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Bacteria, mould and yeast spore inactivation studies by scanning electron microscope observations

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ABSTRACT

Spores are the most resistant form of microbial cells, thus difficult to inactivate. The pathogenic or food spoilage effects of certain spore-forming microorganisms have been the primary basis of sterilization and pasteurization processes. Thermal sterilization is the most common method to inactivate spores present on medical equipment and foods. High pressure processing (HPP) is an emerging and commercial non-thermal food pasteurization technique. Although previous studies demonstrated the effectiveness of thermal and non-thermal spore inactivation, the in-depth mechanisms of spore inactivation are as yet unclear. Live and dead forms of two food spoilage bacteria, a mould and a yeast were examined using scanning electron microscopy before and after the inactivation treatment. *Alicyclobacillus acidoterrestris* and *Geobacillus stearothermophilus* bacteria are indicators of acidic foods pasteurization and sterilization processes, respectively. *Neosartorya fischeri* is a phyto-pathogenic mould attacking fruits. *Saccharomyces cerevisiae* is a yeast with various applications for winemaking, brewing, baking and the production of biofuel from crops (e.g. sugar cane). Spores of the four microbial species were thermally inactivated. Spores of *S. cerevisiae* were observed in the ascus and free form after thermal and HPP treatments.

Different forms of damage and cell destruction were observed for each microbial spore. Thermal treatment inactivated bacterial spores of *A. acidoterrestris* and *G. stearothermophilus* by attacking the inner core of the spore. The heat first altered the membrane permeability allowing the release of intracellular components. Subsequently, hydration of spores, physicochemical modifications of proteins, flattening and formation of indentations occurred, with subsequent spore death. Regarding *N. fischeri*, thermal inactivation caused cell destruction and leakage of intracellular components. Both thermal and HPP treatments of *S. cerevisiae* free spores attacked the inner membrane, altering its permeability, and allowing in final stages the transfer of intracellular components to the outside. The spore destruction caused by thermal treatment was more severe than HPP, as HPP had less effect on the spore core. All injured spores have undergone irreversible volume and shape changes. While some of the leakage of spore contents is visible around the deformed but fully shaped spore, other spores exhibited large indentations and were completely deformed, apparently without any contents inside. This current study contributed to the understanding of spore inactivation by thermal and non-thermal processes.

1. Introduction

Undesirable microorganisms can spoil foods and cause human diseases. Therefore, several techniques are used to control or destroy microbial cells. Certain microorganisms can be present in the vegetative but also in the spore form and these matured spores are difficult to inactivate due to their concrete multilayer membranes, which can withstand multiple environmental conditions. Therefore, spores are commonly used as a target in sterilization and pasteurization processes.

Alicyclobacillus acidoterrestris is an aerobic, non-pathogenic and spore-forming bacteria that has been detected in several spoiled commercial pasteurized acidic fruit juices. Therefore, this organism has been suggested as an ideal target of acidic fruit juice pasteurization (Silva and Gibbs, 2001, 2004; Silva et al., 2014). *A. acidoterrestris* vegetative cells are rod shaped with terminal or sub-terminal endospores (Goto et al., 2007). This bacterium produces compounds that are responsible for juice off-odours. *Geobacillus stearothermophilus* is a non-pathogenic bacteria, which can produce spores under harsh conditions when nutrient concentrations are low. Presence of *G. stearothermophilus* usually results in flat sour spoilage of canned liquid foods (Watanabe

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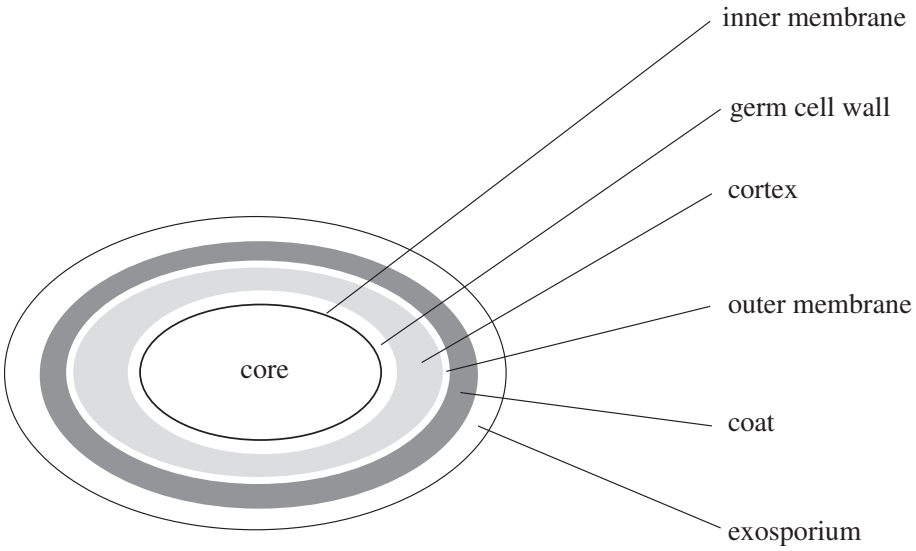

Fig. 1. Structure of a bacterial spore (not drawn to scale). Redrawn from Setlow (2006).

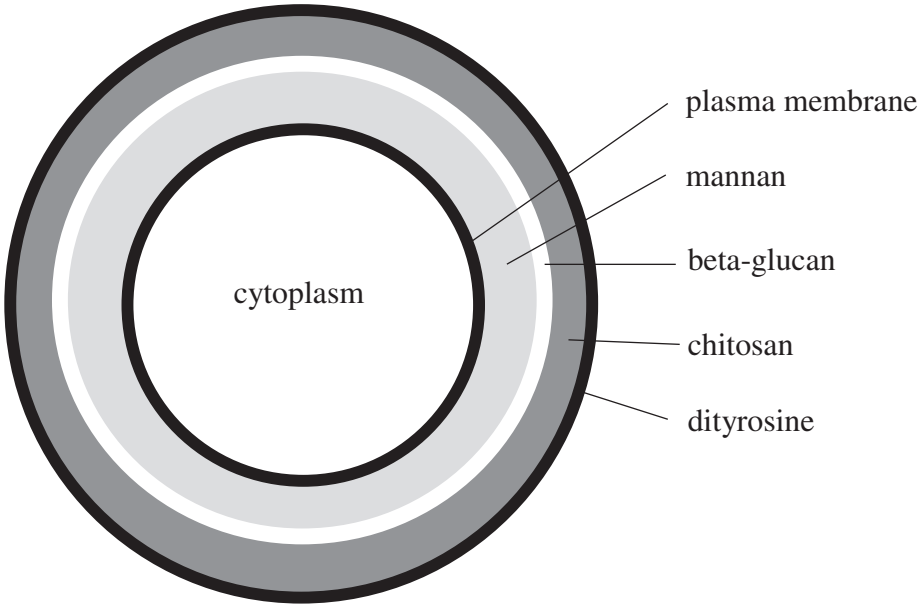

Fig. 2. Structure of a yeast ascospore (not drawn to scale). Modified from Neiman (2005).

et al., 2003). Fig. 1 shows the typical structure of a bacterial endospore. The interior compartment of a bacterial spore is called the core. The spore core is dehydrated with only 10–25% water content, making it very resistant to heat and chemicals. Pyridine-2,6-dicarboxylic acid or dipicolinic acid (DPA) is another component found in the inner core of bacterial spores but not in vegetative cells. This molecule comprises 5–15% of the dry weight of the spore (Molva and Baysal, 2014; Setlow, 2006). Its function is to prevent the denaturation of protein by lowering the core water content (Molva and Baysal, 2014). The outside of the spore core is comprised of a thick, loosely cross-linked peptidoglycan layer called the spore cortex, which prevents hydration of the spore core. The germ cell wall under the cortex will become the cell wall of the bacterium after the endospore germinates. The inner membrane on the other hand, acts as a major permeability barrier against chemicals. The completed spore is encased in a multi-layered protein shell known as the coat which protects the spore (Aronson and Fitz-James, 1976). The spore coat also acts as a permeability barrier (Zhang et al., 2006).

Neosartorya fischeri is a spore-forming phyto-pathogenic mould that is commonly associated with diseases in fruits growing at ground level. It is a ubiquitous fungus which usually proliferates in damp environments, such as soil. It is an extremely rare human pathogenic fungus that can cause keratitis and in some cases pulmonary aspergillosis in transplant patients (Lonial et al., 1997). The information on the structural details of *N. fischeri* spores is very limited. However, it is known that the ascospore (spores produced or contained inside an ascus) walls of *N. fisheri* are usually ornamented with anastomosing ridges (Peterson, 1992). *N. fischeri* spores are known to be extraordinarily resistant to heat (Beuchat, 1986; Conner et al., 1987; Evelyn et al., 2016; Evelyn and Silva, 2017; Girardin et al., 1995) compared to bacteria, yeasts and other moulds (Silva and Gibbs, 2004, 2009; Silva et al., 2014).

Saccharomyces cerevisiae is a spherical shaped non-pathogenic yeast which is well known for its ability to ferment sugars to ethanol, resulting in the production of food and beverages such as beer, wine and baked products. It is also used to produce alcohol for biofuel from sugar cane. *S. cerevisiae* can also produce spores although these spores have a lower resistance to damaging environments compared to bacterial and mould spores (Milani et al., 2015a, 2015b, 2016; Milani and Silva, 2016, 2017). Once *S. cerevisiae* spores are fully formed, the remains of the mother cell collapses around the four mature spores. This event results in the formation of the ascus, consisting of four ascospores enclosed together inside an ascal membrane and wall (Fig. 6), which are