



**HAL**  
open science

## Adaptation of Salmonella to antimicrobials in food-processing environments

Florence Dubois-Brissonnet

► **To cite this version:**

Florence Dubois-Brissonnet. Adaptation of Salmonella to antimicrobials in food-processing environments. Salmonella. Distribution, adaptation, control measures and molecular technologies, InTech, 504 p., 2012, 978-953-51-0661-6. 10.5772/29612 . hal-01991467

**HAL Id: hal-01991467**

**<https://hal.science/hal-01991467>**

Submitted on 23 Jan 2019

**HAL** is a multi-disciplinary open access archive for the deposit and dissemination of scientific research documents, whether they are published or not. The documents may come from teaching and research institutions in France or abroad, or from public or private research centers.

L'archive ouverte pluridisciplinaire **HAL**, est destinée au dépôt et à la diffusion de documents scientifiques de niveau recherche, publiés ou non, émanant des établissements d'enseignement et de recherche français ou étrangers, des laboratoires publics ou privés.



Distributed under a Creative Commons Attribution 4.0 International License

# We are IntechOpen, the world's leading publisher of Open Access books Built by scientists, for scientists

3,900

Open access books available

116,000

International authors and editors

120M

Downloads

Our authors are among the

154

Countries delivered to

TOP 1%

most cited scientists

12.2%

Contributors from top 500 universities



WEB OF SCIENCE™

Selection of our books indexed in the Book Citation Index  
in Web of Science™ Core Collection (BKCI)

Interested in publishing with us?  
Contact [book.department@intechopen.com](mailto:book.department@intechopen.com)

Numbers displayed above are based on latest data collected.  
For more information visit [www.intechopen.com](http://www.intechopen.com)



# Adaptation of *Salmonella* to Antimicrobials in Food-Processing Environments

Florence Dubois-Brissonnet  
AgroParisTech, UMR MicAliS, Massy  
INRA, UMR 1319, MicAliS, Jouy-en-Josas  
France

## 1. Introduction

*Salmonella* remains an important concern in food processing environments as it causes salmonellosis, a major public health problem throughout the world (Anonymous, 2004; Mead et al., 1999). This review deals with the ability of *Salmonella* to survive and adapt to the antimicrobial stresses encountered in food processing environments. It illustrates how bacteria can develop greater tolerance to these stresses and cross-protection with other environmental stresses, thus increasing their persistence throughout the food chain. Two types of antimicrobial agents are primarily encountered in food-processing environments, food preservatives and disinfectants.

Food preservatives are directly included in food products. Their use is the most common method to guarantee microbial food safety until the end of shelf-life. Among these substances, traditional chemical preservatives should be distinguished from naturally-occurring antimicrobial compounds. The former group includes officially approved compounds such as organic acids (lactic, acetic, sorbic, benzoic acids) and their salts, nitrites and nitrates, or sulfites (Roller, 2003). The latter group includes compounds which come from a natural source: whether they are produced by a microorganism, e.g. several weak acids or bacteriocins, or whether they are extracted from a plant or animal product, e.g. essential oil compounds, lactoperoxidase or lactoferrin (Roller, 2003). In most cases, these natural antimicrobials are used in foods for their acidifying or flavoring properties, but they also play an important role in maintaining microbiological food safety. Indeed, it has been long recognized that numerous natural compounds have antimicrobial properties (Naidu, 2000; Roller, 2003), among them, weak organic acids (Doores, 2005) and essential oil compounds (Burt, 2004). The inhibition of Gram-negative bacteria by bacteriocins produced by lactic acid bacteria is limited because of the impermeability of the outer membrane (Abee et al., 1995), even though treatment with EDTA (which permeabilizes the membrane) can render these bacteria sensitive to nisin but not to pediocin (Schved et al., 1994). Nevertheless, microcin L, a bacteriocin produced by *E. coli*, is highly efficient against *S. Typhimurium* (Morin et al., 2011).

Disinfectants are chemical agents used on inanimate surfaces to inactivate all recognized pathogenic microorganisms (Centers for Disease Control and Prevention, USA). They are

applied regularly in food processing environments in order to control surface biocontamination, and must be officially approved (Anonymous, 1998). Numerous disinfectants with different mechanism of action are widely employed by the food industry, such as peroxygens, quaternary ammonium compounds, halogen-releasing agents or aldehydes (McDonnell & Russell, 1999). But regulations are currently being revised (Reach, biocide directive) and some of these molecules would no longer be permitted in the future years (Anonymous, 2006; Anonymous, 2009).

Antimicrobial properties of a compound can be characterized with respect to its bacteriostatic and/or bactericidal activities. The bacteriostatic activity of an antimicrobial is determined by its minimum inhibitory concentration (MIC); i.e. the minimum concentration that can completely inhibit growth of the target pathogen. The bactericidal activity can be evaluated using a survival curve (a single concentration is applied and survivors are enumerated over time) or by establishing the Minimum Bactericidal Concentration (MBC: a range of concentrations are applied for a specific period of time and survivors are then numbered). Food antimicrobials are intended to eradicate or inhibit the growth of pathogenic microorganisms, whereas disinfectants are only designed to kill the microbial population rapidly.

Although bacterial resistance can be characterized regularly using one of these techniques, it is important to know that, like in numerous pathogens, the resistance of *Salmonella* to antimicrobials can evolve as a function of its living conditions. If bacteria are subjected to stressful conditions, they can increase their survivability under conditions that would normally be lethal. Induced tolerance was first demonstrated in *Salmonella* after a heat pre-shock at 48°C which induced an increase in the further thermotolerance of the strain (Mackey & Derrick, 1986). Preservative factors can impose non-lethal stresses upon bacteria in foods, potentially eliciting stress tolerance. Moreover, consumers today are looking for additive-free, fresh and more natural foodstuffs. In this context, combinations of treatments may be a way to enable their use at low doses while maintaining microbial food safety. The term “hurdle technology” is widely employed to describe these combinations of technologies with preservative effects (Leistner, 1995) and, because these treatments are often applied at the same time, the term multifactorial preservation has been proposed as being more appropriate (Roller, 2003). However, because these cumulative processes are all applied at sub-inhibitory concentrations, they may promulgate an adaptive stress response and enable the survival of a greater fraction of the bacterial population.

Moreover, in food processing or farming environments, *Salmonella* biofilms can settle on surfaces, despite disinfection procedures (Vestby et al., 2009). A biofilm is a bacterial community that adheres to a surface and is embedded in a matrix of microbial extracellular polymeric substances (Costerton et al., 1995; Hoiby et al., 2010). Protected by this matrix, *Salmonella* cells in the deeper layers of the biofilm thus become less accessible to the disinfectant because of diffusion limitations and can develop adaptive stress responses to sub-lethal concentrations of disinfectant.

The ability of the pathogen to respond to stressful conditions has been described as bacterial stress adaptation, stress adaptive response, habituation, induced tolerance, acclimatization or stress hardening (Yousef & Courtney, 2003). Numerous physiological responses are

implicated in this increase in bacterial survival and tolerance in harsh environments (Wesche et al., 2009). Many physiological modifications occur concomitantly in the cell, including protein up- or down-regulation, modifications to the cell membrane composition and altered morphology. In addition, these physiological changes following stressful conditions can induce cross resistance to other stressful environmental conditions, modifications to colonization or virulence (Figure 1). In all cases, bacterial stress will exert a considerable impact on the persistence of *Salmonella* throughout the food chain because of the modifications they induce to cell physiology, reactivity and tolerance. This is therefore a major concern in the area of food microbiology because it may constitute an emergent microbiological hazard.

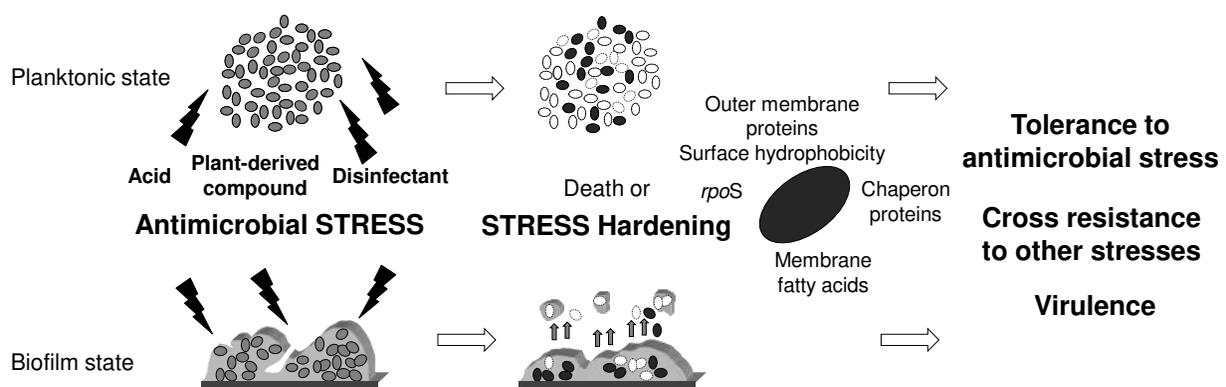


Fig. 1. Adaptation of *Salmonella* to antimicrobial stresses in food processing environments and consequences

Adaptation of *Salmonella* to both food antimicrobials and disinfectants will be discussed in the next three parts. Among the former group, we will focus on the two main groups of natural compounds which have demonstrated their efficiency against *Salmonella* and many other Gram-negative bacteria; i.e. weak acids and essential oil compounds.

## 2. Adaptation of *Salmonella* to weak organic acids

This section reviews the ability of *Salmonella* to grow and survive under acidic conditions. We will also discuss its capacity to develop acid adaptation and the consequences relative to its cross resistance to other stresses and virulence.

*Salmonella* may encounter a variety of acidic stress situations in both natural and industrial environments, as well as during pathogenesis. In foodstuffs, weak organic acids (e.g. acetic, lactic, citric acids, etc.) are either present naturally as constituents of the food, are produced by fermentation or are added during food formulation in order to protect against deterioration. For example, acetic acid is widely employed as a food acidulant to inhibit microbial growth and extend the shelf-life of food. Within the human digestive system, *Salmonella* must endure an extreme pH in the stomach and survive in the presence of weak acids in the intestinal environment before reaching the epithelial cells (Baik et al., 1996).

## 2.1 Growth or survival under acidic conditions

The optimum pH for *Salmonella* growth is generally said to be between 6.5 and 7.5, but the minimum pH value depends on many factors such as the strain and type of acid. For example, *Salmonella* Typhimurium can grow at 25-37°C at pH 4.5 when the pH is adjusted with citric acid, while it has to be over 5.4 with lactic acid and 6.4 with acetic acid (Alvarez-Ordóñez et al., 2010a). These findings can be explained because the undissociated form is that which is primarily responsible for antimicrobial activity, as it is able to penetrate into the cell and reduce the cytoplasmic pH by intracellular dissociation. For a given acid, the lower the pH, the higher the proportion of the undissociated form (Dziezak, 1986). However, the activity of each acid will depend on its pKa; the higher the pKa, the higher is the proportion of undissociated form at a given pH. For example, at pH 5, 34.9% of acetic acid is undissociated, as opposed to 0.41% of citric acid at the same pH. Inhibitory efficiency, thus, decreases as follows: acetic acid (pKa 4.75) > lactic acid (pKa 3.08) > citric acid (pKa1 3.14; pKa2 4.77; pKa3 6.39) (Doores, 2005). Acid preservatives are therefore more efficient in acid or acidified foods. Lactic, acetic, and benzoic acids are authorized in foodstuffs at *quantum satis* concentrations (“the amount which is needed”). They are generally added to foodstuffs at a rate of 1% or higher. Above the pH mode of action, some acids exert specific toxicity; for example propionic, sorbic or benzoic acids, which should therefore be used at lower concentrations such as 0.05% to 0.2% (Mescle & Zucca, 1996).

The minimum growth pH of *Salmonella* is also temperature-dependent. The minimum growth pH is increased by about 1 pH unit at high (45°C) or low (10°C) temperatures, compared to the minimum pH at 25-37°C (Alvarez-Ordóñez et al., 2010a).

Above the minimum pH level, growth kinetics can be characterized by modeling the growth rate versus the concentration of acid (Guillier et al., 2007). Inhibitory activity will depend on the type of acid, its concentration and the pH. Beyond the MIC determination, one parameter is particularly useful for characterizing acid inhibition: the Non-Inhibitory Concentration (NIC) which is the higher concentration at which no inhibition is observed. Different models can be utilized in this respect, including a square-root model based on the Lambert and Pearson model (Lambert & Pearson, 2000). The growth rate ( $\mu_{\max}$ ) as a function of acid concentration ( $c$ ) is expressed as follows, where MIC, NIC and  $\mu_{\max}(c=0)$  are the model parameters (Guillier et al., 2007):

$$\sqrt{\mu_{\max}(c)} = \sqrt{\mu_{\max}(c=0) \cdot g(c)} \quad \text{where} \quad g(c) = \exp \left[ - \frac{c}{\left( \frac{\text{MIC}}{\exp \left( \frac{\ln(\text{NIC}/\text{MIC})}{-e} \right)} \right)} \right]^{\left( \frac{-e}{\ln(\text{NIC}/\text{MIC})} \right)}$$

Depending on the acid, different growth rate inhibition profiles have been generated while acid concentration increases in the medium (Figure 2). There was no pH adjustment. For example, the inhibitory effect of lactic acid on *S. Typhimurium* starts above 36.6 mM, while citric acid is effective up to 9.9 mM. The MIC values for citric, lactic and acetic acids in these conditions are respectively 30 mM, 40 mM and 52 mM (Guillier et al., 2007). In addition to

the MIC, determining the NIC is of great importance because for some compounds, the difference between NIC and MIC values is very small. Thus a small error made when adjusting the concentration in a food product can lead to a level that is the wrong side of the growth-no growth limit.

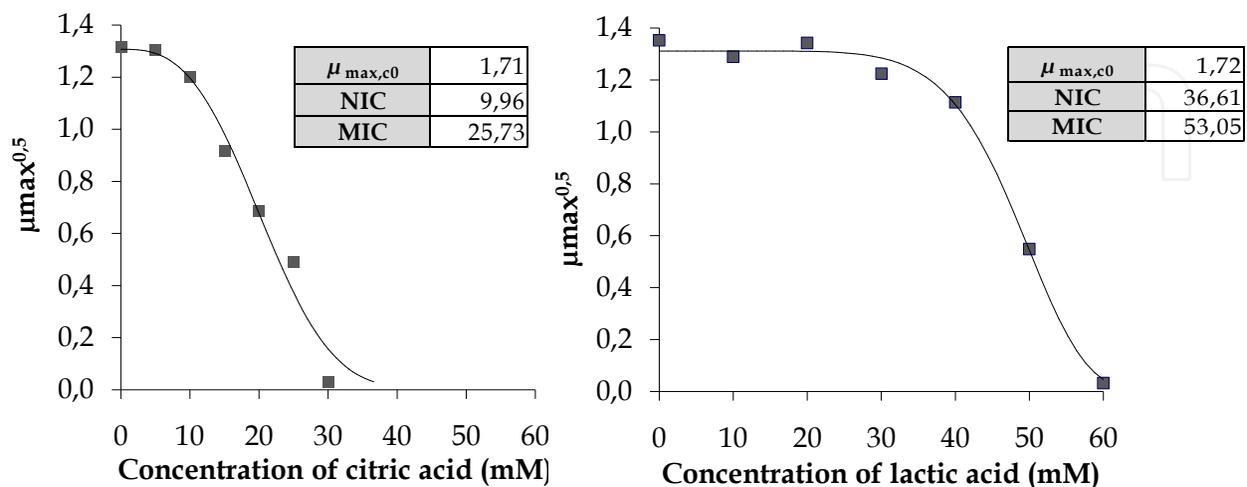


Fig. 2. *S. Typhimurium* growth rate inhibition versus concentration of citric acid or lactic acid (data from Guillier et al., 2007).

Below the minimum growth pH, *Salmonella* can nevertheless survive. The minimum survival pH has usually been stated to be at about 3.0 for *Salmonella*, unlike *E. coli* or *Shigella* that can survive at pH 2 or 2.5 in complex media (Lin et al., 1995). However, different strains of *Salmonella* have been shown to survive at pH 2.5 for prolonged periods of time (de Jonge et al., 2003). The emergence of these acid-resistant strains suggests a preconditioning in acidic conditions, most probably within the intestinal tract of ruminants fed with a carbohydrate-rich diet (de Jonge et al., 2003) as it will be described in the next paragraph.

Furthermore, acid compounds can also have a bactericidal activity against *Salmonella*. This can be evaluated in the same way as other inactivation treatments, using a survival curve or by determining *D*-values (number of minutes required to reduce the number of viable organisms by a factor of 10).

## 2.2 Onset of acid tolerance response (ATR)

Some early studies showed that the exposure of *Salmonella* to mild acidic conditions could protect it against extreme acid conditions (Huhtanen, 1975). When log-phase cells grown at pH 7.6 were shifted to mildly acidic conditions (pH 5.8) for one generation, they were able to develop 100- to 1,000-fold higher tolerance to extreme acid conditions (pH 3.3) than non-adapted cells shifted directly from pH 7.6 to 3.3 (Foster & Hall, 1990). The increased tolerance to a low pH following acid habituation was referred to as the Acid Tolerance Response (ATR) (Foster & Hall, 1990). Even if the majority of studies investigating acid resistance of *Salmonella* were first conducted with inorganic acids, it was later shown that organic acids also have the ability to induce resistance to many other

stresses. Acid shock confers protection on *Salmonella* against the lethal effect of a low pH under different conditions (see some examples in Table 1) obtained with different types of acids (citric, lactic, malic, acetic acids, HCl, etc.), challenge media (laboratory media or foodstuffs), or growth conditions during adaptation. More than just the pH value, it has been shown that the type of acid can influence the amplitude of the ATR. For *S. Typhimurium*, higher ATR values are obtained in order using citric, acetic, lactic, malic acids (Alvarez-Ordóñez et al., 2009). Moreover, food composition can influence the stress responses and subsequent tolerance properties of adapted cells. For example, acid-adapted *S. Typhimurium* was more resistant to extreme acidic conditions when meat extract was used as a challenge medium rather than BHI (Alvarez-Ordóñez et al., 2009). Recently, growth temperature has also been shown to be an important factor affecting the *Salmonella* ATR. Acid tolerance was increased 2-fold in cells grown at 10°C while it increased more than 3.5-fold at a temperature higher than 25°C (Alvarez-Ordóñez et al., 2010a). Moreover, higher ATR obtained with citric acid occurred only at 25-37°C but not at 10°C or 45°C (Alvarez-Ordóñez et al., 2010a). More than preventing growth, maintaining a low temperature may therefore constitute a means of limiting induction of the *Salmonella* ATR. In addition, the ATR may emerge with some strains more than others. For example, *S. Typhimurium* demonstrated a higher degree of acid tolerance than *S. Typhi* (Arvizu-Medrano & Escartin, 2005).

The acid tolerance response is a major concern in terms of food safety as it could permit *Salmonella* survival in acidic environments such as fermented foods, following acid cleaning treatments of industrial surfaces or the gastrointestinal tract. For example, acid-adapted cells were able to survive for a period of two months in cheddar, Swiss and mozzarella cheeses stored at 5°C (Leyer & Johnson, 1992).

### 2.3 ATR mechanism

The ATR mechanism of *Salmonella* has been widely described (Bearson et al., 1997; Foster, 1991; Foster & Hall, 1990; Lee et al., 1994). *Salmonella* possesses both log-phase and stationary-phase ATR systems. The log-phase ATR appears to be a two-stage inducible system (Foster, 1991) with complex regulation systems detailed below. The higher level of acid tolerance provided in stationary-phase appears to differ from log-phase ATR. It has been attributed to a pH-dependant system called stationary-phase ATR, together with a generalized stress response (Lee et al., 1994).

The two-stage system of log-phase ATR consists of pre-acid and post-acid shocks. Pre-acid shock, activated at pH 6, involves an emergency pH homeostatic system, designed to maintain the intracellular pH higher than 5. It induces several amino acid decarboxylases, such as lysine decarboxylase, which decarboxylates intracellular lysine to cadaverine while consuming a proton. Cadaverine is then exchanged for another lysine from the medium *via* a CadB antiporter (Bearson et al., 1997). A higher relative expression of the lysine and arginine decarboxylase systems was demonstrated in acid-adapted *Salmonella Typhimurium* (Alvarez-Ordóñez et al., 2010b). The existence of these homeostatic systems, that can use the extracellular amino acids present in foodstuffs such as meat extract, explains the higher acid tolerance observed in these media. However, prolonged exposure (6h) to mild acid stress causes a loss of homeostasis, resulting in more susceptibility to lethal stress (Greenacre et al., 2003).

Post-acid shock is induced when the pH falls below 4.5. Acid shock proteins (ASP) are required for the adaptive acid tolerance response. Their synthesis is dependent on several regulatory genes: the alternative sigma factor  $\sigma^S$  encoded by *rpoS*, the iron regulator Fur and the two-component signal transduction system PhoPQ (Baik et al., 1996; Bearson et al., 1997; Foster, 1991; Foster, 1999). The alternative sigma factor  $\sigma^S$  has been recognized as a key factor in increasing the stress resistance of *Salmonella* cells in stationary phase but it can also be induced by stresses that bacteria may encounter during processing, such as acid stress (Dodd & Aldsworth, 2002). It controls the expression of about 40 genes/operons involved in generating the physiological changes associated with survival processes, among them ASP synthesis. For example, *S. Typhimurium* produces forty-three ASPs at pH 4.5 (Foster, 1993). At pH values lower than 2.6, the addition of chloramphenicol (which inhibits protein synthesis) has been found to reduce subsequent acid resistance, although the cells were not as sensitive as control cultures grown at pH 7.0 (Humphrey et al., 1993). Some ASPs are chaperon proteins which may protect internal proteins from denaturation (Foster, 1991). The pre-acid shock stage offers the cell an enhanced ability to synthesize APSs following acidification of the medium. The long-term adaptation of *S. Enteritidis* to propionate acid induces the differential expression of over twenty proteins. Of the five over-expressed proteins, two were clearly found to be implicated in acid induced resistance: Dps, a DNA-binding protein from starved cells and CpxR, a transcriptional regulatory protein (Calhoun et al., 2010). These two proteins are normally associated with virulence and pathogenesis.

Moreover, acid adaptation in log-phase has been shown to induce increased production of specific outer membrane proteins and enhanced surface hydrophobicity without lipopolysaccharide alteration (Leyer & Johnson, 1993). These surface modifications may be of considerable concern because they may affect the ability of *Salmonella* to adhere to inert surfaces in food processing environments, and then to form biofilms. Alongside proteome modifications, some other physiological modifications appear following acid adaptation, such as modifications of fatty acid profiles. The proportions of membrane cyclopropane fatty acid (CFA) in *S. Typhimurium* were found to be higher at a pH lower than 6 when compared with those of cells grown at pH 7.5 (de Jonge et al., 2003). CFA levels were also found to be 1.5-fold higher in acid-adapted cells (grown in the presence of acids at pH values of 6.4, 5.4, or 4.5 in BHI) than in non-adapted cells (Alvarez-Ordóñez et al., 2008). It could be speculated that acid-induced RpoS in the exponential phase might increase CFA synthase activity in *S. Typhimurium* (Kim et al., 2005). CFAs are produced by the addition of a methyl group from *S*-adenosyl methionine across a *cis*-double bond of unsaturated fatty acids (UFAs). They are generally known to be preferentially synthesized when cells enter the stationary phase in *Salmonella*, as in numerous other bacteria (Kim et al., 2005). The conversion of UFAs into CFAs induces a decrease in membrane fluidity. This may be a way for stressed cells to limit exchanges with the external medium and conserve energy. The membrane fluidity of *Salmonella* adapted to citric acid was measured by fluorescence anisotropy of a fluorescent probe (DPH) and the results showed that it was lower than that of control cells (Alonso-Hernando et al., 2010). The over-synthesis of CFA is considered to be a major factor in the acid resistance of Gram-negative bacteria (Brown et al., 1997; Chang & Cronan, 1999).

Another concern is that, following acid adaptation, some injured cells may shift into a viable but non-cultivable (VBNC) state, which is one of the current mechanisms that

allows non-sporulating cells to survive. These cells cannot grow on the selective media used to detect pathogens in foods, typically xylose lysine desoxycholate agar for *Salmonella*. They can escape the microbiological controls, but can slowly repair themselves and then re-grow in foodstuffs while being able to maintain their metabolic activity and pathogenicity (Xu et al., 2008).

Acid adaptation appears to be a multiple adaptive response related to the synthesis of several regulatory systems and leading to major modifications of cytoplasmic proteins, together with modifications to membrane protein and fatty acid contents. It is essential to understand these physiological adaptations as they can induce bacterial resistance and hence the persistence of pathogens in the food chain.

#### **2.4 Cross resistance in acid-adapted *Salmonella***

In foods or food processing environments, *Salmonella* can encounter multiple subsequent stressor treatments. Numerous studies have demonstrated that acid adaptation can ensure cross-resistance (or cross-protection) to stresses other than acid stress, such as heat, biocide damage or high osmolarity.

Heat tolerance was the first cross-protection which was demonstrated after *Salmonella* was exposed to acid. For example, a 10-fold difference in survivors was seen between acid-adapted or non-adapted populations after a 20 min challenge at 50°C (Leyer & Johnson, 1993). Heat tolerance was also shown after acid adaptation in orange or watermelon juices (Mazzotta, 2001; Sharma et al., 2005). Several acid shock proteins, including chaperon proteins, are similar to those induced by heat shock, such as GroEL or Dnak (Foster, 1993). This may be one explanation for the cross resistance observed.

Acid adaptation has also been described to enhance tolerance towards osmotic stress (Leyer & Johnson, 1993; Tosun & Gönül, 2003) but it was dependent upon the acid used for adaptation. Acetic acid adaptation provided cells with protection against both NaCl and KCl stresses, while lactic acid adaptation did not protect against osmotic stressors (Greenacre and Brocklehurst, 2006). In the same way, *S. Enteritidis* adapted to acid in marinades did not display any increased resistance to drying processes (Calicioglu et al., 2003).

Acid adaptation provoked dramatic sensitization (~10,000-fold) of *Salmonella* to halogen-based sanitizers including chlorine and iodine (Leyer & Johnson, 1997). This was explained because hypochlorous acid oxidized a higher percentage of cell surface sulfhydryl groups in acid-adapted cells than in non-adapted cells, and sulfhydryl oxidation was correlated to cell inactivation. In the same way, *Salmonella* adapted with lactic acid displays sensitivity to hydrogen peroxide which is concomitant with down-regulation of the OxyR regulon (Greenacre et al., 2006). These results could have an interesting application in maintaining microbial food safety. The weakening of *Salmonella* defenses by acid shock could constitute a potential strategy to enhance the action of halogen-based sanitizers. However, it has been shown that the long-term adaptation of *Salmonella* to propionate induces strong tolerance to the *in vitro* oxidative and nitrosative stresses that may be encountered in mammalian hosts (Calhoun & Kwon, 2010).

Salmonella serotype	Acid pretreatment conditions	Exposure to acid shock	ATR (versus non adapted cells)	Reference
<i>S. Typhimurium</i>	From mid-log phase cells, one cell doubling in Minimal E glucose pH 5.7 (HCl) (vs control at pH 7.0)	Challenge at pH 3.2 - 45 min	16% survival vs 0.25%	(Foster & Hall, 1990)
<i>S. Enteritidis</i>	From mid-exponential phase cells, Lenco broth for 3h at pH 3 (HCl) vs pH 7 (control)	Survival curves at pH 2.5 D-value is the number of minutes required to reduce the number of viable organisms by a factor of 10	D-value 1.9 min vs 0.4 min	(Humphrey et al., 1993)
<i>S. Typhimurium</i>	TSB 37°C 1h in aerobic conditions 100mM propionate vs NaCl (control)	TSB pH 3.0 1h	42% survival vs 0.2%	(Kwon & Rieke, 1998)
<i>S. Typhimurium</i>	2h at 20°C in TSBG in mid-exponential phase Acetic acid pH 5.5 vs TSBG pH 7 (control) Acetic acid pH 5.8 vs TSBG pH 7 Lactic acid pH 5.5 vs TSBG pH 7 Lactic acid pH 5.8 vs TSBG pH 7	Survival curves at pH 3.0 Kmax is the slope of the survival curve	Death rate Kmax 1 h <sup>-1</sup> vs 28.5 h <sup>-1</sup> Kmax 1.7 h <sup>-1</sup> vs 28.5 h <sup>-1</sup> Kmax 2.4 h <sup>-1</sup> vs 28.5 h <sup>-1</sup> Kmax 5.4 h <sup>-1</sup> vs 28.5 h <sup>-1</sup>	(Greenacre et al., 2003)
<i>S. Typhimurium</i>	90 min at 30°C in TSB without dextrose lactic acid pH 4.5 vs TSB pH 7.0 (control) lactic acid pH 5.0 vs TSB pH 7.0	Survival curves at pH 3.5 Kmax is the slope of the survival curve	Death rate Kmax 4.6 h <sup>-1</sup> vs 9.4 h <sup>-1</sup> Kmax 7.7 h <sup>-1</sup> vs 9.4 h <sup>-1</sup>	(Koutsoumanis & Sofos, 2004)
<i>S. Typhimurium</i>	4h at 25°C in PBS pH 5.5 (HCl)	Survival curves in commercial yoghurt at 5°C for 10 days Survival curves at pH 4.0 and 20°C obtained using the Weibull model $\log N = \log N_0 - b \cdot t^n$	Reduction of the population 0.6 log vs. 1.34	(Shen et al., 2007)
<i>S. Enteritidis</i>	Adaptation at pH 5.0 for 2h vs control pH 7.3	Survival curves at pH 4.0 and 4°C obtained using the Weibull model $\log N = \log N_0 - b \cdot t^n$	b: 0.93 vs 2.96; n: 0.86 vs 0.42	(Xu et al., 2008)
<i>S. Enteritidis</i>	Adaptation at pH 5.0 for 2h vs control pH 7.3	Survival curves at pH 4.0 and 4°C obtained using the Weibull model $\log N = \log N_0 - b \cdot t^n$	b: 0.11 vs 0.75; n: 0.99 vs 0.74	(Xu et al., 2008)
<i>S. Typhimurium</i>	Growth until stationary phase in: BHI acetic pH 6.4 - 37°C vs non acidified BHI pH 7.4 BHI citric pH 6.4 - 37°C vs non acidified BHI pH 7.4 BHI HCl pH 6.4 - 37°C vs non acidified BHI pH 7.4 BHI citric pH 4.5 - 37°C vs non acidified BHI pH 7.4 BHI HCl pH 4.5 - 37°C vs non acidified BHI pH 7.4	Survival curve at pH 3 (HCl) The negative reciprocal of the survival curve was used for the D-value	D-value : 15.7 min vs 11.8 min D-value : 22.0 min vs 11.8 min D-value : 15.5 min vs 11.8 min D-value : 24.6 min vs 11.8 min D-value : 22.2 min vs 11.8 min	(Alvarez-Ordóñez et al., 2010a)

Table 1. Examples of conditions for acid adaptation and tolerance in *Salmonella*

## 2.5 Virulence of acid-adapted *Salmonella*

The infectivity of *Salmonella* is based on its ability to overcome numerous lethal environments in order to reach the site of infection. These include the acid barrier of the stomach, the physical barrier of epithelial cells and various immune defenses. To survive in these acidic environments, *Salmonella* has developed elaborate systems to sense stresses and adaptive response to acid stresses. The connection between ATR and virulence is a matter of debate. On the one hand, an acid tolerant *S. Enteritidis* isolate was found to be more virulent in mice than an acid-sensitive isolate (Humphrey et al., 1996). ATR is known to be regulated by RpoS, which also influences the expression of specific virulence factors (Fang et al., 1992; Gahan & Hill, 1999). Mutations in the *rpoS* gene of virulent *S. Typhimurium* strains render them incapable of developing a full ATR and significantly reduce their virulence potential (Fang et al., 1992; Lee et al., 1995). On the other hand, one study demonstrated that low pH environments could select persistent phenotypes of *Salmonella* with increased acid tolerance, but these were less virulent (Karatzas et al., 2008a). Similarly, the long-term adaptation of *Salmonella* to propionate reduced the overall infectivity of the adapted cells by inhibiting the colonization ability of the organism (Calhoun & Kwon, 2010). Moreover, flagella motility, which is fundamental to the virulence process, was shown to be repressed at a low pH in order to allow the conservation of ATP for survival processes (Adams et al., 2001).

## 3. Adaptation of *Salmonella* to plant-derived antimicrobials

Among naturally occurring antimicrobials that can be used in foods, we will focus here on phyto-antimicrobials which have seen a considerable resurgence of interest during the past 10 years among both consumers and industry. Essential oils and their compounds are known to have antimicrobial properties against numerous bacteria and fungi, including *Salmonella* (Burt, 2004; Kim et al., 1995b). Most of these compounds are “generally recognized as safe” (GRAS) and have been registered by the European Commission for their use as flavoring compounds in food products.

### 3.1 Growth inhibition of *Salmonella* by plant-derived antimicrobials

The antibacterial activity of essential oils and their components is due to the permeabilization of the cytoplasmic membrane that leads to a loss of cellular constituents (Burt, 2004; Helander et al., 1998). Its characterization has been widely reported in laboratory media. As previously described, the minimum inhibitory concentration (MIC) and non-inhibitory concentration (NIC) can be determined by modeling growth rate as a function of the antimicrobial concentration (Guillier et al., 2007). As for acid compounds, the growth rate curves versus concentrations have different profiles depending on the compound. For example, menthol is efficient above 0.91 mM for a MIC of 3.3 mM, while the inhibitory effect of carvacrol starts at 0.73 mM for a MIC at 0.90 mM (Guillier et al., 2007). Among 14 natural antimicrobials, phenolic compounds were shown to be the most efficient in inhibiting the growth of *S. Typhimurium*. The MICs of carvacrol, thymol, eugenol, geraniol, menthol,  $\alpha$ -terpineol and *trans*-cinnamaldehyde in BHI at 37°C after 24h were respectively 0.9, 0.9, 3.0, 2.9, 3.3, 4.1 and 3.7 (Guillier et al., 2007). Similar MICs have been found in various media for thymol and carvacrol (Cosentino et al., 1999; Helander et al., 1998; Kim et al., 1995a; Olasupo et al., 2003).

Plant-derived compounds have also been shown to retain a degree of efficacy in foods, but they generally need to be used at higher concentrations to achieve the same level of inhibition. Carvacrol at 3% succeeded in eradicating *Salmonella* in fish cubes after 4 days at 4°C while citral and geraniol were less efficient (Kim et al., 1995b). Basil oil at 50 ppm reduced the number of bacteria in food from 5 to 2 log cfu/g after storage for 3 days (Rattanachaikunsopon & Phumkhachorn, 2010). The susceptibility of bacteria to the antimicrobial effect of essential oils appears to be dependent on the composition and pH of the food, and the storage temperature (Juven et al., 1994; Tassou et al., 1995). Proteins and lipids, among other food constituents, can interact with essential oils, thus limiting their activity. For example, neutralization of the antibacterial effect of thymol was demonstrated by the addition of bovine serum albumin, which probably binds thymol and thus prevents it from penetrating through the bacterial membrane (Juven et al., 1994). Moreover, the activity of thymol on *Salmonella* is greater under anaerobic conditions (Juven et al., 1994).

The concentrations used in food products are to a great extent governed by their effect on the organoleptic properties. The concentrations thus needed to inhibit bacteria often exceed the flavor threshold acceptable to consumers. In that context, multifactorial preservation is highly appropriate for these compounds. Characterization of the synergy between two compounds can be achieved using the isobologram method or by calculating the fractional inhibitory concentration (Najjar et al., 2007), but different experimental designs are necessary if the combinations involve numerous compounds. Some combinations have been shown to be highly effective against the growth of *Salmonella*, such as the use of two plant-derived compounds (cinnamaldehyde-thymol, cinnamaldehyde-carvacrol, thymol-carvacrol) (Zhou et al., 2007b), three compounds (thymol-carvacrol-citral) (Nazer et al., 2005), or plant-derived compounds associated with EDTA, acetic or citric acid (Zhou et al., 2007a).

Some of these compounds can be also used in the vapor phase. For example, allyl isothiocyanate (8.3 µL/liter of air), carvacrol (41.5 µL/liter) or cinnamaldehyde (41.5 µL/liter) were able to inactivate *Salmonella* (decrease of population > 5 log) on sliced tomatoes at 4°C in 10 days (Obaidat & Frank, 2009).

### 3.2 The tolerance response of *Salmonella* to plant-derived compounds

In the context of multifactor preservation, when bacteria are subjected to sub-inhibitory concentrations of antimicrobials, attention should be paid to the potential for bacterial adaptation and the induction of cross-resistance to other treatments. Little is known today about bacterial adaptation to plant-derived compounds, notably in the case of *Salmonella*. Nevertheless, the presence of thymol during the growth of *S. Thompson* induced the up- or down-regulation of many proteins (Di Pasqua et al., 2010). Different chaperon proteins were up-regulated or *de novo* synthesized, such as GroEL and Dnak which are key proteins in protecting cells against stress. Outer membrane proteins were also up-regulated in the presence of thymol (Di Pasqua et al., 2010). Moreover, the membrane fatty acid composition was markedly modified during growth in the presence of plant-derived terpenes such as carvacrol, thymol, citral or eugenol (Dubois-Brissonnet et al., 2011). The saturated fatty acids became more abundant in the cell membrane of *Salmonella* grown in the presence of these terpenes. Membrane saturation appears to be a primary response of bacteria in order to maintain both membrane integrity and functionality. Moreover, compared with control

cells, the cyclization of unsaturated fatty acids (UFA) to cyclopropane fatty acids (CFA) was markedly reduced when cells entered the stationary phase. It was hypothesized that terpenes, that have log  $P_{o/w}$  values of about 3, probably accumulate between the acyl chains of fatty acids, thus limiting the accessibility of *S*-adenosylmethionine to the UFA *cis*-double bond (Dubois-Brissonnet et al., 2011).

A few studies have demonstrated cross-resistance induced by plant-derived compounds. *Salmonella* exposed to sublethal concentrations of tea tree oil displayed reduced susceptibility to a range of antibiotics when compared to non-habituated cultures (McMahon et al., 2007). For example, with a MIC of mupirocin of 64 mg/L in control *S. Enteritidis*, 1024 mg/L was not efficient against adapted cells (McMahon et al., 2007). *S. Typhimurium* cells adapted to carvacrol, thymol, citral or eugenol have all displayed higher tolerance to the bactericidal activity of two disinfectants used in industrial and medical environments, peracetic acid and didecyl dimethyl ammonium bromide (Dubois-Brissonnet et al., 2011). Because these two antimicrobials have different modes of action, it was hypothesized that this induced tolerance could be a general response to the stress induced by slow growth rates and by the reduction in membrane permeability caused by membrane saturation and terpene intercalation (Dubois-Brissonnet et al., 2011).

#### 4. *Salmonella* adaptation to disinfectants – The biofilm implication

Disinfectants are chemical agents used to decontaminate inanimate surfaces. Several groups of disinfectants are regularly used in food processing environments; e.g. halogen-releasing agents, aldehydes, peroxygens or quaternary ammonium compounds (McDonnell & Russell, 1999). The mode of action of these disinfectants has been described extensively in numerous reviews (McDonnell & Russell, 1999; Russell, 2003). Unlike antibiotics, they have a broad spectrum of antimicrobial activity and generally act on several targets in microbial cells, such as outer and cytoplasmic membranes, functional and structural proteins, DNA, RNA and other cytosolic constituents (Russell, 2003).

##### 4.1 *Salmonella* tolerance response to disinfectants

As disinfectants are intended to inactivate rapidly the biocontamination of surfaces, bacterial resistance to a disinfectant is generally evaluated by determining the minimum bactericidal concentration. The MBCs of *Salmonella* to three widely used disinfectants (peracetic acid, benzalkonium chloride and ortho-phthalaldehyde) were evaluated comparative to those of seven other bacterial species (Bridier et al., 2011a). The MBCs, defined here as the concentrations enabling a 5-log reduction in the initial population within 5 minutes at 20°C, were 8.2, 42 and 175 mg/L for the three disinfectants, respectively. Compared with other species, *Salmonella* did not display a remarkable resistance to these three disinfectants. There were only slight intra-specie variations among the ten different *Salmonella* strains, whatever their serotype (Bridier et al., 2011a).

Nevertheless, *Salmonella* is frequently isolated from food-processing environments. For example, *Salmonella* was isolated in 5.3% of 3485 samples of pork and 13.8% of 3573 environmental samples from seven slaughterhouses in four European countries (Hald et al., 2003). In Canada, 37.5% of the 1295 samples collected from 65 abattoirs were positive for *Salmonella* (Bohaychuk et al., 2009). The link between a persistence of *Salmonella* in the

food-processing environment and its adaptation to the disinfectants used in that environment has been discussed. On the one hand, some authors have demonstrated that there was no obvious association between the susceptibility of isolates to disinfectants, their tendencies to persist and the previous use of biocides (Gradel et al., 2005). A few serotypes isolated from Danish broiler houses tended to have higher MICs to some disinfectants, but not necessarily those they had encountered previously (Gradel et al., 2005). Similarly, some Norwegian isolates found to be persistent in fish feed factories were no more resistant to nine disinfectants used in these than isolates from other sources (Moretro et al., 2003). On the other hand, *Salmonella* is known to be able to adapt itself to some disinfectants and to demonstrate increased resistance to disinfection procedures. For example, the benzalkonium chloride (BKC) MIC of *S. Virchow* was shown to rise from 4 to 256 µg/mL after repeated exposure to sublethal concentrations (Braoudaki & Hilton, 2004). This strain was also able to adapt to chlorhexidine. Moreover, the benzalkonium adapted-strain displayed elevated tolerance to both BKC and chlorhexidine; however, the chlorhexidine-adapted strain did not display reciprocal cross-resistance to benzalkonium chloride, suggesting specific resistance mechanisms (Braoudaki & Hilton, 2004). Growth in the presence of increasing sub-inhibitory concentrations of tri-sodium phosphate or acidified sodium chlorite also caused a significant increase in their MICs for *Salmonella* (Alonso-Hernando et al., 2009). Furthermore, 7-day passages of *S. Typhimurium* in sub-inhibitory concentrations of an ammonium quaternary compound containing formaldehyde and glutaraldehyde (AQCFCG) selected variants with reduced susceptibility to antibiotics (Karatzas et al., 2007).

Cross resistance to other environmental stresses was also demonstrated following disinfectant adaptation. Significant increase in thermotolerance and resistance to high pH occurred after 1h shock with 1.5% tri-sodium phosphate (TSP) (Sampathkumar et al., 2004). But sensitivity to acid and hydrogen peroxide concomitantly increased.

The impact of disinfectant adaptation on virulence is dependent on the type of biocide and conditions of stress. AQCFCG-adapted variants displayed an altered expression of several virulence proteins and reduced invasiveness in an epithelial cell line (Karatzas et al., 2008b). Conversely, *S. Typhimurium* LT2 retained its adhesive and invasive abilities after treatment with 5 mg/L peracetic acid for 1h in sewage effluent (Jolivet-Gougeon et al., 2003).

Like for many other stresses, differential protein expression seems concomitant to increased tolerance. In AQCFCG-variants, it has been demonstrated higher levels of the different proteins that protect against stressors such as oxidants or peroxides (Karatzas et al., 2008b). Following chlorine shock, *Salmonella* genes that were associated with stress response, biofilm formation or energy metabolism were also over-expressed (>1.5-fold) (Wang et al., 2010) and hydrogen peroxide treatment induced the synthesis of 30 proteins in *Salmonella*, including that of Dnak, a chaperon protein (Morgan et al., 1986). Moreover, the resistance of BKC-adapted *Salmonella* is related to the up-regulation of an active efflux system (Braoudaki & Hilton, 2005). Similarly, in pre-mentioned variants, bacterial adaptation to disinfectants coincides with the up-regulation of the AcrAB efflux pump, which is responsible for producing the multiple-antibiotic-resistance *mar* strains that are resistant to many agents (Karatzas et al., 2007).

In addition, membrane composition can sometimes be altered with disinfectant adaptation. *S. Enteritidis* grown in the presence of three sanitizers, including a chlorinated product, an alkaline cleaner and a phenolic solution, was shown to increase its short-chain polysaccharide fractions of the LPS (Venter et al., 2006). A reduction in cell hydrophobicity was induced following the adaptation of *S. Enteritidis* to BKC at sub-inhibitory concentrations, although no change to the LPS or outer membrane composition was noticed (Braoudaki & Hilton, 2005). When *Salmonella* cells were exposed to 1.5% TSP for 1 h, significant changes were seen to affect the membrane fatty acid composition: the saturated and cyclic to unsaturated fatty acid ratio increases which leads to a increase in membrane saturation (Sampathkumar et al., 2004). But modifications to membrane fluidity were not related with the induction of resistance following growth in increasing sub-inhibitory concentrations of tri-sodium phosphate (TSP) (Alonso-Hernando et al., 2010).

Because the mechanisms of *Salmonella* adaptation to disinfectants are not fully understood, it is wise to continue the widely accepted practice of rotating the use of disinfectants in the food industry to prevent the development of adaptation and increased resistance.

#### 4.2 Implication of biofilms in the tolerance response of *Salmonella* to disinfectants

The persistence of *Salmonella* in food processing environments is often related to their survival on the surface of equipment. For example, an *S. Enteritidis* strain isolated from an egg conveyor belt was found to be a source of persistent infection in poultry (Stocki et al., 2007). Similarly, *Salmonella* was able to persist in food bowls that had contained raw meat contaminated by this pathogen (Weese & Rousseau, 2006). The survival of microorganisms in food-processing environments is frequently linked to the presence of three-dimensional biofilm structures on surfaces. *Salmonella* produces a biofilm matrix that is mainly composed of fimbriae (curli) and cellulose (Solomon et al., 2005). A study of 111 *Salmonella* strains isolated in Norwegian feed and fish meal factories demonstrated that persistent strains were those that could produce more biofilm than presumably non-persisting strains (Vestby et al., 2009). Images of *Salmonella* biofilms (Figure 3) were acquired using scanning electron microscopy at the MIMA2 microscopy platform (<http://voxel.jouy.inra.fr/mima2>).

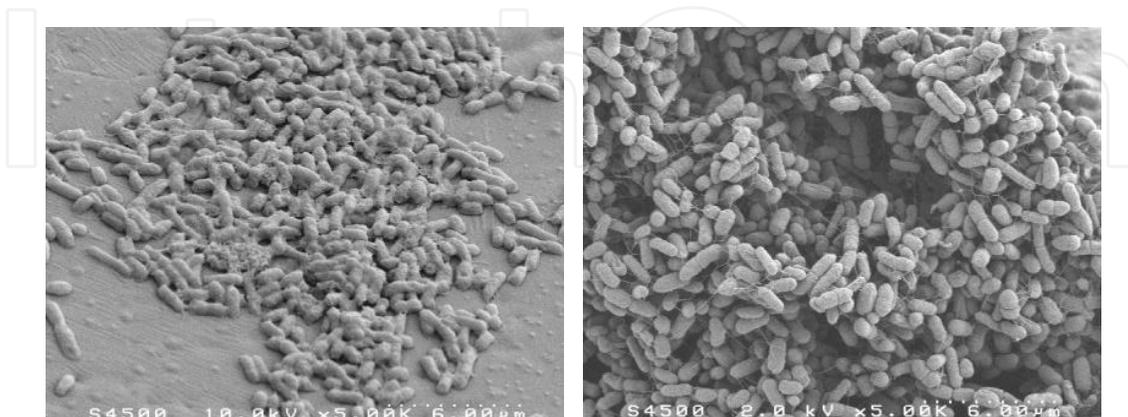


Fig. 3. Images of *Salmonella* biofilms obtained by scanning electron microscopy

Like many other biofilms, *Salmonella* biofilms display specific properties that include increased resistance to biocide treatment (Wong et al., 2010). Reductions in surface-attached

*Salmonella* using 256 ppm of sodium hypochlorite and BKC were respectively decreased 2.1 and 3-fold compared to the planktonic population (Riazi & Matthews, 2011). Sodium hypochlorite was shown to be more efficient than BKC and hydrogen peroxide against *Salmonella* biofilms formed in the Calgary Biofilm Device (Rodrigues et al., 2011). *S. Typhimurium* is more resistant to chlorine treatments when associated with *Pseudomonas fluorescens* in a mixed biofilm (Leriche & Carpentier, 1995).

Rather than a true resistance, biofilm insusceptibility is sometimes referred to as a tolerance because it is mainly induced by physiological adaptation to the biofilm mode of life (Russell, 1999). Biofilm resistance is multifactorial, resulting from the addition of different mechanisms such as diffusion and/or reaction problems affecting the sanitizers in the structure, the appearance of resistant biofilm-specific phenotypes (persister cells), of physiological and genetic heterogeneity, and adaptation to sanitizers (Bridier et al., 2011b). In deeper regions of the three-dimensional structures of biofilms, *Salmonella* may be protected against biocide activity because of the limited reaction-diffusion penetration of antimicrobial agents (Figure 4). Only sub-lethal concentrations of disinfectants can reach the bacteria which may thus develop adaptation mechanisms.

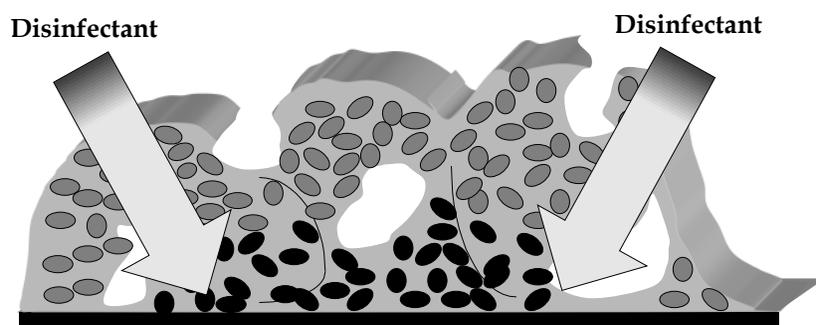


Fig. 4. Limited penetration of antimicrobial agents within the biofilm architecture due to diffusion-reaction problems (black cells can survive and adapt themselves to sub-lethal concentrations of disinfectants)

A few studies have described the adaptation of biofilm cells to disinfectants. One early study showed that when *S. Typhimurium* biofilm was disinfected daily, the proportion of viable but nonculturable cells increased in a 4-day biofilm (Leriche & Carpentier, 1995). Chlorine consumption of such biofilm was increased, suggesting that adaptation to chlorine stress induces a hyper-production of exopolymeric substances that can protect the viable cells. *Salmonella* biofilm cells were also reported to display a greater adaptation than their planktonic counterparts to BCK after continuous exposure to 1  $\mu\text{g}/\text{mL}$  of this disinfectant (Mangalappalli-Illathu & Korber, 2006). Specific proteins involved in energy metabolism, protein biosynthesis, nutrient binding, cold shock and detoxification were up-regulated. Conversely, proteins involved in proteolysis, cell envelope formation, universal stress, heat shock response (Dnak) and broad regulatory functions (Hns) were down-regulated following adaptation (Mangalappalli-Illathu & Korber, 2006).

However, surface-attached *Salmonella* exposed sequentially to 100 ppm chlorhexidine digluconate remained susceptible to the action of the sanitizer. The bacteria were not able to adapt neither develop induced resistance (Riazi & Matthews, 2011). During this experiment, the disinfectant is applied at a much higher concentration than that used by Mangalappalli-

Illathu et al., and it can more easily reach the surface-attached cells which are not embedded in an exopolymeric matrix. This therefore can explain why, in this case, the attached cells were not able to adapt and develop increased resistance.

Very little is known about cross-resistance and virulence abilities of biofilm cells adapted to disinfectant. A recent study has shown that following a disinfection treatment, surviving biofilm *Salmonella* cells demonstrated significantly up-regulated virulence genes (Rodrigues et al., 2011).

Therefore, in order to guarantee microbial food safety, cleaning procedures must be applied regularly in order to prevent biofilm formation, and the concentrations used should be high enough to eradicate surface-attached bacteria. Under these conditions, biofilm formation and adaptation to disinfectants should be limited.

## 5. Conclusion

Because antimicrobials are currently used to ensure microbiological food safety, the potential for the development of bacterial adaptation and induced tolerance to antimicrobial stresses must be taken into account. Although the mechanisms of acid adaptation are now well understood, knowledge on the adaptation of *Salmonella* to naturally occurring compounds and disinfectants needs to be improved. Research must be carried out to monitor the evolution of stress-tolerant pathogens in foods and to generate appropriate methods to detect and control them. Studies should focus on food matrix systems or food-processing plants because these should be considered as highly stressful environments. The ultimate purpose should be to predict and control the behavior of *Salmonella* in terms of its stress tolerance, cross resistance to other stresses, persistence in the environment and virulence in the host.

## 6. Acknowledgments

I thank Dr. R. Briandet (INRA) for helpful discussions and Dr. M. Naïtali (AgroParisTech) for critical reading of the manuscript.

## 7. References

- Abee T, Krockel L, Hill C. (1995). Bacteriocins: modes of action and potentials in food preservation and control of food poisoning. *International Journal of Food Microbiology*, Vol.28, pp.169-185, 0168-1605
- Adams P, Fowler R, Kinsella N, Howell G, Farris M, Coote P, O'Connor CD. (2001). Proteomic detection of PhoPQ- and acid-mediated repression of *Salmonella* motility. *Proteomics*, Vol.1, pp.597-607, 1615-9853
- Alonso-Hernando A, Alonso-Calleja C, Capita R. (2010). Effects of exposure to poultry chemical decontaminants on the membrane fluidity of *Listeria monocytogenes* and *Salmonella enterica* strains. *International Journal of Food Microbiology*, Vol.137, pp.130-136, 0168-1605
- Alonso-Hernando A, Capita R, Prieto M, Alonso-Calleja C. (2009). Adaptation and cross-adaptation of *Listeria monocytogenes* and *Salmonella enterica* to poultry decontaminants. *J Microbiol*, Vol.47, pp.142-146, 1225-8873

- Alvarez-Ordóñez A, Fernández A, Bernardo A, López M. (2009). Comparison of acids on the induction of an Acid Tolerance Response in *Salmonella typhimurium*, consequences for food safety. *Meat Sci*, Vol.81, pp.65-70, 0309-1740
- Alvarez-Ordóñez A, Fernández A, Bernardo A, López M. (2010a). Acid tolerance in *Salmonella Typhimurium* induced by culturing in the presence of organic acids at different growth temperatures. *Food Microbiol*, Vol.27, pp.44-49, 0740-0020
- Alvarez-Ordóñez A, Fernández A, Bernardo A, López M. (2010b). Arginine and lysine decarboxylases and the Acid Tolerance Response of *Salmonella Typhimurium*. *International Journal of Food Microbiology*, Vol.136, pp.278-282, 0168-1605
- Alvarez-Ordóñez A, Fernández A, López M, Arenas R, Bernardo A. (2008). Modifications in membrane fatty acid composition of *Salmonella Typhimurium* in response to growth conditions and their effect on heat resistance. *International Journal of Food Microbiology*, Vol.123, pp.212-219, 0168-1605
- Anonymous. 1998. Directive 98/8/CE of the European parliament and of the council of 16 February 1998 concerning the placing of biocidal products on the market. In: Official Journal of the European Communities.
- Anonymous. 2004. Morbidité et mortalité dues aux maladies infectieuses d'origine alimentaire en France. In: Rapport Institut de Veille Sanitaire -Agence Française de Sécurité Sanitaire des Aliments.
- Anonymous. 2006. Regulation (EC) N° 1907/2006 of the European parliament and of the council of 18 december 2006 concerning the registration, evaluation, authorisation and restriction of chemicals (REACH). In: Official Journal of the European Union.
- Anonymous. 2009. Proposal for a regulation of the European parliament and of the council concerning the placing on the market and use of biocidal products. In: Commission of the European Communities.
- Arvizu-Medrano SM, Escartin EF. (2005). Effect of acid shock with hydrochloric, citric, and lactic acids on the survival and growth of *Salmonella Typhi* and *Salmonella Typhimurium* in acidified media. *Journal of Food Protection*, Vol.68, pp.2047-2053, 0362-028X
- Baik HS, Bearson S, Dunbar S, Foster JW. (1996). The acid tolerance response of *Salmonella typhimurium* provides protection against organic acids. *Microbiology*, Vol.142, pp.3195-3200,
- Bearson S, Bearson B, Foster JW. (1997). Acid stress responses in enterobacteria. *FEMS Microbiol Lett*, Vol.147, pp.173-180,
- Bohaychuk VM, Checkley SL, Gensler GE, Barrios PR. (2009). Microbiological baseline study of poultry slaughtered in provincially inspected abattoirs in Alberta, Canada. *Can Vet J-Rev Vet Can*, Vol.50, pp.173-178, 0008-5286
- Braoudaki M, Hilton AC. (2005). Mechanisms of resistance in *Salmonella enterica* adapted to erythromycin, benzalkonium chloride and triclosan. *Int J Antimicrob Agents*, Vol.25, pp.31-37, 0924-8579
- Braoudaki M, Hilton C. (2004). Adaptive resistance to biocides in *Salmonella enterica* and *Escherichia coli* 0157 and cross-resistance to antimicrobial agents. *Journal of Clinical Microbiology*, Vol.42, pp.73-78,

- Bridier A, Briandet R, Thomas V, Dubois-Brissonnet F. (2011a). Comparative biocidal activity of peracetic acid, benzalkonium chloride and *ortho*- phthalaldehyde on 77 bacterial strains. *Journal of Hospital Infection*, Vol.78, pp.208-213,
- Bridier A, Briandet R, Thomas V, Dubois-Brissonnet F. (2011b). Resistance of bacterial biofilms to disinfectants: a review. *Biofouling*, Vol.27, pp.1017-1032,
- Brown JL, Ross T, McMeekin TA, Nichols PD. (1997). Acid habituation of *Escherichia coli* and the potential role of cyclopropane fatty acids in low pH tolerance. *International Journal of Food Microbiology*, Vol.37, pp.163-173,
- Burt S. (2004). Essential oils: their antibacterial properties and potential applications in foods - a review. *International Journal of Food Microbiology*, Vol.94, pp.223-253,
- Calhoun LN, Kwon YM. (2010). The effect of long-term propionate adaptation on the stress resistance of *Salmonella* Enteritidis. *J Appl Microbiol*, Vol.109, pp.1294-1300, 1364-5072
- Calhoun LN, Liyanage R, Lay JO, Kwon YM. (2010). Proteomic analysis of *Salmonella enterica* serovar Enteritidis following propionate adaptation. *BMC Microbiol*, Vol.10, pp.11, 1471-2180
- Calicioglu M, Sofos JN, Samelis J, Kendall PA, Smith GC. (2003). Effect of acid adaptation on inactivation of *Salmonella* during drying and storage of beef jerky treated with marinades. *International Journal of Food Microbiology*, Vol.89, pp.51-65, 0168-1605
- Chang YY, Cronan JE. (1999). Membrane cyclopropane fatty acid content is a major factor in acid resistance of *Escherichia coli*. *Molecular Microbiology*, Vol.33, pp.249-259, 0950-382X
- Cosentino S, Tuberoso CIG, Pisano B, Satta M, Mascia V, Arzedi E, Palmas F. (1999). In-vitro antimicrobial activity and chemical composition of Sardinian *Thymus* essential oils. *Letters in Applied Microbiology*, Vol.29, pp.130-135,
- Costerton J, Lewandowski Z, Caldwell D, Korber D, Lappin-Scott H. (1995). Microbial biofilms. *Annu Rev Microbiol*, Vol.49, pp.711-745,
- de Jonge R, Ritmeester WS, van Leusden FM. (2003). Adaptive responses of *Salmonella enterica* serovar Typhimurium DT104 and other *S. Typhimurium* strains and *Escherichia coli* O157 to low pH environments. *J Appl Microbiol*, Vol.94, pp.625-632, 1364-5072
- Di Pasqua R, Mamone G, Ferranti P, Ercolini D, Mauriello G. (2010). Changes in the proteome of *Salmonella enterica* serovar Thompson as stress adaptation to sublethal concentrations of thymol. *Proteomics*, Vol.10, pp.1040-1049, 1615-9853
- Dodd CER, Aldsworth TG. (2002). The importance of RpoS in the survival of bacteria through food processing. *International Journal of Food Microbiology*, Vol.74, pp.189-194, 0168-1605
- Doores S. (2005). Organic acids, In: Antimicrobials in food, Boca Raton, CRC Taylor & Francis, p. 91-142,
- Dubois-Brissonnet F, Naitali M, Maf AA, Briandet R. (2011). Induction of fatty acid composition modifications and tolerance to biocides in *Salmonella enterica* serovar Typhimurium by plant-derived terpenes. *Applied and Environmental Microbiology*, Vol.77, pp.906-910,
- Dziezak JD. (1986). Antioxidants and antimicrobial agents. *Food technology*, Vol.40, pp.94-111,

- Fang FC, Libby SJ, Buchmeier NA, Loewen PC, Switala J, Harwood J, Guiney DG. (1992). The Alternative sigma-factor Katf (Rpos) regulates *Salmonella* Virulence. *Proc Natl Acad Sci U S A*, Vol.89, pp.11978-11982, 0027-8424
- Foster JW. (1991). *Salmonella* acid shock proteins are required for the adaptive acid tolerance response. *Journal of Bacteriology*, Vol.173, pp.6896-6902, 0021-9193
- Foster JW. (1993). The acid tolerance response of *Salmonella* Typhimurium involves transient synthesis of key acid shock proteins. *Journal of Bacteriology*, Vol.175, pp.1981-1987, 0021-9193
- Foster JW. (1999). When protons attack: microbial strategies of acid adaptation. *Curr Opin Microbiol*, Vol.2, pp.170-174, 1369-5274
- Foster JW, Hall HK. (1990). Adaptive acidification tolerance response of *Salmonella typhimurium*. *Journal of Bacteriology*, Vol.172, pp.771-778,
- Gahan CGM, Hill C. (1999). The relationship between acid stress responses and virulence in *Salmonella typhimurium* and *Listeria monocytogenes*. *International Journal of Food Microbiology*, Vol.50, pp.93-100,
- Gradel KO, Randall L, Sayers AR, Davies RH. (2005). Possible associations between *Salmonella* persistence in poultry houses and resistance to commonly used disinfectants and a putative role of *mar*. *Veterinary Microbiology*, Vol.107, pp.127-138,
- Greenacre EJ, Brocklehurst TF, Waspe CR, Wilson DR, Wilson PDG. (2003). *Salmonella enterica* serovar Typhimurium and *Listeria monocytogenes* acid tolerance response induced by organic acids at 20 degrees C: optimization and modeling. *Applied and Environmental Microbiology*, Vol.69, pp.3945-3951, 0099-2240
- Greenacre EJ, Lucchini S, Hinton JCD, Brocklehurst TF. (2006). The lactic acid-induced acid tolerance response in *Salmonella enterica* serovar typhimurium induces sensitivity to hydrogen peroxide. *Applied and Environmental Microbiology*, Vol.72, pp.5623-5625, 0099-2240
- Guillier L, Nazer A, Dubois-Brissonnet F. (2007). Growth response of *Salmonella* Typhimurium in presence of natural and synthetic antimicrobials: estimation of MICs from three different models. *Journal of Food Protection*, Vol.70, pp.2243-2250,
- Hald T, Wingstrand A, Swanenburg M, von Altrock A, Thorberg BM. (2003). The occurrence and epidemiology of *Salmonella* in European pig slaughterhouses. *Epidemiol Infect*, Vol.131, pp.1187-1203, 0950-2688
- Helander IM, Alakomi H-L, Latva-Kala K, Mattila-Sandholm T, Pol IE, Smid EJ, Morris LGM, Von Wright A. (1998). Characterization of the action of selected essential oil components on gram-negative bacteria. *J Agric Food Chem*, Vol.46, pp.3590-3595,
- Hoiby N, Bjarsholt T, Givskov M, Molin S, Ciofu O. (2010). Antibiotic resistance of bacterial biofilms. *Int J Antimicrob Agents*, Vol.35, pp.322-332, 0924-8579
- Huhtanen CN. (1975). Use of pH gradient plates for increasing acid tolerance of *Salmonellae*. *Applied Microbiology*, Vol.29, pp.309-312, 0003-6919
- Humphrey TJ, Richardson NP, Statton KM, Rowbury RJ. (1993). Acid habituation in *Salmonella enteritidis* PT4: impact of inhibition of protein synthesis. *Letters in Applied Microbiology*, Vol.16, pp.228-230,
- Humphrey TJ, Williams A, McAlpine K, Lever MS, GuardPetter J, Cox JM. (1996). Isolates of *Salmonella enterica* Enteritidis PT4 with enhanced heat and acid tolerance are

- more virulent in mice and more invasive in chickens. *Epidemiol Infect*, Vol.117, pp.79-88, 0950-2688
- Jolivet-Gougeon A, Sauvager F, Arturo-Schaan M, Bonnaure-Mallet M, Cormier M. (2003). Influence of peracetic acid on adhesion/invasion of *Salmonella enterica* serotype Typhimurium LT2. *Cell Biol Toxicol*, Vol.19, pp.83-93, 0742-2091
- Juven BJ, Kanner J, Schved F, Weisslowicz H. (1994). Factors that interact with the antibacterial action of thyme essential oil and its active constituents. *J Appl Bacteriol*, Vol.76, pp.626-631, 0021-8847
- Karatzas KAG, Hocking PM, Jorgensen F, Mattick K, Leach S, Humphrey TJ. (2008a). Effects of repeated cycles of acid challenge and growth on the phenotype and virulence of *Salmonella enterica*. *J Appl Microbiol*, Vol.105, pp.1640-1648, 1364-5072
- Karatzas KAG, Randall LP, Webber M, Piddock LJV, Humphrey TJ, Woodward MJ, Coldham NG. (2008b). Phenotypic and proteomic characterization of multiply antibiotic-resistant variants of *Salmonella enterica* serovar Typhimurium selected following exposure to disinfectants. *Applied and Environmental Microbiology*, Vol.74, pp.1508-1516, 0099-2240
- Karatzas KAG, Webber MA, Jorgensen F, Woodward MJ, Piddock LJV, Humphrey TJ. (2007). Prolonged treatment of *Salmonella enterica* serovar Typhimurium with commercial disinfectants selects for multiple antibiotic resistance, increased efflux and reduced invasiveness. *J Antimicrob Chemother*, Vol.60, pp.947-955, 0305-7453
- Kim BH, Kim S, Kim HG, Lee J, Lee IS, Park YK. (2005). The formation of cyclopropane fatty acids in *Salmonella enterica* serovar Typhimurium. *Microbiology-(UK)*, Vol.151, pp.209-218, 1350-0872
- Kim J, Marshall MR, Wei C-I. (1995a). Antibacterial activity of some essential oil components against five foodborne pathogens. *Journal of Agricultural and Food Chemistry*, Vol.43, pp.2839-2845,
- Kim JM, Marshall MR, Cornell JA, Preston JF, Wei CI. (1995b). Antibacterial activity of carvacrol, citral, and geraniol against *Salmonella* Typhimurium in culture medium and on fish cubes. *Journal of Food Science*, Vol.60, pp.1364-1374,
- Koutsoumanis KP, Sofos JN. (2004). Comparative acid stress response of *Listeria monocytogenes*, *Escherichia coli* O157: H7 and *Salmonella* Typhimurium after habituation at different pH conditions. *Letters in Applied Microbiology*, Vol.38, pp.321-326, 0266-8254
- Kwon YM, Ricke SC. (1998). Induction of acid resistance of *Salmonella typhimurium* by exposure to short-chain fatty acids. *Applied and Environmental Microbiology*, Vol.64, pp.3458-3463,
- Lambert RJW, Pearson J. (2000). Susceptibility testing : accurate and reproducible minimum inhibitory concentration (MIC) and non-inhibitory concentration (NIC) values. *J Appl Microbiol*, Vol.88, pp.784-790,
- Lee IS, Lin JS, Hall HK, Bearson B, Foster JW. (1995). The stationary-phase sigma-factor sigma(S) (Rpos) is required for a sustained acid tolerance response in virulent *Salmonella* Typhimurium. *Molecular Microbiology*, Vol.17, pp.155-167, 0950-382X

- Lee IS, Slonczewski JL, Foster JW. (1994). A low pH inducible, stationary-phase acid tolerance response in *Salmonella* Typhimurium. *Journal of Bacteriology*, Vol.176, pp.1422-1426, 0021-9193
- Leistner L. (1995). Food preservation by hurdle technology. *Trends in Food Science and Technology*, Vol.6, pp.41-46,
- Leriche V, Carpentier B. (1995). Viable but nonculturable *Salmonella typhimurium* in single- and binary-species biofilms in response to chlorine treatment. *Journal of Food Protection*, Vol.58, pp.1186-1191,
- Leyer GJ, Johnson EA. (1992). Acid adaptation promotes survival of *Salmonella* spp in cheese. *Applied and Environmental Microbiology*, Vol.58, pp.2075-2080, 0099-2240
- Leyer GJ, Johnson EA. (1993). Acid adaptation induces cross-protection against environmental stresses in *Salmonella typhimurium*. *Applied and Environmental Microbiology*, Vol.59, pp.1842-1847,
- Leyer GJ, Johnson EA. (1997). Acid adaptation sensitizes *Salmonella typhimurium* to hypochlorous acid. *Applied and Environmental Microbiology*, Vol.63, pp.461-467,
- Lin JS, Lee IS, Frey J, Slonczewski JL, Foster JW. (1995). Comparative analysis of extreme acid survival in *Salmonella* Typhimurium, *Shigella flexneri*, and *Escherichia coli*. *Journal of Bacteriology*, Vol.177, pp.4097-4104, 0021-9193
- Mackey BM, Derrick CM. (1986). Elevation of the heat resistance of *Salmonella typhimurium* by sublethal heat shock. *J Appl Bacteriol*, Vol.61, pp.389-393,
- Mangalappalli-Illathu AK, Korber DR. (2006). Adaptive resistance and differential protein expression of *Salmonella enterica* serovar Enteritidis biofilms exposed to benzalkonium chloride. *Antimicrobial Agents and Chemotherapy*, Vol.50, pp.3588-3596,
- Mazzotta AS. (2001). Thermal inactivation of stationary-phase and acid-adapted *Escherichia coli* O157: H7, *Salmonella*, and *Listeria monocytogenes* in fruit juices. *Journal of Food Protection*, Vol.64, pp.315-320, 0362-028X
- McDonnell G, Russell AD. (1999). Antiseptics and disinfectants : activity, action, and resistance. *Clinical Microbiology Reviews*, Vol.12, pp.147-179,
- McMahon MAS, Blair IS, Moore JE, McDowell DA. (2007). Habituation to sub-lethal concentrations of tea tree oil (*Melaleuca alternifolia*) is associated with reduced susceptibility to antibiotics in human pathogens. *J Antimicrob Chemother*, Vol.59, pp.125-127, 0305-7453
- Mead PS, Slutsker L, Dietz V, McCaig LF, Bresee JS, Shapiro C, Griffin PM, Tauxe RV. (1999). Food-related illness and death in the United States. *Emerging Infectious Diseases*, Vol.5, pp.609-625,
- Mesle JF, Zucca J. (1996). Les facteurs du développement, In: Microbiologie alimentaire - Tome 1 Aspect microbiologique de la sécurité et de la qualité des aliments, Paris, Tec et Doc Lavoisier,
- Moretro T, Midtgaard ES, Nesse LL, Langsrud S. (2003). Susceptibility of *Salmonella* isolated from fish feed factories to disinfectants and air-drying at surfaces. *Veterinary Microbiology*, Vol.94, pp.207-217, 0378-1135
- Morgan RW, Christman MF, Jacobson FS, Storz G, Ames BN. (1986). Hydrogen peroxide inducible proteins in *Salmonella* Typhimurium overlap with heat shock and other stress proteins. *Proc Natl Acad Sci U S A*, Vol.83, pp.8059-8063, 0027-8424

- Morin N, Lanneluc I, Connil N, Cottenceau M, Pons AM, Sable S. (2011). Mechanism of bactericidal activity of microcin L in *Escherichia coli* and *Salmonella enterica*. *Antimicrobial Agents and Chemotherapy*, Vol.55, pp.997-1007, 0066-4804
- Naidu AS. (2000). Natural food antimicrobial systems, Boca Raton, CRC Press LLC,
- Najjar MB, Kashtanov D, Chikindas ML. (2007). e-poly-l-lysine and nisin A act synergistically against Gram-positive food-borne pathogens *Bacillus cereus* and *Listeria monocytogenes*. *Letters in Applied Microbiology*, Vol.45, pp.13-18,
- Nazer A, Kobilinsky A, Tholozan JL, Dubois-Brissonnet F. (2005). Combinations of food antimicrobials at low levels to inhibit the growth of *Salmonella* sv.Typhimurium: a synergistic effect? *Food Microbiol*, Vol.22, pp.391-398,
- Obaidat MM, Frank JF. (2009). Inactivation of *Salmonella* and *Escherichia coli* O157:H7 on sliced and whole tomatoes by allyl isothiocyanate, carvacrol, and cinnamaldehyde in vapor phase. *Journal of Food Protection*, Vol.72, pp.315-324, 0362-028X
- Olasupo NA, Fitzgerald DJ, Gasson MJ, Narbad A. (2003). Activity of natural antimicrobial compounds against *Escherichia coli* and *Salmonella enterica* serovar Typhimurium. *Letters in Applied Microbiology*, Vol.37, pp.448-451,
- Rattanachaikunsopon P, Phumkhachorn P. (2010). Antimicrobial activity of basil (*Ocimum basilicum*) oil against *Salmonella* Enteritidis in vitro and in Food. *Biosci Biotechnol Biochem*, Vol.74, pp.1200-1204, 0916-8451
- Riazi S, Matthews KR. (2011). Failure of foodborne pathogens to develop resistance to sanitizers following repeated exposure to common sanitizers. *Int Biodeterior Biodegrad*, Vol.65, pp.374-378, 0964-8305
- Rodrigues D, Cerca N, Teixeira P, Oliveira R, Ceri H, Azeredo J. (2011). *Listeria monocytogenes* and *Salmonella enterica* Enteritidis biofilms susceptibility to different disinfectants and stress response and virulence gene expression of surviving cells. *Microb Drug Resist*, Vol.17, pp.181-189, 1076-6294
- Roller S. (2003). Natural antimicrobials for minimal processing of foods, Cambridge, Woodhead Publishing Limited,
- Russell AD. (1999). Bacterial resistance to disinfectants: present knowledge and future problems. *Journal of Hospital Infection*, Vol.43, pp.S57-S68, 0195-6701
- Russell AD. (2003). Similarities and differences in the responses of microorganisms to biocides. *J Antimicrob Chemother*, Vol.52, pp.750-763, 0305-7453
- Sampathkumar B, Khachatourians GG, Korber DR. (2004). Treatment of *Salmonella enterica* serovar Enteritidis with a sublethal concentration of trisodium phosphate or alkaline pH induces thermotolerance. *Applied and Environmental Microbiology*, Vol.70, pp.4613-4620, 0099-2240
- Schved F, Henis Y, Juven BJ. (1994). Response of spheroplasts and chelator permeabilized cells of Gram-negative bacteria to the action of the bacteriocins pediocin Sj-1 and nisin. *International Journal of Food Microbiology*, Vol.21, pp.305-314, 0168-1605
- Sharma M, Adler BB, Harrison MD, Beuchat LR. (2005). Thermal tolerance of acid-adapted and unadapted *Salmonella*, *Escherichia coli* O157 : H7, and *Listeria monocytogenes* in cantaloupe juice and watermelon juice. *Letters in Applied Microbiology*, Vol.41, pp.448-453, 0266-8254

- Shen HW, Yu RC, Chou CC. (2007). Acid adaptation affects the viability of *Salmonella* Typhimurium during the lactic fermentation of skim milk and product storage. *International Journal of Food Microbiology*, Vol.114, pp.380-385, 0168-1605
- Solomon EB, Niemira BA, Sapers GM, Annous BA. (2005). Biofilm formation, cellulose production, and curli biosynthesis by *Salmonella* originating from produce, animal, and clinical sources. *Journal of Food Protection*, Vol.68, pp.906-912, 0362-028X
- Stocki SL, Annett CB, Sibley CD, McLaws M, Checkley SL, Singh N, Surette MG, White AP. (2007). Persistence of *Salmonella* on egg conveyor belts is dependent on the belt type but not on the rdar morphotype. *Poult Sci*, Vol.86, pp.2375-2383, 0032-5791
- Tassou CC, Drosinos EH, Nychas GJE. (1995). Effects of essential oil from mint (*Mentha piperita*) on *Salmonella* Enteritidis and *Listeria monocytogenes* in model food systems at 4 degrees and 10 degrees C. *J Appl Bacteriol*, Vol.78, pp.593-600, 0021-8847
- Tosun H, Gönül SA. (2003). Acid adaption protects *Salmonella* Typhimurium from environmental stresses. *Turkish Journal of Biology*, Vol.27, pp.31-36,
- Venter P, Abraham M, Lues JFR, Ivanov I. (2006). Influence of commercial sanitizers on lipopolysaccharide production by *Salmonella* Enteritidis ATCC 13076. *Journal of Food Protection*, Vol.69, pp.2889-2895, 0362-028X
- Vestby LK, Moretro T, Langsrud S, Heir E, Nesse LL. (2009). Biofilm forming abilities of *Salmonella* are correlated with persistence in fish meal- and feed factories. *BMC Vet Res*, Vol.5, pp.6, 1746-6148
- Wang SY, Phillippy AM, Deng KP, Rui XQ, Li ZX, Tortorello ML, Hang W. (2010). Transcriptomic responses of *Salmonella enterica* serovars Enteritidis and Typhimurium to chlorine-based oxidative stress. *Applied and Environmental Microbiology*, Vol.76, pp.5013-5024, 0099-2240
- Weese JS, Rousseau J. (2006). Survival of *Salmonella* Copenhagen in food bowls following contamination with experimentally inoculated raw meat: effects of time, cleaning, and disinfection. *Can Vet J-Rev Vet Can*, Vol.47, pp.887-889, 0008-5286
- Wesche AM, Gurtler JB, Marks BP, Ryser ET. (2009). Stress, sublethal injury, resuscitation, and virulence of bacterial foodborne pathogens. *Journal of Food Protection*, Vol.72, pp.1121-1138, 0362-028X
- Wong HS, Townsend KM, Fenwick SG, Trengove RD, O'Handley RM. (2010). Comparative susceptibility of planktonic and 3-day-old *Salmonella* Typhimurium biofilms to disinfectants. *J Appl Microbiol*, Vol.108, pp.2222-2228, 1364-5072
- Xu H, Lee HY, Ahn J. (2008). Cross-protective effect of acid-adapted *Salmonella enterica* on resistance to lethal acid and cold stress conditions. *Letters in Applied Microbiology*, Vol.47, pp.290-297, 0266-8254
- Yousef AE, Courtney PD. (2003). Basics of stress adaptation and implications in new-generation foods, In: *Microbial stress adaptation and food safety*, Boca Raton (USA), CRC Press,
- Zhou F, Baoping JI, Zhang H, Jiang H, Yang Z, Li J, Li J, Ren Y, Yan W. (2007a). Synergistic effect of thymol and carvacrol combined with chelators and organic

acids against *Salmonella* Typhimurium. *Journal of Food Protection*, Vol.70, pp.1704-1709

Zhou F, Ji B, Zhang H, Jiang H, Yang Z, Li J, Li J, Yan W. (2007b). The antibacterial effect of cinnamaldehyde, thymol, carvacrol and their combinations against the foodborne pathogens *Salmonella* Typhimurium. *J Food Saf*, Vol.27, pp.124-133

IntechOpen

IntechOpen

© 2012 The Author(s). Licensee IntechOpen. This is an open access article distributed under the terms of the [Creative Commons Attribution 3.0 License](#), which permits unrestricted use, distribution, and reproduction in any medium, provided the original work is properly cited.

IntechOpen

IntechOpen