Food Microbiology 62 (2017) 99–105

Contents lists available at ScienceDirect

Food Microbiology

journal homepage: www.elsevier.com/locate/fm

Short communication

Variability in the adaptive acid tolerance response phenotype of *Salmonella enterica* strains

Alexandra Lianou [a, 1], George-John E. Nychas [b], Konstantinos P. Koutsoumanis [a, *]

[a] Laboratory of Food Microbiology and Hygiene, Department of Food Science and Technology, School of Agriculture, Forestry and Natural Environment, Aristotle University of Thessaloniki, Thessaloniki 54124, Greece
[b] Laboratory of Microbiology and Biotechnology of Foods, Department of Food Science and Human Nutrition, School of Food, Biotechnology and Development, Agricultural University of Athens, Iera Odos 75, Athens 11855, Greece

ARTICLE INFO

Article history:
Received 30 June 2016
Received in revised form
18 September 2016
Accepted 3 October 2016
Available online 4 October 2016

Keywords:
Acid adaptation
Acid tolerance response
Salmonella enterica
Strain variability

ABSTRACT

The objective of this study was the assessment of the stationary-phase, low-pH-inducible acid tolerance response (ATR) of different *Salmonella enterica* strains. For this purpose, 30 strains of the pathogen were grown in tryptone soy broth in the absence (non-adapted cultures) and presence (1% w/v; acid-adapted cultures) of glucose, and then subjected to 4-h acid challenge trials at pH 3.0. Surviving populations of each strain were determined at 1-h intervals, and the Weibull model was fitted to the derived microbiological data. Extensive variability in the acid stress responses of the tested *S. enterica* strains was observed, with the total population reductions (log CFU/ml) attained in 4 h of acid challenge ranging from 0.9 to 5.5 and from 0.6 to 7.0 for the non-adapted and acid-adapted cultures, respectively. As demonstrated by the model scale parameter δ and shape parameter p, the effect of acid adaptation on the inactivation curves was strain-specific. Although acid adaptation resulted in enhanced acid survival for the majority of the tested strains, there were strains exhibiting similar or decreased acid resistance compared to their non-adapted counterparts. Moreover, acid adaptation appeared to decrease the strain variability of δ whereas increasing the strain variability of p: the coefficient of variation of δ among the tested strains was 97.2 and 54.9% for the non-adapted and acid-adapted cultures, respectively, while the corresponding values for p were 12.7 and 48.1%. The data of the present study, which is the first one to systematically evaluate the adaptive ATR of multiple *S. enterica* strains, clearly demonstrate that this phenotype (attempted to be induced by growing the pathogen in the presence of glucose) is strain-dependent.

1. Introduction

Although salmonellosis is known for more than 100 years, it is still a leading cause of foodborne illness in many parts of the world, with its incidence being largely unchanged the last decades (Scallan et al., 2011; CDC, 2015; EFSA-ECDC, 2015). According to surveillance data for 2014, a total of 88,715 confirmed salmonellosis cases were reported by 28 European Union (EU) Member States, resulting in an EU notification rate of 23.4 cases per 100,000 population. *Salmonella enterica* was the second (next to viruses) most frequently reported causative agent in foodborne outbreaks in the EU, being responsible for 20% of all outbreaks (EFSA-ECDC, 2015). Furthermore, *S. enterica* has been associated with the majority of reported fatalities both in the EU and the United States of America (CDC, 2015; EFSA-ECDC, 2015).

In addition to the ongoing strong association of *S. enterica* serotypes Typhimurium and Enteritidis with foodborne human infections (CDC, 2015; EFSA-ECDC, 2015) and the raised incidence of multidrug resistant phenotypes among strains of the organism (Hur et al., 2012), recent considerable changes in the trends of salmonellosis infections have augmented the interest of the scientific community in this pathogen. Alarming epidemiological changes observed during the last decade include the increased prevalence of other serotypes (e.g., Infantis and Javiana) (CDC, 2015) as well as the emergence, and potential association with human disease, of rare serotypes of the pathogen (Graziani et al.,

* Corresponding author.
E-mail address: kkoutsou@agro.auth.gr (K.P. Koutsoumanis).
[1] Present address: Agricultural University of Athens, Department of Food Science and Human Nutrition, Laboratory of Microbiology and Biotechnology of Foods, Athens, Greece.

http://dx.doi.org/10.1016/j.fm.2016.10.011
0740-0020/

2011; Brankatschk et al., 2012; Raguenaud et al., 2012). In this context, the control of *S. enterica* becomes even more challenging, while the need for exemplifying the biological variability underlying its behavior and virulence potential is accentuated.

Given that the ability of pathogenic bacteria to withstand adverse environmental conditions has long been identified as a presumptive determinant of their virulence potential, assessment of the stress tolerance responses of bacterial foodborne pathogens has been the objective of numerous research studies (Alvarez-Ordóñez et al., 2015). Regarding acid stress, a challenge commonly encountered by microorganisms in the food processing environment as well as in the host's gastrointestinal tract, the responses of enterobacteria have been studied extensively, and various techniques and terminologies have been used for their evaluation and description (Buchanan and Edelson, 1999; Samelis et al., 2003; Koutsoumanis and Sofos, 2004). The acid resistance systems that have been described for *S. enterica* include: (i) a pH-independent acid resistance (AR), which is expressed upon entry of the pathogen in stationary phase as part of a generalized stress response; and (ii) low-pH-inducible acid tolerance response (ATR) systems, manifested both at the log- and stationary-phase levels and conferring protection to the pathogen against subsequent exposure to lethal acidic (pH < 4.0) conditions (Foster and Hall, 1990; Lee et al., 1994; Lin et al., 1995). With particular reference to the pH-dependent ATR exhibited by stationary-phase cells of enterobacteria, this is induced via an approach referred to as "acid adaptation" (or "acid habituation") and which involves bacterial growth under mildly acidic conditions (i.e. sublethally low pH) (Buchanan and Edelson, 1999; Samelis et al., 2003; Koutsoumanis and Sofos, 2004; Álvarez-Ordóñez et al., 2010). Induction of acid resistance mechanisms under sublethal acid stress conditions, resulting, among others, in alterations in the virulence characteristics of foodborne pathogens, constitutes an important concern among food microbiologists, food processors and legislators (Theron and Lues, 2007; Perez et al., 2012; Makariti et al., 2015).

Even though the acid stress responses of *S. enterica* have been extensively studied and well characterized, research data regarding the inter-strain variability of the pathogen's acid survival systems are relatively few (Jørgensen et al., 2000; Berk et al., 2005; Lianou and Koutsoumanis, 2013). Taking into account the implications of bacterial adaptive stress responses for food safety, as well as the importance of strain variability of the behavior of foodborne pathogens in microbial risk assessment, the objective of this study was the assessment of the stationary-phase, adaptive ATR phenotype of 30 *S. enterica* strains.

2. Materials and methods

2.1. S. enterica strains and culture conditions

Thirty *S. enterica* strains, mainly isolates of human or bovine origin, belonging to various serotypes (i.e. Enteritidis, Typhimurium, Newport, Heidelberg, Montevideo, Infantis, Agona and Seftenberg) were evaluated in this study (Table 1). These isolates were kindly provided by Dr. Martin Wiedmann (Cornell University, Ithaca, NY, USA), and Dr. Constantin Genigeorgis and Dr. Daniil Sergelidis (Aristotle University of Thessaloniki, Thessaloniki, Greece).

Stock cultures of the strains were kept frozen (−70 °C) onto Microbank™ porous beads (Pro-Lab Diagnostics, Ontario, Canada), while working cultures were stored refrigerated (5 °C) on tryptone soy agar (TSA) slants and were renewed bimonthly. Strains were activated by transferring a loopful from the TSA slants into 10 ml of (i) glucose-free tryptone soy broth (TSB-G; Lab M Limited, Lancashire, UK), and (ii) tryptone soy broth supplemented with 1% (w/v) glucose (Merck, Darmstadt, Germany) (TSB + G), and incubating at 37 °C for 24 h. Culture of the tested strains in TSB-G and TSB + G aimed at obtaining non-adapted and acid-adapted cultures, respectively, with the latter being attained as the result of the pathogen's fermentative growth at the expense of glucose.

2.2. Acid challenge trials

The inherent AR of the non-adapted cultures of the tested strains was evaluated as part of a previous study undertaken in our laboratory (Lianou and Koutsoumanis, 2013), and the obtained data were also utilized in the present study as a reference for the assessment of the adaptive ATR of these strains. In accordance with the procedures described previously for the non-adapted cultures (Lianou and Koutsoumanis, 2013), one-milliliter portions of the acid-adapted cultures of each one of the tested strains were used to inoculate 9 ml of TSB-G acidified to pH 3.0 with HCl (min. 37%, Sigma-Aldrich, Seelze, Germany), to yield an initial concentration of approximately 10^8 CFU/ml. The acid challenge medium was adjusted to the above pH value using a digital pH meter with an epoxy refillable pH probe (Orion 3-Star Benchtop; Thermo Electron Corporation, Beverly, MA, USA), sterilized by autoclaving and, after confirming that its pH value was not considerably altered by the sterilization process, dispensed in test tubes submerged in a water bath (Nüve Sanayi Malzemeleri Ve Ticaret A.Ş., Ankara, Turkey). The bacterial cultures were added to the challenge medium when its temperature was 37 °C, a temperature which was maintained throughout the acid challenge trials, while non-acidified broth samples (pH 7.3 ± 0.2) also were inoculated for the determination of the initial bacterial populations. The total duration of the acid challenge trials was 4 h, and samples from each suspension were taken at 1-h intervals. Appropriate serial decimal dilutions of the bacterial suspensions in quarter strength Ringer's solution (Lab M limited) were surface plated on TSA plates, and surviving populations were determined after incubation of the plates at 37 °C for 72 h. Three independent experiments were conducted for each *S. enterica* strain.

2.3. Data analysis

The microbiological data (log CFU per milliliter) derived from the acid challenge experiments, undertaken both in this study (acid-adapted cultures) and previously (non-adapted cultures; Lianou and Koutsoumanis, 2013), were used to determine the inactivation kinetic parameters for each one of the 30 *S. enterica* strains. In particular, the Weibull model (Mafart et al., 2002) was fitted to the acid challenge data, using the software GInaFiT version 1.6 (Katholieke Universiteit Leuven, Belgium), a freeware Add-in for Microsoft® Excel (Geeraerd et al., 2005):

$$\log_{10}(N_t) = \log_{10}(N_0) - \left(\frac{t}{\delta}\right)^p$$

where N_t (CFU/ml) is the population at time t, N_0 is the population at $t = 0$, δ (time unit) is a scale parameter and p (−) is a shape parameter. The shape parameter p demonstrates the concavity ($p < 1$) or convexity ($p > 1$) of the bacterial inactivation curve, and the scale parameter δ can be denoted as the time for the first decimal reduction of the bacterial population if $p = 1$.

The Weibull model parameters δ and p were evaluated by analysis of variance (ANOVA) using the general linear model procedure of the SPSS 13.0 software package (SPSS Inc., Chicago, IL, USA), and means were separated using the Tukey's HSD test at a significance level of $\alpha = 0.05$.