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Review

The response of foodborne pathogens to osmotic and desiccation stresses in the food chain

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ABSTRACT

In combination with other strategies, hyperosmolarity and desiccation are frequently used by the food processing industry as a means to prevent bacterial proliferation, and particularly that of foodborne pathogens, in food products. However, it is increasingly observed that bacteria, including human pathogens, encode mechanisms to survive and withstand these stresses. This review provides an overview of the mechanisms employed by *Salmonella* spp., Shiga toxin producing *E. coli*, *Cronobacter* spp., *Listeria monocytogenes* and *Campylobacter* spp. to tolerate osmotic and desiccation stresses and identifies gaps in knowledge which need to be addressed to ensure the safety of low water activity and desiccated food products.

Contents

1. Introduction

In the food industry, salt, in combination with other "mild technologies" is often used as a general preservative and an antibacterial agent because of its inhibitory effects on bacterial growth in ready-to-eat

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(RTE) meat, seafood, and fermented foods such as salami, cheese, baked goods, fruit and vegetables (Desmond, 2006). In addition, salt is often considered an essential additive to enhance the flavour, texture and shelf life of meat products (Ruusunen and Puolanne, 2005). Salt can cause damage to bacterial cells by disrupting the maintenance of osmotic balance between the cytoplasmic and intracellular environments (Csonka, 1989). Hyperosmotic solutions of sugars have been used for the dehydration and reformulation of ready to eat fruits (Torreggiani and Bertolo, 2004). Osmotic dehydration treatment has also been adopted as a partial dewatering process by immersion of fruit and vegetable tissues in hypertonic solutions (Rahman, 2008). Osmotic dehydration represents a mild process to improve the fresh-cut product stability and quality, along with other preservation technologies (i.e. sanitation, refrigeration, modified atmosphere packaging (Torreggiani and Bertolo, 2004)). Besides the diffusion of water from the vegetable tissue simultaneous solutes' counter-diffusion into the tissue is usually observed (Kowalska and Lenart, 2001) and the sucrose concentration seems to cause a hindrance of microbial cell adhesion to fruit surfaces (Gianotti et al., 2001).

Bacteria may encounter osmotic stress during a shift to a hyperosmotic solution or due to dehydration. Changes in osmolarity pose significant stress on bacterial cells by causing either swelling in hypotonic environments or dehydration and shrinkage under hypertonic environments (Csonka, 1989; Sleator and Hill, 2002). The term water potential represents the work involved in moving 1 mol of water from some point in a system (at constant pressure and temperature) to a pool of pure water at atmospheric pressure and at the same temperature as the system under consideration, while matric water potential generally is applied to considerations of water interactions at surfaces and interfaces (Abee and Wouters, 1999; Potts, 1994). When water molecules are associated with interfaces (including foods) such as the surfaces of colloidal particles (solid particles that range from −0.002 to 1 μm in diameter, e.g., proteins, ribosomes, some bacteria, and viruses) in an aqueous solution, they have less tendency to react chemically in bulk solution or to escape to the surrounding vapour phase. Interfaces thus lower the thermodynamic activity of the water, especially near the surface of the colloid (Potts, 1994). Interfaces together with solutes lower the water activity (a_w), so that there is an additive effect in solutions containing solutes and colloids.

Moreover, water efflux occurs when bacterial cells are exposed to a gas phase with an a_w that is lower than the cell compartment. If there is a considerable difference between the water activities of the two compartments, exposure of the cells for a limited time may lead to rapid shrinkage of the cytoplasm. However, if the a_w of the gas phase is sufficient to permit growth, albeit slow growth, the cells may achieve a water balance through de novo synthesis of compatible solutes.

The removal of a substantial fraction of the bulk water from cells through a drying stress is termed desiccation, and such desiccation can be achieved through either rapid or slow drying. There is one fundamental distinction between matric and osmotic systems: the immediate environment of a cell under desiccation (matric stress) is the atmosphere; i.e., the surfaces of their cell walls are exposed to a gas phase, while cells under osmotic stress are bathed in an aqueous solution, albeit in one of diminished a_w (Potts, 1994).

The question of desiccation also represents a challenge for food preservation, surface disinfection, pathogen transmission and, at the same time, an opportunity for the production of probiotics and dried cultures for the dairy, beer and wine industries. Regarding the former it is important to diminish microbial viability as efficiently as possible, while for the latter viability needs to be kept high (Nocker et al., 2012). Shrinkage of the cell's capsular layers, increase in intracellular salt concentrations and macromolecules due to a decrease in cell volume are the main consequences of desiccation (Potts, 1994). Other effects include changes in biophysical properties (such as surface tension), reduced fluidity of membrane lipids, and damage to proteins and DNA. Moreover, one of the molecular mechanisms of damage leading to death in desiccation-sensitive cells upon drying is free-radical attack to phospholipids, DNA and proteins. Regulation of the antioxidant defence system is complex and its role in desiccation tolerance is not yet firmly established, although in cyanobacteria cells of *Nostoc commune*, adapted to intense solar irradiation, Fe-superoxide dismutase was demonstrated to be the third most abundant solute (Shirkey et al., 2000). In general, as recently confirmed by Nocker et al. (2012), Gram-negative species are greatly more susceptible to drying than the Gram-positive species. The reasons for the higher resistance of Gram-positive bacteria are thought to be related to their smoother surfaces, the thicker peptidoglycan layer and the lack of lipopolysaccharides (Miyamoto-Shinohara et al., 2008).

Disaccharides and extracellular polysaccharides show a clearly protective effect against desiccation. In particular trehalose and sucrose, form 'supersaturated solid solutions' (such as glasses) with high viscosity, that form when the sugar solution becomes highly concentrated due to water loss (Koster, 1991). The benefits of such glasses for cells undergoing desiccation include filling space to prevent cellular collapse and continuance of hydrogen bonding at the interface between the glass and the cells (Koster, 1991). Membrane lipids, protected by the formation of hydrogen bonds between the sugar and the phospholipid head groups, contribute to the maintenance of normal lipid structure in the membranes (Welsh and Herbert, 1999). Similar effects apply to proteins (Leslie et al., 1995).

It is well known that the presence of extracellular polysaccharides in biofilms protects cells from desiccation and other stresses. Potts (1994) described that the shrink–swell behaviour of extracellular polysaccharides under conditions of different water potentials affected the pore sizes and passage of solutes. The authors hypothesised that the low permeability of extracellular polysaccharides results in a 'hydraulic decoupling' during rapid wetting or drying events and therefore effectively shields extracellular polysaccharide-embedded cells from adverse effects of extreme fluctuations in hydrated conditions. Additionally, Nocker et al. (2012) demonstrated that magnesium chloride concentrations ≥50 mM dramatically increase bacterial susceptibility to desiccation in the case of Gram-negative bacteria, and to a lesser extent also for Gram-positive bacteria.

The ability to survive and/or proliferate under stresses such as osmotic and desiccation stress is well known to contribute to the persistence of pathogens both in foods and food-processing environments, elevating the risk of transmission of pathogens through the food chain to humans. However, a further aspect sometimes overlooked is the possibility that exposure to osmotic stress along the food chain may lead to cross-protection against subsequent stresses faced in food production or during transit in the GI tract. For example, some mechanisms known to contribute to osmotic stress resistance, such as compatible solute transporters and cold shock proteins, can contribute to *L. monocytogenes*' ability to grow at low temperature (Sleator et al., 2003). It has also been demonstrated by phenotypic data that growth at low temperature provides cross-protection to subsequent salt stress (Bergholz et al., 2012) and that at 37 °C, exposure of *L. monocytogenes* to osmotic stress increases its resistance to subsequent exposure to bile salts (Begley et al., 2002). Another example is represented by the accumulation of compatible solutes such as glycine betaine and carnitine, the mechanism used by bacterial cells to overcome osmotic stress, which mainly stabilises enzymes and proteins, thus ensuring their continuous function in adverse conditions. It was demonstrated by Jørgensen et al. (1995) that the heat resistance of *L. monocytogenes* increased with the time it had been exposed to salt in a rich medium. Heating in 9% NaCl compared with a medium without added NaCl resulted in an 8-fold increase in heat resistance, while growth for 48 h and heating in the same menstruum gave a 22-fold increase. Furthermore, *Listeria* requires osmolyte uptake systems to maintain the full bile tolerance in vitro and the presence of carnitine contributes significantly to bile tolerance (Watson et al., 2009).

It is clear that bacteria, including foodborne pathogens, have evolved a number of complex interplaying systems to tolerate desiccation and