Survival characteristics of monophasic Salmonella Typhimurium 4,[5],12:i:- strains derived from pig feed ingredients and compound feed

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Abstract
The presence of Salmonella in animal feed or feed ingredients at the feed mill or on-farm is a cause for concern, as it can be transmitted to food-producing animals and subsequently to humans. The objective of this study wasto determine the survival characteristics of five feed ingredient- and feed-derived monophasic Salmonella Typhimurium 4,[5],12:i:- strains. The first part of the study investigated thermal inactivation using an immersed heating coilapparatus. A Weibull model provided a good fit, with low RMSE values (0.04–0.43) and high R2 values (0.93–0.99) obtained. There was considerable inter-strain variation in heat resistance, with D-values ranging from 397.83 to689 s at 55 °C, 11.35– to 260.95 s at 60 °C and 1.12 to 6.81 at 65 °C. Likewise, z-values ranged from 2.95 to 5.44 °C. One strain demonstrated a significantly higher thermal tolerance, even though it had been isolated from a mealfeed. However, overall the strains investigated do not appear to be that much more heat resistant than Salmonella previously studied. The second part of this study involved assessing the ability of the five Salmonella strains tosurvive during storage over a 28-day period in pelleted weaner pig feed treated with 0.3% sodium butyrate. While a mean reduction in the Salmonella count of 0.79 log10 CFU was seen in the treated feed during the storage period, areduction (albeit only 0.49 log10 CFU) was also observed in the control feed. Although there was no overall effect of treatment, sodium butyrate resulted in reductions in Salmonella counts of 0.75 and 0.22 log10 CFU at days 14 and 24of feed storage, respectively but at the end of the 28-day storage period counts were 0.25 log10 CFU higher in the treated feed. Therefore, the sodium butyrate used appears unsuitable as an agent for feed treatment perhaps due tothe protective coating on the particular feed additive used. Overall, the results of this study enhance knowledge about the behaviour and survival characteristics of monophasic Salmonella. Typhimurium 4,[5],12:i:- strains in animalfeed and may assist the feed industry and pig producers in implementing effective intervention strategies for their control.

Keywords: Thermal inactivation; Storage; Pig feed; D-value; z-value

Abbreviations: RMSE, root mean squared error; CFU, colony forming units; aw, water activity; EFSA, European Food Safety Authority; TSA, tryptone soya agar; TSB, tryptone soya broth; MRD, maximum recovery diluent; XLD, xylose lysine deoxycholateagar; SAS, Statistical Analysis System

1 Introduction

Salmonella is a leading cause of gastroenteritis in humans and continues to be of significant public health concern (Majowicz et al., 2010). Animal feed is a well-documented vector for the entry of Salmonella into the food chain (EFSA, 2008; Crump, Griffin, & Angulo, 2002). Recovery of Salmonella from animal feed and ingredients is not uncommon in the EU. In 2008, prevalence in compound feed was reported to range from 0 to 6% (EFSA, 2008). More recent studies havefound similar contamination rates i.e. 3.2% in a Spanish study of different animal feeds (Torres, Piquer, Algarra, de Frutos, & Sobrino, 2011), 1.5% in a UK poultry feed study (Davies & Wales, 2010) and 0.95% in a survey of pig feed conductedby our group in Ireland (Burns et al., 2015). The latter isolated monophasic variants of Salmonella Typhimurium (4,[5],12:i:-) (i.e. those that lack the expression of flagellar Phase 2 antigens) from pig feed ingredients and compound pig feed(Burns et al., 2015). This is a cause for concern considering that the occurrence of monophasic variants in human cases of illness in the EU has increased rapidly from 360 in 2007– to 5932 in 2012, along with the number of countries reportingthis serotype (EFSA, 2014). It is also worrying that this serotype has been isolated from a wide range of animals and foods of animal origin, with pigs appearing to be a common reservoir (Hopkins et al., 2010).The physiology of Salmonella lends itself well to survival on a wide range of feeds and feed ingredients (Maciorowski, Herrera, Jones, Pillai, & Ricke, 2007), as it has developed diverse mechanisms to survive at low water activity (aw),low pH and low concentrations of available carbon, nitrogen, and phosphorus, the latter by means of a starvation stress response (Spector, 1998) and a stationary phase acid tolerance response, respectively (Lee, Slonczewski, & Foster, 1994).One study reported Salmonella survival for 26 months in poultry feed (Davies & Wray, 1997), while another demonstrated survival times of up to 3 years in pig and poultry feeds (D'Aoust & Sewell, 1986). Survival may be influenced by factorssuch as the serovar/strain of Salmonella, growth phase of cells, presence of antimicrobials, aw, feed structure, acidity and storage temperature (Andino & Hanning, 2015; EFSA, 2008); these may enhance or reduce survival, depending onwhether conditions are favourable or not. Certain serotypes are isolated more often from feed and feed mills, as a result of their physiology and in particular their ability to survive in dry environments (Binter et al., 2011). However, due to therecent emergence of monophasic variants of S. Typhimurium, only a few studies to date have investigated their phenotypic traits (Bugarel et al., 2012; Mandilara, Lambiri, Polemis, Passiotou, & Vatopoulos, 2013; Seixas, Machado, Bernardo,Vilela, & Oliveira, 2014). As a result, only limited information is available on their survival characteristics, with no data available for survival in animal feed.Control of Salmonella spp in animal feed may require multiple interventions, such as heat treatment, irradiation and chemical treatment with organic acids or their salts, formaldehyde, terpenes or essential oils (Himathongkham, Pereira,& Riemann, 1996; Jones, 2011; Koyuncu et al., 2013; Wales, Allen, & Davies, 2010). The use of heat treatment to accomplish microbial population reductions is the most common and is based on the destructive effects of appropriatetime–-temperature combinations. In Ireland, any feed intended for poultry must be subjected to heat treatment to produce a minimum temperature of 75 °C at the core for 1 min as specified by S.I. No. 364/1991. A guidance note for the control ofSalmonella in pigs issued by the Department of Agriculture, Food and the Marine (Anon, 2007) specifies a similar heat treatment for pig feed, although this is not a legal requirement. Excessive heating during processing, however, can lead todestruction of essential amino acids. With the exception of S. Senftenberg and some other heat resistant serotypes, D-values (decimal reduction times i.e. the time taken at a given temperature to produce a 10-fold reduction in viable cellnumbers) for Salmonella are typically 0.18–10 min at 60 °C and <1 min at 70 °C (Scientific Committee on Veterinary Measures Relating to Public Health, 2003). Typical z-values (change in temperature necessary to produce a 10-fold reduction inthe D-value) range from 4 to 5 °C (Scientific Committee on Veterinary Measures Relating to Public Health, 2003). However, factors such as aw, fat, carbohydrate and protein content, presence of salts, pH, number of organisms, inhibitorycompounds, temperature and duration of heating may all influence the effectiveness of heat treatments (Olsen & Nottingham, 1980). The advantage of chemical treatment is that residual effects contribute to the control of Salmonella on storedfeed, (Koyuncu, Andersson, & Haggblom, 2010) and in the gastrointestinal tract of the animal post-consumption (Berge & Wierup, 2012). However, one study suggests that they interfere with Salmonella detection rather than killing the organism(Carrique-Mas, Bedford, & Davies, 2007).The objective of this study was to examine the survival of feed- and feed ingredient-derived monophasic variants of S. Typhimurium in terms of their thermal tolerance and ability to persist on stored feed treated with a sodium butyratefeed additive, as little is known about the survival characteristics of these variants, particularly in animal feed.

2 Material and methods

2.1 Bacterial strains, culture conditions and preparation of inocula

The five monophasic S. Typhimurium 4,[5],12:i:- strains used in this study were selected as they were the only Salmonella isolates recovered from feed mill samples taken in a previous study (Burns et al., 2015). Three of the isolates were recovered fromcommercial compound feed sampled from storage bins at the feed mills (one from finisher meal sampled from one feed mill and one each from dry sow meal and dry sow pelleted feed, both sampled at the same feed mill). The remaining two isolates originated infeed ingredients sampled at mill intakes (one from wheat sampled at another commercial mill and one from soybean meal sampled at a home compounder). Details of these five strains are listed in Table 1. All were maintained on Protect™ cryoprotectant beads(Technical Service Consultants Limited, Lancashire, UK) at −80 °C. Each was resuscitated by streaking a Protect™ bead onto tryptone soya agar (TSA; Oxoid, Basingstoke, UK) and incubating at 37 °C for 22 ± 2 h. A single colony was then inoculated into 25 mLtryptone soya broth (TSB; Oxoid) and incubated at 37 °C for 18 ± 2 h. These cultures were centrifuged at 10,000 g for 10 min at 4 °C and the supernatant discarded. The pellet was then re-suspended in 25 mL TSB, creating an inoculum containing ~8–9log10 CFU/mL. Inocula were stored at 4 °C for a maximum of 1 h prior to use.

Table 1 Characteristics of monophasic Salmonella Typhimurium 4,[5],12:i-strains used in the present study.

Teagasc strain ID	Origin[a]	Serotype	Antibiotic resistance profile[b]	MLVA no. of repeats					MLVA type
				STTR9	STTR5	STTR6	STTR10	STTR3	
2278	Finisher meal feed (Mill C)	4,5,12:i	ASSuT	3	11	9	NA	0211	B
2888	Soybean meal (Mill E)	4, 12:i:-	TGm	3	11	9	NA	0211	B
3836	Wheat (Mill B)	4, 12:i:-	ACSSuTTmGm	3	13	16	NA	0211	A
3844	Dry sow meal feed (Mill D)	4, 12:i:-	ACSSuTTmGm	3	13	16	NA	0211	A
3845	Dry sow pelleted feed (Mill D)	4, 12:i:-	ACSTCpCe	3	13	16	NA	0211	A

a Isolates originated in pig feed and pig feed ingredients sampled at commercial feed mills (Mills B, C & D) and one home compounder (Mill E) (Burns et al., 2015).

b Ampicillin (A), Chloramphenicol (C), Trimethoprim/Sulfamethoxazole (Tm), Gentamicin (Gm), Nalidixic acid (Na), Sulfisoxazole (Su), Ciprofloxacin (Cp), Streptomycin (S), Tetracycline (T) and Ceftiofur (Ce).

2.2 Thermal inactivation experiments

Thermal inactivation experiments were carried out for each of the monophasic S. Typhimurium strains using an immersed heating coil apparatus (Sherwood instruments, Lynnwood, MA, USA). This apparatus, originally designed by Cole and Jones(1990), has a narrow bore stainless steel coil fully submerged in a thermostatically controlled water bath. The three treatment temperatures used were 55, 60 and 65 °C. The apparatus was adjusted to the target temperature and allowed to equilibrate for at least2 h prior to commencement of a run. In line with the manufacturer's recommendations, 10 mL of Salmonella inoculum (prepared as outlined in Section 2.1) was injected into the coil apparatus using a disposable syringe, and treated aliquots (400 µL) weredispensed automatically at pre-determined time intervals into sterile glass vials on a revolving carousel. The collection vials were pre-filled with 1.6 mL of cooled TSB to aid dilution. A flushing step was used between samples to remove sample which may haveremained at the tip of the coil tubing. An unheated aliquot of TSB was also collected before commencement of each thermal inactivation cycle to serve as the T0 sample. Samples were collected every 480 s at 55 °C, every 30 s at 60 °C and every 3s at 65 °C andimmediately cooled on ice. Salmonella was enumerated by preparing a 10-fold dilution series in maximum recovery diluent (MRD; Oxoid) and spread-plating 100 µL aliquots of appropriate dilutions in duplicate onto TSA. Aliquots were also spread-plated onto aselective agar, xylose lysine deoxycholate agar (XLD; Oxoid), in order to examine the level of sub-lethal injury caused by heating. Both XLD and TSA plates were incubated at 37 °C for 22 ± 2 h. Following incubation, colonies were counted to obtain the numberof surviving Salmonella cells at each time point for each temperature. Three replicates were performed for each Salmonella isolate at each temperature.

2.3 Survival of Salmonella in pig feed during storage

Commercially produced first stage weaner pig feed which had been finely ground and subsequently pelleted to a diameter of 3 mm was used. The feed was produced with and without supplementation with 0.3% sodium butyrate (Adimix®; Nutriad,Kasterlee, Belgium). This inclusion rate was recommended by the manufacturer. Prior to use, 90 g samples of each feed were taken and from these 25 g was confirmed as Salmonella-negative by analysing for the presence of Salmonella spp. according tostandard microbiological procedures (EN ISO 6579:2002/Cor 1:2004) with modified brilliant green agar (Oxoid) used for additional selective plating. Control and sodium butyrate-treated feed was then inoculated with each of the five monophasic S. Typhimuriumisolates in triplicate as follows. Feed (2 kg) was transferred to sterile 10 L stainless steel containers and 4.5 mL of Salmonella inoculum [prepared as outlined in Section 2.1, except that isolates were resuscitated from frozen stocks on plate count agar (Oxoid)] wasadded in order to give a final inoculum of ~4 log10 CFU/g feed. This was done using an atomizer followed by immediate mixing and a ~4 h post-inoculation drying period at room temperature (~20 °C). Preliminary tests had shown that, using this approach, theinoculum was evenly distributed in the feed (data not shown). Each stainless steel container was then stored at 10 °C (average temperature for Ireland over the last 3 years three years as calculated from Met Eireann data recorded at Dublin airport) in order tosimulate environmental conditions for handling and storage of pig feed on Irish commercial pig farms. Since pig feeds are generally stored for less than 1 month from production to consumption, a period of 28 days was chosen over which to evaluate the survivalof Salmonella. Duplicate 25 g samples of inoculated feed were sampled on day 0 and thereafter intermittently over the 28-day storage period. The samples were homogenized for 90sec with 225 mL of buffered peptone water (Oxoid) in a stomacher at normalspeed. Salmonella was enumerated in these samples by making 10-fold serial dilutions of the suspension in sterile tubes containing 9 mL of MRD. Aliquots (0.1 mL) from each dilution (10−1 to 10−3) were spread-plated on XLD agar. After incubating plates at37 °C for 24 ± 2 h, presumptive Salmonella colonies were enumerated. At each time point, up to 5 colonies per XLD plate were confirmed as Salmonella using a Salmonella latex agglutination kit (Oxoid). The aw values of all samples were measured using anAqualab model CX-2 water activity meter (Labcell, Alton, UK), calibrated daily using distilled water (aw = 1.000 ± 0.003) and a saturated solution of sodium chloride (aw = 0.755 ± 0.001 at 20 °C). The pH of the buffered peptone water homogenate of all samples(prepared as outlined above) was measured using an Orion ROSS™ epoxy body, flat surface, combination pH electrode (Thermo Scientific, Beverly, USA).

2.4 Statistical analysis

The thermal inactivation kinetics of the monophasic S. Typhimurium 4,[5],12:i-strains were determined using a regression analysis of the microbial inactivation data. The Microsoft Excel Addin tool, GInaFiT, was employed to obtain the a D-value and shape factor (β) (Geeraerd, Valdramidis, & Van Impe, 2005) by fitting microbial inactivation data to the Weibull model [Eq-1]

$$\log(Nt) = \log(Ne) - \left[\frac{t}{D}\right]^{\beta}$$

Where Nt (CFU/mL) was the number of microorganisms at time t (min), N0 (CFU/mL) the initial number of microorganisms, D (min) the time for the first decimal reduction and β [-] the scale and shape of the inactivation curve. For evaluation of the fitting capacity of the models the statistical criterion of the adjusted coefficient of multiple determination R2 adj and the root mean squared error (RMSE) was used.

$$R^2_{adj} = 1 - \left[\frac{nt - 1}{nt - np}\right] \cdot \frac{SSE}{SSTO}$$

Herein, *SSTO* is the total sum of squared errors $\sum (yi - \overline{y})^2$ and SSE the sum of squared errors $\sum (yexp(ti) - y(ti, pls))$.

$$RMSE = \sqrt{\frac{\sum_{i=1}^{nt} (yexp(ti) - y(ti, pls))^2}{nt - np}}$$

where $y_{exp}(t)$ denoted the experimental observations, $y(t,p_{ls})$ the predicted values, n_t the total number of data points, n_p the number of estimated model parameters.

The z-value was calculated by using the [Eq-4].

$$z\ value = \frac{T2 - T1}{\log D1 - \log D2}$$

where D1 and D2 are decimal reduction time (min) at temperature T1 and T2 (°C), respectively.

For the survival of Salmonella in pig feed, the data were analysed as a three-way factorial combination of treatment, strain and day. The variables tested were strains (2278, 2888, 3836, 3844 and 3845), feed treated with and without sodium butyrate and number of days (0–28). Both the thermal inactivation and feed storage data were analysed using the mixed procedure of Statistical Analysis System (SAS; SAS Institute Inc., 2011). Means comparisons were carried out to describe significant effects and a Tukey adjustment was used for multiple comparisons. Residual checks were made to ensure that the assumptions of the analysis were met and, where appropriate, the response was log-transformed. Statistical significance was assumed at $P < 0.05$.

3 Results

3.1 Thermal inactivation of monophasic S. Typhimurium strains

The thermal inactivation curves for the five feed- and feed ingredient-derived monophasic S. Typhimurium 4,[5],12:i:- strains at temperatures of 55, 60 and 65 °C following recovery on XLD and TSA are shown in Figs. 1–3. The Weibull model was shownto be a good fit to the survivor curves, with R2 values ranging from 0.92 to 0.99 and small RMSE values i.e. ranging from 0.04 to 0.43 at all temperatures (Table 2). There was considerable variation noted in the shape of the survivor curves at each temperature, asdescribed by the shape factor (β). When β = 1 it indicates a linear curve, when β > 1 the curves have a concave, downward shape, indicating the presence of shoulders (population surviving longer at the start of heating) and when β is < 1 the survivor curveshave an upward concavity, indicating a tailing or resistant population at the end of thermal treatment. In general, shoulder populations (β > 1) were more common at 55 and 60 °C and tailing (β < 1) was more common at 65 °C (Table 2). However, there was interstrainvariation in the β values obtained at each temperature; for example, when recovered on TSA, strain 3845 had a higher β value than three of the other strains (P < 0.05) at 55 °C whereas at 60 and 65 °C strain 2278 had a higher β value than all otherstrains (P < 0.05) (Table 2). Strain 2278 in particular showed considerable tailing at 55 °C and shoulders at 60 °C (Figs. 1 and 2). For some strains there were significant differences in the β values obtained when XLD was used as recovery medium versus TSAand, in general, the β values were lower for XLD than TSA at 60 and 65 °C (P < 0.05).

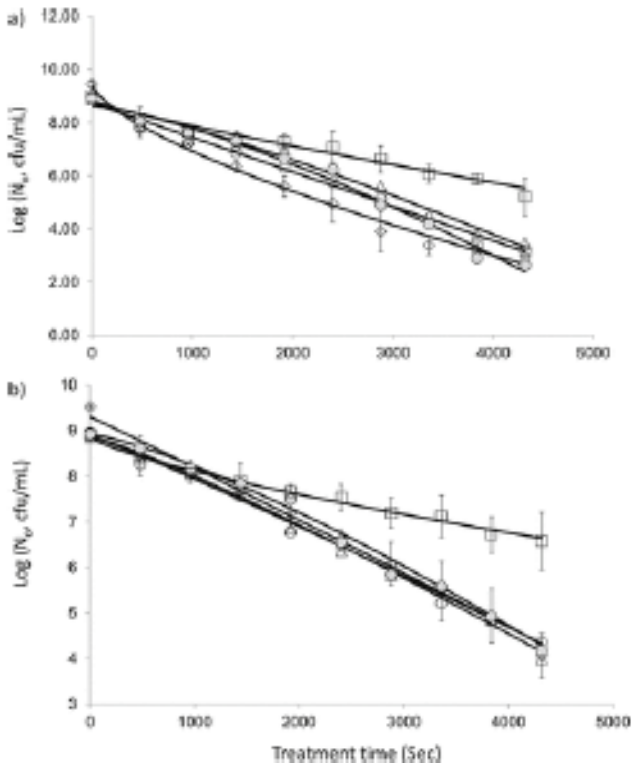

Fig. 1 Thermal inactivation curves at 55 °C for monophasic Salmonella Typhimurium 4,[5],12:i-strains [(□) 2278; (◊) 2888; (▵) 3836; (○) 3844, (●) 3845] plated on XLD (a) and TSA (b) fitted to Weibull model. Values are the mean of 3 replicates, with SE indicated by error bars. Log (No, CFU/mL):
Log value of initial count.

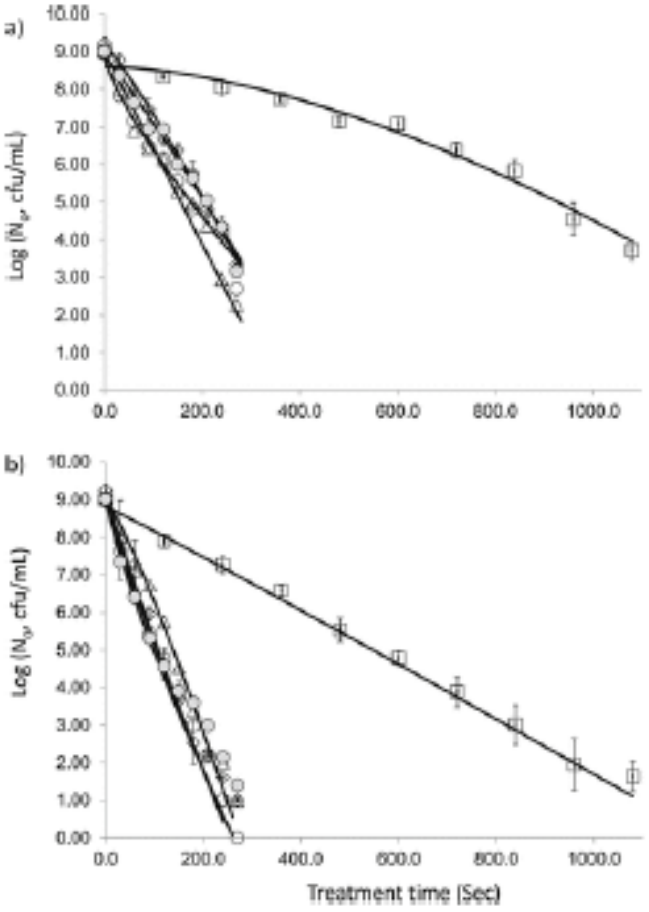

Fig. 2 Thermal inactivation curves at 60 °C for monophasic Salmonella Typhimurium 4,[5],12:i-strains [(□) 2278; (◊) 2888; (▵) 3836; (○) 3844, (●) 3845] plated on XLD (a) and TSA (b) fitted to Weibull model. Values are the mean of 3 replicates, with SE indicated by error bars. Log (No, CFU/mL): Log value of initial count.

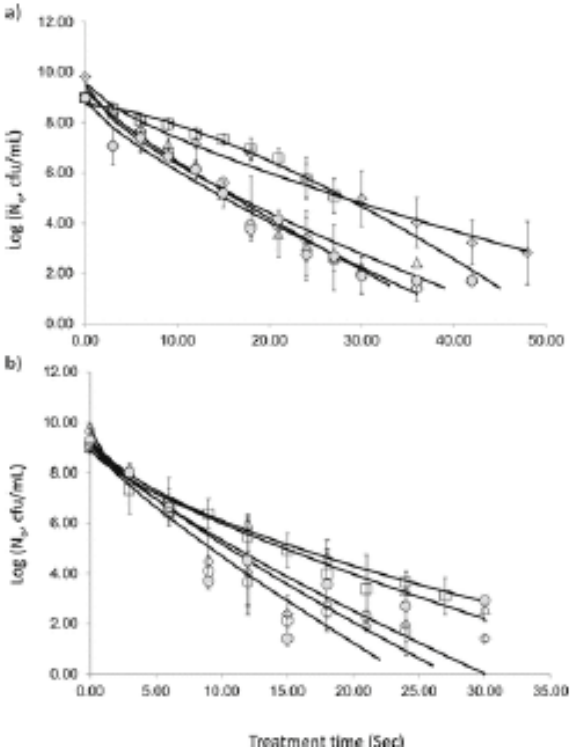

Fig. 3 Thermal inactivation curves at 65 °C for monophasic Salmonella Typhimurium 4,[5],12:i-strains [(□) 2278; (◊) 2888; (▵) 3836; (○) 3844, (●) 3845] plated on XLD (a) and TSA (b) fitted to Weibull model. Values are the mean of 3 replicates, with SE indicated by error bars. Log (No, CFU/mL):
Log value of initial count.

Table 2 Weibull model parameters and statistical parameters for the survival of monophasic Salmonella Typhimurium 4,[5],12:i-strains following thermal inactivation using TSA (tryptone soya agar) and XLD (xylose lysine deoxycholate agar) as recovery media.

	TSA					XLD				
Strain	Log_{10} CFU/mL (N_o)[1]	D-value (sec)[2]	β[3]	RMSE[4]	R^2[5]	Log_{10} CFU/mL (N_o)	D-value (sec)	β	RMSE	R^2
Temperature of 55 °C										
2278	8.88 (0.19)^{b}	456.8 (168.99)^{b}	0.73 (0.19)^{r}	0.04	0.95	8.84 (0.15)^{b}	397.22 (83.02)^{bc}	0.85 (0.05)^{rv}	0.12	0.93
2888	9.30 (0.11)^{a}	397.83 (65.57)^{bA}	1.02 (0.05)^{vX}	0.08	0.98	9.44 (0.06)^{a}	74.71 (11.05)^{de}	0.68 (0.01)^{rY}	0.99	0.98
3836	8.88 (0.18)^{b}	544.05 (54.66)^{ab}	1.15 (0.01)^{wx}	0.07	0.98	8.67 (0.13)^{c}	565.83 (131.42)^{ab}	1.24 (0.18)^{w}	0.14	0.98
3844	8.88 (0.19)^{b}	488.63 (70.10)^{b}	1.08 (0.04)^{v}	0.08	0.97	8.75 (0.12)^{bc}	332.45 (162.05)^{c}	1.00 (0.20)^{v}	0.11	0.99
3845	8.92 (0.22)^{b}	689.0 (147.96)^{a}	1.29 (0.06)^{wX}	0.07	0.98	8.64 (0.07)^{c}	603.7 (48.79)^{a}	1.36 (0.09)^{wW}	0.15	0.98
Temperature of 60 °C										
2278	8.60 (0.20)^{c}	260.95 (25.53)^{aA}	1.67 (0.12)^{wW}	0.16	0.97	8.82 (0.08)^{b}	66.79 (21.77)^{de}	1.03 (0.15)^{wx}	0.12	0.99
2888	9.18 (0.16)^{a}	27.35 (8.61)^{a}	1.12 (0.13)^{vW}	0.09	0.99	9.25 (0.25)^{a}	8.64 (2.99)^{b}	0.91 (0.14)^{vX}	0.34	0.98
3836	8.97 (0.05)^{b}	17.16 (2.98)^{b}	1.00 (0.09)^{v}	0.21	0.98	8.98 (0.40)^{ab}	21.49 (9.29)^{b}	1.19 (0.22)^{w}	0.21	0.99
3844	8.81 (0.07)^{b}	11.35 (2.99)^{b}	0.79 (0.16)^{v}	0.14	0.93	8.96 (0.17)^{ab}	9.02 (1.61)^{b}	0.90 (0.01)^{v}	0.20	0.99
3845	8.86 (0.12)^{b}	28.02 (1.99)^{bA}	1.10 (0.08)^{wW}	0.13	0.97	9.04 (0.08)^{ab}	6.31 (1.60)^{de}	0.81 (0.11)^{vX}	0.50	0.99
Temperature of 65 °C										
2278	8.74 (0.25)^{c}	6.81 (3.10)^{a}	1.50 (0.37)^{wW}	0.12	0.96	8.90 (0.21)^{c}	0.82 (0.38)^{a}	0.76 (0.10)^{wx}	0.17	0.97
2888	9.54 (0.12)^{a}	1.12 (0.47)^{bA}	0.72 (0.09)^{v}	0.21	0.96	9.23 (0.25)^{bc}	0.60 (0.53)^{de}	0.78 (0.28)^{wx}	1.29	0.92
3836	9.12 (0.17)^{b}	1.96 (0.53)^{bA}	1.04 (0.16)^{vW}	0.43	0.98	9.75 (0.03)^{a}	0.25 (0.12)^{de}	0.58 (0.06)^{vX}	0.19	0.98
3844	9.12 (0.07)^{b}	1.39 (0.26)^{bA}	0.91 (0.07)^{v}	0.15	0.98	9.14 (0.07)^{bc}	0.60 (0.27)^{de}	0.83 (0.17)^{w}	0.51	0.98
3845	9.01 (0.35)^{b}	1.18 (0.30)^{bA}	0.87 (0.10)^{v}	0.40	0.97	9.36 (0.35)^{b}	0.59 (0.28)^{de}	0.80 (0.21)^{wx}	0.84	0.98

1Log10 CFU/mL (No): Log value of initial count. Values are the means of three replicate experiments and values in parentheses are standard deviations.
2D-values: decimal reduction times i.e. the time taken at a given temperature to produce a 10-fold reduction in viable cell numbers. Values are the means of three replicate experiments and values in parentheses are standard deviations.
3RMSE: root mean square error.
4β: shape factor. Values are the means of three replicate experiments and values in parentheses are standard deviations.
5R2: regression coefficient.

abcd, wxyzFor each temperature, values within a column that share a common superscript are not significantly different at P < 0.05. Where no letters appear within a column there were no significant differences.
ABCD, WXYZWithin rows, values that share a common superscript are not significantly different at P < 0.05. Where no letters appear within a row there were no significant differences.

The decimal reduction (D) values calculated by the Weibull model for the five Salmonella strains at the three heating temperatures are presented in Table 2. There were some slight differences in initial counts for the different Salmonella strains at eachtemperature (P < 0.05). As the heating temperature increased, the D-values decreased. The results show that the recovery method had a significant impact, especially at the higher temperatures, with higher D values reported from TSA than XLD for strain 2888 at55 °C, strains 2278 and 3845 at 60 °C and all but one strain at 65 °C (P < 0.05). There was considerable inter-strain variation in D-values at all heating temperatures; at 55 °C the value obtained for strain 3845 was higher than that obtained for all except oneother strain using both recovery media (P < 0.05). While at 60 °C strain 2278 was the most heat resistant, with higher D-values than all but one other strain using TSA and all other strains using XLD (P < 0.05). The same was found at 65 °C when TSA was used,with 2278's D-value higher than that of all other strains (P < 0.05); however, using XLD its D-value was higher than that of only one other strain (P < 0.05).

Based on the z-values (change in temperature required for one log10 reduction in the D-value), strain 2278 was more thermotolerant than the other four strains based on the TSA recovery method (P < 0.001; Table 3), whereas strain 3845 was the leastresistant, with a lower z-value than all but one other strain (P < 0.001; Table 3). Using the XLD recovery method the most thermotolerant strain was 2888, with a higher z-value than two other strains (P < 0.05; Table 3), and the least was strain 3836, but its z-valuewas only lower than that of strain 2278 (P < 0.05; Table 3).