 250, 300, and 350 MPa for different treatment times up to 30 min, and temperature never exceeded 40 °C.

2.8 Pulsed electric field (PEF) treatments

The PEF equipment used in this investigation was supplied by ScandiNova (Modulator PG, ScandiNova, Uppsala, Sweden). The equipment and treatment chamber have been previously described by Saldaña et al. (2009). Prior to PEF treatments, 0.1 mL of the microbial cell suspension were dissolved in citrate-phosphate McIlvaine buffer (pH 7.0 and 1 mS/cm of conductivity) at a concentration of approximately 10^7 cells/ml. Samples were placed with a sterile syringe in the treatment chamber, which had a gap of 0.25 cm. Treatments were based on square pulses with a width of 3 µs and a frequency of 1 Hz. Electric field strengths were set at 20, 25, and 30 kV/cm. Under these experimental conditions, the energy per pulse was 1.20, 1.88, and 2.70 kJ/kg. Treatments of up to 50 pulses (150 µs) were applied. Under these conditions, the final temperature of the treatment media was always below 35 °C.

2.9 Ultraviolet C light (UV-C) treatments

UV-C treatments were carried out in a microtiter plate under static conditions. Microtiter plates were coated with 0-2 layers of a microplate sealing film (BREATHseal, Greiner bio-one, Frickenhausen, Germany) and located at a distance of 17.50 to 24.50 cm from a 32 W UV-C lamp (VL-208G, Vilber, Germany). Fluence was measured by means of a UVX radiometer (UVP, LLC, Upland, CA). Under these experimental conditions, fluences between 0.20 and 1.10 ± 0.2 mW/cm^2 were attained. The treatment medium was citrate-phosphate McIlvaine buffer of pH 7.0, and the initial concentration was of approximately 10^7 cells/ml. Treatment times of up to 180 seconds were applied and temperature never exceeded 30 °C.

2.10 Recovery after different treatments and survival counting

After treatments, samples were adequately diluted in Buffered Peptone Water (Oxoid; BPW) and plated in the recovery medium, TSA-YE. Plates were incubated for 24 h at 37 °C, after which the number of colony-forming units (CFU) per plate was counted.

2.11 Curve fitting and statistical analysis

Survival curves were obtained by plotting the logarithm of the survival fraction (Log_{10} N/N_0) versus treatment time (hours for NaCl determinations; minutes for acid, heat, HHP, and peroxide treatments; seconds for UV treatments and µs for PEF treatments). Since deviations from linearity were observed in survival curves to the majority of agents/technologies, GInaFiT, the Geeraerd inactivation model-fitting tool was used to fit survival curves and calculate resistance parameters (Geeraerd, Valdramidis, & Van Impe, 2005).

$$Log_{10}(N_t) = Log_{10}\left[\left(10^{Log_{10}(N_0)} - 10^{Log_{10}(N_{res})}\right) \cdot e^{-k_{max}t} \cdot \left(\frac{e^{k_{max}\cdot S_l}}{1+\left(e^{k_{max}\cdot S_l}-1\right)\cdot e^{-k_{max}\cdot t}}\right) + 10^{Log_{10}(N_{res})}\right] \quad \text{(Eq. 1)}$$

In this equation, N_t represents the number of survivors, N_0 the initial count, and t the treatment time.

This model describes the survival curves by means of three parameters: shoulder length (S_l), defined as the time before exponential inactivation begins; inactivation rate (K_{max}), defined as the slope of the exponential portion of the survival curve; and N_{res} which describes residual population density (tail). Therefore, the traditional decimal reduction time value (D-value) can be calculated from the K_{max} parameter using equation 2.

$$D\text{-value} = 2.303/K_{max} \quad \text{(Eq. 2)}$$

Standard deviations (SD), statistical significance of differences ($p < 0.05$), Iterative Grubbs' test (Alpha = 0.05), Pearson's correlation coefficient and statistical analysis (unpaired t-test -with and without Welch's correction- and one way ANOVA; $p<0.05$) were calculated using GraphPad PRISM® statistical software (GraphPad Prism version 7.00 for Windows, GraphPad Software, San Diego, California, USA). Principal component analysis (PCA) was carried out using InfoStat statistical software (InfoStat version 2018, Córdoba, Argentina).

3. RESULTS AND DISCUSSION

In this study, the variability in resistance of 15 *Salmonella* strains belonging to 11 different serovars against seven different preservation technologies and environmental stresses was studied. The selected serovars included 9 out of the 20 the most common serotypes associated with human infection in Europe throughout the most recent years (EFSA, 2018). The other two serovars (*S.* Gallinarum and *S.* Senftenberg strain 775W) were chosen because of their well-known specific characteristics –avian host-specificity and high heat resistance, respectively- that have been described elsewhere (Eswarappa, Janice, Balasundaram, Dixit, & Chakravortty, 2009; Ng, Bayne, & Garibaldi, 1969). Five strains of *S.* Typhimurium were included in the study to enable comparison between intra-serovar and inter-serovar variability in stress resistance among salmonellae. Among all the strains, *S.* Typhimurium SL1344 was considered as the reference strain throughout the whole study, since it is a well characterized strain (Humphrey, Clark, Humphrey, & Jepson, 2011).

Given the considerable number of determinations to be obtained (more than 450 survival curves), it was decided to obtain the microbial suspensions and to carry out resistance assays to chemical agents in microtiter plates instead of conventional flasks or tubes, as described in the Materials and Methods section. A preliminary study

indicated that both the growth kinetics and the resistance of *Salmonella* cells to all chemical agents herein evaluated were comparable for cells grown in microtiter plates and in conventional agitated flaks (data not shown). Once the methodology had been established, survival curves to the 7 agents under study were obtained. These survival curves (representing the Log_{10} of the survival fraction *vs* treatment time) showed different profiles. Thus, for instance, survival curves to hydrogen peroxide and HHP displayed shoulders, whereas those to NaCl and PEF showed tails. Therefore, the non-linear Geeraerd model (Geeraerd, Herremans, & Van Impe, 2000) was required to describe them accurately, and the corresponding resistance parameters (N_0; S_l; K_{max}, N_{res}) were calculated. The mean values of these parameters (and their standard deviation), together with the goodness-of-fit parameters, are included in Table 1. The traditional decimal reduction time value (*D*) of each survival curve was calculated from its corresponding K_{max} (Eq. 2). In addition, in order to facilitate comparisons between strains and/or agents, it was decided to use the *2D*-value parameter (the time required to reduce bacterial counts in 2 Log_{10} cycles). This parameter was chosen because it takes into account simultaneously the duration of the shoulder phase and the inactivation rate in the linear portion of the curve and also because not all the treatments (at the intensities here applied) achieved 3 Log_{10} cycles of inactivation (Cebrián, Mañas, & Condón, 2016). Anyway, it should be noted that similar conclusions can be drawn if the *1D*-value (time to inactivate the first Log_{10} cycle) or *3D*-values (when it was possible to calculate it) are compared (data not shown).

3.1 Acid Resistance

The *2D*-values of the 15 studied strains when exposed to acid pH (2.5) varied from 20.52 to 34.48 min (average value= 26.52 min). *S.* Hadar was the most resistant and *S.* Typhimurium 7162 the most sensitive strain (Fig. 1A). Figure 1A also includes the 95

% confidence interval of the mean of the calculated *2D*-values for the whole set of strains under study (discontinuous line) as a measurement of inter-serovar variability in resistance, and the 95 % confidence interval of the mean of the *2D*-values calculated for the 5 *S.* Typhimurium strains (continuous line) as an intra-serovar variability measurement. Although the number of strains used to determine these confidence intervals is different (15 *vs* 5), it can be observed that the variability in resistance to acid conditions among *S.* Typhimurium strains was greater (at least comparable) than inter-serovar variability. In addition, no significant differences ($p>0.05$) were found when the acid resistance of the strains belonging to *S.* Typhimurium (5 strains) *vs* that of strains belonging to other serovars (10 strains) was compared (unpaired t-test with Welch correction). In other words, the differences in acid resistance observed among *Salmonella* strains would probably be more linked to strain-specific characteristics than to serovar-specific ones. Nevertheless, it should be remarked these conclusions should be taken with caution since the number of strains (15) and serovars (10) studied in this work is quite low and further studies including a higher number of strains and serovars would be required to validate them. While the influence of pH on growth and survival of microorganisms has been widely studied, few studies are available on the variability in acid resistance among multiple strains of *Salmonella enterica*. Rodríguez, Aguirre, Lianou, Parra-Flores, & García de Fernando (2016) studied the influence of the type of substrate and acid, including citric acid as in our study, on microbial resistance to acid conditions, and they found notable differences among bacterial genus. Among the studied microorganisms they included *S.* Enteritidis 4300, and the calculated *D*-value was in the range of those observed in this study (5.62 min at pH 2.56 *vs* 9.09 at pH 2.5). In other studies, where a larger number of serovars was evaluated, the medium was acidified with HCl. Although this implies that resistance values are not directly

comparable with those obtained in this study, it should be noted that both Berk, Jonge, Zwietering, Abee, & Kieboom (2005) and Lianou & Koutsoumanis (2013) reported a considerable variability in acid resistance among the tested strains, greater than that observed in this study. Such differences between our results and those previously reported might be due to the number of strains studied or the chosen strains, yet might also be due to the different type of acid used, since it is well known that the mode of action of organic and inorganic acids and the resistance mechanisms of bacteria against each of them are very different (Spector & Kenyon, 2012).

In order to determine if these conclusions were valid for a wider pH range, we studied the influence of treatment medium pH (from 2.0 to 3.0) on the *2D*-values of the most pH-resistant and pH-sensitive serovars. *S*. Typhimurium SL1344 was likewise included in this set of experiments as a reference strain. As can be observed in Figure 1B, which represents the Log_{10} of the *2D*-values of each strain *vs* treatment medium pH, the influence of treatment medium pH on the resistance of the three serovars was very similar, strongly suggesting that the conclusions drawn from the experiments carried out at pH 2.5 would be valid for a wider range of pH, at least between 2.0 and 3.0.

3.2 Hydrogen peroxide resistance

Resistance to 30 mM hydrogen peroxide was also determined for the 15 strains, and the obtained results are displayed in Figure 2A. In this case *S*. Senftenberg was the most resistant strain (*2D*-value 66.52 minutes), and the least resistant one was *S*. Enteritidis 4300 (*2D*-value 43.83 minutes). These values are in the range of those reported in previous research works (Sagarzazu, Cebrián, Pagán, Condón, & Mañas (2013); Wahlig et al., 2019). As described for acid resistance, intra-serovar variability in hydrogen peroxide resistance exceeded inter-serovar variability and no significant differences were found when comparing the hydrogen peroxide resistance of the 5 *S.* Typhimurium

strains *vs.* the other 10 non-*S.* Typhimurium strains. To the best of our knowledge, no previously published study has dealt specifically with the heterogeneity of hydrogen peroxide resistance within the genus *Salmonella*. On the other hand, as can be deduced from Figure 2B, a modification of the concentration of H_2O_2 had the same effect on the *2D*-values calculated for the most and the least H_2O_2 resistant strains, as well as for *S.* Typhimurium SL1344.

3.3 NaCl resistance

2D-values in NaCl-added medium for the strains under study varied from 5.39 to 9.03 hours, these are values corresponding to *S.* Enteritidis 4300 and *S.* Saintpaul, respectively. According to the results obtained, intra-serovar variability was as large or even larger than inter-serovar variability (Fig. 3A). A similar result was observed by Lianou & Koutsoumanis (2011) when they evaluated the growth capacity (growth rate, μ_{max}) of 60 *Salmonella* strains at different concentrations of NaCl. On the other hand, results obtained here indicate that, despite the observed differences among *2D*-values, there were no significant differences ($p>0.05$) in NaCl resistance among the studied *S.* Typhimurium strains. A similar result was obtained by Cebrián, Arroyo, Mañas, & Condón (2014), who determined the maximum non-inhibitory concentration of NaCl for four *S.* Typhimurium strains and found hardly any differences among them. Nevertheless, and conversely to what it was observed for acid and hydrogen peroxide resistance, significant differences ($p<0.05$) were found when comparing the NaCl resistance of *S.* Typhimurium strains vs. the other 10 *Salmonella* strains. This would mean that NaCl resistance might be, at least to some extent, serovar-dependent, being that of *S.* Typhimurium strains among the highest of the serovars here studied. Further work would be required in order to validate this conclusion.

Regarding the influence of NaCl concentration on the resistance of *Salmonella* (*2D*-value), increasing the NaCl concentration resulted in a decrease in the *2D*-values in the three strains studied (Figure 3B). However, whereas in the range between 20 and 30 % the magnitude in decrease was similar for all three strains, above that concentration the decrease was much more marked for the more NaCl-resistant ones. This strongly suggests that differences in NaCl resistance among *Salmonella* strains would depend on the NaCl concentration used.

3.4 Heat Resistance

Conversely to acid resistance, large differences in heat resistance were observed between the most and the least heat-resistant serovar. Thus, the *2D*-value to heat (58 ºC) varied between 1.62 min and 23.46 min for serovars Saintpaul and Senftenberg (strain 775W), respectively (Fig. 4A). In parallel, intra-serovar differences in heat resistance were much smaller than the differences observed when comparing different serovars. However, these observations are mainly due to the extraordinary thermal resistance of *S.* Senftenberg strain 775W, which has already been documented. This particular strain is considered a singularity, not only when compared with other *Salmonella* serovars, but also with other strains belonging to the serovar Senftenberg (Ng et al., 1969). Therefore, if this strain is excluded from the analysis, one can conclude that inter-serovar variability in resistance to heat would be lower than intra-serovar variability. Remarkably, the heat resistance parameters (*D*-values) and the variability in heat resistance determined here are comparable to those previously reported, even though other strains, growth methods, and treatment mediums were used. Thus, Juneja, Eblen, & Ransom (2001) evaluated the heat resistance of 35 *Salmonella* strains in chicken broth at 58 ºC, reporting *D*-values between 1.29 and 2.98 minutes. Similarly, Quintavalla, Larini, Mutti, & Barbuti, (2001) reported that the *D*-values of 94 *S.*

enterica strains belonging to different serovars determined in culture broth at 58 ºC ranged between 0.79 and 2.67 min. The variability in heat resistance among strains obtained in this study is also similar to that determined in the meta-analysis carried out by van Asselt & Zwietering (2006). As pointed out by den Besten, Wells-Bennik, & Zwietering, (2018) if *S.* Senftenberg 775W is excluded from analysis, the variability in heat resistance among *Salmonella* serovars is, in general terms, lower than among strains of other species.

The influence of treatment temperature on microbial heat resistance is usually estimated *via* the calculation of the z value (the inverse of the slope of the line obtained when the Log_{10} of the *D*-values is represented *vs* its corresponding treatment temperature). In this case, we calculated the z_{2D} (increase in temperature required to reduce the 2*D*-value 10-fold) for the most and the least heat-resistant strain, as well as for *S.* Typhimurium SL1344, and no significant differences ($p>0.05$) were found among them (Fig. 4B). Therefore, it is feasible to conclude that the relative resistance of the different *Salmonella* strains would be similar regardless of treatment temperature, within the range studied here.

3.5 HHP resistance

As it can be observed in figure 5A, *S.* Typhimurium SL1344 displayed the highest baroresistance (*2D*-value at 300 MPa = 8.83 min), and *S.* Infantis the lowest (*2D*-value at 300 MPa = 5.79 min). The average 2*D*-value was of 6.98 min for all the strains/serovars, and of 7.05 min for the *S.* Typhimurium strains but, in spite of this slightly higher average *2D*-value, no significant differences ($p>0.05$) were found when comparing the baroresistance of *S.* Typhimurium strains (5 strains) *vs* that of strains belonging to other serovars (10 strains). As for all agents, except heat, the 95 % confidence interval of the mean of the *2D*-values calculated for *S.* Typhimurium strains

was broader than that corresponding to the whole set of strains. These results agree with those obtained by Sherry et al. (2004) who observed that resistance to high pressure was relatively uniform among the serovars studied. In contrast, Tamber (2018), who studied the HHP resistance of 99 *S. enterica* strains from 24 serovars, found that after exposure to 600 MPa for 3 minutes, differences of up to 5 Log_{10} cycles in the number of survivors were found between the most and the least baroresistant strains. Further work will be required to ascertain whether these differences are a result of differences among process parameters and experimental conditions applied in the studies, or whether they may reflect inherent differences among the tested strains. In any case, Tamber (2018) also observed that, despite the close genetic relationships between the strains of some serovars, the distribution of resistance patterns differed among strains, suggesting that there was no significant relationship between pressure tolerance and the serovar.

Since our reference strain (*S.* Typhimurium SL1344) was already the most HHP-resistant one, we included the second most resistant one, *S.* Newport, in the experiments designed to determine the influence of pressure on the *2D*-values. For the three strains, a marked and similar decrease in resistance was observed after raising pressure from 250 to 300 MPa, but not to 350 (Fig. 5B). This could be attributed to the presence of tails in survival curves to HHP, which may interfere with the estimation and interpretation of the *2D* parameter. Patterson, Quinn, Simpson, & Gilmour (1995) analyzed that, when calculating *D*-values corresponding to high hydrostatic pressure treatments, difficulties could arise due to the surviving tail populations, and this effect was noticeable when pressure was greater than 350 MPa. In any case, the observed trends were similar for all three strains, indicating that, as for acid, peroxide and heat, conclusions drawn for selected pressure would be valid for the entire range under study.

3.6 Resistance to PEF

The estimated *2D*-value (μs) for the tested strains varied from 26.16 to 49.83, for *S.* Virchow and *S.* Stanley, respectively (Fig. 6A), i.e. an approximately 2-fold variation between the most and the least resistant strains. Variability in PEF resistance among *Salmonella* serovars has scarcely been studied. The results obtained for the Typhimurium strains are similar to those obtained by Saldaña et al. (2009). Similarly, up to a 2-fold difference in the calculated *5D*-values was observed when comparing the resistance to PEF of *S.* Senftenberg 775W, *S.* Typhimurium STCC 443 and *S.* Enteritidis STCC 4300 in the range between 19 and 28 kV/cm (Álvarez, Mañas, Condón, & Raso, 2003). As for most of the previously studied agents, the 95 % confidence interval of the mean of the *2D*-values calculated for the 5 *S.* Typhimurium strains was similar to that calculated for the whole set of strains (15) but it should be noted that the PEF resistance of the *S.* Typhimurium strains was in the upper range. Furthermore, significant differences ($p<0.05$) were found when comparing the PEF resistance *S.* Typhimurium strains vs. the other 10 *Salmonella* strains, thus suggesting that this trait might be both strain and serovar dependent. Finally, as can be seen in Figure 6B, the influence of electric field strength on the resistance of the three serovars under study (the most and the less resistant ones, along with strain SL1344) was analogous.

3.7 UV-C resistance

The *2D*-value to UV-C (0.47 mW/cm^2) treatments for the tested strains ranged from 49.73 to 70.20 seconds. *S.* Gallinarum and *S.* Newport were the most sensitive, and *S.* Infantis was the most resistant one. The differences in resistance among strains of *S.* Typhimurium were comparable to those observed when comparing different serovars (Fig. 7A) but statistical analysis suggests that differences in UV resistance might be determined by both the strain and the serovar. In any case, the *2D*-value varied less than

1.5-fold. Gayán et al. (2012) also observed a 1.4-fold difference in the *4D*-values to UV light among five strains of *Salmonella*, revealing that *S.* Typhimurium STCC 878 and *S.* Enteritidis 4300 were the most resistant and the most sensitive strain, respectively, among the strains they studied. Gabriel & Nakano (2009) also reported that in phosphate-buffered saline (PBS) buffer the *S.* Enteritidis strain they tested was less resistant to UV-C than *S.* Typhimurium. Kim & Yuk (2017) similarly tested the resistance of 18 *Salmonella* strains to 405 nm LED light indicating that efficacy of 405 nm LED illumination may depend on serotype and strain within the same serotype. In addition, as can be seen in Figure 7B, the *2D*-values of the three selected strains showed a similar trend when fluence was modified.

3.8. Comparative study

In order to establish meaningful comparisons among strains and agents/technologies, we applied the iterative Grubbs' test to the obtained data (*2D*-values) in order to identify potential outliers that could exert a disproportionate influence on further data analysis and lead to non-valid conclusions. Grubbs' test detected a single outlier: the *2D*-value to heat of *S.* Senftenberg 775W. This value was therefore excluded from subsequent analysis. This was a true outlier value, since the elevated heat resistance of this strain has been documented elsewhere (Ng et al., 1969).

As described above, one of the major objectives of this investigation was to quantify and compare variability in resistance to different stresses/technologies among different *Salmonella* strains. Since the *2D*-values obtained for each agent/technology cannot be directly compared because of the varying time scale of survival curves, these resistance parameters were normalized by dividing them by the average *2D*-value of the resistance of all the *Salmonella* strains studied. These normalized values were used to build figure 8, which illustrates the variability in resistance of the 15 strains studied to each of the 7

agents investigated. As can be observed in the figure, resistance to UV was the most homogeneous one. Conversely, *Salmonella* resistance to heat and PEF resistance were much more heterogeneous. When comparing these two latter technologies it should be noted that, although the difference between the maximum and the minimum *2D*-values was higher for heat (whiskers length), the 25th and 75th percentiles (box length) were more separated for PEF, thereby indicating that the frequency distribution of heat values would have a higher kurtosis (i.e. a higher probability of including outliers). On the other hand, the dispersion of resistance values of almost all treatments showed a symmetrical distribution around the median, except for NaCl resistance values, for which the dispersion of resistance values displayed a positive asymmetric right-skewed distribution.

These results are similar to those previously reported by Cebrián et al. (2016) who concluded that the differences in resistance among strains of the genus *Salmonella* were smaller for UV than for the other agents studied (heat, PEF, and HHP), and that, conversely to other microorganisms and provided that *S.* Senftenberg 775W is excluded from analysis, variability in resistance to PEF and HHP is comparable to that of heat. Furthermore, as already pointed out by den Besten et al. (2018) for heat, all these data suggest that the variability in stress resistance among *Salmonella* serovars would generally be lower than among strains of other species.

It should be noted that this comparison was established using results obtained under very specific fixed experimental conditions: bacteria were grown to stationary growth phase under optimal conditions, and treatments were applied in buffer/laboratory media at neutral pH, and with a very high water activity. Although results obtained here indicate that the range of experimental conditions under which these conclusions are valid would be broader (pH 2.0-3.0; 55-64 ºC; 250-350 MPa; 10-100 mM H_2O_2; 20-

30% NaCl; 20-30 kV/cm; 0.2-1.1 mW/cm^2), results already indicate that, for instance, if resistance to NaCl were studied at higher NaCl concentrations (33 %), the observed variability in resistance would be of lower magnitude. Similarly, Lianou & Koutsoumanis (2011) already observed that the magnitude of differences in growth rate (μ_{max}) among *Salmonella* strains depended highly on growth conditions (composition of the growth medium). On the other hand, our results indicate that variability among experimental replicates (biological replicates) was lower than intra-serovar and inter-serovar variability, with very few exceptions.

Our experimental design also allowed us to determine whether any positive or negative association between *Salmonella* resistance to the different stresses could be ascertained. For this purpose, Pearson's correlation test was performed (Table 2). Result indicate a positive correlation between resistance to osmotic and oxidative stress (r= 0.565, p-value= 0.035). Further analysis of results corroborated the existence of this relation: *S.* Enteritidis 4300, *S.* Infantis, *S.* Newport, and *S.* Virchow are the most sensitive serovars to the two environmental stresses, and *S.* Saintpaul, *S.* Typhimurium 443 and *S.* Typhimurium 7162 are the most resistant (Table 1 and Figures 2A and 3A). A positive correlation was also observed between PEF and UV-C resistances (r= 0.558, p-value=0.038). The most resistant strains to both technologies would be *S.* Typhimurium SL1344 and *S.* Typhimurium 4954, and the most sensitive strains would be *S.* Newport, *S.* Virchow, and *S.* Gallinarum. It should also be noted that, as pointed out above, the same conclusions can be drawn if the *1D* or the *3D*-values are used to establish these comparisons, with the only exception that if *1D*-values are compared a positive correlation between acid and UV resistance is observed.

Based on our results, there would be no correlation between resistance to heat and acid pH (r = 0.233, p-value 0.423). This finding contrasts with the fact that the existence of

cross-protection phenomena between pH and heat has already been described in *Salmonella* spp. (Álvarez-Ordóñez, Fernández, López, Arenas, & Bernardo, 2008). It also contrasts with the results of Humphrey, Slater, McAlpine, Rowbury, & Gilbert (1995), who observed that the most heat-resistant *S.* Enteritidis PT4 isolates were also more resistant to acid, H_2O_2, and desiccation. Nevertheless, similar results to those reported herein were obtained by Lianou & Koutsoumanis (2013), and by Gill, Tamber & Yang (2019).

According to our PCA analysis, the two principal components explain 53.8% of the variability of the data (Figure 9). CP1 would be positively correlated with UV and PEF resistance, and negatively with pH and HHP, whereas CP2 would be positively correlated with NaCl, H_2O_2 and PEF resistance (Table figure 9). Thus, strains with a higher PEF and UV resistance are located more on the right on the x-axis (CP1), whereas those more resistant to NaCl and H_2O_2 are higher on the y-axis (CP2). In this plot, it can also be observed that strains displaying similar resistance profiles are located close to one another (e.g. the *S.* Typhimurium STCC 443 and *S.* Stanley). These observations are very similar to the Pearson's test results, since both indicate an association between UV and PEF, as well as between NaCl and H_2O_2 resistance, along with certain further trends, such as a positive association between PEF and NaCl resistance, and negative correlations between PEF and acid resistance, and between HHP and UV resistance.

Altogether, these results demonstrate that *Salmonella* strains that are the most resistant to a given stress are not necessarily more resistant to other types of stresses, as also has been previously demonstrated for *Salmonella* by other authors such as Sherry et al. (2004), Lianou & Koutsoumanis (2013) and Gill, Tamber & Yang (2019). This can be easily explained by the different modes of action and cellular targets of each of the

technologies/agents studied here (Cebrián et al., 2016; Sherry et al., 2004). Nevertheless, since an association between NaCl and hydrogen peroxide resistance, as well as between PEF and UV resistance, was found, further work will be required to elucidate the underlying mechanisms.

It should be noted that the mode of action of NaCl and hydrogen peroxide on bacterial cells are assumed to be quite different. Thus, NaCl is a water-depressing solute that imposes a hyperosmotic stress on cells and that, once inside the cytoplasm, can inhibit enzyme activity by perturbing the hydrophobic–electrostatic balance between the forces maintaining protein structure, and can exert Na^+-specific toxic effects such as the inhibition of certain enzymatic activities and ionic channels of the bacterial cell (Murguía, Bellés, & Serrano, 1996; Stewart, Cole, Legan, Slade, & Schaffner, 2005). Hydrogen peroxide acts indirectly though the generation of oxidative species (such as the hydroxyl radical) via the Fenton reaction, which can cause oxidative damages to various cellular components, including DNA and proteins (Imlay & Linn, 1988; Juven & Pierson, 1996). A potential explanation of the relationship between both agents might be found in the results of Mandal & Kwon (2017), who observed that more than 30% of the genes involved in desiccation resistance in *Salmonella* Typhimurium were also involved in hydrogen peroxide (H_2O_2, 1mM) resistance. Nevertheless, the same authors also indicated that much less genes (15 %) were shared between osmotic stress (3% NaCl), and hydrogen peroxide resistance. In any case, the stressor concentrations used in their study are much lower than in ours, and further work would be required to determine if their results are valid under our conditions.

Similarly, whereas the main targets of PEF are the cellular envelopes (Mañas & Pagán, 2005), the effect of UV light on genetic material is the main factor responsible for the latter technology's ability to inactivate microorganisms (Gayán, Condón, & Álvarez,

2014), although other cellular components such as proteins can also undergo damage. Regarding this second association (PEF-UV) it should be noted that membrane fluidity has been proposed as a factor which plays a role in microbial resistance to UV (Gayán, Mañas, Álvarez, & Condón, 2013), in such a way that a more fluid membrane would render a more UV-sensitive cell. However, the role of membrane fluidity in PEF resistance, although widely discussed, still remains to be clarified (Cebrián et al., 2016).

The development of cross-resistance responses is commonly attributed to the activation/induction of general stress sigma factors such as RpoS in the case of *Salmonella* (Hengge, 2011). In the same way, it has been hypothesized that differences in stress resistance among strains could be due, among other factors, to a potential association between stress sensitivity and mutations in the *rpoS* gene, or with a decreased level of expression of RpoS-dependent genes (Jørgensen et al., 2000). Since it has been demonstrated that the deletion of *rpoS* leads to a decrease in resistance of *E. coli* to all the agents tested here (Notley-McRobb, King, & Ferenci, 2002), and a similar role for *rpoS* would be expected in *Salmonella* (Robbe-Saule, Algorta, Rouilhac, & Norel, 2003), if an increased expression of RpoS-controlled genes was the cause for increased resistance to a particular stress, it should be accompanied with an increased resistance to all agents tested because our experiments were carried out with stationary growth phase cells. Most agents exert a plethora of effects on bacterial cells, i.e. most of them are regarded as multi-target agents, and even those that share a cellular target (such as PEF and HHP, for instance) have widely differing mechanisms of action. Our results might also be explained by specific resistance mechanisms playing a greater role than general stress mechanisms in *Salmonella* resistance, thereby masking the influence of general stress response (RpoS) controlled mechanisms. In fact, the combination of these three factors – different mechanisms of action plus multi-target technologies plus

specific resistance mechanisms playing a major role in resistance – would probably explain the obtained results, even for agents with very similar modes of action and targets, such as heat and HHP (Sherry et al., 2004). Furthermore, even for a single agent such as HHP, Tamber (2018) indicated that the response of *S. enterica* strains was heterogeneous and multifactorial, making it impossible to identify a unique mechanism capable of explaining the observed differences in resistance, and thereby hampering the prediction of individual *S. enterica* strains' response to HHP.

Finally, it is worth noting that further examination of figure 9 reveals that *S.* Typhimurium strains clustered together in the PCA biplot -right on the x axis and high on the y axis- and quite apart from most of the strains from other serovars here studied. This would mean that *S.* Typhimuirum strains were among the most PEF, UV, hydrogen peroxide and NaCl resistant *Salmonella* strains and that these strains would be displaying a differentiated stress-resistance phenotype -at least for some agents-, what would be reasonable given their closer genetic background. These conclusions are consistent with those drawn in sections 3.2 to 3.7 and seem to indicate that resistance to some agents such as PEF, UV and NaCl, might be, at least to some extent, a serovar-dependent characteristic. In any case it should be noted that the number of strains here studied is limited and that further studies, including a higher number of strains and from a wide range of *Salmonella* serovars would be required to validate the conclusions drawn from these results.

4. Conclusions

The resistance of 15 strains belonging to 11 serovars of *Salmonella enterica* subsp. *enterica* to several different environmental stresses (acid, hydrogen peroxide, NaCl and heat) and non-thermal food preservation technologies (HHP, PEF, UV) was determined and compared. For most agents tested, intra-serovar (*S.* Typhimurium) variability in

resistance was comparable to inter-serovar variability, despite the similar genetic backgrounds of strains belonging to the same serovar. If *S.* Senftenberg 775W is excluded from the analysis, differences in resistance (*2D*-values) among strains varied less than 2.4-fold for all agents, including heat. Results reported herein also indicate that *Salmonella* strains that are the most resistant to a given stress are not necessarily more resistant to other types of stress. Nevertheless, the statistical analysis of the whole set of data reveals a positive correlation between the resistance of *Salmonella* strains to oxidative and osmotic stress, as well as between UV and PEF resistance. Further work will be required to fully elucidate the mechanisms responsible for these two phenomena.

The results obtained in this work would be especially helpful in defining safe food preservation processes and in improving quantitative microbiological risk assessments of *Salmonella* in food products.

ACKNOWLEDGMENTS

This work was supported by the European Regional Development Fund; and MINECO-CICYT (AGL2017-84084-R).

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Figure

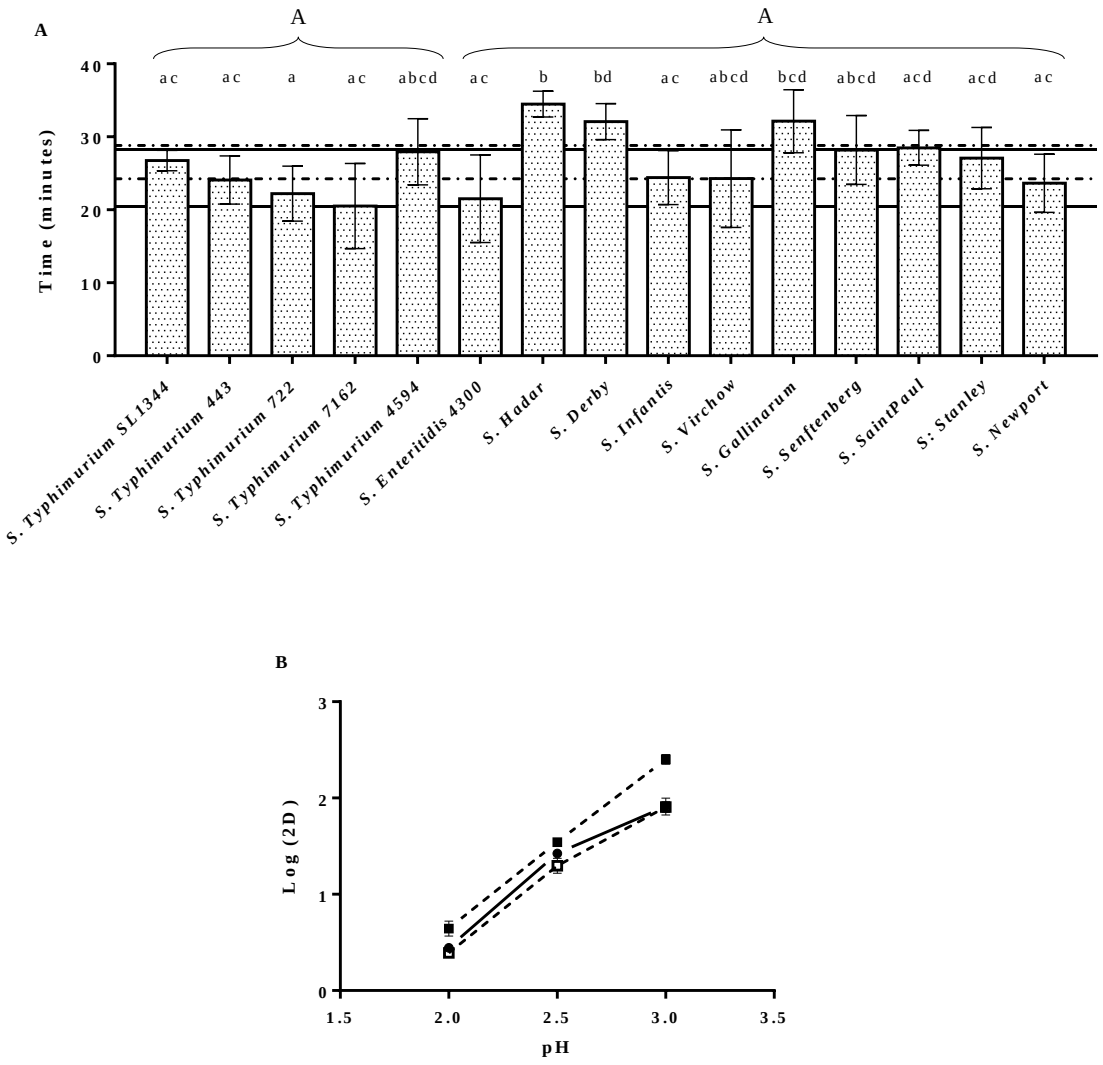


Fig. 1. A) *2D*-values of the 15 strains of *Salmonella* to acid pH (2.5). Discontinuous and continuous lines correspond to the 95 % confidence interval of the mean *2D*-value of all the *Salmonella* strains (inter-serovar variability) and of *S.* Typhimurium strains (intra-serovar variability), respectively. Different letters indicate statistically significant differences between strains (lowercase letters) or groups (Typhimurium *vs* Non-Typhimurium; uppercase letters). B) Influence of treatment medium pH on the resistance of the 3 serovars selected: *S.* Typhimurium SL1344 (●, continuous line), *S.* Hadar (■, discontinuous line) and *S.* Typhimurium 7162 (□, discontinuous line). Error bars represent the standard deviations.

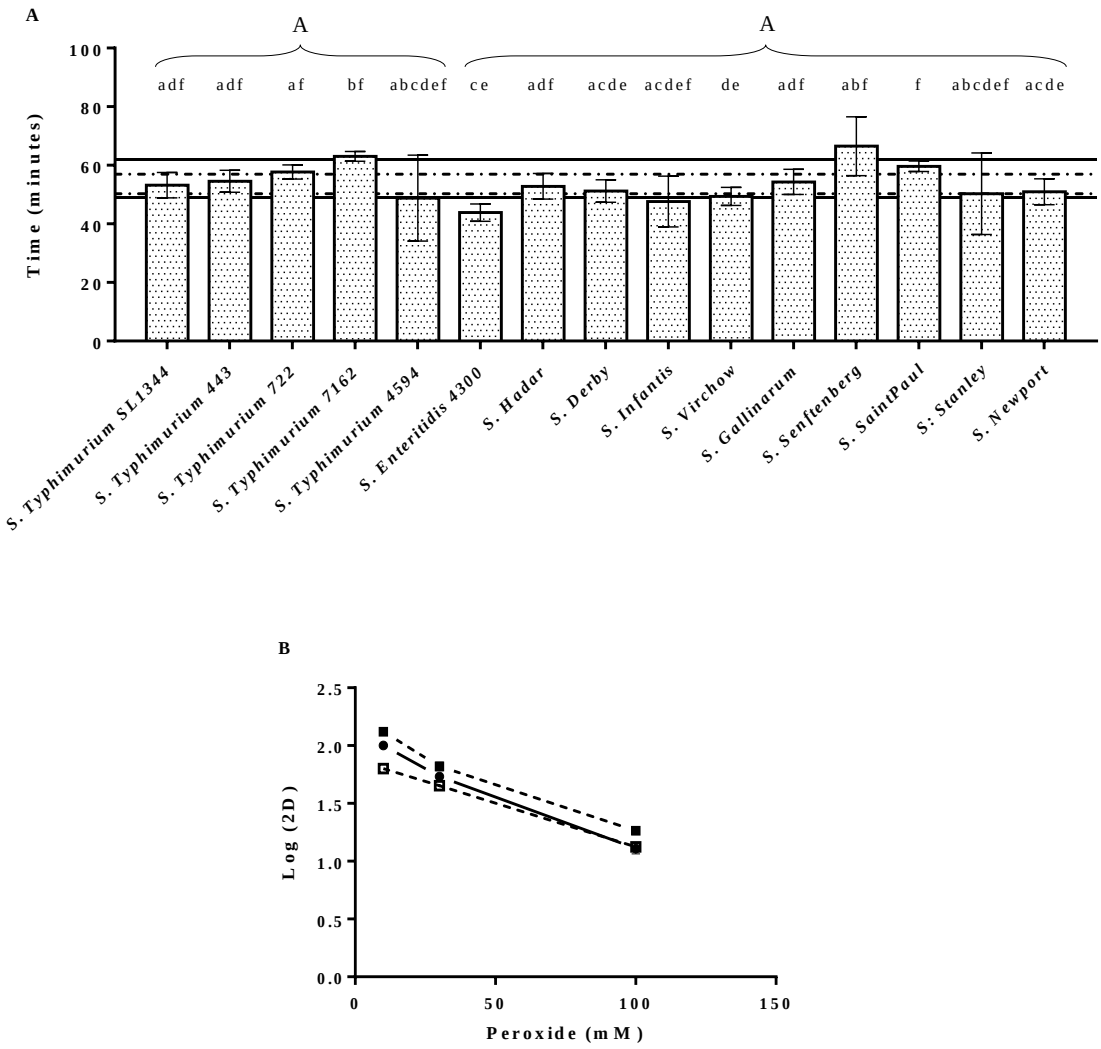


Fig. 2. A) *2D*-values of the 15 strains of *Salmonella* to hydrogen peroxide (30 mM). Discontinuous and continuous lines correspond to the 95 % confidence interval of the mean *2D*-value of all the *Salmonella* strains (inter-serovar variability) and of *S.* Typhimurium strains (intra-serovar variability), respectively. Different letters indicate statistically significant differences between strains (lowercase letters) or groups (Typhimurium *vs* Non- Typhimurium; uppercase letters). B) Influence of the hydrogen peroxide concentration on the resistance of the 3 serovars selected: *S.* Typhimurium SL1344 (●, continuous line), *S.* Senftenberg (■, discontinuous line) and *S.* Enteritidis 4300 (□, discontinuous line). Error bars represent the standard deviations.

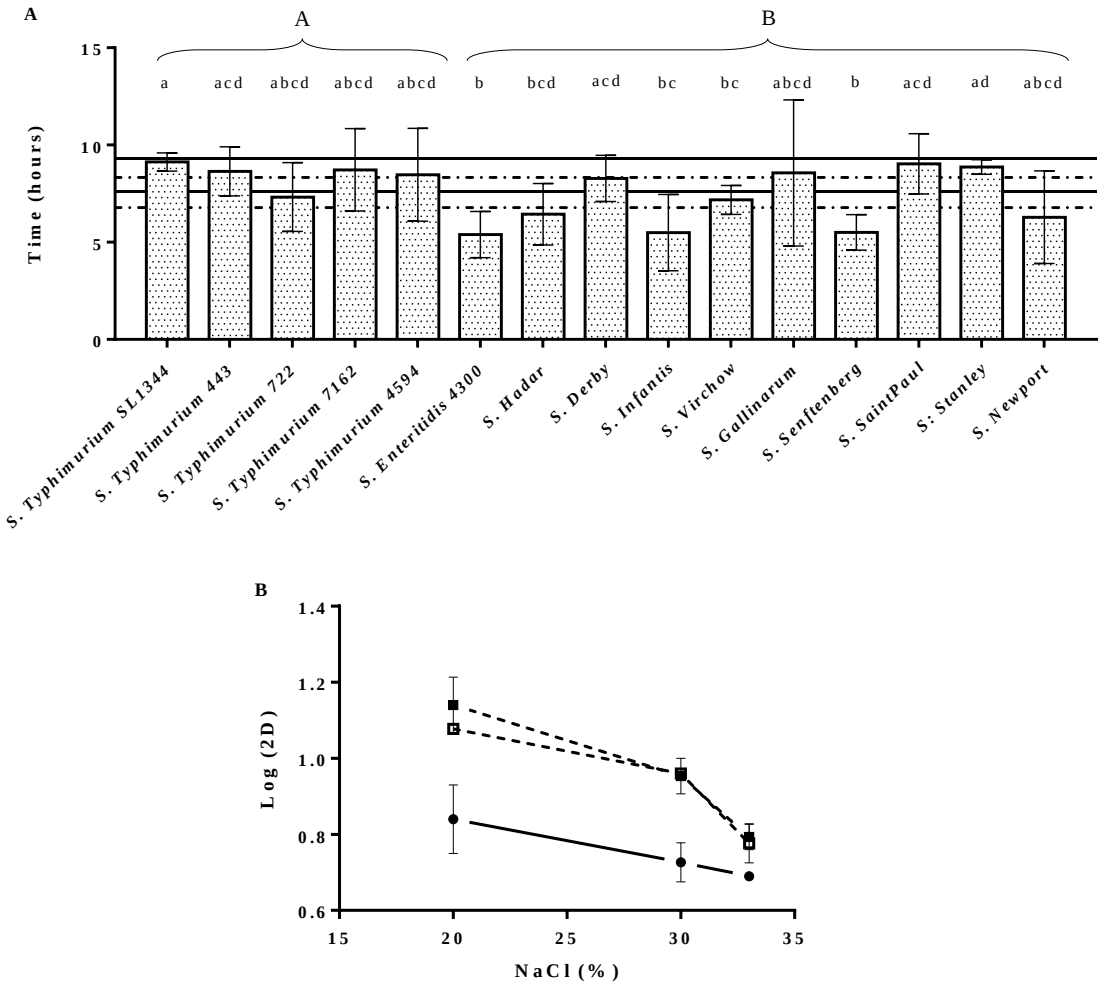
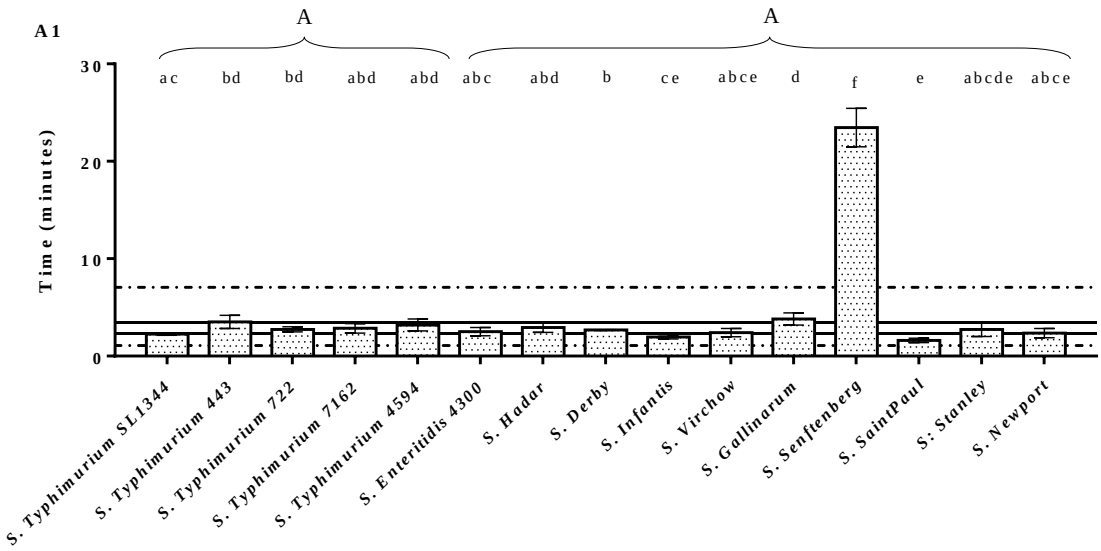
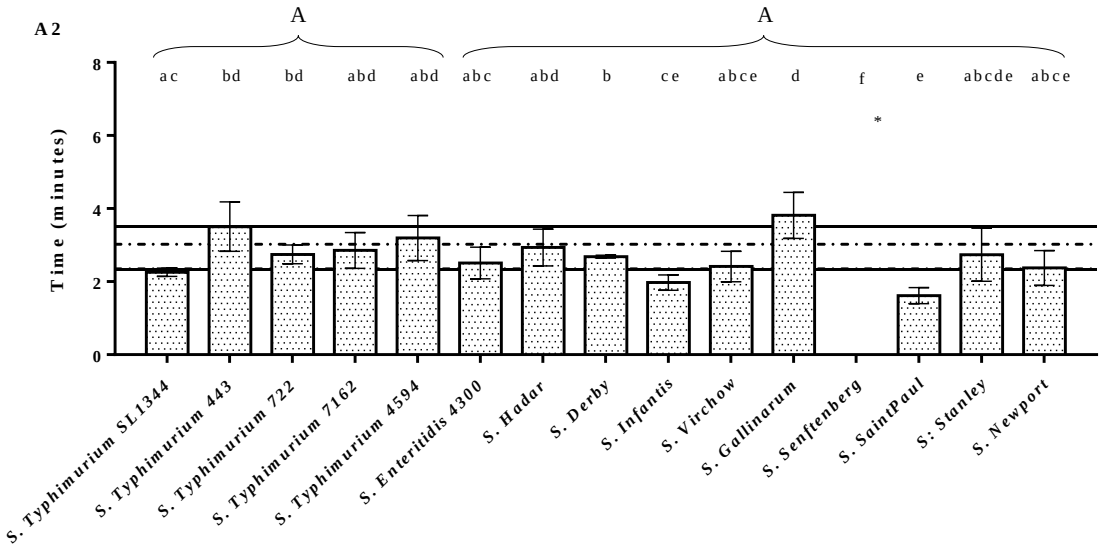


Fig. 3. A) *2D*-values of the 15 strains of *Salmonella* to sodium chloride (30 %). Discontinuous and continuous lines correspond to the 95 % confidence interval of the mean *2D*-value of all the *Salmonella* strains (inter-serovar variability) and of *S.* Typhimurium strains (intra-serovar variability), respectively. Different letters indicate statistically significant differences between strains (lowercase letters) or groups (Typhimurium *vs* Non- Typhimurium; uppercase letters). B) Influence of sodium chloride concentration on the resistance of the 3 serovars selected: *S.* Typhimurium SL1344 (●, continuous line), *S.* Saintpaul (■, discontinuous line) and *S.* Enteritidis 4300 (□, discontinuous line). Error bars represent the standard deviations.


A1
A
A
ac bd bd abd abd abc abd b ce abce d f e abcde abce
Time (minutes)
30
20
10
0
S. Typhimurium SL1344
S. Typhimurium 443
S. Typhimurium 722
S. Typhimurium 7162
S. Typhimurium 4594
S. Enteritidis 4300
S. Hadar
S. Derby
S. Infantis
S. Virchow
S. Gallinarum
S. Senftenberg
S. SaintPaul
S. Stanley
S. Newport


A2
A
A
ac bd bd abd abd abc abd b ce abce d f e abcde abce
*
Time (minutes)
8
6
4
2
0
S. Typhimurium SL1344
S. Typhimurium 443
S. Typhimurium 722
S. Typhimurium 7162
S. Typhimurium 4594
S. Enteritidis 4300
S. Hadar
S. Derby
S. Infantis
S. Virchow
S. Gallinarum
S. Senftenberg
S. SaintPaul
S. Stanley
S. Newport

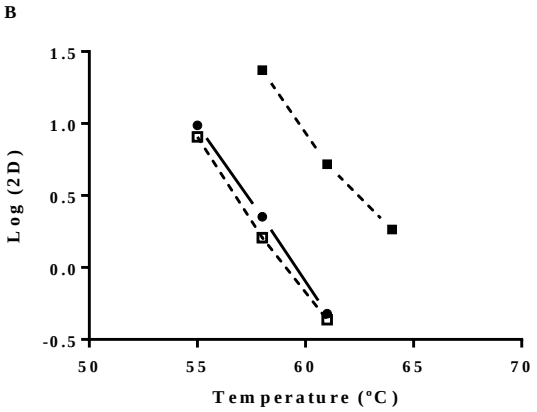

B
Log (2D)
1.5
1.0
0.5
0.0
-0.5
50
55
60
65
70
Temperature (°C)

Fig. 4. A1) *2D*-values of the 15 strains of *Salmonella* to heat (58 ºC) and A2) *2D*-values excluding *S.* Senftenberg from the analysis. Discontinuous and continuous lines correspond to the 95 % confidence interval of the mean *2D*-value of all the *Salmonella* strains (inter-serovar variability) and of *S.* Typhimurium strains (intra-serovar variability), respectively. Different letters indicate statistically significant differences between strains (lowercase letters) or groups (Typhimurium *vs* Non- Typhimurium; uppercase letters). B) Influence of treatment medium temperature on the resistance of the 3 serovars selected: *S.* Typhimurium SL1344 (●, continuous line), *S.* Senftenberg (■, discontinuous line) and *S.* Saintpaul (□, discontinuous line). Error bars represent the standard deviations.

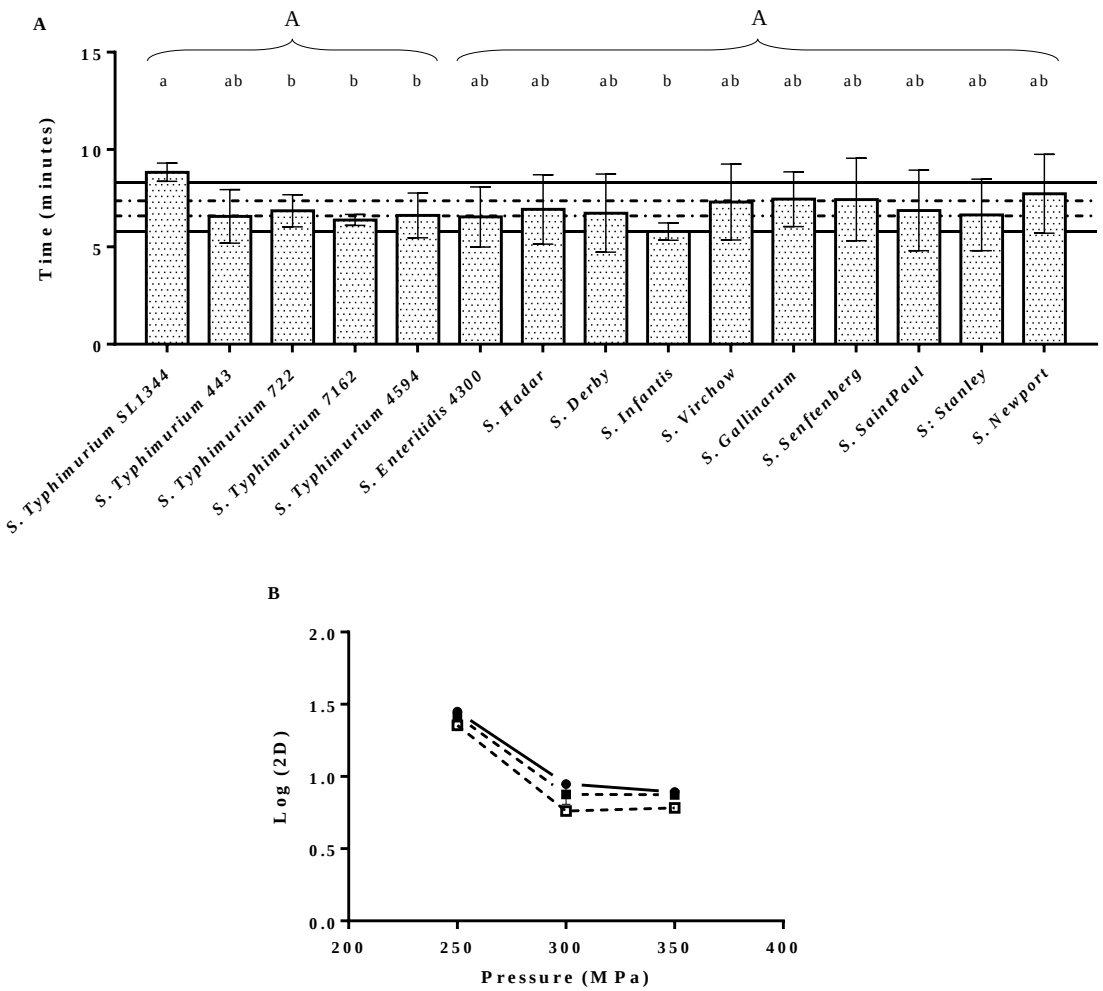


Fig. 5. A) *2D*-values of the 15 strains of *Salmonella* to high hydrostatic pressure (300 MPa). Discontinuous and continuous lines correspond to the 95 % confidence interval of the mean *2D*-value of all the *Salmonella* strains (inter-serovar variability) and of *S.* Typhimurium strains (intra-serovar variability), respectively. Different letters indicate statistically significant differences between strains (lowercase letters) or groups (Typhimurium *vs* Non- Typhimurium; uppercase letters). B) Influence of the pressure on the resistance of the 3 serovars selected: *S.* Typhimurium SL1344 (●, continuous line), *S.* Newport (■, discontinuous line) and *S.* Infantis (□, discontinuous line). Error bars represent the standard deviations.

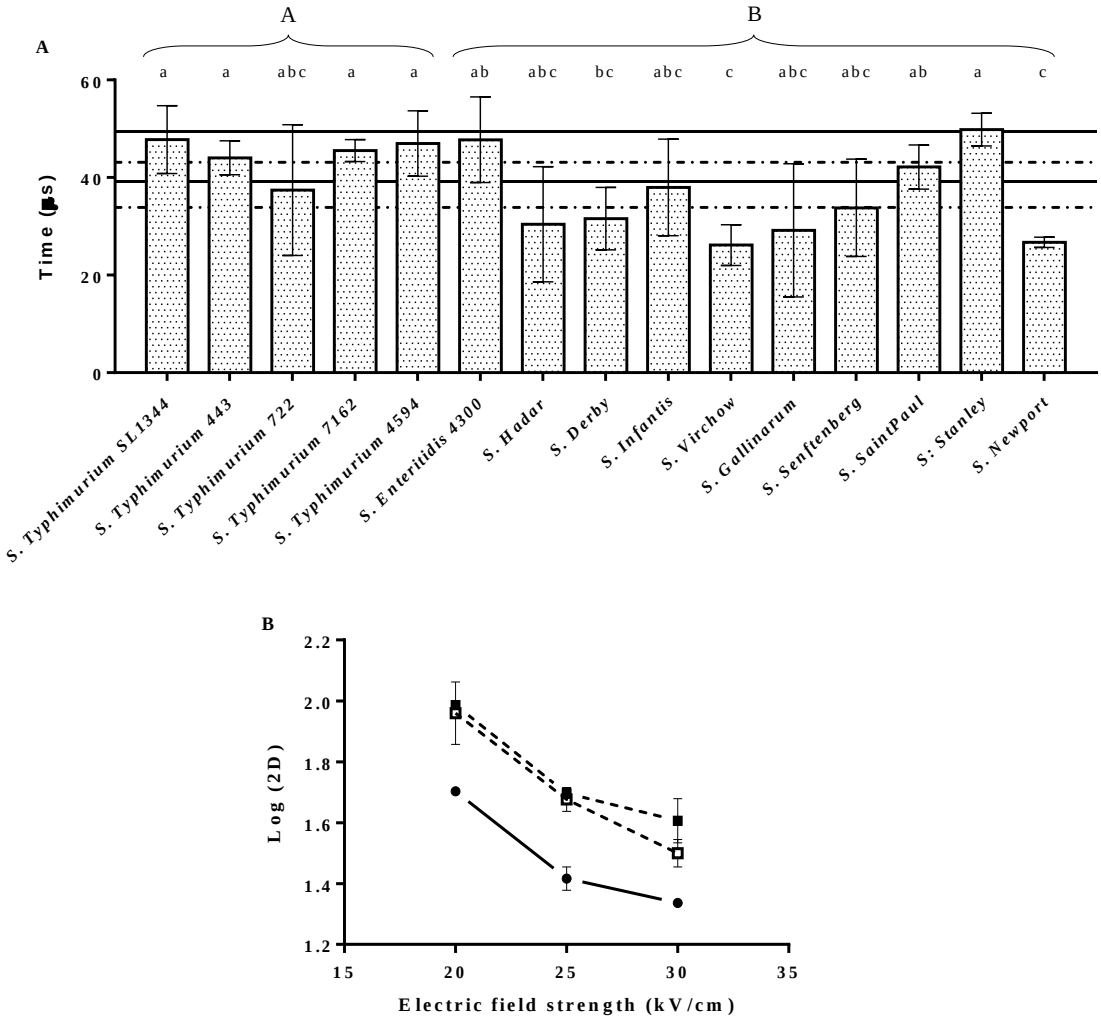


Fig. 6. A) *2D*-values of the 15 strains of *Salmonella* to pulsed electric fields (25 kV/cm). Discontinuous and continuous lines correspond to the 95 % confidence interval of the mean *2D*-value of all the *Salmonella* strains (inter-serovar variability) and of *S.* Typhimurium strains (intra-serovar variability), respectively. Different letters indicate statistically significant differences between strains (lowercase letters) or groups (Typhimurium *vs* Non- Typhimurium; uppercase letters). B) Influence of sodium chloride concentration on the resistance of the 3 serovars selected: *S.* Typhimurium SL1344 (●, continuous line), *S.* Stanley (■, discontinuous line) and *S.* Virchow (□, discontinuous line). Error bars represent the standard deviations.

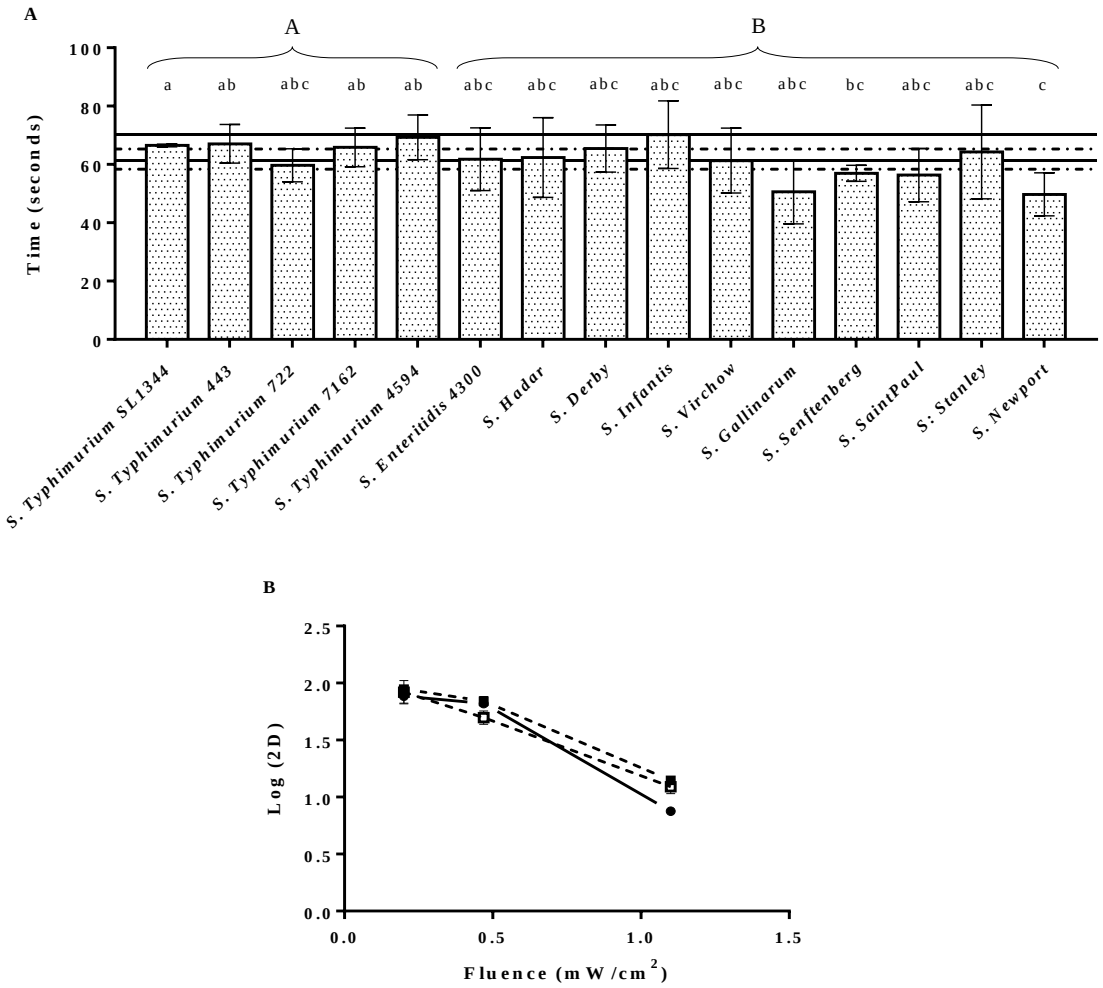


Fig. 7. A) *2D*-values of the 15 strains of *Salmonella* to UV-C (0.47 mW/cm2). Discontinuous and continuous lines correspond to the 95 % confidence interval of the mean *2D*-value of all the *Salmonella* strains (inter-serovar variability) and of *S.* Typhimurium strains (intra-serovar variability), respectively. Different letters indicate statistically significant differences between strains (lowercase letters) or groups (Typhimurium *vs* Non- Typhimurium; uppercase letters). B) Influence of UV-C fluence on the resistance of the 3 serovars selected: *S.* Typhimurium SL1344 (●, continuous line), *S.* Infantis (■, discontinuous line) and *S.* Gallinarum (□, discontinuous line). Error bars represent the standard deviations.

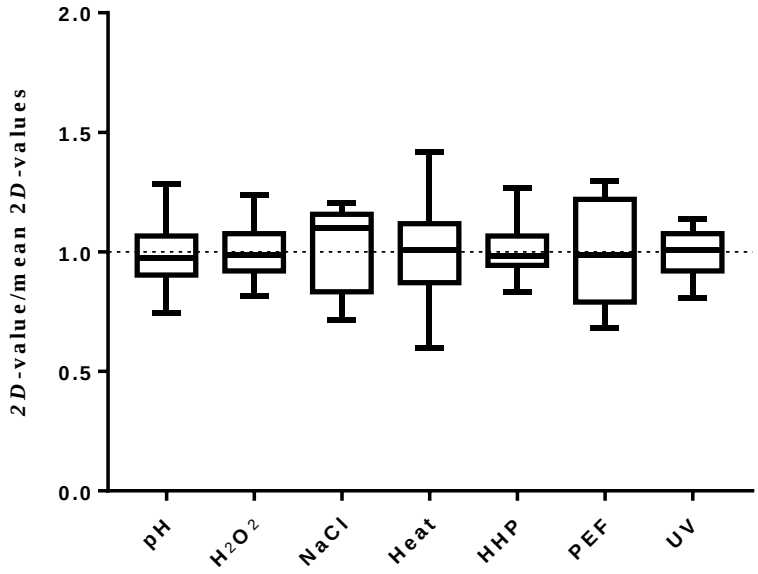
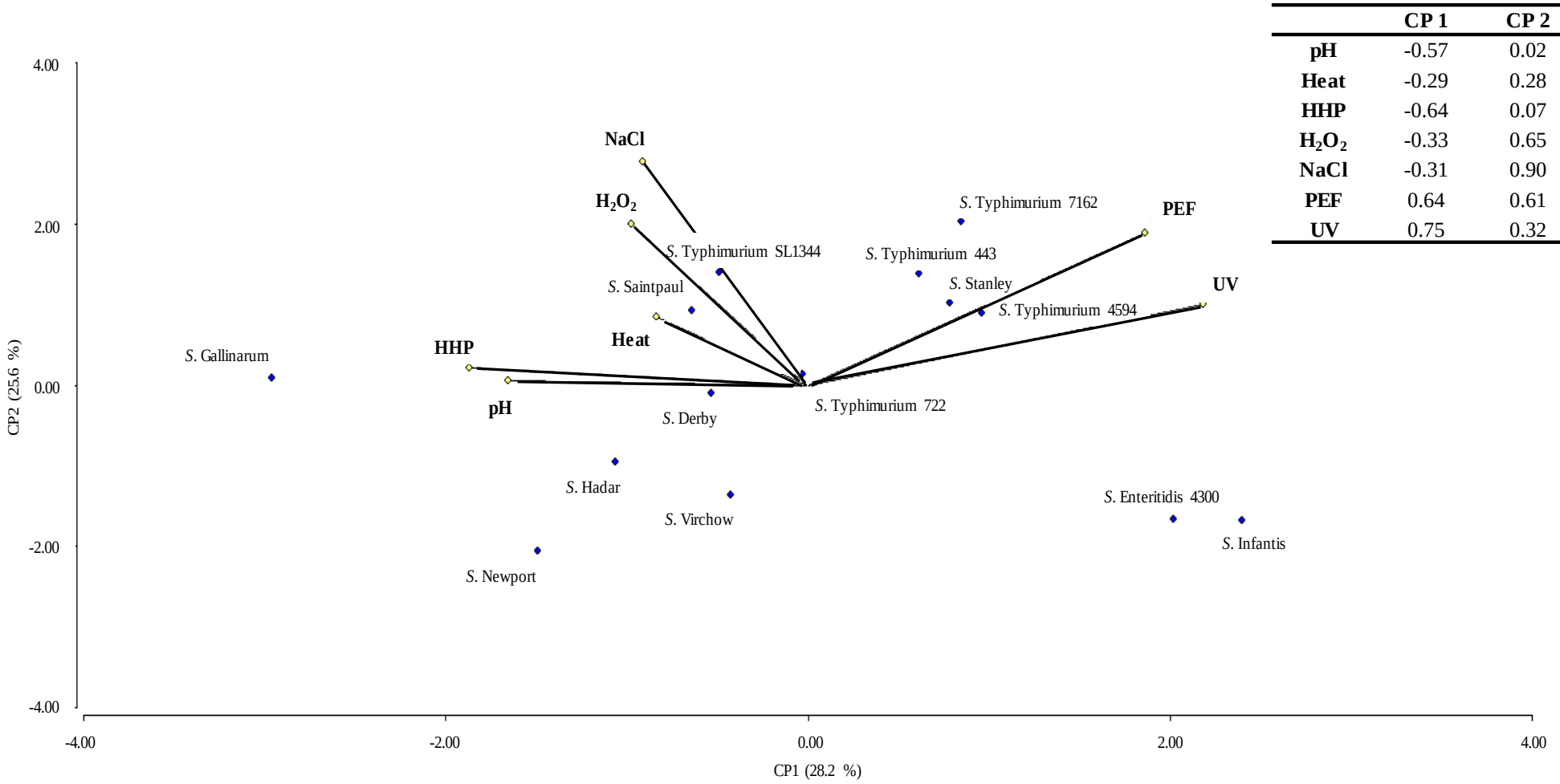


Fig. 8. Variability in resistance to different environmental stresses and non thermal food preservation technologies among the *Salmonella* strains studied. The *2D*-value to heat of *S.* Senftenberg has been excluded from the analysis as described in the results section.



	CP 1	CP 2
pH	-0.57	0.02
Heat	-0.29	0.28
HHP	-0.64	0.07
H_2O_2	-0.33	0.65
NaCl	-0.31	0.90
PEF	0.64	0.61
UV	0.75	0.32

Fig. 9. Bioplot representation of the principal component analysis, showing the distribution of *Salmonella* serovars along components 1 and 2.

Table 1. Resistance (K_{max}. S_l and N_{res}) and goodness of the fit (R^2, *RMSE*) parameters calculated after fitting the survival curves to the 7 agents investigated of the 15 *Salmonella* strains studied to the Geeraerd's model. Values presented correspond to the mean and SD of the means (in parentheses) of the resistance parameters and to the range of values obtained for the goodness of the fit values (all calculated from 3 independent replicates).

	pH					H_2O_2					NaCl				
	K_{max} (min^{-1})	S_l (min)	N_{res} (CFU/ml)	R^2	RMSE	K_{max} (min^{-1})	S_l (min)	N_{res} (CFU/ml)	R^2	RMSE	K_{max} (min^{-1})	S_l (min)	N_{res} (CFU/ml)	R^2	RMSE
***S.* Typhimurium SL1344**	0.217 (0.351)	4.940 (4.281)	-	0.94 - 0.99	0.053 - 0.478	0.126 (0.023)	15.68 (8.795)	-	0.99 - 1.00	0.099 - 0.178	0.523 (0.032)	-	4.28 (0.313)	0.97 - 0.99	0.106 - 0.160
***S.* Typhimurium 443**	0.300 (0.165)	8.662 (3.731)	-	0.99 - 1.00	0.011 - 0.087	0.143 (0.027)	21.50 (3.589)	-	0.98 - 0.99	0.000 - 0.233	0.553 (0.073)	-	3.53 (1.348)	0.92 - 0.98	0.194 - 0.296
***S.* Typhimurium 722**	0.430 (0.286)	8.633 (10.32)	-	0.97 - 0.99	0.067 - 0.912	0.126 (0.008)	20.92 (0.096)	-	0.99 - 1.00	0.000 - 0.152	0.659 (0.185)	-	2.46 (0.795)	0.98 -0.99	0.122 - 0.146
S. Typhimurium 7162	0.433 (0.246)	6.843 (9.650)	-	0.97 - 1.00	0.027 - 0.656	0.125 (0.014)	25.81 (5.560)	-	0.99 - 1.00	0.000 - 0.075	0.577 (0.139)	-	3.14 (0.989)	0.98 - 1.00	0.063 - 0.172
***S.* Typhimurium 4954**	0.260 (0.066)	9.339 (4.825)	-	0.99 - 1.00	0.086 - 0.204	0.159 (0.047)	18.14 (16.54)	-	0.92 - 1.00	0.000 - 0.510	0.585 (0.175)	-	3.15 (0.847)	0.97 - 1.00	0.084 - 0.171
***S.* Enteritidis 4300**	0.283 (0.129)	3.339 (3.049)	-	0.93 - 1.00	0.133 - 0.632	0.212 (0.034)	21.74 (5.730)	-	0.99 - 1.00	0.000 - 0.257	0.881 (0.172)	-	2.32 (1.054)	0.96 - 1.00	0.069 - 0.367
***S.* Hadar**	0.186 (0.021)	9.583 (0.962)	-	0.98 - 1.00	0.017 - 0.106	0.184 (0.012)	27.73 (5.766)	-	0.97 - 0.99	0.462 - 0.107	0.748 (0.191)	-	2.82 (0.581)	0.97 - 1.00	0.085 - 0.325
***S.* Derby**	0.177 (0.047)	4.347 (7.529)	-	0.94 - 1.00	0.035 - 0.496	0.160 (0.060)	20.06 (13.43)	-	0.99 - 1.00	0.024 - 0.302	0.568 (0.085)	-	2.66 (0.622)	0.97 - 0.98	0.179 - 0.227
***S.* Infantis**	0.257 (0.051)	5.958 (5.025)	-	0.98 - 1.00	0.000 - 0.292	0.179 (0.029)	21.43 (7.432)	-	0.99 - 1.00	0.192 - 0.267	0.913 (0.320)	-	2.26 (0.446)	0.96 - 0.97	0.252 - 0.383
***S.* Virchow**	0.250 (0.070)	4.865 (5.214)	-	0.99 - 1.00	0.067 - 0.319	0.143 (0.035)	15.88 (5.779)	-	0.98 - 0.99	0.084 - 0.348	0.647 (0.069)	-	2.13 (0.229)	0.98 - 0.99	0.140 - 0.206
***S.* Gallinarum**	0.153 (0.023)	1.927 (1.670)	-	0.96 - 0.99	0.078 - 0.303	0.159 (0.049)	24.21 (6.453)	-	0.99 - 1.00	0.071 - 0.228	0.633 (0.327)	-	2.12 (0.214)	0.96 -0.98	0.221 - 0.353
***S.* Senftenberg**	0.297 (0.203)	7.615 (6.951)	-	0.95 - 0.99	0.100 - 0.483	0.122 (0.028)	27.29 (1.240)	-	0.99 - 1.00	0.113 - 0.132	0.894 (0.133)	-	3.24 (0.971)	0.97 - 0.99	0.109 - 0.261
***S.* Saintpaul**	0.217 (0.006)	7.197 (2.871)	-	0.99 - 1.00	0.013 - 0.115	0.105 (0.007)	15.72 (0.914)	-	0.99 - 1.00	0.142 - 0.188	0.537 (0.094)	-	3.51 (0.389)	0.96 - 0.98	0.087 - 0.360
***S.* Stanley**	0.303 (0.145)	9.762 (3.241)	-	0.99 - 1.00	0.054 - 0.269	0.129 (0.038)	11.81 (0.415)	-	0.98 - 1.00	0.140 - 0.327	0.522 (0.021)	-	2.44 (0.760)	0.94 - 0.98	0.148 - 0.428
***S.* Newport**	0.200 (0.035)	0.136 (0.132)	-	0.99 - 1.00	0.078 - 0.280	0.112 (0.024)	8.489 (8.485)	-	0.96 - 0.99	0.185 - 0.470	0.819 (0.345)	-	2.50 (0.522)	0.97 - 0.99	0.071 - 0.467

Table 1. Continuation

	Heat					HHP					PEFl				
	K_{max} (min^{-1})	S_l (min)	N_{res} (CFU/ml)	R^2	RMSE	K_{max} (min^{-1})	S_l (min)	N_{res} (CFU/ml)	R^2	RMSE	K_{max} (min^{-1})	S_l (min)	N_{res} (CFU/ml)	R^2	RMSE
***S.* Typhimurium SL1344**	2.823 (0.261)	0.536 (0.279)	-	0.95 - 0.99	0.120 - 0.580	0.027 (0.522)	-	-	0.94 - 0.98	0.177 - 0.316	0.102 (0.018)	-	4.356 (0.226)	0.99 - 1.00	0.031 - 0.251
***S.* Typhimurium 443**	1.540 (0.358)	0.391 (0.525)	-	0.97 - 0.98	0.149 - 0.250	1.379 (0.932)	2.139 (1.174)	-	0.97 - 0.99	0.113 - 0.253	0.109 (0.012)	-	4.271 (0.529)	0.99 - 1.00	0.076 - 0.128
***S.* Typhimurium 722**	1.903 (0.272)	0.291 (0.329)	-	0.97 - 0.99	0.161 - 0.312	0.913 (0.262)	1.502 (1.239)	-	0.94 - 0.99	0.004 - 0.009	0.137 (0.043)	-	4.367 (0.099)	0.99 - 1.00	0.055 - 0.270
S. Typhimurium 7162	1.812 (0.094)	0.307 (0.457)	-	0.92 - 0.99	0.185 - 0.574	0.922 (0.145)	1.304 (0.525)	-	0,92 - 0,99	0.013 - 0.134	0.106 (0.005)	-	4.483 (0.476)	0.99 - 1.00	0.040 - 0.073
***S.* Typhimurium 4954**	1.507 (0.210)	0.094 (0.163)	-	0.96 - 0.98	0.191 - 0.338	1.126 (0.674)	1.639 (1.147)	-	0.92 - 0.98	0.029 - 0.231	0.103 (0.019)	-	4.174 (0.346)	0.99 - 1.00	0.028 - 0.183
***S.* Enteritidis 4300**	1.893 (0.316)	0.028 (0.042)	-	0.97 - 0.99	0.146 - 0.377	0.766 (0.233)	0.170 (0.294)	-	0.94 - 0.95	0.380 - 0.419	0.102 (0.018)	-	4.353 (0.204)	0.99 - 1.00	0.062 - 0.216
***S.* Hadar**	1.883 (0.336)	0.438 (0.290)	-	0.99 - 1.00	0.142 - 0.229	0.962 (0.407)	1.322 (1.258)	-	0.92 - 094	0.470 - 0.561	0.168 (0.059)	-	3.542 (0.700)	0.93 - 1.00	0.159 - 0.515
***S.* Derby**	2.540 (0.540)	0.815 (0.363)	-	0.93 - 0.99	0.087 - 0.405	0.887 (0.220)	1.316 (1.874)	-	0.95 - 0.99	0.147 - 0.325	0.152 (0.035)	-	3.645 (0.392)	0.98 -1.00	0.058 - 0.455
***S.* Infantis**	2.472 (0.234)	0.099 (0.171)	-	0.98 - 0.99	0.229 -0.328	1.146 (0.292)	1.555 (1.255)	-	0.95 - 0.98	0.337 - 0.418	0.129 (0.039)	-	3.780 (0.379)	0.98 - 1.00	0.079 - 0.388
***S.* Virchow**	2.043 (0.132)	0.155 (0.268)	-	0.96 - 0.99	0.128 - 0.557	1.006 (0.424)	1.985 (2.780)	-	0.94 - 0,99	0.294 - 0.351	0.181 (0.027)	-	3.775 (0.278)	0.98 - 1.00	0.087 - 0.409
***S.* Gallinarum**	1.693 (0.434)	0.980 (0.638)	-	0.96 - 0.99	0.120 - 0.349	0.635 (0.133)	-	-	0.96 - 0.98	0.076 - 0.339	0.180 (0.067)	-	3.059 (0.696)	0.97 - 0.99	0.289 - 0.501
***S.* Senftenberg**	0.196 (0.015)	-	-	0.96 - 0.98	0.028 - 0.146	0.650 (0.160)	-	-	0.94 - 0.97	0.045 - 0.234	0.149 (0.052)	-	3.772 (0.428)	0.99 - 1.00	0.022 - 0.285
***S.* Saintpaul**	3.293 (1.104)	0.123 (0.213)	-	0.96 - 0.99	0.223 - 0.535	1.032 (0.698)	1.099 (1.011)	-	0.93 - 0.96	0.063 - 0.336	0.114 (0.014)	-	4.309 (0.202)	0.99 - 1.00	0.099 - 0.223
***S.* Stanley**	1.923 (0.174)	0.330 (0.572)	-	0.96 - 0.97	0.300 - 0.664	0.977 (0.362)	1.494 (1.357)	-	0.93 - 0.97	0.000 - 0.268	0.096 (0.006)	-	4.005 (0.620)	0.99 - 1.00	0.001 - 0.082
***S.* Newport**	2.483 (0.441)	0.480 (0.733)	-	0.93 - 0.99	0.219 - 0.591	0.680 (0.148)	0.748 (1.296)	-	0.93 - 0.99	0.068 - 0.446	0.178 (0.008)	-	4.223 (0.177)	0.95 - 0.99	0.216 - 0.535

* Values in parentheses represent the SD of the means.

Table 1. Continuation

	UV-C				
	K_{max} (min^{-1})	S_l (min)	N_{res} (CFU/ml)	R^2	RMSE
***S.* Typhimurium SL1344**	0.070 (0.002)		-	0.95 - 0.96	0.398 - 0.413
***S.* Typhimurium 443**	0.074 (0.003)	5.084 (4.489)	-	0.99 - 1.00	0.011 - 0.035
***S.* Typhimurium 722**	0.078 (0.007)	-	-	0.98 - 1.00	0.081 - 0.210
S. Typhimurium 7162	0.074 (0.010)	2.677 (4.637)	-	0.98 - 0.99	0.145 - 0.170
***S.* Typhimurium 4954**	0.083 (0.022)	10.64 (9.256)	-	0.99 - 1.00	0.062 - 0.147
***S.* Enteritidis 4300**	0.080 (0.006)	3.763 (6.518)	-	0.92 - 1.00	0.087 - 0.196
***S.* Hadar**	0.093 (0.004)	12.57 (14.46)	-	0.92 - 0.98	0.294 - 0.433
***S.* Derby**	0.071 (0.008)	-	-	0.92 - 0.92	0.623 - 0.723
***S.* Infantis**	0.067 (0.012)	-	-	0.93 - 0.99	0.137 - 0.232
***S.* Virchow**	0.080 (0.014)	3.739 (5.287)	-	0.98 - 1.00	0.102 - 0.148
***S.* Gallinarum**	0.112 (0.026)	8.086 (2.254)	-	0.93 - 0.99	0.353 - 0.685
***S.* Senftenberg**	0.090 (0.007)	5.439 (4.841)	-	0.99 - 1.00	0.048 - 0.090
***S.* Saintpaul**	0.122 (0.048)	13.26 (11.49)	-	0.95 - 1.00	0.129 - 0.463
***S.* Stanley**	0.098 (0.057)	6.793 (11.77)	-	0.95 - 0.99	0.133 - 0.275
***S.* Newport**	0.102 (0.017)	3.463 (5.999)	-	0.96 - 0.98	0.392 - 0.496

* Values in parentheses represent the SD of the means.

Table 2. Pearson correlation coefficient values calculated for the *2D* resistance values of the 15 *Salmonella* strains to the different environmental stresses and non-thermal food preservation technologies studied. Values in parentheses correspond to the p-value.

	pH	**H_2O_2**	**NaCl**	**Heat**	**HHP**	**PEF**	**UV**
pH		-0.043 (0.883)	0.128 (0.662)	0.233 (0.423)	0.181 (0.537)	-0.340 (0.234)	-0.128 (0.663)
H_2O_2	-0.043 (0.883)		**0.565 (0.035)**	0.061 (0.837)	0.075 (0.799)	0.043 (0.885)	-0.176 (0.548)
NaCl	0.128 (0.662)	**0.565 (0.035)**		0.099 (0.735)	0.233 (0.422)	0.446 (0.110)	0.233 (0.423)
Heat	0.233 (0.423)	0.061 (0.837)	0.099 (0.735)		-0.040 (0.892)	-0.061 (0.836)	-0.043 (0.885)
HHP	0.181 (0.5357)	0.075 (0.799)	0.233 (0.422)	-0.040 (0.892)		-0.184 (0.528)	-0.403 (0.153)
PEF	-0.340 (0.234)	0.043 (0.885)	0.446 (0.110)	-0.061 (0.836)	-0.184 (0.528)		**0.558 (0.038)**
UV	-0.128 (0.663)	-0.176 (0.548)	0.233 (0.423)	-0.043 (0.885)	-0.403 (0.153)	**0.558 (0.038)**	

*Graphical Abstract

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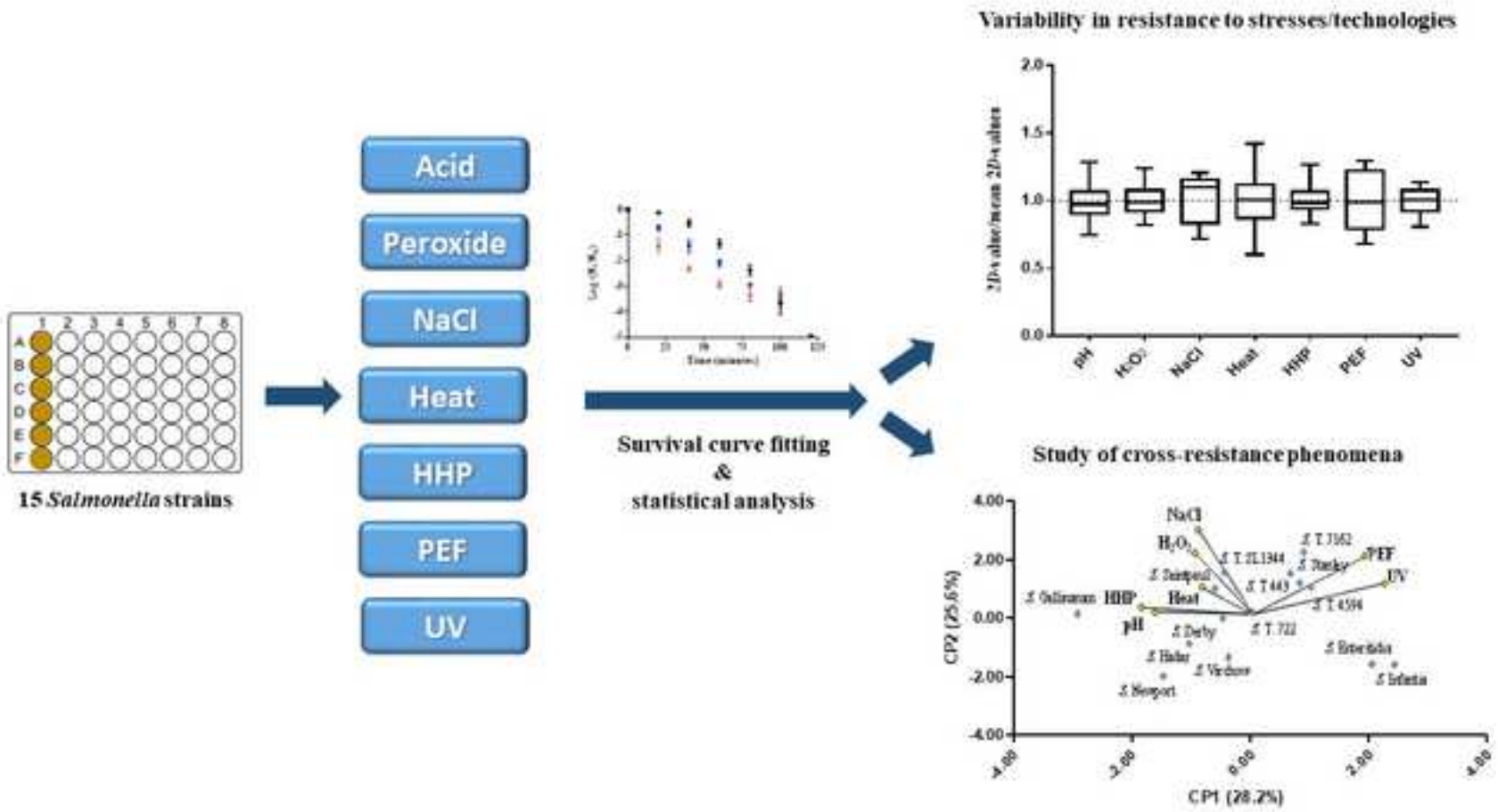

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