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MINI-REVIEW

Desiccation-induced cell damage in bacteria and the relevance for inoculant production

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Abstract

Plant growth–promoting bacteria show great potential for use in agriculture although efficient application remains challenging to achieve. Cells often lose viability during inoculant production and application, jeopardizing the efficacy of the inoculant. Since desiccation has been documented to be the primary stress factor affecting the decrease in survival, obtaining xerotolerance in plant growth–promoting bacteria is appealing. The molecular damage that occurs by drying bacteria has been broadly investigated, although a complete view is still lacking due to the complex nature of the process. Mechanic, structural, and metabolic changes that occur as a result of water depletion may potentially afflict lethal damage to membranes, DNA, and proteins. Bacteria respond to these harsh conditions by increasing production of exopolysaccharides, changing composition of the membrane, improving the stability of proteins, reducing oxidative stress, and repairing DNA damage. This review provides insight into the complex nature of desiccation stress in bacteria in order to facilitate strategic choices to improve survival and shelf life of newly developed inoculants.

Key Points

- *Desiccation-induced damage affects most major macromolecules in bacteria.*
- *Most bacteria are not xerotolerant despite multiple endogenous adaption mechanisms.*
- *Sensitivity to drying severely hampers inoculant quality.*

Keywords Desiccation · Xerotolerance · Inoculant · Plant growth–promoting bacteria · Oxidative stress · Membrane stress

Introduction

Plant-associated bacteria have become an attractive ecological alternative for fertilizers and pesticides to improve crop production (Vessey 2003; Backer et al. 2018; Majeed et al. 2018). Current research in this field has mainly focused on finding new plant growth–promoting bacteria and identifying their mode of action. While a wide array of mechanisms to promote plant growth have been discovered, the application of plant growth–promoting bacteria in agriculture remains limited. A main reason is that, despite clear beneficial plant phenotypes under laboratory conditions, the efficacy in the field remains unreliable (Stephens and Rask 2000; Kaminsky et al. 2019). Kaminsky et al. (2019) propose that the difficulty of producing a successful inoculant is due to the need of multiple, sometimes conflicting, traits: an important one being the survival of the microorganism. Inoculants are designed to enrich the targeted plant environment with active beneficial microorganisms. Therefore, keeping the bacterial cells viable during the inoculant production, storage, and application is considered to be crucial and one of the main aspects determining the success of the inoculant (Bashan et al. 2014; Kaminsky et al. 2019). Desiccation is considered to be one of the main causes of cell death in inoculants and occurs at different stages during the process of inoculant production (Deaker et al. 2004; Berninger et al. 2018). In order to easily preserve the bacteria for longer

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periods of time, inoculants are dried to halt bacterial metabolism (Meng et al. 2008), although many bacteria do not survive this process. According to Berninger et al. (2018), the drying procedure is generally the most crucial process determining viability. However, desiccation also occurs when the inoculant is coated on seeds or during application of the inoculant on plant or in soil. Finally, in the soil, the cells can also experience multiple wet-drying cycles as a result of environmental fluctuations affecting their relative abundance in the rhizosphere (Berninger et al. 2018).

To increase desiccation tolerance, also named xerotolerance, in plant growth–promoting bacteria, general knowledge about desiccation-induced damage and responses to desiccation is crucial. However, despite the relevance for inoculant production, desiccation research in plant growth–promoting bacteria is scarce. Therefore, in order to obtain a complete view, information concerning desiccation tolerance in other bacterial species is included in this study. Besides inoculants, desiccation research has been conducted in many bacterial species and is scattered over multiple application domains. Environmental research is primarily concerned with how bacteria persist in and adapt to environments with multiple wet-dry cycles (Šťovíček et al. 2017; Hernández et al. 2019). In the food industry, bacterial desiccation research is executed in order to prevent pathogenic contamination of low water content food (Burgess et al. 2016; Esbelin et al. 2018). Desiccation tolerance in (human) probiotics is also important in obtaining a stable delivery method in food, feed, and pharmaceutical industries (Huang et al. 2017).

This minireview provides an overview of the molecular stresses that occur during the process of desiccation in bacteria and how they respond in order to prevent cell death. The link between the production of inoculants and bacterial desiccation is discussed together with currently applied methods to improve the shelf life of inoculants.

The desiccation process

In bacteria under hydrated conditions, water molecules are present in high abundancy both extracellularly and intracellularly (Potts 1994). It was estimated that the dry weight of bacterial cells is between 31 and 57% (Bratbak and Dundas 1984). Virtually all other molecules are water (Bratbak and Dundas 1984). When cells are dehydrated, the drying process can be roughly divided into two steps. In the initial drying phase, extracellular unbound water is removed. The external concentration of solutes and salts therefore increases and causes an osmotic imbalance with the cell cytoplasm (Potts et al. 2005; Vriezen et al. 2007). The loss of turgor pressure leads to the displacement of water across the plasma membrane and results in shrinking of the cell. The strong reduction of water in the cytoplasm increases the concentration of intracellular molecules including metabolites and ions affecting cellular metabolism (Potts et al. 2005; Lebre et al. 2017). In addition, crowding resulting from this hyperosmotic stress reduces the cellular mobility of macromolecules, further altering its metabolism (Vriezen et al. 2007; Mika et al. 2010). Parry and colleagues showed that the bacterial cytoplasm can obtain glass-like properties and suggest that this originates from extreme molecular crowding (Parry et al. 2014). This phenomenon is enhanced by a lowered metabolic state and primarily impairs the movement of large macromolecules like the chromosome (Parry et al. 2014). Due to a metabolic imbalance in aerobic bacteria, reactive oxygen species (ROS) like superoxide anion ($^{\circ}O_2^-$), hydrogen peroxide (H_2O_2), and hydroxyl radical (°OH) accumulate, causing oxidative stress (França et al. 2007). During desiccation, oxidative damage primarily happens not only as a result of hydroxyl and peroxyl radicals but also from longer-lived forms derived from protein hydroperoxides (Fredrickson et al. 2008).

Water that is bound to macromolecules corresponds to 15% of the total water content of the cell (Persson and Halle 2008). In the second stage of drying, this hydration shell of macromolecules is (partly) lost (França et al. 2007). The solute surrounding the cell solidifies and contact between the cellular molecules and air increases (Dupont et al. 2014). In particular, in the presence of solar radiation, desiccated cells are further damaged because of formation of ROS (França et al. 2007; Dupont et al. 2014). Even when cells are stored under desiccated form, they still lose viability. Many studies have shown that the bacterial death rate is dependent on storage conditions such as relative humidity and temperature (Achour et al. 2001; Vriezen et al. 2007; Hernández et al. 2009; Rojas-Tapias et al. 2014). When cellular metabolism is shut down and macromolecules are immobilized, damaged macromolecules and ROS that have accumulated during the drying and storage phases do not cause immediate cell death. When rehydrated, damaged cellular molecules regain mobility and potentially cause lethal damage. Viable cells however repair accumulated damage and restore an active metabolism (Vriezen et al. 2007). Furthermore, when rehydrated, a hypo-osmotic shock is possible, dependent on the rehydration medium and speed of rehydration (Vriezen et al. 2007; Dupont et al. 2014).

Desiccation of bacteria is a complex phenomenon due to the multiple mechanical and structural alterations of the cell that occur during the process. The complex nature of the consequences of desiccation makes it difficult to pinpoint a main cause of death in bacteria and a clear understanding of this phenomenon is therefore still lacking. Multiple studies show that the removal of water affects a wide array of macromolecules. The membrane, proteins, and DNA of the cell undergo changes causing cellular stress in a multitude of ways.

Damage to the plasma membrane

During the initial stage of drying, the removal of water and shrinkage of the cell lead to mechanical stress on the plasma membrane (Fig. 1). While the surface area of the plasma membrane remains constant, the volume of the cell decreases, forcing the plasma membrane to deform (Dupont et al. 2014). Even though little attention has been given to this phenomenon in bacteria, it has been proven to cause ruptures in the plasma membrane of yeast cells during rapid dehydration, leading to cell death (Simonin et al. 2007; Dupont et al. 2010).

During mild desiccation, the packaging density of the heads of the phospholipids rises (Crowe et al. 1992; Halverson and Firestone 2000). This results in higher Van der Waals forces between the hydrocarbon chains causing the melting temperature to rise. Therefore, lipids transform from their liquid crystalline phase into a gel phase (Crowe et al. 1992). Variations of gel-to-liquid phase conversions in phospholipids can lead to fusion and/or breaking of the membrane causing leakage when rehydrated (Lebre et al. 2017). Further dehydration lowers the phase transition temperature and can cause changes from the typical lamellar liquid crystalline structure to a hexagonal II phase, where inverted micelles form between the bilayers (Halverson and Firestone 2000). The lamellar bilayer structure is also stabilized by the presence of water due to its hydrophilic properties (Dupont et al. 2014).

During desiccation, the cell membrane also becomes more susceptible to ROS. Phospholipids mainly suffer from peroxidation and de-esterification as a result of free radicals (França et al. 2007). The polar groups also affect iron autoxidation, causing ROS to be generated that can attack the hydrophobic side (França et al. 2007; García 2011).

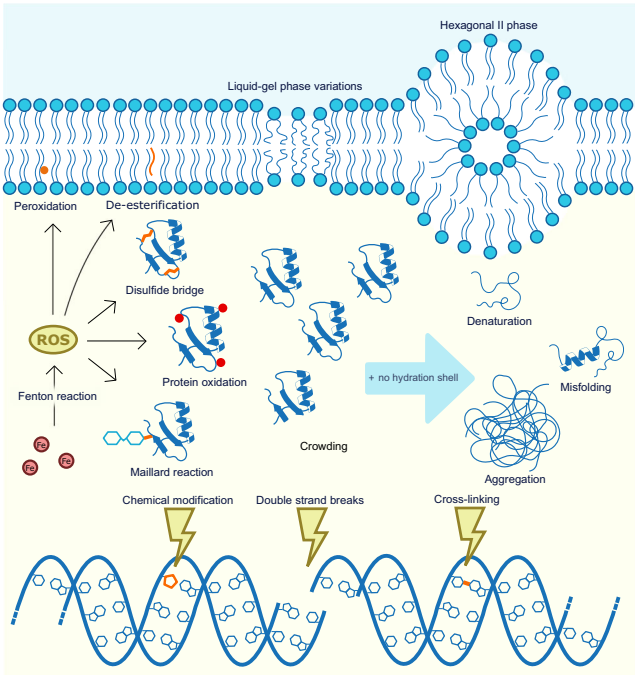

Fig. 1 Molecular damage occurring in bacteria during desiccation. Desiccation induces gel formation of the membrane. Variation between the liquid crystalline and gel phase can cause leakage of the cell. During extreme desiccation, the hexagonal II phase can occur. Reactive oxygen species accumulate during dehydration and damage the membrane through peroxidation and de-esterification. Iron can further enhance ROS formation through the Fenton reaction. ROS can induce the formation of unwanted disulfide bridges, Maillard reactions, and oxidation of proteins. Together with crowding and the removal of the hydration shell, damaged proteins can misfold or denaturate and result in formation of protein aggregates. Desiccation-induced DNA damage occurs through covalent modifications (e.g., the formation of hydantoin rings), double-strand breaks, and cross-linking

Damage to proteins

The activity of proteins highly depends on their native structure. Damaged proteins that lose their native state can lose their functionality, causing metabolic stress. The exposure of aggregation-prone hydrophobic regions that normally occur in the core of the protein can also cause protein aggregation. Protein aggregation has previously been shown to occur in desiccated yeast cells (Tapia and Koshland 2014). Besides inducing loss of functionality, protein aggregates have also been shown to induce ROS formation, to promote molecular crowding, to induce lipid peroxidation, to cause leakage, and to rearrange the cell membrane (Bednarska et al. 2013). Protein damage is speculated to be the primary stress leading to desiccation-induced cell death (Morano 2014; Tapia and Koshland 2014).

Removal of unbound water can damage proteins by altering important properties like stability and association rates due to molecular crowding (Tokuriki et al. 2004; Cheung et al. 2005; Wieczorek and Zielenkiewicz 2008; Miklos et al. 2011; Wang et al. 2012; Köhn and Kovermann 2019). Water lubricates and therefore facilitates conformational changes in proteins by increasing their flexibility, allowing them to fold into a functional state (Paciaroni et al. 2005). In addition, the hydrophilic nature of water provides structural stability of macromolecules by interaction with their hydrophilic groups (Dupont et al. 2014). Removal of water as a solvent can therefore result in severe changes in the conformation of proteins, making them lose their native state, as the absence of the hydrophilic environment causes hydrophobic regions to be no longer embedded within the protein core (Tanford 1997).

Oxidative stress leads to protein oxidation and is proposed to be the main determinant of desiccation sensitivity in bacteria (Fredrickson et al. 2008; García 2011). Mostly irreversible carbonyl groups are produced on protein side chains and lead to misfolding and potentially aggregation (Tyedmers et al. 2010). Carbonylated proteins are resistant to degradation due to further reactions with lysine residues (Stadtman and Levine 2006). Furthermore, production of reactive aldehydes due to oxidative stress can also damage proteins through the Maillard

reaction (Lebre et al. 2017). Here, covalent bonds between the carbonyl group of reducing sugars and primary amines cause irreversible polymerization (Billi and Potts 2002; Potts et al. 2005). Unwanted disulfide bridge formation and oxidation of thiols and sulfonic acid can also occur (França et al. 2007).

Damage to DNA

During dehydration, DNA damage occurs through covalent modifications (e.g., through Maillard reactions), cross-linking, and double-strand breaks (Humann and Kahn 2015; Lebre et al. 2017). DNA damage caused by oxidative stress is complex and a comprehensive overview is lacking but primarily happens through chemical modification, cross-linking, and other lesions (Potts et al. 2005; França et al. 2007; García 2011). Pyrimidines can be degraded to hydantoin rings, which cannot be replicated by DNA polymerases anymore (Potts et al. 2005). Because DNA protection and repair mechanisms are slowed down, DNA damage accumulates causing cell death (Lebre et al. 2017).

Adaptation to desiccation

In order to survive desiccation, bacteria must adapt to the multiple stresses occurring during the drying, storing, and rehydration processes (Fig. 2). Despite a strong selection pressure, complete xerotolerance in organisms in general is uncommon which may be the consequence of a trade-off between desiccation tolerance and growth (Billi and Potts 2002; Alpert 2006). However, many bacteria have been shown to adapt to these conditions and to withstand desiccation (Lebre et al. 2017). Since the metabolism is severely impaired when water activity is low, production of protective molecules and metabolic reorganization are expected to happen at the start of the drying process (Vriezen et al. 2007). Indeed, in a transcriptomic study of LeBlanc and colleagues monitoring the changes in expression during the drying process of *Rhodococcus jostii*, the largest changes in gene expression happen during the initial drying phase (between 1 and 3 h of drying) (LeBlanc et al. 2008). Surprisingly, these changes are mostly downregulation of genes while the largest changes in upregulation happen during the end phase of the drying step (6 to 12 h). Similar results were found in *Anabaena* sp. in which 12% of all genes were downregulated in the beginning of the drying process (1–3 h) (Katoh et al. 2004). The largest change in upregulation also happened during the end stage of drying (6 to 10 h). The window during which the late stage of drying is measured is large in these studies and roughly ranges from cells containing half of the initial water content to complete desiccation. Transcriptomic studies that include more time points at this late stage of drying might therefore be very valuable. Since most lethal stresses are thought to occur when

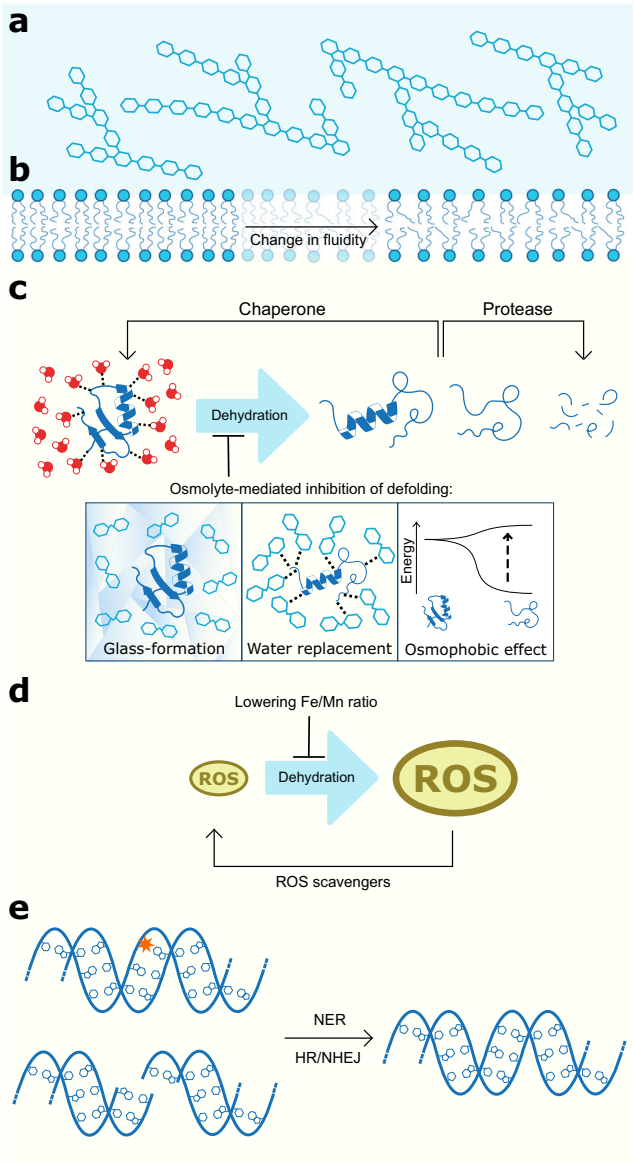

Fig. 2 Adaptation mechanisms to desiccation found in bacteria. **a** Upregulation of production of exopolysaccharides and biofilm formation can protect bacteria through water retainment, gel formation in a desiccated state, lowering of oxidative stress, and/or cell aggregation. **b** A change of cell membrane composition can alter membrane fluidity, thereby preventing unwanted phase transitions (like the hexagonal II phase), and alter susceptibility to oxidative stress. **c** Production of molecules affecting faith and stability of proteins. At low water content, protein denaturation is decreased through osmolytes by forming glass-like structures, replacing hydrogen bound of water and increasing the energy needed for denaturation through the osmophobic effect. Chaperones can promote proper folding while proteases degrade damaged proteins. **d** The formation of reactive oxygen species (ROS) is reduced by lowering the Fe/Mn ratio. Lowering the iron content reduces Fenton reaction and therefore ROS formation, while a high manganese content reduced oxidative stress since it can be used as a cofactor in complexes that keep radical levels low. ROS scavenging proteins and molecules on the other hand directly remove potentially harmful radicals. **e** Modifications of DNA can be repaired by nucleotide excision repair (NER). Double-strand breaks can be repaired by non-homologous end joining (NHEJ) or homologous recombination (HR)

cells are completely desiccated and the metabolism is abolished during this stage, the bacteria must sense relatively mild stresses early in the drying process and make preventive adjustments to withstand further desiccation. Aside from transcriptomic studies performed with desiccated cells (Katoh et al. 2004; Cytryn et al. 2007; LeBlanc et al. 2008; Li et al. 2012; Kocharunchitt et al. 2014; Srikumar et al. 2018), changes in gene expression in cells subjected to low water activity or hyperosmotic stress would therefore also be relevant for desiccation research. Current knowledge about bacterial adaptation to desiccation, based on gene expression studies and mutant analyses, is reviewed here.

Compatible solutes and non-reducing saccharides

Osmolytes or compatible solutes replace potassium after an initial hyperosmotic shock and are taken up from the environment or synthesized intracellularly (Whatmore et al. 1990; Paul 2013). These organic molecules accumulate intracellularly to counterbalance the osmotic pressure but are not toxic at high concentrations. Compatible solutes are usually neutral betaines, polyols, sugars, and amino acids and do not drastically alter the metabolic activity of the cell (Galinski 1995). Besides restoring the osmotic imbalance, compatible solutes can also hinder protein damage by preventing unfolding and aggregation at lower water contents. Different modes of actions have been proposed and may occur simultaneously to protect proteins during desiccation. Many mono- and disaccharides like sucrose, glucose, and trehalose can offer protection through the formation of biological glasses at a low water content (Potts 1994). These are amorphous solids with an extremely high viscosity. The resulting immobilization and physical separation of the dehydrated proteins and other macromolecules protect them by keeping them in place and in their native form (Crowe et al. 1998). Furthermore, the vitreous state reduces the release of ROS and prevents lipid oxidation and Maillard reactions (França et al. 2007). Under low relative humidity, the glass transition temperature is decreased, making it possible for compatible solutes to form glasses (Potts 1994). When cells are rehydrated, the glass transition temperature rises again, making the compatible solutes melt and metabolic activity resume (Crowe et al. 1998). The replacement theory is a second proposed mechanism by which osmolytes can protect macromolecules. Sugars like trehalose can directly replace the hydrogen bonds when water is removed and therefore keep the proteins in their native state (Crowe et al. 1998). These two modes of action have attracted most attention but the less explored hypothesis of preferential exclusion could also explain the protective effect of osmolytes (Potts 1994). Here, osmolytes like glycine, betaines, and ectoine can form structures that are disadvantageous for external molecules to bind (Arakawa and Timasheff 1985). This creates an osmophobic effect which makes it thermodynamically more favorable for proteins to keep their native state because conformational changes result in a larger surface area and more chances of interacting with the osmolyte (Bolen and Baskakov 2001; Kurz 2008). This process is mainly important at intermediate to low water contents, in which the hydration shell, and therefore the activity, remains stable for a longer period of time (Arakawa and Timasheff 1985; Potts 1994).

Different types of osmolytes have been shown to be involved in the adaptation to desiccation and their effect is often strain-specific (Lebre et al. 2017). Trehalose has attracted a lot of attention since it is known to be the most effective osmoprotectant among sugars (Crowe et al. 1992, 1996). It restores osmotic imbalance and stabilizes organic molecules by water replacement. The high flexibility of the glycosidic link between the two D-glucose residues allows for a better interaction with the irregular polar groups of macromolecules (França et al. 2007). During mild desiccation, sugars like trehalose can keep the phase transition temperature low (Eleutheria et al. 1993; Potts 1994). Due to the very high glass transition state of trehalose, stable glasses can be formed more easily and thereby stabilize the cell when desiccated (Crowe et al. 1998; Lebre et al. 2017). Studies have shown that trehalose can also reduce oxidative stress, even when desiccated (Pereira et al. 2003; França et al. 2007), and prevent protein aggregation in yeast (Tapia and Koshland 2014). Both externally added and intracellularly synthesized trehalose have been proven to be beneficial for survival during desiccation in multiple species, further supporting its importance (García De Castro et al. 2000; Manzanera et al. 2002; Streeter 2003; McIntyre et al. 2007). Furthermore, trehalose biosynthesis genes are upregulated in multiple species upon desiccation (Cytryn et al. 2007; Li et al. 2012; Srikumar et al. 2018). Even though trehalose is the most investigated osmolyte, a wide array of osmolytes have been reported to be produced and/or imported by bacteria. Multiple studies have proven the role of osmolytes like ectoine, hydroxyectoine, glycine betaine, and other betaines in xerotolerance (Manzanera et al. 2002, 2004; Oren 2008; LeBlanc et al. 2008; Klähn and Hagemann 2011; Li et al. 2012; Finn et al. 2013; Srikumar et al. 2018).

Chaperones and proteases

Chaperones assist in folding and assembly of proteins into their native structure and avoid protein aggregation (Kim et al. 2013). When (re-)folding occurs unsuccessfully, proteases can clear misfolded proteins by proteolytic degradation. Molecular chaperones act during multiple types of abiotic stresses, including desiccation (Lebre et al. 2017). Chaperones DnaK and DnaJ have been shown to be important to withstand desiccation in *Salmonella typhimurium* in a transposon screen (Mandal and Kwon 2017). Enrichment of

chaperones and proteases (GrpE and ClpB) is also present in desiccated *Acinetobacter baumanni* cells (Gayoso et al. 2014). Furthermore, gene expression of chaperones has also been shown to be upregulated during hyperosmotic shock in multiple bacteria and after desiccation in *Bradyrhizobium japonicum* (Gottesman et al. 1997; Motohashi et al. 1999; Zolkiewski 1999; Domínguez-Ferreras et al. 2006; Cytryn et al. 2007). The effect of chaperones is often tested during short-term desiccation. Chaperones are thought to only confer tolerance to short-term desiccation, as shown in yeast, because protein damage during long-term desiccation inactivates these proteins, making them lose their effect (Tapia and Koshland 2014).

ROS-scavenging molecules

Removal of ROS occurs though peroxidases, catalases, and superoxide dismutases.

Superoxide dismutases are frequently found in bacteria and convert superoxide anions into oxygen and hydrogen peroxide, which can be further degraded by catalases and peroxidases (França et al. 2007; Imlay 2008). Superoxide anions are not very reactive but oxidize catalytic iron atoms of enzymes causing a release of iron, resulting in Fenton reactions (Imlay 2003). The induction of genes encoding superoxide dismutase (*chrC* and *sodF*) has been shown to be upregulated during desiccation in *B. japonicum* (Cytryn et al. 2007). Furthermore, the abundance of superoxide dismutase and catalase H is higher in dried *A. baumanii* cells (Gayoso et al. 2014) and the activity of superoxide dismutase, catalase, and peroxidase has been shown to peak when *Anabaea* sp. is dried (Singh et al. 2013). Deletion of the catalase biosynthesis gene *katE* has been shown to negatively impact xerotolerance in *A. boumannii* (Farrow et al. 2018). However, a direct effect of the presence of superoxide dismutases and peroxidases on desiccation tolerance has not yet been documented.

Antioxidants like glutathione, ascorbic acid, and vitamin E prevent oxidation and thereby avoid damage by ROS (França et al. 2007). Glutathione is an antioxidant that can directly protect cells from ROS through its sulfhydryl group or by being a cofactor of peroxidases. In *Listeria monocytogenes*, insertion of a transposon in a glutathione synthesis gene was found to cause lower survival after desiccation (Hingston et al. 2015). In a quantitative proteomic study, glutathione peroxidases also have been shown to be more abundant in desiccated *A. baumanii* cells (Gayoso et al. 2014).

Change of metal concentrations

The concentrations of iron and manganese have a large impact on the effects of desiccation. A low concentration of intracellular iron in the cell improves desiccation tolerance because Fenton and Haber-Weiss reactions, which produce hydroxyl and peroxyl radicals, are iron dependent (García 2011). Fredrickson and colleagues found a clear link between Mn/Fe concentrations and desiccation tolerance (Fredrickson et al. 2008). In *Ensifer meliloti*, hyperosmotic stress strongly reduces the expression of genes involved in iron uptake, which could be a preventive measure to reduce Fenton reactions during desiccation (Rüberg et al. 2003; Domínguez-Ferreras et al. 2006). Manganese on the other hand can form complexes that keep the concentration of iron-dependent superoxides and peroxyl radicals low (Fredrickson et al. 2008). Complexes of manganese with cellular ligands such as phosphate, lactate, and bicarbonate can scavenge superoxide and hydrogen peroxide (Archibald and Fridovich 1981; Archibald and Fridovich 1982; Stadtman et al. 1990). Manganese is also a common cofactor in multiple superoxide dismutases, like SodB in *E. meliloti* (Santos et al. 1999). A high Mn/Fe ratio has been proven to reduce iron-mediated protein oxidation (Daly et al. 2007). In cyanobacteria, manganese-containing catalases are expressed when cells are desiccated and a manganese importer has been shown to be important for desiccation tolerance in *S. typhimurium* (Rajeev et al. 2013; Mandal and Kwon 2017).

Change of cell membrane composition

Appropriate homeoviscous adaptation of the membrane is needed to maintain the correct fluidity to keep the membrane in a liquid crystalline phase. Cis-unsaturated fatty acids are preferred since they have a higher fluidity and a lower melting temperature (Halverson and Firestone 2000; Siliakus et al. 2017). Upon extreme desiccation, lower membrane fluidity is preferred to avoid a hexagonal II phase (Lebre et al. 2017). High saturation and high trans/cis ratios of monounsaturated fatty acids are found in the membrane of desiccated cells to reduce the fluidity (Kieft et al. 1994; Halverson and Firestone 2000). Moreover, polyunsaturated phospholipids are more susceptible to peroxidation, making them more sensitive to oxidative stress (França et al. 2007; García 2011). Short and branched chain fatty acids are also important for desiccation tolerance. In some rhizobia, more saturated short-chain fatty acids are present when desiccated (Boumahdi and Hornez 1999; Boumahdi et al. 2001). Furthermore, *L. monocytogenes* mutants having less branched-chain fatty acids are more susceptible to desiccation (Hingston et al. 2015). Short and branched-chain fatty acids are known to increase the fluidity of the membrane. However, because these are synthesized de novo, they do not allow rapid adaptation (Siliakus et al. 2017). Finally, an increase in cyclopropane fatty acids in desiccated cells has also been shown in *Escherichia coli* and *Pseudomonas aureofaciens* (Kieft et al. 1994; Kocharunchitt et al. 2014).

The polar heads may also have an impact on desiccation tolerance. Bacteria mostly contain phosphatidylglycerol (PG),

cardiolipin (CL), and phosphatidylethanolamine (PE) (López-Lara et al. 2003). In *E. coli*, synthesis genes of CL and PE are upregulated during desiccation (Kocharunchitt et al. 2014). Anionic phospholipids like PG and CL are more abundant than zwitterionic phospholipids when grown with low water activity as the probability to form non-bilayer structures is reduced (Russell et al. 1995).

Besides phospholipids, lipopolysaccharides (LPS) have been shown to be important for desiccation in multiple studies (Vriezen et al. 2007; Cytryn et al. 2007; Garmiri et al. 2008; Mandal and Kwon 2017). In dehydrated *Salmonella enterica* cells, conversion of lipid A laurate to palmitoleate is promoted, causing membrane fluidity to increase (Gruzdev et al. 2012). In a transposon-based screen in *Rhizobium leguminosarum*, two *fab* genes responsible for high molecular weight LPS biosynthesis were demonstrated to be important for survival against desiccation (Vanderlinde et al. 2009). Furthermore, several genes of *B. japonicum* related to LPS were upregulated when dried (Cytryn et al. 2007).

Restoring DNA damage

DNA repair is important during desiccation (Imlay 2008; Humann et al. 2009; Humann and Kahn 2015). To date, a direct link between three pathways for DNA repair and desiccation tolerance has been reported. The first pathway is the nucleotide excision repair (NER) pathway, which is a versatile repair pathway that can repair bulky adducts and helix-distorting lesions (primarily cyclobutane pyrimidine dimers). *E. meliloti* mutants of *uvrA*, *uvrB*, and *uvrC*, which are part of the nucleotide excision repair (NER) pathway, show higher sensitivity to prolonged desiccation (Humann et al. 2009). Surprisingly, cyclobutane pyrimidine dimers were not found in desiccated *E. meliloti* cells (Humann and Kahn 2015).

Double-strand breaks can be repaired by homologous recombination (HR) and non-homologous end joining (NHEJ). HR is the conserved mechanism and is primarily active during growth since it requires a second intact chromosome. Analysis of *recA* mutants highlights the importance of HR for desiccation in multiple species (Aranda et al. 2011; Dupuy et al. 2017). NHEJ has been acquired by some bacteria to act during non-growing conditions. In bacteria, NHEJ is conducted by Ku proteins that bind DNA ends and LigD that ligates them together (Pitcher et al. 2007a; Shuman and Glickman 2007). The importance of NHEJ for desiccation tolerance in *E. meliloti* has been shown using *ku* mutants (Dupuy et al. 2017). In *Mycobacterium smegmatis*, both mechanisms were also needed for desiccation tolerance, further highlighting the importance of double-strand breaks during desiccation (Pitcher et al. 2007b). Multiple DNA repair genes, like *mutL* and *recF*, were also found to be upregulated when *B. japonicum* cells were desiccated (Cytryn et al. 2007).

Exopolysaccharides

Exopolysaccharides (EPS) are involved in desiccation tolerance in many bacteria. Adding EPS externally before the drying process and removing EPS result in increase and decrease in desiccation tolerance respectively (Tamaru et al. 2005; Knowles and Castenholz 2008; Nocker et al. 2012). However, addition of EPS is not found to increase survival in every study (Ophir and Gutnick 1994). EPS biosynthesis genes are also upregulated in desiccated *B. japonicum*, *L. monocytogenes*, and *S. enterica* cells (White et al. 2006; Cytryn et al. 2007; Truelstrup Hansen and Vogel 2011). A causal link with desiccation tolerance has been shown in multiple species using deletion mutants of EPS biosynthesis genes (Ophir and Gutnick 1994).

Exopolysaccharides can protect bacteria against desiccation in various ways. EPS has a hygroscopic nature and therefore can withhold water for longer periods (Lebre et al. 2017). Low permeability can also reduce water loss from the cell during rapid drying and prevent toxic compounds from entering the cell (Vriezen et al. 2007). EPS is also expected to be able to form biological glass; however, this was not demonstrated experimentally yet. Crowe and colleagues propose that it forms a gel instead, and can have similar protective effects when desiccated (Crowe et al. 1998). Furthermore, EPS has been shown to be important for tolerance against oxidative stress (Lehman and Long 2013). Not only can it decrease hydrogen peroxide levels, for example by non-enzymatic scavenging, but it can also chelate iron (thereby reducing Fenton reactions) (Chowdhury et al. 2011; Cho et al. 2013; Lehman and Long 2013). EPS can also promote aggregation of bacterial cells, potentially protecting the cells against desiccation (Monier and Lindow 2003; Sorroche et al. 2010, 2012; Dorken et al. 2012). EPS is also a major constituent of biofilms, for which a positive correlation with desiccation tolerance has been shown in multiple studies (Iibuchi et al. 2010; Lebre et al. 2017; Du et al. 2018).

General metabolic changes

Desiccation induces different metabolic changes. Shutting down the metabolism could be a strategy to reduce the amount of ROS (França et al. 2007). Strong reduction in metabolic activity causes cells to enter a dormant state that is stress resistant. This has been shown to occur during dehydration in *A. baumannii* (Gayoso et al. 2014; Lebre et al. 2017). Bacteria like *Bacillus subtilis* are able to withstand different kinds of extreme abiotic stresses by forming spores; however, the reason for desiccation tolerance is yet unknown (Lebre et al. 2017). Surprisingly, other bacteria upregulate their metabolism during desiccation. In *Cronobacter sakazakii* and *B. japonicum*, genes involved in the pentose phosphate pathway are highly upregulated when dried (Cytryn et al. 2007;

Srikumar et al. 2018). Other energy-generating pathways like glycolysis, Krebs cycle, and oxidative phosphorylation are upregulated in multiple species (Katoh et al. 2004; Gruzdev et al. 2012; Kocharunchitt et al. 2014; Srikumar et al. 2018). A higher amount of NADPH could protect against desiccation-induced oxidative stress (Juhnke et al. 1996; Boada et al. 2000) while ATP could act as hydrotrope and thereby prevent protein aggregation (Patel et al. 2017).

The general stress response is activated by multiple stressors to adapt the cell to multiple stresses and is known to be activated in a stationary phase. In many bacteria, desiccation tolerance drastically increases when dried during stationary phase, indicating the relevance of this stress response (Vriezen et al. 2007). Deletion mutants with a disabled general stress response have been shown to have a lower survival after desiccation in *E. meliloti* (Humann et al. 2009). The general stress response induces a wide array of stress-related genes that may cross-protect the cell against desiccation. In rhizobia for example, the general stress response regulates more than 100 genes (Schlüter et al. 2013; Jans et al. 2013), and induces the synthesis of multiple molecules involved in xerotolerance like trehalose, superoxide dismutase, and catalase (Sauviac et al. 2007; Flechard et al. 2009, 2010; Jans et al. 2013). Furthermore, RelA, a regulator of the stringent stress response (which is usually activated during nutrient limitation and regulates a wide array of stress-related genes (Vercruysse et al. 2011)), is proven to be important for desiccation tolerance in *E. meliloti* (Humann et al. 2009).

Industrial drying processes for inoculant production

Keeping bacteria in a viable state during inoculant production is crucial to promote their beneficial effect on the plant. Viability highly depends on the drying procedure. Therefore, a correct drying method and corresponding protective measures should therefore be carefully considered.

Despite its relatively high cost, freeze-drying is a popular method to obtain a dry inoculant (Santivarangkna et al. 2007). In a first step, a cell culture is frozen, causing extra- and intracellular water molecules to change to their solid state. Extracellular water freezes first. Intracellular water freezes around below − 5 °C or flows outside the cell due to changes in osmolarity and vapor pressure and freezes externally (El-kest and Marth 1992). The freezing step is preferably done quickly such that the amount of large ice crystals that potentially puncture the cell membrane is limited (Morgan et al. 2006) and cell morphology is maintained (El-kest and Marth 1992). In the primary drying phase, the frozen culture is exposed to an extremely low pressure, causing the ice to sublimate and to be removed from the sample. This happens at low temperature to prevent collapse of ice by melting (Morgan et al. 2006). The secondary drying phase is performed to remove the bound water and happens at higher temperatures (Trelea et al. 2016). Because the sample is frozen, the desiccation stress during the drying process is very limited. However, when stored, macromolecules can experience lethal damage probably through Maillard reactions and oxidative stress caused by direct contact with air (Achour et al. 2001; Carvalho et al. 2008; Stephan et al. 2016). When dried cultures are rehydrated, water molecules flow back into the cell causing molecules to regain mobility and cell activity can be initiated.

Spray-drying is a more cost-friendly and sustainable way and is frequently used to create dry formulations (García 2011; Huang et al. 2017). Here, an atomized liquid culture is dried by exposing the drops to a hot air flow which causes the bacteria to dry within a few seconds (Huang et al. 2017). A co-current system is frequently used in heat-sensitive samples in order to make the inlet air flow, which has the higher temperature, in contact with the wet product and the dry product in contact with the lowest temperature (Peighambardoust et al. 2011). Even though this procedure reduces heating of the sample, a main lethal stress still comes from high temperature. The inlet temperature of the hot air flow can exceed over 100 °C and even though this quickly cools down, the outlet temperature is still between 60 and 85 °C. This causes severe heat stress during the falling rate stage resulting in lethal damage (Fu and Chen 2011; Huang et al. 2017). The high temperature further induces protein denaturation and can break polymers into smaller units (Fu and Chen 2011). Spray-drying at a high temperature damages the plasma membrane by forming holes possibly due to phase transition (Fu and Chen 2011; Huang et al. 2017).

Exposure to air at ambient temperature can be used to dry cultures to obtain inoculants at a very low cost (Santivarangkna et al. 2007). In fluidized bed drying, in which a culture mixed with a solid matrix placed on a mechanically shaking bed is exposed to an upward moving forced air flow of around 40 °C, the drying process takes a few hours (Berninger et al. 2018). This technique is often used in combination with spray-drying, which can not only reduce the operating cost, but also lower the drying temperature during spray-drying, which increases the survival (Peighambardoust et al. 2011). Alternatively, a slow removal of moisture can be obtained by air-drying, when typical temperatures around 30 °C are implemented (Kosanke et al. 1997; Berninger et al. 2018). Since this process takes from a few hours to multiple days, the bacteria can alter their metabolism to adapt during the drying process.

Without adjustments, survival after desiccation is often not sufficient to obtain high cell concentrations for inoculation. Even with attempts to increase the survival and shelf life, this is still a common problem that limits the quality of the inoculant. Even though multiple protective methods have been

described in literature, many are expected to be kept secret by companies. It is crucial to match the protective technique with the drying technique and strain. Bacteria are unable to adjust their metabolism to upcoming desiccation stress in drying techniques like freeze-drying and spray-drying. In this case, an appealing strategy is to expose the bacteria to sublethal stresses that induce adaptation to the lethal stresses they are about to experience. This technique, called pre-stressing, has been described to be used in commercially available inoculants like Trident. Which sublethal stress results in the highest survival is strain dependent and some optimization will be required. Commonly, hyperosmotic stress or heat stress is applied; however, oxidative stress and pH stress have also shown to increase desiccation tolerance (Desmond et al. 2002; Prasad et al. 2003; Schoug et al. 2008; Liu et al. 2014). Published transcriptomic data might therefore be very useful in selecting the most promising stresses to test in an industrially relevant setting. For example, sublethal stresses that induce the production of osmolytes, EPS, and/or oxygen-scavenging molecules might be interesting. Induction of the general stress response, for example through nutrient starvation during the stationary phase, positively affects the survival in many cases as it upregulates the expression of a wide array of these protective molecules (Fu and Chen 2011). Alternatively, protective molecules could be supplemented by a helper strain. This approach has been proven to be effective in multiple studies (Kozyrovska et al. 2005; Prasanna et al. 2014). Despite the fact that they could also positively alter the metabolism, helper strains are primarily used for the production of extracellular protective molecules like EPS. Finally, a popular method to increase survival is by adding protectants to the medium prior to drying. An overview of published xeroprotectants is given by Berninger et al. (2018) and the effect on xerotolerance has been shown to highly depend on the strain and drying techniques (Fu and Chen 2011; Berninger et al. 2018). However, some patterns are visible. Sugars like trehalose and sucrose are for example commonly used to increase desiccation tolerance in all drying techniques (Carvalho et al. 2008; Strasser et al. 2009; Huang et al. 2017). Besides xerotolerance, sugars can also provide protection against freezing or heat stress and therefore further increase survival during freeze- and spray-drying, respectively (Obuchi et al. 2000; Buitink et al. 2000). Even though trehalose is considered one of the most effective, the industrial application is limited due to its high cost. Therefore, cheaper non-reducing disaccharides are often considered. Another noteworthy protectant is skimmed milk (Lian et al. 2002; Wang et al. 2016). Even though the protective mechanisms have not been elucidated, it is hypothesized that its high lactose content prevents the occurrence of damage during dehydration (Riveros et al. 2009). Finally, bacteria can be physically protected from environmental stresses like desiccation through encapsulation. This technique has become very popular in drying probiotics, since the protective coating can also protect against the adverse conditions like low pH occurring in the stomach (Fu and Chen 2011), and is also emerging in microbial inoculant production (John et al. 2011; Schoebitz et al. 2013).

Conclusion and future perspectives

Water is essential for life and bacteria are no exception. In contrast to most abiotic stress conditions in which bacteria have managed to adapt to thrive in extreme environments, growth under desiccated form does not exist. Removal of water increases hyperosmotic pressure, molecular crowding in the cytoplasm, oxidative stress, and changes in physical properties of molecules, causing bacteria to experience extreme stress which often results in cell death. Studies showed that all major biomolecules are damaged; however, a consensus about which type of damage generally causes cell death is lacking. Adaptation to desiccation happens primarily though the prevention of the desiccation-induced damage. ROS-scavenging molecules and lowered intracellular iron concentrations reduce oxidative stress, while chaperones and sugars can keep proteins in their native form. Changes in membrane fluidity also prevent unwanted phase variations of the membrane. DNA damage is mainly restored by repair mechanisms like homologous recombination, non-homologous end joining, and nucleotide excision repair. Finally, EPS can retain water and therefore prolong the hydrated state or prevent complete dehydration.

A standardized method of measuring desiccation tolerance does not exist, making the comparison between different studies difficult. Some studies investigate desiccation tolerance by measuring tolerance or growth under hyperosmotic conditions. Many other studies utilize a fast drying process in which the bacteria are concentrated and exposed to air on a filter. Some apply a slow drying procedure under ambient conditions, while others dry by industrial methods like spray-drying. In a desiccated state, the conditions like relative humidity of the environment, light, and time spent in a desiccated state also vary between studies. The way desiccation tolerance is measured however highly influences the results of the study and should therefore be carefully considered. Slow drying procedures for example allow bacteria to detect and respond to desiccation to confer tolerance, while in fast drying procedures this is not the case. Even though all are referred to as desiccation, these different drying conditions should not be considered equals. Elaborate studies looking at different drying conditions (e.g., drying speed) are limited and could be very valuable to obtain more insights about how these parameters affect desiccation tolerance.

Despite the high number of studies that investigate molecular damage occurring during desiccation and the responses of

bacteria to this stress condition, knowledge on how to reach xerotolerance is lacking for most plant growth–promoting bacteria. Pre-stressing or addition of xeroprotectants is commonly applied to obtain a higher survival in inoculants but the shelf life of inoculants still remains a limiting factor. Viability of the plant growth–promoting bacteria is essential to confer improved plant growth and therefore further research is needed to facilitate the improvement of the quality of inoculants.

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Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

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References

Achour M, Mtimet N, Cornelius C, Zgouli S, Mahjoub A, Thonart P, Hamdi M (2001) Application of the accelerated shelf life testing method (ASLT) to study the survival rates of freeze-dried *Lactococcus* starter cultures. J Chem Technol Biotechnol 76:624–628. https://doi.org/10.1002/jctb.427

Alpert P (2006) Constraints of tolerance: why are desiccation-tolerant organisms so small or rare? J Exp Biol 209:1575–1584. https://doi.org/10.1242/jeb.02179

Arakawa T, Timasheff SN (1985) The stabilization of proteins by osmolytes. Biophys J 47:411–414. https://doi.org/10.1016/S0006-3495(85)83932-1

Aranda J, Bardina C, Beceiro A, Rumbo S, Cabral MP, Barbé J, Bou G (2011) *Acinetobacter baumannii* RecA protein in repair of DNA damage, antimicrobial resistance, general stress response, and virulence. J Bacteriol 193:3740–3747. https://doi.org/10.1128/JB.00389-11

Archibald FS, Fridovich I (1981) Manganese, superoxide dismutase, and oxygen tolerance in some lactic acid bacteria. J Bacteriol 146:928–936

Archibald FS, Fridovich I (1982) The scavenging of superoxide radical by manganous complexes: *in vitro*. Arch Biochem Biophys 214:452–463. https://doi.org/10.1016/0003-9861(82)90049-2

Backer R, Rokem JS, Ilangumaran G, Lamont J, Praslickova D, Ricci E, Subramanian S, Smith DL (2018) Plant growth-promoting rhizobacteria: context, mechanisms of action, and roadmap to commercialization of biostimulants for sustainable agriculture. Front Plant Sci 9:1473. https://doi.org/10.3389/fpls.2018.01473

Bashan Y, de Bashan LE, Prabhu SR, Hernandez JP (2014) Advances in plant growth-promoting bacterial inoculant technology: formulations and practical perspectives (1998–2013). Plant Soil 378:1–33. https://doi.org/10.1007/s11104-013-1956-x

Bednarska NG, Schymkowitz J, Rousseau F, Van Eldere J (2013) Protein aggregation in bacteria: the thin boundary between functionality and toxicity. Microbiology 159:1795–1806. https://doi.org/10.1099/mic.0.069575-0

Berninger T, González López Ó, Bejarano A, Preininger C, Sessitsch A (2018) Maintenance and assessment of cell viability in formulation of non-sporulating bacterial inoculants. Microb Biotechnol 11:277–301. https://doi.org/10.1111/1751-7915.12880

Billi D, Potts M (2002) Life and death of dried prokaryotes. Res Microbiol 153:7–12

Boada J, Roig T, Perez X, Gamez A, Bartrons R, Cascante M, Bermúdez J (2000) Cells overexpressing fructose-2,6-bisphosphatase showed enhanced pentose phosphate pathway flux and resistance to oxidative stress. FEBS Lett 480:261–264. https://doi.org/10.1016/S0014-5793(00)01950-5

Bolen D, Baskakov IV (2001) The osmophobic effect: natural selection of a thermodynamic force in protein folding. J Mol Biol 310:955–963. https://doi.org/10.1006/jmbi.2001.4819

Boumahdi M, Hornez (1999) Influence of growth phases and desiccation on the degrees of unsaturation of fatty acids and the survival rates of rhizobia. J Appl Microbiol 87:611–619

Boumahdi M, Mary P, Hornez JP (2001) Changes in fatty acid composition and degree of unsaturation of (brady)rhizobia as a response to phases of growth, reduced water activities and mild desiccation. Antonie Van Leeuwenhoek 79:73–79

Bratbak G, Dundas I (1984) Bacterial dry matter content and biomass estimations. Appl Environ Microbiol 48:755–757

Buitink J, van den Dries IJ, Hoekstra FA, Alberda M, Hemminga MA (2000) High critical temperature above Tg may contribute to the stability of biological systems. Biophys J 79:1119–1128. https://doi.org/10.1016/S0006-3495(00)76365-X

Burgess CM, Gianotti A, Gruzdev N, Holah J, Knøchel S, Lehner A, Margas E, Esser SS, Sela (Saldinger) S, Tresse O (2016) The response of foodborne pathogens to osmotic and desiccation stresses in the food chain. Int J Food Microbiol 221:37–53. https://doi.org/10.1016/j.ijfoodmicro.2015.12.014

Carvalho AS, Silva J, Ho P, Teixeira P, Malcata FX, Gibbs P (2008) Effects of various sugars added to growth and drying media upon thermotolerance and survival throughout storage of freeze-dried *Lactobacillus delbrueckii* ssp. *bulgaricus*. Biotechnol Prog 20:248–254. https://doi.org/10.1021/bp034165y

Cheung MS, Klimov D, Thirumalai D (2005) Molecular crowding enhances native state stability and refolding rates of globular proteins. Proc Natl Acad Sci 102:4753–4758. https://doi.org/10.1073/pnas.0409630102

Cho E, Choi JM, Kim H, Tahir MN, Choi Y, Jung S (2013) Ferrous iron chelating property of low-molecular weight succinoglycans isolated from *Sinorhizobium meliloti*. BioMetals 26:321–328. https://doi.org/10.1007/s10534-013-9615-5

Chowdhury SR, Basak RK, Sen R, Adhikari B (2011) Optimization, dynamics, and enhanced production of a free radical scavenging extracellular polysaccharide (EPS) from hydrodynamic sediment attached *Bacillus megaterium* RB-05. Carbohydr Polym 86:1327–1335. https://doi.org/10.1016/J.CARBPOL.2011.06.050

Crowe JH, Hoekstra FA, Crowe LM (1992) Anhydrobiosis. Annu Rev Physiol 54:579–599. https://doi.org/10.1146/annurev.ph.54.030192.003051

Crowe LM, Reid DS, Crowe JH (1996) Is trehalose special for preserving dry biomaterials? Biophys J 71:2087–2093. https://doi.org/10.1016/S0006-3495(96)79407-9

Crowe JH, Carpenter JF, Crowe LM (1998) The role of vitrification in anhydrobiosis. Annu Rev Physiol 60:73–103. https://doi.org/10.1146/annurev.physiol.60.1.73

Cytryn EJ, Sangurdekar DP, Streeter JG, Franck WL, Chang W, Stacey G, Emerich DW, Joshi T, Xu D, Sadowsky MJ (2007) Transcriptional and physiological responses of *Bradyrhizobium japonicum* to desiccation-induced stress. J Bacteriol 189:6751–6762. https://doi.org/10.1128/JB.00533-07

Daly MJ, Gaidamakova EK, Matrosova VY, Vasilenko A, Zhai M, Leapman RD, Lai B, Ravel B, Li SMW, Kemner KM, Fredrickson JK (2007) Protein oxidation implicated as the primary determinant of bacterial radioresistance. PLoS Biol 5:e92. https://doi.org/10.1371/journal.pbio.0050092

Deaker R, Roughley RJ, Kennedy IR (2004) Legume seed inoculation technology—a review. Soil Biol Biochem 36:1275–1288. https://doi.org/10.1016/J.SOILBIO.2004.04.009

Desmond C, Ross RP, O'Callaghan E, Fitzgerald G, Stanton C (2002) Improved survival of *Lactobacillus paracasei* NFBC 338 in spray-dried powders containing gum acacia. J Appl Microbiol 93:1003–1011

Domínguez-Ferreras A, Pérez-Arnedo R, Becker A, Olivares J, Soto MJ, Sanjuán J (2006) Transcriptome profiling reveals the importance of plasmid pSymB for osmoadaptation of *Sinorhizobium meliloti*. J Bacteriol 188:7617–7625. https://doi.org/10.1128/JB.00719-06

Dorken G, Ferguson GP, French CE, Poon WCK (2012) Aggregation by depletion attraction in cultures of bacteria producing exopolysaccharide. J R Soc Interface 9:3490–3502. https://doi.org/10.1098/rsif.2012.0498

Du X, Wang X, Dong X, Li P, Wang S (2018) Characterization of the desiccation tolerance of *Cronobacter sakazakii* strains. Front Microbiol 9:2867. https://doi.org/10.3389/FMICB.2018.02867

Dupont S, Beney L, Ritt J-F, Lherminier J, Gervais P (2010) Lateral reorganization of plasma membrane is involved in the yeast resistance to severe dehydration. Biochim Biophys Acta Biomembr 1798:975–985. https://doi.org/10.1016/j.bbamem.2010.01.015

Dupont S, Rapoport A, Gervais P, Beney L (2014) Survival kit of *Saccharomyces cerevisiae* for anhydrobiosis. Appl Microbiol Biotechnol 98:8821–8834. https://doi.org/10.1007/s00253-014-6028-5

Dupuy P, Gourion B, Sauviac L, Bruand C (2017) DNA double-strand break repair is involved in desiccation resistance of *Sinorhizobium meliloti*, but is not essential for its symbiotic interaction with *Medicago truncatula*. Microbiology 163:333–342. https://doi.org/10.1099/mic.0.000400

Eleutheria ECA, de Araujo PS, Panek AD (1993) Role of the trehalose carrier in dehydration resistance of *Saccharomyces cerevisiae*. Biochim Biophys Acta, Gen Subj 1156:263–266. https://doi.org/10.1016/0304-4165(93)90040-F

El-kest SE, Marth EH (1992) Freezing of *Listeria monocytogenes* and other microorganisms: a review. J Food Prot 55:639–648. https://doi.org/10.4315/0362-028X-55.8.639

Esbelin J, Santos T, Hébraud M (2018) Desiccation: an environmental and food industry stress that bacteria commonly face. Food Microbiol 69:82–88. https://doi.org/10.1016/j.fm.2017.07.017

Farrow JM, Wells G, Pesci EC (2018) Desiccation tolerance in *Acinetobacter baumannii* is mediated by the two-component response regulator BfmR. PLoS One 13:e0205638. https://doi.org/10.1371/journal.pone.0205638

Finn S, Condell O, McClure P, Amézquita A, Fanning S (2013) Mechanisms of survival, responses and sources of *Salmonella* in low-moisture environments. Front Microbiol 4:331. https://doi.org/10.3389/fmicb.2013.00331

Flechard M, Fontenelle C, Trautwetter A, Ermel G, Blanco C (2009) *Sinorhizobium meliloti rpoE2* is necessary for H_2O_2 stress resistance during the stationary growth phase. FEMS Microbiol Lett 290:25–31. https://doi.org/10.1111/j.1574-6968.2008.01401.x

Flechard M, Fontenelle C, Blanco C, Goude R, Ermel G, Trautwetter A (2010) RpoE2 of *Sinorhizobium meliloti* is necessary for trehalose synthesis and growth in hyperosmotic media. Microbiology 156:1708–1718. https://doi.org/10.1099/mic.0.034850-0

França MB, Panek AD, Eleutherio ECA (2007) Oxidative stress and its effects during dehydration. Comp Biochem Physiol Part A Mol Integr Physiol 146:621–631. https://doi.org/10.1016/j.cbpa.2006.02.030

Fredrickson JK, Li SW, Gaidamakova EK, Matrosova VY, Zhai M, Sulloway HM, Scholten JC, Brown MG, Balkwill DL, Daly MJ (2008) Protein oxidation: key to bacterial desiccation resistance? ISME J 2:393–403. https://doi.org/10.1038/ismej.2007.116

Fu N, Chen XD (2011) Towards a maximal cell survival in convective thermal drying processes. Food Res Int 44:1127–1149. https://doi.org/10.1016/J.FOODRES.2011.03.053

Galinski EA (1995) Osmoadaptation in bacteria. Adv Microb Physiol 37:272–328

García AH (2011) Anhydrobiosis in bacteria: from physiology to applications. J Biosci 36:939–950

García De Castro A, Bredholt H, Strøm AR, Tunnacliffe A (2000) Anhydrobiotic engineering of gram-negative bacteria. Appl Environ Microbiol 66:4142–4144

Garmiri P, Coles KE, Humphrey TJ, Cogan TA (2008) Role of outer membrane lipopolysaccharides in the protection of *Salmonella enterica* serovar Typhimurium from desiccation damage. FEMS Microbiol Lett 281:155–159. https://doi.org/10.1111/j.1574-6968.2008.01093.x

Gayoso CM, Mateos J, Méndez JA, Fernández-Puente P, Rumbo C, Tomás M, Martínez de Ilarduya Ó, Bou G (2014) Molecular mechanisms involved in the response to desiccation stress and persistence in *Acinetobacter baumannii*. J Proteome Res 13:460–476. https://doi.org/10.1021/pr400603f

Gottesman S, Wickner S, Maurizi MR (1997) Protein quality control: triage by chaperones and proteases. Genes Dev 11:815–823

Gruzdev N, McClelland M, Porwollik S, Ofaim S, Pinto R, Saldinger-Sela S (2012) Global transcriptional analysis of dehydrated *Salmonella enterica* serovar Typhimurium. Appl Environ Microbiol 78:7866–7875. https://doi.org/10.1128/AEM.01822-12

Halverson LJ, Firestone MK (2000) Differential effects of permeating and nonpermeating solutes on the fatty acid composition of *Pseudomonas putida*. Appl Environ Microbiol 66:2414–2421. https://doi.org/10.1128/AEM.66.6.2414-2421.2000

Hernández A, Zamora J, González N, Salazar E, Sánchez MDC (2009) Anhydrobiosis quotient: a novel approach to evaluate stability in desiccated bacterial cells. J Appl Microbiol 107:436–442. https://doi.org/10.1111/j.1365-2672.2009.04216.x

Hernández M, Klose M, Claus P, Bastviken D, Marotta H, Figueiredo V, Enrich-Prast A, Conrad R (2019) Structure, function and resilience to desiccation of methanogenic microbial communities in temporarily inundated soils of the Amazon rainforest (Cunia Reserve, Rondonia). Environ Microbiol 21:1702–1717. https://doi.org/10.1111/1462-2920.14535

Hingston PA, Piercey MJ, Truelstrup Hansen L (2015) Genes associated with desiccation and osmotic stress in *Listeria monocytogenes* as revealed by insertional mutagenesis. Appl Environ Microbiol 81:5350–5362. https://doi.org/10.1128/AEM.01134-15

Huang S, Vignolles M-L, Chen XD, Le Loir Y, Jan G, Schuck P, Jeantet R (2017) Spray drying of probiotics and other food-grade bacteria: a review. Trends Food Sci Technol 63:1–17. https://doi.org/10.1016/J.TIFS.2017.02.007

Humann JL, Kahn ML (2015) Genes involved in desiccation resistance of rhizobia and other bacteria. In: de Bruijn FJ (ed) Biological nitrogen fixation. John Wiley & Sons, Inc, New Jersey, pp 397–404

Humann JL, Ziemkiewicz HT, Yurgel SN, Kahn ML (2009) Regulatory and DNA repair genes contribute to the desiccation resistance of *Sinorhizobium meliloti* Rm1021. Appl Environ Microbiol 75:446–453. https://doi.org/10.1128/AEM.02207-08

Iibuchi R, Hara-kudo Y, Hasegawa A, Kumagai S (2010) Survival of *Salmonella* on a polypropylene surface under dry conditions in relation to biofilm-formation capability. J Food Prot 73:1506–1510. https://doi.org/10.4315/0362-028X-73.8.1506

Imlay JA (2003) Pathways of oxidative damage. Annu Rev Microbiol 57:395–418. https://doi.org/10.1146/annurev.micro.57.030502.090938

Imlay JA (2008) Cellular defenses against superoxide and hydrogen peroxide. Annu Rev Biochem 77:755–776. https://doi.org/10.1146/annurev.biochem.77.061606.161055

Jans A, Vercruysse M, Gao S, Engelen K, Lambrichts I, Fauvart M, Michiels J (2013) Canonical and non-canonical EcfG sigma factors control the general stress response in *Rhizobium etli*. Microbiologyopen 2:976–987. https://doi.org/10.1002/mbo3.137

John RP, Tyagi RD, Brar SK, Surampalli RY, Prévost D (2011) Bio-encapsulation of microbial cells for targeted agricultural delivery. Crit Rev Biotechnol 31:211–226. https://doi.org/10.3109/07388551.2010.513327

Juhnke H, Krems B, Kötter P, Entian KD (1996) Mutants that show increased sensitivity to hydrogen peroxide reveal an important role for the pentose phosphate pathway in protection of yeast against oxidative stress. Mol Gen Genet 252:456–464

Kaminsky LM, Trexler RV, Malik RJ, Hockett KL, Bell TH (2019) The inherent conflicts in developing soil microbial inoculants. Trends Biotechnol 37:140–151. https://doi.org/10.1016/J.TIBTECH.2018.11.011

Katoh H, Asthana RK, Ohmori M (2004) Gene expression in the *Cyanobacterium Anabaena* sp. PCC7120 under desiccation. Microb Ecol 47:164–174. https://doi.org/10.1007/s00248-003-1043-6

Kieft TL, Ringelberg DB, White DC (1994) Changes in ester-linked phospholipid fatty acid profiles of subsurface bacteria during starvation and desiccation in a porous medium. Appl Environ Microbiol 60:3292–3299

Kim YE, Hipp MS, Bracher A, Hayer-Hartl M, Ulrich Hartl F (2013) Molecular chaperone functions in protein folding and proteostasis. Annu Rev Biochem 82:323–355. https://doi.org/10.1146/annurev-biochem-060208-092442

Klähn S, Hagemann M (2011) Compatible solute biosynthesis in cyanobacteria. Environ Microbiol 13:551–562. https://doi.org/10.1111/j.1462-2920.2010.02366.x

Knowles EJ, Castenholz RW (2008) Effect of exogenous extracellular polysaccharides on the desiccation and freezing tolerance of rock-inhabiting phototrophic microorganisms. FEMS Microbiol Ecol 66:261–270. https://doi.org/10.1111/j.1574-6941.2008.00568.x

Kocharunchitt C, King T, Gobius K, Bowman JP, Ross T (2014) Global genome response of *Escherichia coli* O157 : H7 Sakai during dynamic changes in growth kinetics induced by an abrupt downshift in water activity. PLoS One 9:e90422. https://doi.org/10.1371/journal.pone.0090422

Köhn B, Kovermann M (2019) Macromolecular crowding tunes protein stability by manipulating solvent accessibility. ChemBioChem 20:759–763. https://doi.org/10.1002/cbic.201800679

Kosanke JW, Osburn MS, Smith RS (1997) Process for preparation of bacterial agricultural products. US patent 5695541

Kozyrovska NO, Negrutska VV, Kovalchuk MV, Voznyuk TN (2005) *Paenibacillus* sp. as a promising candidate for development of a novel technology of inoculant production. Biopolym Cell 21:312–318. https://doi.org/10.7124/bc.0006F7

Kurz M (2008) Compatible solute influence on nucleic acids: many questions but few answers. Saline Syst 4:6. https://doi.org/10.1186/1746-1448-4-6

LeBlanc JC, Gonçalves ER, Mohn WW (2008) Global response to desiccation stress in the soil actinomycete *Rhodococcus jostii* RHA1. Appl Environ Microbiol 74:2627–2636. https://doi.org/10.1128/AEM.02711-07

Lebre PH, De Maayer P, Cowan DA (2017) Xerotolerant bacteria: surviving through a dry spell. Nat Rev Microbiol 15:285–296. https://doi.org/10.1038/nrmicro.2017.16

Lehman AP, Long SR (2013) Exopolysaccharides from *Sinorhizobium meliloti* can protect against H_2O_2-dependent damage. J Bacteriol 195:5362–5369. https://doi.org/10.1128/JB.00681-13

Li H, Bhaskara A, Megalis C, Tortorello ML (2012) Transcriptomic analysis of *Salmonella* desiccation resistance. Foodborne Pathog Dis 9:1143–1151. https://doi.org/10.1089/fpd.2012.1254

Lian W-C, Hsiao HC, Chou CC (2002) Survival of bifidobacteria after spray-drying. Int J Food Microbiol 74:79–86. https://doi.org/10.1016/s0168-1605(01)00733-4

Liu J, Jiang W, Li F, Wang L, Zeng J, Li Q, Wang Y, Yang Q (2014) Effect of drying conditions on the particle size, dispersion state, and mechanical sensitivities of Nano HMX. Propellants. Explos Pyrotech 39:30–39. https://doi.org/10.1002/prep.201300050

López-Lara IM, Sohlenkamp C, Geiger O (2003) Membrane lipids in plant-associated bacteria: their biosyntheses and possible functions. Mol Plant-Microbe Interact 16:567–579. https://doi.org/10.1094/MPMI.2003.16.7.567

Majeed A, Muhammad Z, Ahmad H (2018) Plant growth promoting bacteria: role in soil improvement, abiotic and biotic stress management of crops. Plant Cell Rep 37:1599–1609. https://doi.org/10.1007/s00299-018-2341-2

Mandal RK, Kwon YM (2017) Global screening of *Salmonella enterica* serovar Typhimurium genes for desiccation survival. Front Microbiol 8:1723. https://doi.org/10.3389/fmicb.2017.01723

Manzanera M, García de Castro A, Tøndervik A, Rayner-Brandes M, Strøm AR, Tunnacliffe A (2002) Hydroxyectoine is superior to trehalose for anhydrobiotic engineering of *Pseudomonas putida* KT2440. Appl Environ Microbiol 68:4328–4333. https://doi.org/10.1128/AEM.68.9.4328-4333.2002

Manzanera M, Vilchez S, Tunnacliffe A (2004) High survival and stability rates of *Escherichia coli* dried in hydroxyectoine. FEMS Microbiol Lett 233:347–352. https://doi.org/10.1111/j.1574-6968.2004.tb09502.x

McIntyre HJ, Davies H, Hore TA, Miller SH, Dufour J-P, Ronson CW (2007) Trehalose biosynthesis in *Rhizobium leguminosarum* bv. *trifolii* and its role in desiccation tolerance. Appl Environ Microbiol 73:3984–3992. https://doi.org/10.1128/AEM.00412-07

Meng XC, Stanton C, Fitzgerald GF, Daly C, Ross RP (2008) Anhydrobiotics: the challenges of drying probiotic cultures. Food Chem 106:1406–1416. https://doi.org/10.1016/J.FOODCHEM.2007.04.076

Mika JT, Van Den Bogaart G, Veenhoff L, Krasnikov V, Poolman B (2010) Molecular sieving properties of the cytoplasm of *Escherichia coli* and consequences of osmotic stress. Mol Microbiol 77:200–207. https://doi.org/10.1111/j.1365-2958.2010.07201.x

Miklos AC, Sarkar M, Wang Y, Pielak GJ (2011) Protein crowding tunes protein stability. J Am Chem Soc 133:7116–7120. https://doi.org/10.1021/ja200067p

Monier J-M, Lindow SE (2003) Differential survival of solitary and aggregated bacterial cells promotes aggregate formation on leaf surfaces. Proc Natl Acad Sci 100:15977–15982. https://doi.org/10.1073/pnas.2436560100

Morano KA (2014) Anhydrobiosis: drying out with sugar. Curr Biol 24:R1121–R1123. https://doi.org/10.1016/J.CUB.2014.10.022

Morgan CA, Herman N, White PA, Vesey G (2006) Preservation of micro-organisms by drying; a review. J Microbiol Methods 66:183–193. https://doi.org/10.1016/j.mimet.2006.02.017

Motohashi K, Watanabe Y, Yohda M, Yoshida M (1999) Heat-inactivated proteins are rescued by the DnaK.J-GrpE set and ClpB chaperones. Proc Natl Acad Sci U S A 96:7184–7189

Nocker A, Fernández PS, Montijn R, Schuren F (2012) Effect of air drying on bacterial viability: a multiparameter viability assessment. J Microbiol Methods 90:86–95. https://doi.org/10.1016/j.mimet.2012.04.015

Obuchi K, Iwahashi H, Lepock JR, Komatsu Y (2000) Calorimetric characterization of critical targets for killing and acquired thermotolerance in yeast. Yeast 16:111–119. https://doi.org/10.1002/(SICI)1097-0061(20000130)16:2<111::AID-YEA507>3.0.CO;2-V

Ophir T, Gutnick DL (1994) A role for exopolysaccharides in the protection of microorganisms from desiccation. Appl Environ Microbiol 60:740–745

Oren A (2008) Microbial life at high salt concentrations: phylogenetic and metabolic diversity. Saline Syst 4:2. https://doi.org/10.1186/1746-1448-4-2

Paciaroni A, Cinelli S, Cornicchi E, De Francesco A, Onori G (2005) Fast fluctuations in protein powders: the role of hydration. Chem Phys Lett 410:400–403. https://doi.org/10.1016/J.CPLETT.2005.05.098

Parry BR, Surovtsev IV, Cabeen MT, O'Hern CS, Dufresne ER, Jacobs-Wagner C (2014) The bacterial cytoplasm has glass-like properties and is fluidized by metabolic activity. Cell 156:183–194. https://doi.org/10.1016/j.cell.2013.11.028

Patel A, Malinovska L, Saha S, Wang J, Alberti S, Krishnan Y, Hyman AA (2017) ATP as a biological hydrotrope. Science 356(80):753–756. https://doi.org/10.1126/science.aaf6846

Paul D (2013) Osmotic stress adaptations in rhizobacteria. J Basic Microbiol 53:101–110. https://doi.org/10.1002/jobm.201100288

Peighambardoust SH, Golshan Tafti A, Hesari J (2011) Application of spray drying for preservation of lactic acid starter cultures: a review. Trends Food Sci Technol 22:215–224. https://doi.org/10.1016/J.TIFS.2011.01.009

Pereira E d J, Panek AD, ECA E (2003) Protection against oxidation during dehydration of yeast. Cell Stress Chaperones 8:120–124

Persson E, Halle B (2008) Cell water dynamics on multiple time scales. Proc Natl Acad Sci 105:6266–6271. https://doi.org/10.1073/pnas.0709585105

Pitcher RS, Brissett NC, Picher AJ, Andrade P, Juarez R, Thompson D, Fox GC, Blanco L, Doherty AJ (2007a) Structure and function of a mycobacterial NHEJ DNA repair polymerase. J Mol Biol 366:391–405. https://doi.org/10.1016/j.jmb.2006.10.046

Pitcher RS, Green AJ, Brzostek A, Korycka-Machala M, Dziadek J, Doherty AJ (2007b) NHEJ protects mycobacteria in stationary phase against the harmful effects of desiccation. DNA Repair (Amst) 6:1271–1276. https://doi.org/10.1016/J.DNAREP.2007.02.009

Potts M (1994) Desiccation tolerance of prokaryotes. Microbiol Mol Biol Rev 58:755–805

Potts M, Slaughter SM, Hunneke F-U, Garst JF, Helm RF (2005) Desiccation tolerance of prokaryotes: application of principles to human cells. Integr Comp Biol 45:800–809. https://doi.org/10.1093/icb/45.5.800

Prasad J, McJarrow P, Gopal P (2003) Heat and osmotic stress responses of probiotic *Lactobacillus rhamnosus* HN001 (DR20) in relation to viability after drying. Appl Environ Microbiol 69:917–925. https://doi.org/10.1128/aem.69.2.917-925.2003

Prasanna R, Triveni S, Bidyarani N, Babu S, Yadav K, Adak A, Khetarpal S, Pal M, Shivay YS, Saxena AK (2014) Evaluating the efficacy of cyanobacterial formulations and biofilmed inoculants for leguminous crops. Arch Agron Soil Sci 60:349–366. https://doi.org/10.1080/03650340.2013.792407

Rajeev L, da Rocha UN, Klitgord N, Luning EG, Fortney J, Axen SD, Shih PM, Bouskill NJ, Bowen BP, Kerfeld CA, Garcia-Pichel F, Brodie EL, Northen TR, Mukhopadhyay A (2013) Dynamic cyanobacterial response to hydration and dehydration in a desert biological soil crust. ISME J 7:2178–2191. https://doi.org/10.1038/ismej.2013.83

Riveros B, Ferrer J, Bórquez R (2009) Spray drying of a vaginal probiotic strain of *Lactobacillus acidophilus*. Dry Technol 27:123–132. https://doi.org/10.1080/07373930802566002

Rojas-Tapias D, Ortega Sierra O, Rivera Botía D, Bonilla R (2014) Preservation of *Azotobacter chroococcum* vegetative cells in dry polymers. Univ Sci 20:201. https://doi.org/10.11144/Javeriana.SC20-2.pacv

Rüberg S, Tian Z-X, Krol E, Linke B, Meyer F, Wang Y, Pühler A, Weidner S, Becker A (2003) Construction and validation of a *Sinorhizobium meliloti* whole genome DNA microarray: genome-wide profiling of osmoadaptive gene expression. J Biotechnol 106:255–268. https://doi.org/10.1016/J.JBIOTEC.2003.08.005

Russell NJ, Evans RI, ter Steeg PF, Hellemons J, Verheul A, Abee T (1995) Membranes as a target for stress adaptation. Int J Food Microbiol 28:255–261. https://doi.org/10.1016/0168-1605(95)00061-5

Santivarangkna C, Kulozik U, Foerst P (2007) Alternative drying processes for the industrial preservation of lactic acid starter cultures. Biotechnol Prog 23:302–315. https://doi.org/10.1021/bp060268f

Santos R, Bocquet S, Puppo A, Touati D (1999) Characterization of an atypical superoxide dismutase from *Sinorhizobium meliloti*. J Bacteriol 181:4509–4516

Sauviac L, Philippe H, Phok K, Bruand C (2007) An extracytoplasmic function sigma factor acts as a general stress response regulator in *Sinorhizobium meliloti*. J Bacteriol 189:4204–4216. https://doi.org/10.1128/JB.00175-07

Schlüter JP, Reinkensmeier J, Barnett MJ, Lang C, Krol E, Giegerich R, Long SR, Becker A (2013) Global mapping of transcription start sites and promoter motifs in the symbiotic α-proteobacterium *Sinorhizobium meliloti* 1021. BMC Genomics 14:156. https://doi.org/10.1186/1471-2164-14-156

Schoebitz M, López MD, Roldán A (2013) Bioencapsulation of microbial inoculants for better soil–plant fertilization. A review. Agron Sustain Dev 33:751–765. https://doi.org/10.1007/s13593-013-0142-0

Schoug Å, Fischer J, Heipieper HJ, Schnürer J, Håkansson S (2008) Impact of fermentation pH and temperature on freeze-drying survival and membrane lipid composition of *Lactobacillus coryniformis* Si3. J Ind Microbiol Biotechnol 35:175–181. https://doi.org/10.1007/s10295-007-0281-x

Shuman S, Glickman MS (2007) Bacterial DNA repair by non-homologous end joining. Nat Rev Microbiol 5:852–861. https://doi.org/10.1038/nrmicro1768

Siliakus MF, van der Oost J, Kengen SWM (2017) Adaptations of archaeal and bacterial membranes to variations in temperature, pH and pressure. Extremophiles 21:651–670. https://doi.org/10.1007/s00792-017-0939-x

Simonin H, Beney L, Gervais P (2007) Sequence of occurring damages in yeast plasma membrane during dehydration and rehydration: mechanisms of cell death. Biochim Biophys Acta Biomembr 1768:1600–1610. https://doi.org/10.1016/j.bbamem.2007.03.017

Singh P, Tiwari A, Singh SP, Asthana RK (2013) Desiccation induced changes in osmolytes production and the antioxidative defence in the cyanobacterium *Anabaena* sp. PCC 7120. Physiol Mol Biol Plants 19:61–68. https://doi.org/10.1007/s12298-012-0145-3

Sorroche FG, Rinaudi LV, Zorreguieta Á, Giordano W (2010) EPS II-dependent autoaggregation of *Sinorhizobium meliloti* planktonic cells. Curr Microbiol 61:465–470. https://doi.org/10.1007/s00284-010-9639-9

Sorroche FG, Spesia MB, Zorreguieta A, Giordano W (2012) A positive correlation between bacterial autoaggregation and biofilm formation in native *Sinorhizobium meliloti* isolates from Argentina. Appl Environ Microbiol 78:4092–4101. https://doi.org/10.1128/AEM.07826-11

Srikumar S, Cao Y, Yan Q, Van Hoorde K, Nguyen S, Cooney S, Gopinath GR, Tall BD, Sivasankaran SK, Lehner A, Stephan R, Fanning S (2018) RNA sequencing-based transcriptional overview of xerotolerance in *Cronobacter sakazakii* SP291. Appl Environ Microbiol 85. https://doi.org/10.1128/AEM.01993-18

Stadtman ER, Levine RL (2006) Protein oxidation. Ann N Y Acad Sci 899:191–208. https://doi.org/10.1111/j.1749-6632.2000.tb06187.x

Stadtman ER, Berlett BS, Chock PB (1990) Manganese-dependent disproportionation of hydrogen peroxide in bicarbonate buffer. Proc Natl Acad Sci U S A 87:384–388. https://doi.org/10.1073/PNAS.87.1.384

Stephan D, Da Silva A-PM, Bisutti IL (2016) Optimization of a freeze-drying process for the biocontrol agent *Pseudomonas* spp. and its influence on viability, storability and efficacy. Biol Control 94:74–81. https://doi.org/10.1016/J.BIOCONTROL.2015.12.004

Stephens JH, Rask H (2000) Inoculant production and formulation. Field Crop Res 65:249–258. https://doi.org/10.1016/S0378-4290(99)00090-8

Šťovíček A, Kim M, Or D, Gillor O (2017) Microbial community response to hydration-desiccation cycles in desert soil. Sci Rep 7:45735. https://doi.org/10.1038/srep45735

Strasser S, Neureiter M, Geppl M, Braun R, Danner H (2009) Influence of lyophilization, fluidized bed drying, addition of protectants, and storage on the viability of lactic acid bacteria. J Appl Microbiol 107:167–177. https://doi.org/10.1111/j.1365-2672.2009.04192.x

Streeter JG (2003) Effect of trehalose on survival of *Bradyrhizobium japonicum* during desiccation. J Appl Microbiol 95:484–491. https://doi.org/10.1046/j.1365-2672.2003.02017.x

Tamaru Y, Takani Y, Yoshida T, Sakamoto T (2005) Crucial role of extracellular polysaccharides in desiccation and freezing tolerance in the terrestrial cyanobacterium *Nostoc commune*. Appl Environ Microbiol 71:7327–7333. https://doi.org/10.1128/AEM.71.11.7327-7333.2005

Tanford C (1997) How protein chemists learned about the hydrophobic factor. Protein Sci 6:1358–1366. https://doi.org/10.1002/pro.5560060627

Tapia H, Koshland DE (2014) Trehalose is a versatile and long-lived chaperone for desiccation tolerance. Curr Biol 24:2758–2766. https://doi.org/10.1016/J.CUB.2014.10.005

Tokuriki N, Kinjo M, Negi S, Hoshino M, Goto Y, Urabe I, Yomo T (2004) Protein folding by the effects of macromolecular crowding. Protein Sci 13:125–133. https://doi.org/10.1110/ps.03288104

Trelea IC, Fonseca F, Passot S (2016) Dynamic modeling of the secondary drying stage of freeze drying reveals distinct desorption kinetics for bound water. Dry Technol 34:335–345. https://doi.org/10.1080/07373937.2015.1054509

Truelstrup Hansen L, Vogel BF (2011) Desiccation of adhering and biofilm *Listeria monocytogenes* on stainless steel: survival and transfer to salmon products. Int J Food Microbiol 146:88–93. https://doi.org/10.1016/J.IJFOODMICRO.2011.01.032

Tyedmers J, Mogk A, Bukau B (2010) Cellular strategies for controlling protein aggregation. Nat Rev Mol Cell Biol 11:777–788. https://doi.org/10.1038/nrm2993

Vanderlinde EM, Muszynski A, Harrison JJ, Koval SF, Foreman DL, Ceri H, Kannenberg EL, Carlson RW, Yost CK (2009) *Rhizobium leguminosarum* biovar *viciae* 3841, deficient in 27-hydroxyoctacosanoate-modified lipopolysaccharide, is impaired in desiccation tolerance, biofilm formation and motility. Microbiology 155:3055–3069. https://doi.org/10.1099/mic.0.025031-0

Vercruysse M, Fauvart M, Jans A, Beullens S, Braeken K, Cloots L, Engelen K, Marchal K, Michiels J (2011) Stress response regulators identified through genome-wide transcriptome analysis of the (p)ppGpp-dependent response in *Rhizobium etli*. Genome Biol 12:R17–R19. https://doi.org/10.1186/gb-2011-12-2-r17

Vessey JK (2003) Plant growth promoting rhizobacteria as biofertilizers. Plant Soil 255:571–586. https://doi.org/10.1023/A:1026037216893

Vriezen JAC, de Bruijn FJ, Nüsslein K (2007) Responses of rhizobia to desiccation in relation to osmotic stress, oxygen, and temperature. Appl Environ Microbiol 73:3451–3459. https://doi.org/10.1128/AEM.02991-06

Wang Y, Sarkar M, Smith AE, Krois AS, Pielak GJ (2012) Macromolecular crowding and protein stability. J Am Chem Soc 134:16614–16618. https://doi.org/10.1021/ja305300m

Wang J, Huang S, Fu N, Jeantet R, Chen XD (2016) Thermal aggregation of calcium-fortified skim milk enhances probiotic protection during convective droplet drying. J Agric Food Chem 64:6003–6010. https://doi.org/10.1021/acs.jafc.6b02205

Whatmore AM, Chudek JA, Reed RH (1990) The effects of osmotic upshock on the intracellular solute pools of *Bacillus subtilis*. J Gen Microbiol 136:2527–2535. https://doi.org/10.1099/00221287-136-12-2527

White AP, Gibson DL, Kim W, Kay WW, Surette MG (2006) Thin aggregative fimbriae and cellulose enhance long-term survival and persistence of *Salmonella*. J Bacteriol 188:3219–3227. https://doi.org/10.1128/JB.188.9.3219-3227.2006

Wieczorek G, Zielenkiewicz P (2008) Influence of macromolecular crowding on protein-protein association rates–a Brownian dynamics study. Biophys J 95:5030–5036. https://doi.org/10.1529/biophysj.108.136291

Zolkiewski M (1999) ClpB cooperates with DnaK, DnaJ, and GrpE in suppressing protein aggregation. A novel multi-chaperone system from *Escherichia coli*. J Biol Chem 274:28083–28086