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Evaluation of the strain variability of *Salmonella enterica* acid and heat resistance

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ABSTRACT

The inherent acid and heat resistances of 60 *Salmonella enterica* strains were assessed in tryptone soy broth without dextrose acidified to pH 3.0 or heated at 57 °C. A total of 360 inactivation curves were generated. Regarding the acid challenge experiments, the inactivation rate (k_{acid}), estimated using the log–linear model, ranged from 0.47 to 3.25 h^{-1}. A log–linear model with a "survival tail" was used to describe the thermal inactivation of the strains, and the estimated inactivation rate (k_{heat}) ranged from 0.42 to 1.33 min^{-1}. The strain variability of k_{acid} was considerably higher than that of k_{heat} with the coefficient of variation of this kinetic parameter among the tested strains being 39.0% and 18.3%, respectively. No correlation was observed between the estimated k_{acid} and k_{heat} values of the 60 *S. enterica* strains. Furthermore, no trends among the tested strains related to origin, serotype or antibiotic resistance profile were evident. The present study is the first one to comparatively evaluate the inherent acid and heat resistance profiles of multiple *S. enterica* strains. Beyond their value in strain selection for use in food safety challenge studies, the collected data should be useful in describing and integrating the strain variability of *S. enterica* acid and heat resistance profiles in quantitative microbial risk assessment.

1. Introduction

Bacteria are continuously exposed to environmental stresses, both in their natural habitats as well as in the context of interventions/processes aiming at their inactivation. From a food microbiology perspective, "bacterial stress" is typically defined as a physical, chemical or nutritional condition which, by being insufficiently severe to assure bacterial death, results in sublethally injured bacteria. Nonetheless, the above definition may be expanded to include stresses of various levels of severity, the highest of which being conditions resulting in lethality experienced by the entire or a fraction of bacterial population (Wesche et al., 2009). Since thermal processing of foods constitutes the most traditional and widely practiced intervention for improving their microbiological safety and quality, exposure to high temperatures is one of the most common stresses experienced by foodborne pathogens. Furthermore, enterobacteria need to resist a series of adverse conditions within host organisms in order for their disease-causing activity to be elicited, one of which being the low-pH conditions encountered both in the host's stomach as well as within macrophage phagolysosomes upon invasion of the intestinal M cells (Audia et al., 2001). *Salmonella enterica* is a facultative intracellular pathogen known for its outstanding ability to cope successfully with environmental extremes encountered both in its natural habitat and within infected hosts (Foster and Spector, 1995). Indeed, assessing the relationship between the stress responses of this organism and its pathogenicity/virulence has been the objective of several studies (Humphrey et al., 1996; Bearson et al., 1998; Berk et al., 2005). Given the above and that the control of foodborne salmonellosis continues to be a great challenge for food microbiologists and food safety authorities (CDC, 2011; EFSA-ECDC, 2012), research data on the stress responses of *S. enterica* are still expected to be valuable for the purpose of food safety and public health advancement.

The acid stress responses of enterobacteria, particularly *Escherichia coli*, have been studied extensively, with various techniques and terminologies being used for their evaluation and description (Gorden and Small, 1993; Lin et al., 1995; Buchanan and Edelson, 1999a; Samelis et al., 2003; Koutsoumanis and Sofos, 2004). With regard to the acid survival systems that have been described for *S. enterica*, these include a pH-independent acid resistance (AR), expressed as part of a generalized stress resistance upon entry into stationary phase, as well as low-pH-inducible acid tolerance response (ATR) systems manifested both at the log- and stationary-phase levels (Lee et al., 1994; Lin et al., 1995; Bearson et al., 1998; De Jonge et al., 2003; Samelis et al., 2003; Koutsoumanis and Sofos, 2004). Concerning its thermal resistance, *S. enterica* is generally

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regarded as a relatively heat sensitive foodborne pathogen; however, its responses to thermal treatments have been shown to be highly variable (Doyle and Mazzotta, 2000). There are several research findings indicating that exposure of bacterial pathogens, including *S. enterica*, to low-pH conditions can offer them cross-protection against subsequent heat treatments (Lou and Yousef, 1996; Buchanan and Edelson, 1999b; Wilde et al., 2000; Álvarez-Ordóñez et al., 2008). Hence, the comparative evaluation of the acid and heat survival behaviors of *S. enterica* may provide information useful for elucidating the physiological responses of this organism.

Given that the inherent differences in microbial behavior among identically treated strains of the same species have been recognized as a significant source of biological variability in microbiological studies (Whiting and Golden, 2002), one of the most important factors that need to be considered when assessing the environmental stress responses of foodborne pathogens is the strain(s) tested. Considerable intra-species variability of survival under lethal acidic and/or heat conditions has been demonstrated for several foodborne pathogens including *E. coli* (Whiting and Golden, 2002), *Listeria monocytogenes* (Lianou et al., 2006) and *Staphylococcus aureus* (Rodríguez-Calleja et al., 2006). With reference to *S. enterica*, there were very early research data indicating the strain-dependent character of its thermal resistance (Ng et al., 1969), an observation which was further supported by the findings of subsequent investigations undertaken both in laboratory media and food products (Humphrey et al., 1995; Murphy et al., 1999; Juneja et al., 2001b; Quintavalla et al., 2001; Juneja et al., 2003; Alvarez et al., 2006). Considerable is the strain variability that has been observed with regard to the acid stress responses of this organism too (Bacon et al., 2003b; De Jonge et al., 2003; Samelis et al., 2003; Berk et al., 2005). Nevertheless, only few of the aforementioned studies have evaluated the survival behavior of multiple *S. enterica* strains specifically looking into their inherent acid/heat resistance profiles. Moreover, a positive correlation between the acid and heat stress responses of *S. enterica* has been implied (Humphrey et al., 1995; Berk et al., 2005); however, since this observation has been made only occasionally and with regard to certain strains of the pathogen, additional research is needed if such a correlation is to be ascertained.

The present study was conducted to evaluate the inactivation kinetic behavior of 60 *S. enterica* strains under acid and heat challenge conditions aiming at (i) characterizing the strain variability of these behaviors, and (ii) assessing the relationship between the inherent acid and heat resistance profiles of this pathogen. Potential trends among the tested strains related to serotype, origin and antibiotic resistance profile also were assessed, and the importance of the findings in describing and integrating strain variability in quantitative microbial risk assessment is discussed.

2. Materials and methods

2.1. S. enterica strains

Sixty strains were evaluated in the present study, primarily isolates of human or animal (almost exclusively bovine) origin, belonging to various serotypes commonly associated with human infections: Typhimurium (18 strains), Enteritidis (10 strains), Newport (9 strains), Heidelberg (8 strains), Montevideo (4 strains), Seftenberg (4 strains), Agona (3 strains), Infantis (3 strains) and Derby (1 strain). The above isolates were kindly provided by Dr. Martin Wiedmann (Cornell University, Ithaca, NY, USA), Dr. Constantin Genigeorgis (Aristotle University of Thessaloniki, Thessaloniki, Greece) and Dr. Daniil Sergelidis (Aristotle University of Thessaloniki). A table including the original designation, serotype, origin and source of each one of the 60 isolates has been provided previously (Lianou and Koutsoumanis, 2011a). Information regarding their antibiotic resistance profile was available (provided by the strains' donors) for 42 of the tested strains, with 18 of them being characterized as pansusceptible, five being resistant to one antibiotic, and 19 exhibiting resistance to more than one antibiotics (i.e., two to nine different antibiotics).

Stock cultures of the strains were kept frozen (−70 °C) onto Microbank™ porous beads (Pro-Lab Diagnostics, Ontario, Canada). Working cultures were stored refrigerated (5 °C) on tryptone soy agar (TSA; Lab M Limited, Lancashire, United Kingdom) slants and were renewed bimonthly. Strains were activated by transferring a loopful from the TSA slants into 10 ml of tryptone soy broth without dextrose (TSB-G; Lab M Limited) and incubating at 37 °C for 24 h.

2.2. Acid and heat challenge trials

One-milliliter portions of the 24-h cultures of each one of the tested strains were used to inoculate 9 ml of the challenge media to yield an inoculum concentration of approximately 8 log CFU/ml. For the acid challenge trials, the medium used was TSB-G acidified to pH 3.0 with HCl (min. 37%; Sigma–Aldrich, Seelze, Germany). The pH of the challenge medium was adjusted to this value using a digital pH meter with an epoxy refillable pH probe (Orion 3-Star Benchtop; Thermo Electron Corporation, Beverly, MA, USA), and was also measured after autoclaving to assure that its value was not considerably altered by the sterilization process. The acid challenge medium was dispensed in test tubes submerged in a water bath (Nüve Sanayi Malzemeleri Ve Ticaret A.Ş., Ankara, Turkey) and was prewarmed at 37 °C prior to inoculation with the strains' cultures, with this temperature being maintained throughout the acid challenge trials. With reference to the heat challenge trials, the medium used was TSB-G heated at 57 °C; the broth was dispensed in test tubes submerged in a water bath (Nüve Sanayi Malzemeleri Ve Ticaret A.Ş.) and inoculated with the strains' cultures when its temperature reached 57 °C. The temperature of the heat challenge medium was monitored throughout the duration of the trials using an electronic temperature-monitoring device (HOBO® U12-014 data logger equipped with a TC6-J thermocouple; Onset Computer Corporation, Bourne, MA, USA). Non-acidified and non-heated broth samples also were inoculated for determination of the initial bacterial populations. The total duration of the acid and heat challenge trials was 4 h and 20 min, respectively, and samples from each bacterial suspension were taken at 1-h and 5-min intervals, respectively. The selection of the above challenge durations and sampling time intervals was based on the findings of preliminary experiments. Appropriate serial decimal dilutions of the suspensions' samples in quarter strength Ringer's solution (Lab M Limited) were surface plated on TSA. Surviving populations were determined after incubation of plates at 37 °C for a total of 72 h (colonies were counted at 48 h of incubation and the counts were confirmed at 72 h). Three independent experiments were conducted for each strain and challenge condition ($n = 3$).

2.3. Microbial inactivation models

The microbiological data (log CFU per milliliter) derived from the challenge experiments were used to determine the corresponding inactivation kinetic parameters for each one of the *S. enterica* strains. More specifically, the acid challenge data were fitted to the log–linear model (Bigelow and Esty, 1920) which, for identification purposes, was reformulated as (Geeraerd et al., 2005):