International Journal of Food Microbiology 187 (2014) 33–40

Contents lists available at ScienceDirect

International Journal of Food Microbiology

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

Survival and death kinetics of *Salmonella* strains at low relative humidity, attached to stainless steel surfaces

Edyta Margas [a,b,*], Nicolas Meneses [c], Beatrice Conde-Petit [c], Christine E.R. Dodd [b], John Holah [a]

[a] Campden BRI, Station Road, Chipping Campden, GL556LD, Gloucestershire, UK
[b] The University of Nottingham, Food Sciences, Sutton Bonington Campus, Sutton Bonington, Leicestershire LE12 5RD, UK
[c] Buhler AG, Corporate Technology, Gupfenstrasse 5, 9240 Uzwil, Switzerland

ARTICLE INFO

Article history:
Received 14 March 2014
Received in revised form 15 June 2014
Accepted 29 June 2014
Available online 4 July 2014

Keywords:
Salmonella
Survival
Desiccation
Surface attachment
Low water activity

ABSTRACT

Salmonella is a major pathogen of concern for low water activity foods and understanding its persistence in dry food processing environments is important for producing safe food. The studies sought to assess the survival of 15 isolates of *Salmonella* on stainless steel surfaces. Additionally, the aim was to select a suitable model to describe and understand the strains' survival kinetics. *Salmonella* isolates were dried onto stainless steel surfaces, placed in controlled temperature (25 °C) and humidity (33%) conditions and their viability assessed at times from 1 h to 30 days. The highest survival rate was associated with *S.* Typhimurium DT104, *S.* Muenchen, and *S.* Typhimurium (NCTC 12023), where, after 30 days, the reduction ranged from 1.3 $\log_{10}$ cfu/surface to 1.6 $\log_{10}$ cfu/surface. The lowest survival was linked to a *S.* Typhimurium strain used in European Standard disinfectant approval tests and *S.* Typhimurium isolated from whey powder. For most of the strains, following an initial reduction in viability in the first hours (<72 h), no further reduction was seen over the 30 day period; therefore a 2-population Weibull model was fitted to model the survival kinetics.

The overall survival was neither serotype nor time related. All strains had two different subpopulations, one more resistant to desiccation than the other.

The results indicate the possibility of the long term survival of *Salmonella* on environmental surfaces (at least 30 days) and suggest the most suitable model to describe and predict survival kinetics. The results also identify strains that may be used to study stress response mechanisms and potential factory control measures in future studies.

1. Introduction

Historically, low water activity foods ($a_w < 0.85$) were of little concern with regard to microbiological safety due to their product characteristics. However, during the last 40 years *Salmonella* outbreaks have been linked to a number of food products with low water activity including: cereal, seeds, nuts, chocolate, dried fruits and vegetables, halva, dried milk products, spices, salami, tahini and tea (www.cdc.gov/foodsafety/outbreaks/). These outbreaks are usually very specific and are characterised as widely geographically spread, affecting a large number of people and lasting for relatively long periods of time. Beuchat and Komitopoulou (2011) and the Centres for Disease Control and Prevention website (CDC, 2014) described at least 40 large, documented outbreaks of *Salmonella* associated with dry foods. There is, therefore, a growing concern for the potential presence of *Salmonella* in such products. As the manufacture of many of these foods contains a thermal processing step, post process environmental contamination is thought to be the major source for contamination. Indeed, the same *Salmonella* type has been isolated from products produced over a number of years (e.g. 10 years from cereals produced at the same plant; CDC, 2008). The assumption has been that the particular *Salmonella* strain isolated had survived in the plant for this time period and had contaminated products, presumably when Good Manufacturing Practices (GMPs) were out of control. Driven by these contamination incidents, guidelines on implementation of better controls have been published (Beuchat and Komitopoulou, 2011; Holah et al., 2011; Podolak et al., 2009; Scott et al., 2009). However, there are still a number of bottlenecks in the dry food industry which are making implementation of these controls difficult (poor agriculture practices, lack of segregation, hygienic design of production equipment or lack of dry decontamination methods).

Evidence of *Salmonella* survival has been found in food processing environments. A study focused on contaminated chocolate by Craven et al. (1975) showed that the cause of the contamination was the uncontrolled airborne spread of dust in the factory environment. Samples

* Corresponding author at: Buhler AG, Corporate Technology, Gupfenstrasse 5, 9240 Uzwil, Switzerland. Tel.: +41719554394.
E-mail addresses: sbxem3@nottingham.ac.uk, edyta.margas@buhlergroup.com (E. Margas), nicolas.meneses@buhlergroup.com (N. Meneses), beatrice.conde-petit@buhlergroup.com (B. Conde-Petit), Christine.dodd@nottingham.ac.uk (C.E.R. Dodd), john.holah@campdenbri.co.uk (J. Holah).

http://dx.doi.org/10.1016/j.ijfoodmicro.2014.06.027
0168-1605/

taken in an oil meal plant also detected *Salmonella* on the processing floor, in dust in the air and on equipment (Morita et al., 2006). In several studies *Salmonella* was isolated from filters, processing floors, mixers, air and brooms in feed mills or crushing plants over a long period of time, indicating *Salmonella*'s ability to survive in dry processing environments (Binter et al., 2011). It has been reported that once a production line is contaminated with *Salmonella* spp., the microorganism will establish itself on the equipment and facility surfaces. In studies conducted by Gounadaki et al. (2008) *Salmonella* species were recovered from 26.5% of the environmental and equipment samples from dry sausage processing facilities. It is evident, therefore, that *Salmonella* can be present in the food processing environment and potentially contaminate the product.

The ability of *Salmonella* to survive for long periods of time in low a_w products e.g. dried almond hulls (Uesugi and Harris, 2006), halva (Kotzekidou, 1998), peanut butter (Burnett et al., 2000), dry milk, cocoa powder and poultry feed (Juven et al., 1984) or even on walnut kernels (Blessington et al., 2012) has been documented. However, whilst there is some information on the survival of *Salmonella* in dried products there is limited information on the survival of *Salmonella* in the dry food processing environment, both in terms of the length of survival and the possible factors contributing to such survival. Uesugi et al. (2007) demonstrated the potential for long-term (5 years) environmental persistence of *Salmonella* in almond orchards. However, this was an open environment and may have been contaminated on an ongoing basis from an external source. Resistance of *Salmonella* strains to dry conditions was confirmed by Janning et al. (1994). In addition, Kusumaningrum et al. (2003) showed that *Salmonella* can remain viable on dry stainless steel surfaces and present the potential for contamination for considerable time periods.

The subspecies of most concern, in relation to food safety, is *Salmonella enterica* subsp. *enterica*. More than 99% of *Salmonella* isolated from humans belong to this subspecies (Old, 1992). The total number of *S. enterica* subsp. *enterica* serovars is approximately 2579 (Grimont and Weill, 2007), however the number of serovars associated with outbreaks in low a_w foods, is only ~30 (Beuchat and Komitopoulou, 2011; CDC, 2014). Therefore, it is important to assess if these strains can also survive in the low relative humidities of dry food processing factories and thus identify the need for appropriate control mechanisms.

Wet cleaning and disinfection is the preferred method in the food industry to control microbial contamination. Dry processing food factories are often dry cleaned only (which does not always remove all surface soiling and microorganisms) or wet cleaned periodically (e.g. following every batch or each time a different allergenic ingredient is processed). For example, in the dry milk industry, the typical production time is approximately 30 days before wet cleaning is undertaken. Therefore, due to the existing dry cleaning methods not being sufficient for microbial eradication it is important for the food industry to know if *Salmonella* can survive for prolonged periods of time on surfaces without water or until the next wet cleaning occurrence (e.g. for 30 days).

In order to ensure that the results would be as useful and applicable to the food industry as possible, the same *Salmonella* serovars as those from the foods which caused recent *Salmonella* outbreaks were used. Additionally, heat- and disinfectant-resistant (Anon, 2001) laboratory test strains were used to investigate if they were also resistant to desiccation. The studies aimed to (a) establish survival kinetics for 15 *Salmonella* strains, (b) assess any differences between and within serotype isolates, (c) show if *Salmonella* cells attached to stainless steel surfaces, with traceable amounts of nutrients, can survive for prolonged periods of time, and (d) choose the best kinetic model to describe the survival.

2. Materials and methods

2.1. Salmonella strains

A total of 15 *S. enterica* subsp. *enterica* serovars were obtained from the Campden BRI culture collection (Table 1). Identification and characterisation of the strains were carried out by ribotyping (Campden BRI, Dept. Food Microbiology) prior to experimental use. Four isolates of *S.* Typhimurium were used to compare differences in survival rate between the same serotype and six isolates from cereal manufacturing environments were also used to compare differences in survival of strains isolated from the same environment.

2.2. Surfaces

Stainless steel discs (2 cm diameter, Grade 2B, 1.4301 (EN 10088-1, EN 10 088-2)) were used (Resurgem Engineering Co Ltd, UK). They were soaked in hot water with detergent (Fairy liquid, Procter & Gamble) for 60 min. Each disc was then cleaned with a sponge and rinsed with sterile distilled water. They were then left to dry exposed to the air for 30 min, wiped with alcohol wipes (Izo-wipes; Johnson Diversey), and sterilised in an autoclave at 121 °C for 20 min.

2.3. Test suspensions

Cultures were maintained at −80 ºC in cryo vials containing glycerol (Pro-Lab Diagnostics, Microbank). Working cultures of each serotype were sequentially prepared by adding a cryo bead to 150 ml Nutrient Broth (NB, Oxoid 0001) which was incubated for 24 h at 37 °C in a shaking incubator at 100 rpm. The broth was then centrifuged at 3000 *g* for 10 min (Camlab, ALC centrifuge, PK 120). The degree of cell washing necessary to remove any nutrients from the cell solution, which could have an impact on subsequent cell survivability, was assessed during initial experiments. The survival of *S.* Typhimurium 'HR' was accessed

Table 1
Salmonella isolates used for the experiments.

No.	Given code	Name	Source
1.	FH/Sa/159	*Salmonella* Agona	Cotton seeds
2.	FH/Sn/161	*Salmonella* Napoli	Chocolate confectionary, refrigerated 7 years (1986 outbreak Italy)
3.	FH/Sm/164	*Salmonella* Muenchen	Cocoa bean environment
4.	FH/Se/162	*Salmonella* Enteritidis	Cereal
5.	FH/St/163	*Salmonella* Tennessee	Sesame seeds
6.	FH/St/165	*Salmonella* Typhimurium	Whey powder
7.	FH/St/167	*Salmonella* Typhimurium DT104	HPA, Colindale
8.	FH/St/168	*Salmonella* Typhimurium	NCTC 12023, heat resistant "HR"
9.	FH/St/68	*Salmonella* Typhimurium	NCTC (PHILS), NC000 74-17, European standard disinfectant test strain "DS"
10.	FH/S1/172	*Salmonella* Lomita	Cereals environment "1"
11.	FH/S2/173	*Salmonella* Lomita	Cereals environment "2"
12.	FH/S3/174	*Salmonella* Cubana	Cereals environment "1"
13.	FH/S7/178	*Salmonella* Cubana	Cereals environment "2"
14.	FH/S5/176	*Salmonella* Mbandaka	Cereals environment "1"
15.	FH/S6/177	*Salmonella* Mbandaka	Cereals environment "2"