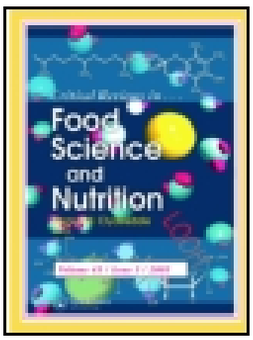

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REVIEW

Salmonella enterica: A hidden risk for dry-cured meat consumption?

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ABSTRACT

Meat curing, fermentation, and drying are both preservation technologies, and traditional manufacturing practices. Despite being considered a safe food, due to the several hurdles that prevent pathogen growth, dry-cured meat manufacturing may not ensure complete pathogen elimination. Besides, the final products are still susceptible to microbial contamination. *Salmonella enterica* is noteworthy among the pathogenic microorganisms that can contaminate these products. To survive hypertonic/hyperosmotic, acid, and low a_w/desiccation stresses intrinsically associated with dry-curing, *Salmonella* has evolved with highly sophisticated mechanisms, comprising sensors/receptors, signaling cascade systems, and enzymes/transcription factors that ensure their tolerance and survival despite many harsh environmental conditions. Links between osmotic and acid stresses, such as the dissociable sigma factor of RNA polymerase, which regulates gene transcription, and mutual membrane receptors like the two-component system EnvZ/OmpR, which senses abiotic conditions, lead to stress cross-protection. Furthermore, virulence gene expression seems to be triggered by sublethal stresses on pre-adapted *Salmonella* cells, increasing their adherence and invasiveness of host cells. These are evidence that the ability to tolerate stresses enhances *Salmonella* pathogenicity and compromises the safety of dry-cured meats, by sheltering the pre-exposed and, subsequently, more virulent, stressed bacterial cells.

KEYWORDS

Stress responses; Sublethal abiotic stresses; Virulence; Osmotic and acid stresses; Food safety

1. Introduction

Meat processing and the development of meat-derived products were initially driven by the need to preserve meat. Fermentation, drying, and salting are highlighted as the oldest of these processes (Bosse et al., 2018). The production of low-moisture, salty, and fermented meat products is currently widespread, with several kinds of products found in different countries. Overall, dry-curing involves the use of sodium chloride, alone or combined with nitrate and nitrite, that can be mixed into minced meats (sausages), rubbed on the surface of entire pieces (hams), or used to prepare a brine for soaking entire, but small, pieces (loins) (Holck et al., 2017; Morales-Partela et al., 2017; Sadeghi-Mehr, Lautenschlaeger, and Drusch, 2016).

The manufacturing of dry-cured meats involves several steps, such as salting, fermentation, drying, and ripening. Due to these different steps, dry-cured meats exhibit hurdles that prevent microbial growth, such as high salt content (high osmolarity), reduced moisture, low water activity (a_w), and acidic pH (Coroller et al., 2015; Morales-Partera et al., 2017). The final product results in meat products with different flavors and textures, as well as extended shelf-lives. Dry-cured meats, in general, are ready to eat (RTE) products, intended for consumption without prior cooking or thermal processing (Henriksen, 2014). Although the intrinsic hurdles of the dry-cured processing reduce and inactivate foodborne pathogen growth, dry-cured products contain no step in their manufacturing process specifically designed to eliminate these microorganisms, or to deal with post-processing contamination thus leading to potential public health issues (ESFA and ECDC, 2017)

Salmonellosis is one of the major worldwide foodborne diseases (Petrovska et al., 2016), and *Salmonella* is considered important foodborne pathogen, the most common cause of confirmed bacterial outbreaks in the United States of America (USA) in 2015 (CDC, 2017). *Salmonella* food contamination can occur at any point in the food production chain, from livestock feed to food manufacturing and processing (Cunha Neto et al., 2018; Panzenhagen et al., 2018). In 2016, *Salmonella* was detected in 1.8% of meat products intended to be eaten raw in European Union countries (ESFA and ECDC, 2017). Despite the usually low levels of *Salmonella* contamination in raw materials, lack of control during processing or storage conditions may result growth of the pathogen to high levels, leading to an unsuccessful prevention of this pathogen in the final dry-cured

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product (Birk et al., 2016). Moreover, if the raw materials are pathogen-free, contamination may originate from the processing equipment and post-processing, handling, and packaging steps. In this case, even though adequate dry-curing manufacturing imposes harsh conditions for foodborne pathogen growth, *Salmonella* may still be present in the final product (Sadeghi-Mehr et al., 2016). *Salmonella* persistence in dry-cured meats can be attributed to tolerance mechanisms acquired by bacteria cells against the harsh characteristics of those food matrices. Although physiological adaptation to novel environments is advantageous for bacteria, the persistence of enteric pathogens in dry-cured meat products can facilitate the transmission of high-infectivity foodborne pathogens to human beings, increasing the number of outbreaks.

In this context, this review aims to address the risks associated with *Salmonella* survival in different dry-cured meat products. Possible routes of contamination and consideration of pathogen survival mechanisms under the main stress conditions encountered by these microorganisms in dry-cured meat matrices, as well as the links between mechanisms of acquired persistence and enhanced virulence, are explored. The gaps between known stress response mechanisms and how they could lead to cell persistence advantages and higher *Salmonella* virulence are also discussed.

2. Cured meat and meat products: An overview

The term cured meat is vastly employed to describe several products worldwide. Curing generally means using a curing salt, normally sodium chloride, and nitrate/nitrite, to produce a distinctive color and flavor in the meat product, although the formulation and technology process varies depending on the country of origin of the product (Toldra, 2006). From a technological point of view, cured products can be classified into two groups, according to their processing methods: dry-curing and wet or pickle-curing (Bosse et al., 2018). Dry-curing consists in the diffusion of the curing salt though the meat moisture, without water addition (Toldra and Flores, 1998). After salting, the product is aged (ripened) for a period ranging from weeks to years, depending on the time necessary to reach the a_w characteristic of each meat product. This process is widely used in countries located in the Mediterranean basin, where long ripening time periods are carried out in order to achieve the distinct characteristics of products from this region (Toldra and Flores 1998). In wet or pickle-curing, a brine is used to diffuse the curing salts into the meat, either by soaking the meat, injecting the brine into the piece or by mixing it with minced meat. In this type of process, very common in Northern Europe, the meat products are heated, and, optionally, smoked, after the curing process (Flores, 1997).

The dry-curing technology can be applied to entire pieces or minced meats. To cure ham or loins, the meats are rubbed with the mixture of salts and ingredients. The drying step of entire pieces require considerably long periods of time, e.g. over 6 months in Mediterranean hams, such as the Spanish Serrano and Italian Parma (Toldra and Flores 1998), with - shorter periods - for Northern hams, i.e. as 1-3 months for German Westphalia (Flores, 1997). The curing of minced meat is usually used to produce dry or semi dry-cured sausages, where curing salts, chopped meat, spices, and, optionally, a starter culture, are mixed and this mixture is stuffed into natural or synthetic casings. Sausage fermentation usually takes 1-7 days, followed by 2-24 weeks of drying, depending on type of sausage (Vignolo, Fontana and Fadda, 2010).

Cured meat products are RTE regarded as shelf-stable and safe, due to microbial growth barriers (hurdles) inherent to their manufacturing i.e., decreased a_w, pH lowering, and increased acid content. However, as these products lack a manufacturing step specifically designed to eliminate pathogenic microorganisms, the safety of these meat products relies on pathogen inhibition by product manufacturing conditions (Barbuti and Parolari, 2002). Cooked cured products are an exception among these meat products, as they are treated by thermal exposure during manufacturing, and will not be discussed in detail in this review.

3. *Salmonella* survival and persistence in dry-cured meat products

Salmonella is recognized as the leading cause of human foodborne infections and outbreaks with an estimated 80.3 million cases of salmonellosis are attributed to contaminated foods worldwide each year (Finn et al., 2013), mainly by the ingestion of contaminated food from animal origin, such as eggs, meats and their derived products (Ferrari, Panzenhagen, Conte-Junior, 2017). For dry-cured meats and meat products the risk of *Salmonella* contamination starts at manufacturing. Contamination of raw meats has been traced to poor sanitation practices and non-compliance to good manufacturing practices (Bremer et al., 2004), mainly in pork, where animals are the primary source of *Salmonella* contamination. Although the intermediary slaughtering stages, such as equipment, tools, and even post-processing handling are also potential sources of microbiological contamination (Andres and Davies 2015; Cabral et al., 2017; Finn et al., 2013; Oliveira et al., 2017).

Fermented sausages are a group of dry-cured meat products widely produced and consumed in Europe (Di Cagno et al., 2008). Fermented sausages can be classified in dry or semi-dry fermented sausages according to their a_w and moisture to protein ratio (Ockerman and Basu, 2007). Although fermented style sausages owe their safety to the same combination of intrinsic factors such as a_w, pH, and lactic acid content, they differ among themselves due to distinct conditions of fermentation and drying (Barbuti and Parolari, 2002). Dry-fermented sausages present low a_w (commonly between 0.85 and 0.91), moderate low pH (from 5.2 to 5.8), and the lactic acid content varying from 0.5 to 1.0%. On the other hand, semi-dry fermented sausages show higher a_w values (> 0.91), combined to low pH (4.7 to 5.4), and lactic acid content between 0.5 and 1.3% (Vignolo, Fontana and Fadda, 2010). Despite of these hurdles *Salmonella* outbreaks associated with dry fermented sausages has been reported (Andreoli et al., 2017; Bone et al., 2010;

Bremer et al., 2004; CDC, 1995; Emberland et al., 2006; Gossner et al., 2012; Luzzi et al., 2017; Kuhn et al., 2011; Omer et al., 2018; Scaltriti et al., 2015; Scavia et al., 2013).

Salmonella raises enormous concerns for public health authorities in both developing and developed countries, and its absence in RTE foods is required in compliance to regulatory requirements of the European Commission, 2005 and the United States Food Safety and Inspection Service (USDA/FSIS), 2010. Since the occurrence of pathogens in the raw materials must be taken in account, the manufacturing process steps of dry-fermented sausages including fermentation, drying and ripening, should be taken in account to ensure the safety of the final product (Barbuti and Parolari, 2002). For this reason, the USDA-FSIS has established the guidelines for RTE sausages manufacturers, as explained by Porto-Fett et al., (2010), where the governmental agency recognizes that the manufacturing process must include one of the following options for curing manufacture aiming at *Salmonella* control: I) use heat as detailed in CRF 318.17; II) use a validated 5D inactivation treatment; III) conduct a hold-and-test program for finished product; IV) conduct tests and apply a validated 2D process in the raw material, and V) use other methods that would ensure equivalent 5D inactivation (USDA-FSIS, 2003).

Numerous studies have addressed microbiological challenge tests to assess the safety of fermented sausages by studying the behavior of *Salmonella* throughout the manufacturing process. Dry-cured fermented sausages, such as salami and many other products from Mediterranean countries, are air dried for long periods (ripened) (Talon, Leroy, and Lebert, 2007). Although traditional, the salami manufacturing process may never be proven to ensure product safety by extinguishing pathogenic bacteria. In a recent study designed to simulate the poor standardized conditions commonly found in the production of commercial salami, 13% of salami produced from *Salmonella* contaminated raw ground meat still tested positive for the presence of the pathogen. Although the short curing periods of 20 to 48 days adopted by industries were pointed as the cause, 3% of samples re-tested after an extended curing period of 49-86 days still tested positive. The absence of the pathogen was only achieved after an additional 8 days of curing (Bonardi et al., 2017). Although this study reinforced the importance of adequate ripening periods during the manufacture of low acid dry-fermented sausages, the absence of microbial contamination in the raw material can be pointed out as crucial for the safety and quality of the final product.

The fermentation process of dry-cured sausages, on the other hand, can be properly established, since the microbiota involved (also called technological microbiota) is well-defined. Natural fermentation of dry-cured sausages, as in several dry-cured meat products mainly relies on two groups, lactic acid bacteria (LAB), and coagulase-negative staphylococci (Talon, Leroy, and Lebert 2007). However, spontaneous meat fermentation promoted by indigenous technological microbiota may not ensure *Salmonella* growth inhibition in meat batter, increasing the risk of food poisoning. *Salmonella* Typhimurium present in raw meat can reassume growth after freezing (-18 °C) despite a 72-h fermentation step by indigenous microbiota (Birk et al., 2016). On the other hand, when a consortium formed by *Staphylococcus xylosus* and *Pediococcus pentosaceus* was used, *S.* Typhimurium growth was inhibited and $>$ 1 log CFU/g reduction of the pathogen were achieved (Birk et al., 2016). In fact, the practice of adding tailored starter cultures initially driven by the idea of standardize the processing and enhance the sensorial quality of dry-fermented products has proven valuable to ensure control of pathogen growth in fermented products. The biosafety effect of the starter cultures goes beyond meat acidification, since many LAB species are able to produce low molecular peptides with bacteriostatic or bactericidal effect, bacteriocins, which inhibit the growth of spoilage and/or pathogenic bacteria) (Oliveira et al., 2017).

Besides fermentation, a significant concern regarding the manufacture of dry-fermented sausages is the extent of the ripening period adopted for these products, since these periods are usually defined, in order to reduce costs and attend market demands, rather than assure the safety of the final product (Consigliere, Meloni, Mazzette, 2017). A study on *S.* Typhimurium throughout the manufacturing process of French style dry-cured fermented sausage was conducted by Coroller et al., (2014), using a modeling approach. The authors observed that the sausage formulation, which included variations in NaCl, dextrose, lactose and starters (fast or moderate acidification cultures), did not influence the growth evolution of *S.* Typhimurium. A growth period was observed during the first two days of manufacturing, followed by inactivation of *S.* Typhimurium at a rate of approximately 1 log CFU/g at each 5 days during the first 15 days of manufacturing, displaying lower rates at the end of the manufacturing process. The obtained model allowed to infer that the main factor in *S.* Typhimurium inactivation was the starter culture that reduced both the growth and the resistance of this pathogen to pH and lactic acid, enabling a reduction of 6 log CFU/g throughout the whole manufacturing period (35 days).

Another dry-cured meat product that may lead to consumer risks is dry-cured ham. The premise of risk for dry-cured ham relies, mainly, on post-processing contamination (Alba et al., 2012), as many manufacturing processes, like the ones applied in Mediterranean countries, can last as long as years (Flores, 1997) with no reported pathogen survival throughout this process. For products with shorter manufacturing periods, however, such as dry-cured hams from northern Europe or country-hams from the United States of America, product safety may depend on preservative effects of additional steps included in the manufacturing process, such as fermentation and smoking.

Reynolds et al., (2001) investigated the manufacturing process of country style ham to evaluate its efficacy in reducing *Salmonella* levels. The authors followed commercial process parameters, using nitrate/nitrite, without a smoking step, and the final product was obtained after 69 days and stored for 51 days, totaling 120 days of microbiological

evaluation. The manufacturing process lead to a reduction of 5.5 log CFU/g in the *Salmonella* population in the final product (at the 69th day), with absence of the pathogen only at the end the 120 day observed storage period.

Sadeghi-Mehr et al., (2016) evaluated the manufacturing and storage of dry-cured formed ham, a novel product that unlike traditional hams is made up of multiple small pieces of meat. The increasing concern about this product is its high surface area, which becomes a contamination route from the manufacturing process to the inside of the product. The authors carried out a study to assess contamination cases occurring both during processing and in post-processing (slicing and storage) of the dry-cured formed-ham. The manufacturing lasted 35 days, and the product reached an a_w of 0.919, where *Salmonella* inactivation values reached 2.86 log CFU/g. In the storage trial reduction levels reached 2.39 log CFU/g after 35 days of vacuum storage at 4 °C. Indicating that *Salmonella* absence after production and storage of dry-cured formed ham was dependent of the quality of the raw material and good manufacturing practices, once the manufacturing itself was not enough to cause pathogen absence.

As mentioned previously, dry-cured ham production usually requires long drying and ripening periods, which are time-consuming and costly, and have driven the search for strategies to shorten the workflow. Stollewerk et al., (2014) evaluated the effects of NaCl replacement, acidification (by injection of starter cultures alongside brine injection) or smoking on the growth of *Salmonella* in dry-cured ham produced with a QDS® (a patented technology to shorten the drying/ripening phases of dry-cured meat products), simulating a contamination of 40 - 50 CFU/g during the slicing process, before the QDS® step. A rapid a_w drop was achieved utilizing the drying technology, leading to an a_w between 0.93-0.94, however the absence of *Salmonella* in the product was only achieved at the 112th day of storage, or after smoking.

To mitigate the risk of infections and ensure the commercialization of safe products, raw material quality and control during the manufacturing process is indispensable, and systems such as the Hazard Analysis Critical Control Point (HACCP) should be applied (Oliveira et al., 2017). Moreover, concerns about the safety of the dry-cured meat products have increased, whether due to developments of novel products, changes in the manufacturing processes, or new sale trends as packed sliced products. For this reason, non-thermic preservation technologies have emerged as an option to ensure the microbial safety of the final product (Alba et al., 2012; Bover-cid et al., 2012; Bover-cid et al., 2017; Cabeza et al., 2009; Ganan et al., 2013; Marcos, Aymerich, Garriga, 2005; Stollewerk et al., 2012; Stollewerk et al., 2014).

4. *Salmonella* stresses responses and tolerance in dry-cured matrices

Once in the dry-cured matrix, the microorganism undergoes an array of stresses and must adapt rapidly to adverse and fluctuating conditions to survive. Bacterial responses to stresses begin after an environmental change is sensed, which triggers adaptive cell responses. These cell adaptations are collectively known as stress tolerance responses, defined as induced resistance to ordinary lethal stress following exposure to mild sub-lethal stress conditions (Álvarez-Ordóñez, et al., 2015). Tolerance responses are often associated to transcription regulation of genes encoding stress proteins, which protect cell molecules and structures, enhancing bacteria survival in inhospitable environment (Álvarez-Ordóñez et al., 2008).

The manufacturing process of dry-cured meat products provides an adequate set of conditions for pathogens to develop stress tolerance responses, due to the gradual changes in intrinsic factors within the food matrices (Coroller et al., 2015). In these conditions, *Salmonella* survival mechanisms (physiological and stress tolerance responses) are triggered, where desiccation/osmotic and acid stresses are the major stresses regarding pathogen persistence.

Salmonella possesses several survival mechanisms, ranging from surface structure modifications to modulation of genes responsible for persistence. Each abiotic stress triggers a different set of coordinated modifications in the cell. However, overlaps in the signaling cascades among different stresses exist, resulting in stress cross-protection, where exposure to an incurred stress can generate tolerance against a subsequently other (Gruzdev, Pinto and Sela, 2011). When it comes to cross protection caused by dry-cured matrices, alternative sigma factors as σ^S (*RpoS*) and σ^E (*RpoE*) play a major role. They are part of general *Salmonella* stress responses to maintain cell viability under abiotic stresses, such as nutrient deprivation, hyperosmolarity and acid stress. Sigma factors are dissociable subunits of the RNA polymerase holoenzyme that can redirect DNA transcription as part of cellular response to stresses or changes in growth conditions. Each sigma factor controls the expression of a specific subset of genes (regulon), with either a defined primary function (e.g., genes related to acid shock), or multiple functions (e.g., genes related to stationary-phase general stress response). Moreover, alternative sigma factors are also involved in regulating the expression of virulence and virulence-associated genes. Virulence genes encode pathogen proteins necessary for bacteria to establish infection in a host organism, and virulence-associated genes can contribute to bacterial survival in the host environment by enhancing bacteria capacity to spread to new individuals or to survive passage through a host organism (Kazmierczak, Wiedmann, and Boor, 2005).

4.1. Osmotic and desiccation stresses in dry-cured meat matrices

The water activity in dry-cured meat products is lowered by both the addition of osmotic active substances, i.e., salts (NaCl and NO_3^-/NO_2^-), and the desiccation steps included in the manufacturing process that expose opportunist pathogens to osmotic and desiccation stresses. Although scarce

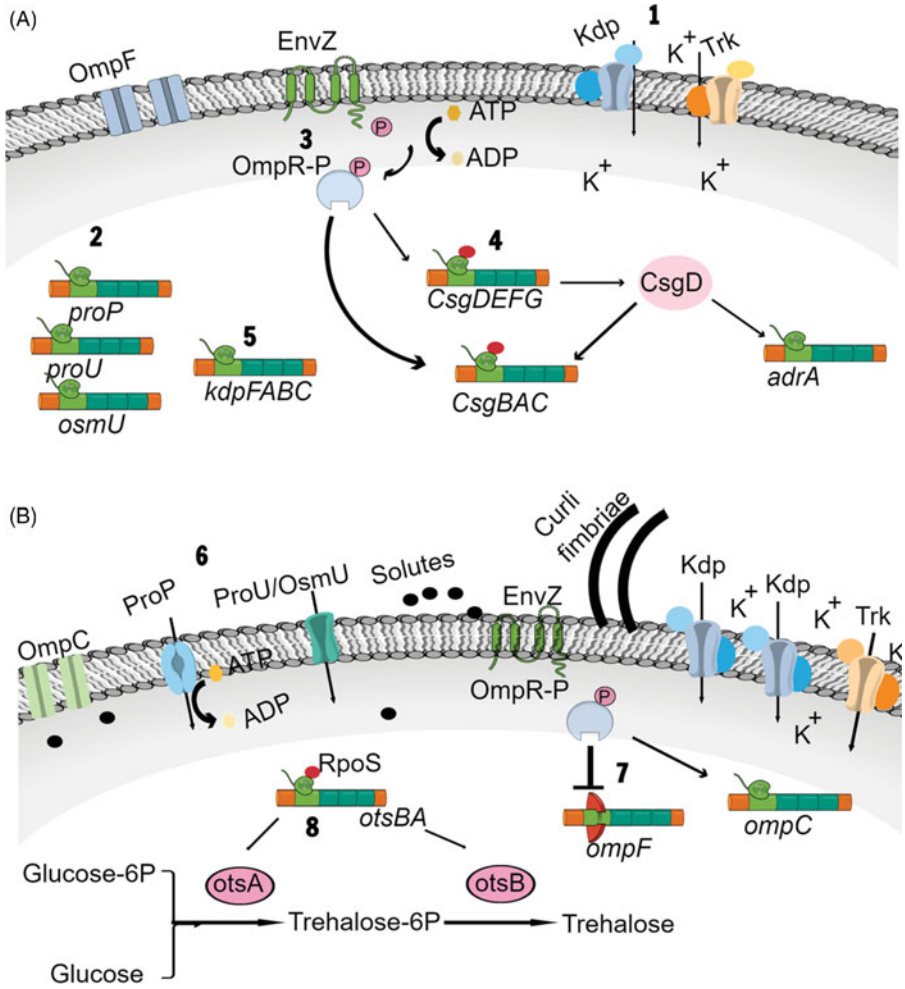

Figure 1. Summary of *Salmonella* physiological and transcriptional response to osmotic upshift **(A)** represents the short-term response to hyperosmotic stress. (1) While the K^+ uptake occurs through the Trk transporter (the low affinity transport system). (2) The expression of cell transport systems ProU, ProP, and OsmU begins. (3) The osmotic upshift is sensed by EnvZ, a histidine kinase, who phosphorylates the response regulator OmpR. (4) The phosphorylated regulator OmpR-P, alongside with the alternative sigma factor RpoS (σ^S), attached to RNA polymerase, activates *csgDEFG* transcription and the resulting regulatory protein, CsgD, activates both *csgBAC* and *adrA* transcription, increasing biosynthesis of curli fimbriae and cellulose in the cell. (5) The sensed osmotic upshift also leads to Kdp system expression (a high-affinity K^+ uptake system) **(B)** the long-term response to hyperosmotic stress. (6) ProU, OsmU and ProP perform the active transport of compatible solutes from the surrounding extracellular medium. (7) The phosphorylated regulator OmpR-P promotes *OmpC* up-regulation and *OmpF* repression, makeing OmpC the major porin docked in the cell membrane. (8) The binding of σ^S to RNA polymerase activates the transcription of the *otsBA* operon, which encodes the enzymes required for conversion of glucose into trehalose.

knowledge in the scientific literature is available on *Salmonella* physiological and genetic adaptations to survive desiccation stress when compared to other well-studied stresses, it is clear that regulatory overlaps among stress response networks, including the osmotic one, exist (Gruzdev et al., 2011; Li, Bhaskara, Megalis, Tortorello, 2012).

4.1.1. Salmonella *protective surface structures*

The first defense of *Salmonella* against desiccation are cell surface structures, where exopolysaccharides exert a protective effect on microbial colonies, by absorbing water and acting as a water reservoir. In addition, the immobilized water slows drying rates, protecting cells against changes in environmental water content (Garmiri et al., 2008; Ophir and Gutnick, 1994). Another protective structure against desiccation is the O chain polysaccharide (OPS) element of the outer lipopolysaccharide membrane in Gram-negative bacteria. OPS synthesis and the expression of codifying genes have been demonstrated to attenuate damaging desiccation effects in *Salmonella* Typhimurium (Garmiri et al., 2008). White et al., (2006) observed that *Salmonella* strains capable of producing curli (a thin aggregative fimbriae) and extracellular cellulose exhibited greater survival rates against desiccation. It was hypothesized that curli fimbriae play a critical role in organizing the extracellular matrix, while extracellular cellulose is a natural protector against oxidative agents that can also trap additional polysaccharides on the cell surface, enhancing protection (White et al., 2006).The expression of both curli fimbriae and cellulose is dependent on the regulatory protein CsgD, which activates transcription of the *csgBAC* operon encoding structural curli subunits, and the *adrA* gene, a positive effector of cellulose biosynthesis (Brombacher et al., 2006). *csgD* expression is controlled by the alternative sigma factor σ^S and the response regulator OmpR is also required for transcriptional activation of the csgD promoter (Römling et al., 1998).

OmpR is activated after cell exposure to osmolarity upshift. The same phenomenon seems to be associated to desiccation state, where osmolarity upshift is caused by water removal. Furthermore, CsgD has been considered the master regulator of biofilm production, due to the role it plays in the expression of several determinants involved in biofilm formation (Fàbrega and Vila, 2013)

4.1.2. Salmonella *osmoregulation following salt-curing meat with sodium chloride and/or nitrate/nitrite salts*

As a consequence of exposure to the hyperosmotic environment within dry-cured meats or desiccation during the dry-curing processing, *Salmonella* suffers a loss of water resulting in considerable shrinkage of cytoplasm volume, known as plasmolysis, that leads to the inhibition of several physiological processes, including nutrient uptake (Roth, Leckie, and Dietzler, 1985), macromolecule synthesis (Csonka, 1989) and even DNA replication (Meury, 1988). *Salmonella* cells attempt to maintain their turgor pressure by accumulating or releasing molecules that do not interfere in cellular processes, for this reason known as compatible solutes, able to maintain proper balance between internal and external osmolarities, a shared general response found in all microorganisms. (Balaji et al., 2005; Csonka, 1989). Compatible solutes can accumulate in the cell by *de novo* synthesis or by transport from the extracellular media (Csonka, 1989).

Salmonella cells osmoregulation can be divided into two distinct phenomena: a short-term, transient response, that occurs quickly after an osmotic upshift and long-term response, guaranteeing microorganism survival in high intracellular osmolarities (Csonka and Hanson, 1991). The short and long-term *Salmonella* cell responses to osmotic upshift are summarized in Figure 1. The transient osmotic response involves the accumulation of potassium ions (K^+), through two main transport systems for K^+ influx, namely the Kdp system, a high-affinity K^+ transport system activated few minutes after the osmotic upshift, and the constitutive Trk system, a low-affinity transport system (Balaji et al., 2005; Spector and Kenyon, 2012). Besides already being enrolled in *Salmonella* osmotic response, the induction of the Kdp system has also been observed in *Salmonella* under desiccation conditions, implied in post-dehydration persistence (Gruzdev et al., 2012). The Kdp system comprises three components: KdpA, a membrane-spanning protein, KdpB, an integral membrane protein and KdpC, a peripheral membrane protein. The Trk system also comprises three components: TrkA, a peripheral membrane protein, TrkE, a membrane-associated protein and TrkG, a membrane-spanning protein (Burgess et al., 2016).

What triggers the initial response to osmotic stress is not yet satisfactorily understood, whether loss of turgor pressure suggested by the "turgor control model" or due to the increase in intracellular ionic strength, suggested by the "ionic strength model" (Balaji et al., 2005). Although several similarities between these models exist, in the turgor model, the transcription of *kdpABC* genes is under the positive regulation of the two-component system kdpD/kdpE, which would sense turgor loss in the cell, and all other responses would be secondary consequences of K^+-glutamate accumulation (Balaji et al., 2005; Csonka, 1989). On the other hand, in the ionic strength mode, the induction of the *kdp* operon, along with all other osmotic responses, is primarily induced by the ionic strength increase (Balaji et al., 2005; Poolman et al., 2002). Independently of the primary trigger, and in accordance with Csonka (1989), Balaji et al., (2005) reported that the mechanism adopted by *Salmonella* in osmotic imbalance conditions is dependent on the chemical nature of the solute, since the *kdp* operon was induced 170-fold when exposed to 0.3 M NaCl in comparison to 0.6 M sucrose.

The long-term osmoregulation response relies on the uptake or *de novo* synthesis of osmoprotective compounds (compatible solutes), such as proline, glycine-betaine, ectoine (Csonka, 1989) and trehalose (Howells et al., 2002). The main osmoprotectant transport systems in *Salmonella* are ProU (ProVWX) and OsmU, both ABC-transporters, and ProP, a H^+ symporter, member of the major facilitator permease superfamily (Frossard et al., 2012). The up-regulation of the *proU, proP* and *osmU* genes under osmotic stress (Balaji et al., 2005; Frossard et al., 2012) has been documented and the same phenomena have also been reported in *Salmonella* under desiccation stress (Finn, Händler, et al., 2013; Li et al., 2012).

The disaccharide trehalose is an important compatible solute for *Salmonella* osmoadaptation, since it accumulates in *Salmonella* by *de novo* synthesis and may become the main osmoprotectant if exogenous compounds are not available (Kempf and Bremer, 1998). Trehalose plays a role in maintaining protein structure and function, by "replacing water" in extreme desiccation conditions, preventing denaturation (Crowe and Hoekstra, 1992; Diniz-Mendes et al., 1999). This molecule also stabilizes cell membrane lipids, by inhibiting the fusion between vesicles during drying and by depressing the phase transition temperature of the dry lipids, maintaining them in a liquid crystalline phase (Crowe and Hoekstra, 1992; Kempf and Bremer, 1998). Trehalose biosynthesis involves the enzymes trehalose-6-phosphate synthase (OtsA) and trehalose-6-phosphate phosphatase (OtsB), encoded by the *otsBA* operon, whose expression is dependent on the alternative sigma factor σ^S. Indeed, several studies report that σ^S plays a key role in the survival of *Salmonella* under several stress conditions including osmotic, heat, oxidative, starvation and acid stresses (Table 1) (Fang et al., 1992; Ibanez-Ruiz et al., 2000; McMeechan et al., 2007), strengthening the concept of the cross-protection to abiotic stresses. Alongside σ^S, the alternative sigma factor σ^E, involved in *Salmonella* growth in high osmolarity environments, regulates promotor recognition by RNA polymerase in response to other stresses, such as heat, oxidation, and starvation. σ^E also plays a role in tolerance dehydration and long-term *Salmonella* persistence (Table 1) (Gruzdev et al., 2012; McMeechan et al., 2007).

De novo synthesis and up-regulation of osmoprotectant transport systems genes can be accompanied by the passive diffusion of osmoprotectants when *Salmonella* is exposed to high osmotic pressure. Passive diffusion is regulated by

Table 1. Regulatory systems/proteins involved in the resistance and virulence of *Salmonella enterica* serovars subjected to different abiotic stresses.

Regulator	Acid	Osmotic	Desiccation	Heat	Oxidative	Role in virulence	References
σ^S	Yes	Yes	Yes	Yes	Yes	Yes	(Fàbrega and Vila, 2013; Fang et al., 1992; Ibanez-Ruiz et al., 2000; McMeechan et al., 2007)
σ^E	Yes	No	No	Yes	Yes	Yes	(Gruzdev et al., 2012; Kazmierczak et al., 2005; Humphreys, Stevenson, Bacon, Weinhardt, and Roberts,1999)
Fur	Yes	No	No	No	No	Yes	(John W Foster,1993; Troxell et al., 2011)
PhoPQ system	Yes	No	No	No	No	Yes	(B. L. Bearson et al., 1998; Soncini et al., 1996)
OmpR-EnvZ system	Yes	Yes	No	No	No	Yes	(Bang et al., 2000; Feng, Oropeza, Walthers, et al., 2003; L. C. Wang et al., 2012)
CsgD	No	No	Yes	No	No	Yes	(Fàbrega and Vila,2013; Römling et al., 1998)

changes in the rate of outer membrane porins - OmpF and OmpC - via the two-component system EnvZ/OmpR (Wang et al., 2012). At low osmolarity, OmpF is the major porin, however, when an increase in osmolarity is sensed by EnvZ kinase the OmpR is phosphorylated (Feng et al., 2003; Wang et al., 2012). This, in turn, causes *ompC* up-regulation and *ompF* repression, making OmpC the predominant porin (Feng et al., 2003). The difference between these porins is the size of the pore, where OmpC exhibits a smaller pore and allows for a slow flux, blocking larger molecules and facilitating the diffusion of small hydrophilic molecules (Feng et al., 2003). Although porin shifts were not detected in *Salmonella* during desiccation (Li et al., 2012; Mandal and Kwon, 2017), the mechanism is relevant in the dry-curing of meat products, where the use of humectants such as NaCl and nitrate/nitrate provoke an osmotic upshift.

4.2. Salmonella *acid stress and pH homeostasis response in dry-cured meat*

Acidic stress is commonly encountered by foodborne pathogens in dry-cured meat products, mainly due to the conversion process of muscle to meat, resulting in a drop in pH, after *rigor mortis,* to approximately pH 5.4 depending on the muscle (Kylä-Puhju et al., 2004). Furthermore, fermentation in many dry-cured meat matrices result in the production of organic acids, causing a drop in pH below 5.0 (Mataragas et al., 2015). Acid stress is one of the most studied stresses, and acid tolerance response was first characterized in *Salmonella enterica* serovar Typhimurium. (Álvarez-Ordóñez et al., 2012; Foster and Hall, 1990).

After exposure to an acid environment *Salmonella* cells attempt to maintain intracellular pH at a constant level by the modulation of proton pumps (sodium and potassium-proton antiporters), which extrude H^+ from the cytoplasm to low pH environments (Audia, Webb, and Foster, 2001). Along with these pumps, Álvarez-Ordonez et al., (2010) reported that the lysine and arginine decarboxylase systems also play a role in the maintenance of intracellular *Salmonella* Typhimurium pH, by converting lysine into cadaverine, and arginine into agmatine, with the use of one proton in each transformation and subsequently expulsion of the biogenic amines from the cell (Álvarez-Ordóñez et al., 2010).

4.2.1 Acid tolerance response (ATR)

Salmonella acquired acid tolerance was first described by Foster and Hall, in 1990, when observing *Salmonella* Typhimurium resistance to lethal pH, after exposure to a mild acidic condition. Tolerance response involves the expression of acid shock proteins (ASPs), whose function is to prevent and repair macromolecular damages caused by acid stress (Audia et al., 2001). The ATR in *Salmonella* is affected by several factors, such as the genetic background within distinct serovars and even within strains from the same serovar (Lianou and Koutsoumanis, 2013); incubation temperature; chemical nature of the acidulant (organic or inorganic) and cell growth phase (logarithmic or stationary phase) when exposed to the stress (Álvarez-Ordóñez et al., 2012; Arvizu-Medrano and Escartín, 2005; Audia et al., 2001). *Salmonella* Typhimurium proteomic analyses showed that 60 ASPs in log phase and 48 ASPs in stationary phase were expressed, but only five of them overlap, evidencing the existence of two separate ATR systems, the log-phase acid tolerance response (LP ATR) and the stationary phase acid tolerance response (SP ATR) (Audia et al., 2001). Although the individual effect of each ASP is important, the biosynthesis activation of the protein set is equally relevant. *Salmonella* maintain several regulons that enable acid adaptation, mainly those controlled by Fur (ferric uptake

regulator), PhoPQ, σ^S, and OmpR/EnvZ. Since it discovery, acid tolerance response has been investigated for many *Salmonella* serovars besides Typhimurium, with evidence pointing to a higher dissimilarities among strains than serovars (Lianou, Nychas, Koustsoumanis, 2017).

4.2.1.1. Acid tolerance response in log-phase cells (LP ATR).

The ATR mechanism is induced in *Salmonella* log phase cells following adaptation to pH decreases from 5.8 to 4.3 (Audia et al., 2001; Foster, 1993; Lianou and Koutsoumanis, 2013). Among *Salmonella* regulons necessary to withstand and adapt to cytoplasm acidification, those regulated by Fur, PhoPQ and σ^S are essential for the log phase dependent acid-tolerance response.

Fur acts as a negative gene regulator for exogenous iron assimilation but is able to induce the expression of a ASP set, which is transcribed after 20-40 min exposure to pH 5.8-4.3, This rapid and transient mechanism allows *Salmonella* to survive at extremely low pH, such as 3 (Foster, 1993). Fur increases ASP transcription in an iron-independent manner, since it can separately sense Fe^{2+} and H^+ ions, through histidine residues in distinct protein binding domains. Therefore, proteins regulated by Fur in response to iron or low pH form two distinct clusters, with the exception of seven proteins that are influenced by both (Foster and Hall, 1992; Hall and Foster, 1996). Briefly, it should be noted that Fur is essential for cell response to low pH caused by the production of organic acids, as in the case of acids from fermentative steps of dry-cured meat manufacturing (Bearson, Wilson, Foster 1998).

A sustained log phase ATR, which is σ^S-dependent, is induced after exposure for at least 60 minutes to mild pH conditions and replaces the transient ATR response. After exposure, intracellular σ^S levels increase, mainly due to the increased translation of *RpoS* mRNA (Audia and Foster, 2003). However, another mechanism to guarantee high σ^S levels during the log-phase also exists, by the cease of the proteolytic degradation of σ^S by ClpX (a protease regulated by MviA) (Bearson et al., 1996). Since σ^S competes for the RNA polymerase core, the higher availability of σ^S subunits results in the induction of the σ^S regulon (Rychlik and Barrow, 2005), increasing the transcription of at least 10 σ^S-dependent ASPs (Audia et al., 2001).

The two-component system PhoPQ is another important regulator in the ATR log phase. It is composed by PhoQ, a membrane bound sensor protein, and PhoP, a transcriptional regulator (Rychlik and Barrow, 2005). PhoQ senses Mg^{2+} and Ca^{2+} and under low magnesium concentrations, phosphorylates the transcriptional sensor PhoP, which induces the *phoPQ* operon itself, and the genes within the *PhoPQ* regulon. *PhoP*-induced genes include those involved in Mg^{2+} homeostasis and virulence (Soncini et al., 1996). Although the main signal controlling *phoPQ* regulon is the low Mg^{2+} concentrations, it can be also induced by moderate acid pH, even under high Mg^{2+} levels (Bearson et al., 1998). The *phoPQ*-dependent ATR in *Salmonella* Typhimirium is the response against inorganic acids (Bearson et al., 1998), consistent with the fact that induction of PhoPQ-dependent genes is required for *Salmonella* survival in the macrophage phagolysosome, where the stress is caused by an increase in H^+ cytoplasmic concentrations (Audia et al., 2001; Rychlik and Barrow, 2005).

4.2.1.2. Acid tolerance response in stationary phase cells (SP ATR).

As mentioned previously, stationary-phase cells display a distinct ATR from log phase cells, with few protein overlaps. However, as in log-phase ATR, the stationary phase possesses two-independent ATR systems. Non-adapted stationary-phase cells grown at pH 7 display an innate resistance to pH 3, being 1,000-fold more tolerant to 1 h acid exposure than log-phase cells (Lee, Slonczewski, and Foster, 1994). That innate system is σ^S-dependent and is part of the general stress response, induced as cells enter the stationary growth phase, triggered by nutrient deprivation. Yet, this system is only capable of maintaining cells alive at pH 3 for 4 h exposure (Lee et al., 1994). To survive longer periods in acid environments, the cell must induce the acid stationary phase ATR, which is dependent on a preadaptation to pH 5.5 to 4.5. Differently from log-phase ATR systems, the acid-induced stationary-phase ATR is Fur-, PhoPQ- and σ^S-independent (Lee et al., 1994), but OmpR-dependent (Bang et al., 2000).

The two-component Envz/OmpR system is associated with the osmotic stress response, where it regulates OmpC and OmpF porins. Besides its role in osmotic stress, OmpR it is the major regulator of acid-induced stationary-phase ATR, since *ompR* mutants lead to sensitive stationary-phase cells, with almost no inducible ATR (Bang et al., 2000). Bang et al., (2000, 2002) elucidated that OmpR is an ASP that auto regulates the *ompR envZ* operon in response to low pH, through the binding of OmpR-P to promoter sequences and activation of target genes transcription. *ompR* can be transcribed by the activation of two distinct promoter sequences, one activated at neutral and the other, at acidic pH. Transcription of *ompR* from the acid promoter is repressed by the histone-like protein H-NS and requires OmpR-P for induction (Bang et al., 2002). The same authors also observed that OmpR-P levels required for its auto induction rely on phosphorylation from both the sensor kinase EnvZ and the alternative phosphodonor molecule acetyl-phosphate (Ac-P). After these findings, it was suggested that the acid stress alters DNA supercoiling in the *ompR* promoter, allowing OmpR-P to bind and displace H-NS, which in turn increases *ompR* transcription (Audia et al., 2001).

5. Effects of the dry-cured meat matrix on *Salmonella* virulence factors

Taken together, the stressful conditions imposed by the dry-cured meat matrices are usually considered preservative food conditions, where the combination of single non-lethal stresses (hurdles) lead to microorganism inactivation (Leistner, 2000). However, as aforementioned *Salmonella* could become more tolerant from exposure to these sub-lethal stresses. Furthermore, the notion that the adaptation

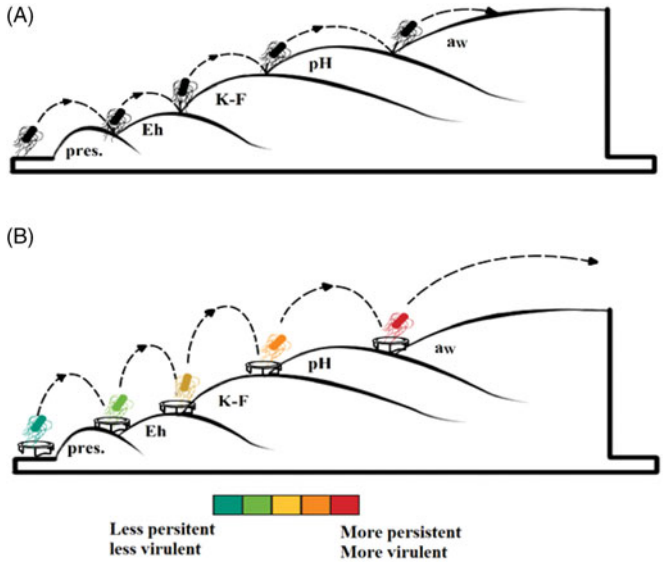

Figure 2. Representation of abiotic stress (hurdles) effects during dry-cured meat preservation (pres: preservatives; Eh: redox potential; K-F: competitive endogenous flora; pH: acidification; a_w: water activity) **(A) Represents the effect of hurdle technology as stated by** Leistner and Gorris (1995)**.** The collective effect of individual hurdles encountered in dry-cured meat derivatives lead to *Salmonella* inactivation and food preservation. **(B) Represents the alternative hypothesis proposed herein.** Here, each hurdle triggers tolerance to subsequent stresses, leading to *Salmonella* cells more persistence and virulent by the cross-protection effect, and conjugated regulation of stress response and virulence trais (represented by the trampoline).

to one stress can lead to cross-protection towards other stresses (Table 1) could make the food preservation by hurdle technology harder to achieve, once more persistent bacteria arise (Figure 2).

Virulence can be defined as the pathogen ability to overcome body defenses. In this regard, *Salmonella* display several virulence strategies. During its life cycle, *Salmonella* can be exposed to a variety of stresses in the environment, in the food matrix, and in the gastrointestinal tract during host infection. As a foodborne pathogen, *Salmonella* infection in humans occurs by the ingestion of contaminated food or water. After the ingestion of the contaminated food, the pathogen encounters several barriers in the host digestive tract that can compromise its survival. The first host defense against bacterial infection is the stomach environment, where the pH of gastric juice decreases to 1-2 when in fasting state (Birk et al., 2016). Once the pathogen has passed through the stomach, it enters the intestine, an inhospitable environment where it has to face antimicrobial peptides released by the digestion of dietary proteins, the detergent-like activity of bile, low oxygen availability, and high osmolarity caused by sugars and other absorbed nutrients (Aguila et al., 2016; Rychlik and Barrow, 2005). After surviving all these host defenses, *Salmonella* must compete against the gut commensal microbiome for space and nutrients in order to colonize the intestine, after adhering and invading epithelial cells (Fàbrega and Vila, 2013; Rychlik and Barrow, 2005). Invasion can occur in phagocytic or non-phagocytic cells, where *Salmonella* cells are engulfed in nutrient deficient vesicles, called *Salmonella*-containing vacuoles (Fàbrega and Vila, 2013).

Although initially low, the stomach pH transiently increases to 5-7 in the postprandial period (Birk et al., 2016). It has been suggested that food composition influences bacterial survival, where high-fat content food provides bacterial protection during the gastric passage, lowering the infective dose, as observed in the *Salmonella* Typhimurium outbreak caused by chocolate poisoning in Norway (Kapperud et al., 1990). The buffering capacity of the food should also be considered for high protein food matrices, such as dry-cured meats that could protect bacteria, due to their buffering capacity delaying the stomach pH decrease (Birk et al., 2012; 2016). In addition, Henriksen (2016) suggested that the induction of acid tolerance response it is an important factor in *Salmonella* survival during the gastric passage. In sum, these effects (meat buffering and ATR) suggest that besides becoming more resistant *Salmonella* within dry-cured meat might, in fact, become more virulent, since the number of cells surviving the host barriers are higher, increasing the risk of infection.

5.1. Virulence determinants: An overview

The most relevant virulence determinants in *Salmonella* are: the pSLT virulence plasmid; flagella; fimbriae (curli, Pef, Lpf, Std); chaperones; adhesins; actin rearrangement and inhibition; induction of pro-inflammatory responses; biofilm-related proteins and the highly conserved DNA regions known as *Salmonella* pathogenicity islands (SPI) (Fàbrega and Vila, 2013).

Virulence plasmids vary within *Salmonella* serovars, however all of them display a highly conserved 8kb region containing the *spv* locus, which encodes the *spvR* regulatory gene and four structural *spvABCD* genes. The *spv* locus is responsible for survival and growth of *Salmonella* in the host once these genes are not expressed during exponential growth *in* vitro but are rapidly induced following *Salmonella* entry into mammalian cells, including macrophages (Marcus et al., 2000). Moreover, activation of the *spv* genes σ^S-dependent is observed in stationary phase cells (Fierer et al., 1993).

To date, a total of five SPIs have been identified (Dos Santos, Ferrari, Conte-Junior, 2018). SPI-1 encodes several effector proteins involved in epithelial cell invasion, in special the type 3 secretion system (T3SS1), also known as "the needle complex." SPI-2 is known for its key importance to *Salmonella* survival and replication within the *Salmonella*-containing vacuoles. The role of the remaining SPIs is not fully elucidated, but SPI-3 is required for *Salmonella* intra-macrophage survival and growth in low Mg^{2+} media. SPI-4 complements SPI-1 and encodes a type 1 secretion system, SIEE (a non-fimbriae adhesin) and is associated to inflammatory responses, contributing to long-term persistence. Moreover, SPI-5 encodes effector proteins shown to contribute to systemic infection in a murine model (Fàbrega and Vila, 2013; Gerlach et al., 2007; Wang et al., 2016).

5.2. Relation between stress and virulence

Certain regulatory proteins involved in *Salmonella* stress responses promoted by dry-cured meat matrix also play a role in the modulation of virulence genes (Table 1), which may result in stressed and are more virulent *Salmonella* cells.

5.2.1. Stress proteins related with virulence response

Besides controlling the expression of a wide range of genes related to several stresses, the σ^S regulon is also associated with *Salmonella* virulence. The transcription of the virulence plasmid *spv* in *Salmonella* Typhimurium, required for systemic infection, is controlled by σ^S. On the other hand, Nickerson and Curtiss (1997) demonstrated that σ^S does not contribute to *Salmonella* Typhimurium adherence, invasion or survival, in a study on an embryonic intestinal epithelial cell line, since a *rpoS* mutation failed to decrease these capabilities in wild-type cells (Nickerson and Curtiss, 1997). This suggests that σ^S does not contribute to infection leading to gastroenteritis. However, the same authors demonstrated the importance of σ^S regulation on *Salmonella* Typhimurium chromosomal genes involved in invasion and colonization of murine Peyer's patch cells, which implicate σ^S in systemic infection (Nickerson and Curtiss, 1997). Moreover, Fang et al., (1992) demonstrate that σ^S modulate the expression of the *spvRABCD* virulence plasmid genes. Also consistent with the hypothesis of the role of σ^S in *Salmonella* virulence Robbe-Saule et al., (2003), found no σ^S mutant strain among 75 clinical isolates of *Salmonella* Typhimurium suggesting that those mutants may be naturally counter-selected due to the partially loss of its pathogenicity.

σ^E is a member of the subfamily of sigma factors, whose function is to sense and respond to changes in the periplasm and extracellular media and displays a protective effect against a variety of stresses (Table 1) (Kazmierczak, Wiedmann, and Boor, 2005). Control of *htrA* expression, a gene required for macrophage survival, seems to be the main σ^E role in *Salmonella* virulence, although it does not account for the entire σ^E virulence regulation. The lack of σ^E by mutation of the *rpoE* gene leads to lower *Salmonella* Typhimurium survival in Peryer's patch cells and lower capacity to translocate to deeper tissues when compared to *htrA* mutants (Humphreys et al., 1999).

The PhoPQ two-component system responsible for *Salmonella* ATR has been demonstrated to regulate hundreds of genes encoding the majority of virulence properties, including intracellular survival, invasion, lipid A structure, antimicrobial peptide resistance, and phagosomes (Prost and Miller, 2008). The PhoPQ system can indirectly modulate *hila,* a gene known as a master regulator of the SPI1 (Bajaj et al., 1996; Fàbrega and Vila, 2013). Miller, Kukral and Mekalanos (1989) found that deletion of *phoP* or *phoQ* gene cause a decrease in *Salmonella* survival in mouse macrophages. According, Miller and Mekalanos (1990) showed that without proper regulation, an indistinct activation of phoP is not enough to account for the virulence and survival in macrophages.

The two-component regulatory system EnvZ-OmpR senses the changes in extracellular osmolarity and also plays a role in virulence by activation of SPI-2 gene expression. The activation begins with a signal from the macrophage environment, sensed by the EnvZ kinase, which then activates the response regulator OmpR. The activated OmpR has reported effects on SPI-1 and also induces the transcription of *ssrAB* genes, the regulatory system required for SPI-2 gene expression (Fàbrega and Vila, 2013).

Fur, the iron-regulator protein, acts as a *hilA up-activator*. Fur is thought to act solely as a gene repressor, but when it represses the nucleoid-associated protein H-NS, it stops HilD repression, which allows for *hilA* expression, activating SPI1 structural gene expression (Ellermeier and Slauch, 2008; Troxell et al., 2011). In a similar fashion Fur control the expression of SPI-2 through repression of ssrB response regulator (Choi et al., 2014). A clear link between stress response regulation and *Salmonella* virulence exists, since an overlap in regulatory proteins and signaling cascades for both processes occurs.

5.2.2. Virulence status of Salmonella cells exposed to sub-lethal stresses in dry-cured meat products.

Since *Salmonella* encounters different challenges during host infection, it is hypothesized that the previous contact of the pathogen with similar stresses by exposure to dry-cured meat matrices would result in more virulent cells. However, virulence traits are strictly controlled (Choi et al., 2014). Therefore, pathogen pre-adaptation to sub-lethal conditions, similar to the ones encountered within the host, may not necessarily lead to changes in virulence traits. The question is whether or not *Salmonella* pre-adaptation to abiotic stresses within the dry-cured meat matrices, with subsequent expression of virulence regulatory traits, leads to the development of highly virulent bacterial cells.

Studies on cell lineages have been carried out to search for links between *Salmonella* stress tolerance response and virulence. In one study, the influence of acid adaptation on *Salmonella* Typhimurium virulence was evaluated, indicating that mutations in different ATR genes affect *Salmonella* Typhimurium strain pathogenicity following oral infection in mice. The *fur* mutant strains display a 1 to 3-log increase in the LD_{50}, an effect that depends on the genetic background of the strains. It has also been demonstrated that *fur* mutations led to a decrease in *Salmonella* Typhimurium ability to invade intestinal epithelial cells, as a consequence of the lack of induction of *hilA* by Fur, as described previously. The attenuation of acid-sensitive mutants indicates a link between the pathogen pre-development of an acid stress response and their virulence, supporting the hypothesis that *Salmonella* pre-adapted to acid stress can lower its minimum infective dose (Wilmes-Riesenberg et al., 1996).

Acid-adapted *Salmonella* Typhimurium showed higher survival in synthetic gastric fluid (pH 2-3) (Fratamico, 2003). Xu, Lee and Ahn (2010) found that *Salmonella* Typhimurium exposed to media acidified with lactic acid pH (5-6) increase the expression of *stn* and *invA* genes

responsible for enterotoxin and invasiveness, respectively. Furthermore, experiments using cell lineages in culture also corroborate the present hypothesis. Acid-adapted *Salmonella* Typhimurium isolates showed increased replication in the J774A.1 macrophage cell-lineage when compared to non-acid-adapted cells (Fratamico, 2003). Rishi, Pathak and Ricke (2005) observed that *Salmonella* Typhimuirum cells exposed to short chain fatty acids (SCFA) exhibited higher intracellular survival in mice macrophage than non-stressed cells. In accordance to Durant et al., (2000a), who studied the stationary phase of *Salmonella* Typhimurium exposed to SCFA, indicating that they presented increased adherence and invasion in Hep-2 cell line. On the other hand, Durant et al., (2000b) demonstrated that exposure of log-phase *Salmonella* Typhimurium to SCFA decreased adherence and invasion in the Hep-2 cell line. Taken together, these results point to an increased risk for *Salmonella* outbreaks related to dry-cured meats, as these cells would be in stationary growth phase, under stress condition and in the presence SCFA, as the result of lactic acid bacteria fermentation.

The influence of stresses, other than acid, on *Salmonella* virulence was evaluated by Tartera and Metcalf (1993), who studied the effect of media osmolarity on *Salmonella* Typhi adherence and invasion in Henle 407 and Caco2 cell lines. The authors observed that adherence and invasion increased concurrently with media osmolarity. In accordance with previous findings, Chakroun et al., (2018) found that subjecting *Salmonella* Typhimurium to osmotic stress (seawater with 4% salinity) resulted in increased adherence and invasion in Caco-2 cells. Furthermore, the authors demonstrated that *Salmonella* Typhimurium grown in Luria-Bertani media supplemented with 0.3 M of NaCl expressed *sopB*, *sopE2*, and *hilA* genes in higher levels than inside Caco-2 cells. Similarly, Huang et al., (2007) also found that high osmolarity media induced the expression of SPI-1 genes.

Although virulence genes are thought to be expressed only inside the host, and repressed in outside environments (Vasanthakrishnan et al., 2015), due to strict regulation, studies showing that virulence related genes are expressed in stress abiotic conditions similar to those encountered in food matrices offer an alternative hypothesis. In sum, evidence indicate that *Salmonella* can become more persistent to stresses and present enhanced virulent traits after exposure to sublethal stresses commonly found in dry-cured meats.

6. Concluding remarks

Although dry-cured meat products are traditionally considered as a safe food, several pathways may lead to *Salmonella* outbreaks in these products. *Salmonella* is an opportunistic microorganism able to colonize immunodeficient hosts, increasing the risk of outbreaks by the consumption of contaminated dry-cured meat products. If contamination occurs during manufacturing, the absolute elimination of the pathogen in the final product is unlikely. Moreover, the trend in commercialization for ready-to-eat sliced products adds a risk source for all meat products, due to the chance of post-processing contamination. For this reason, it should be emphasized that preventing contamination is the most effective way to assure microbiological safety and quality of dry-cured meat products, especially ensuring compliance with manufacturing practices and avoiding contamination caused by selection of raw materials and ingredients.

The recipe for *Salmonella* adaptability to withstand adverse environments is a series of well-regulated stress response systems that reprogram its phenotypes. *Salmonella* display overlapping systems, leading to cross-protection among abiotic stresses. These overlaps include the transcriptional regulation of several genes triggered by sublethal stresses, such as those encoding transmembrane sensors, intracellular signaling effectors, osmoprotectant biosynthesis or transport/diffusion to the cytoplasm, and alternative sigma factor recognition and activation. Phenotype plasticity is clearly established between exposure to stress and the effectiveness of pre-adapted *Salmonella* to survive through the gastric passage, present adhesiveness, pathogen invasion and colonization and multiplication within macrophages. However, questions still remain as to how specific is the association between stress response and enhanced virulence, and what role does genetic background play in this virulence.

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