AN APPROACH TO DESICCATION-TOLERANT BACTERIA IN STARTER CULTURE PRODUCTION

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1. Introduction

Water is necessary for life on Earth. At the cell scale, water serves as solvent for organic and inorganic solutes, metabolites and as substrate for, or product of metabolic activity. In addition, it is well established that water plays an important role in maintenance of structural and functional integrity of biological membranes and macromolecules (Crowe et al., 1987). Nevertheless, a number of organisms are able to survive almost complete desiccation : a phenomenon known as anhydrobiosis (Crowe et al., 1992).

Desiccation-tolerance is not a property of "normal growth" conditions, but rather an ability to survive adverse hydration conditions. During the dry period, the cells are not active, but in a dormant state, ready to resume activity when the hydration conditions are favourable again (Kaprelyants et al., 1993). These anhydrobiotic organisms (plant seeds, soil dwelling rotifers, crustacean cysts, nematodes, bacterial spores,...) attain a desiccated state very resistant to ionising radiation, heat, UV radiation and may persist in the dry form for decades (Aguilera and Karel, 1997; Brown, 1976; Potts, 1994; Jawad et al., 1998). These properties may be used as indirect indicators of desiccation tolerance (Sanders and Maxcy, 1979; Weekers et al., 1999a). Some bacterial species are also able to withstand desiccation without formation of differentiated forms through the accumulation of “protective components” such as disaccharides.

The drying resistance of bacteria is relevant for the conservation of starter cultures and has important economical consequences (Rapoport and Beker, 1987). The preservation of bacteria in a desiccated form has a main advantage on a frozen form : a lower cost in storage and transport. The disadvantages of dried cultures are considerable loss of activity and poor shell-life under uncontrolled conditions (Lievense and Van't

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Riet, 1993; Lievense and Van't Riet, 1994). For these reasons bacteria can be selected according to their desiccation-tolerance. In this strategy, desiccation tolerance is used as the main selective pressure and is considered as the most important property for the production of starter cultures. Therefore products such as a starter culture for the bioremediation of xenobiotic-contaminated soils are stored in a dry form (Weekers et al., 1999a; Weekers et al., 1998; Weekers et al., 1996). The xenobiotic catabolic activity may be introduced afterward into the drought-tolerant strains by the mean of natural conjugation in order to broaden the potential applications of the product (Weekers et al., 1999b).

In this paper, the new approach of desiccation-tolerant bacteria selection is described. Mechanisms of damages to the cells due to desiccation and adaptations towards drought-tolerance are proposed for undifferentiated cells. The strains selected according to the new strategy serve as examples and a comparison is made between the desiccation-tolerant strains, some sensitive strains and a drought-tolerant reference, *Deinococcus radiodurans* (ATCC13939) on a desiccation-tolerance point of view. The importance of drought-tolerance for technological applications of bacteria is emphasized.

2. Selection of desiccation-tolerant bacteria

Bacterial strains were isolated according to their desiccation-tolerance. Their potential technological applications such as growth on xenobiotic compounds were looked at afterward and were improved with plasmids. Soil bacteria were isolated from dried xenobiotic-polluted soil samples according to their desiccation-tolerance and were compared to reference strains chosen with equivalent technological properties i.e. the ability to decompose recalcitrant xenobiotic compounds (table 1). *Deinococcus radiodurans,* ATCC13939 served as a drought-tolerant reference. *Deinococcaceae* developed a very effective DNA repair ability what provides them resistance to ionising radiation. Mattimore and Battista (1995) have shown that *D. radiodurans* was also resistant to desiccation, since functions necessary to survive desiccation were also necessary to survive ionising radiation.

Under different drying conditions with or without protective additives and with different drying technologies, the bacteria selected from desiccated soil samples exhibited a better tolerance to desiccation than the references. Whatever the drying technique was, the survival ratio between the soil strains and the references was 4 to 65 folds higher. The survival was as good as the one of *Deinococcus radiodurans* with most techniques, but was lower after storage in the dry form. The difference in behaviour among the strains may arise from variations in sensitivity of the different targets of the desiccation damages or from the mechanisms of drought-tolerance that each strain utilises.

Table 1 . Survival of the strains to various desiccation processes : freeze-drying (w or w/o. trehalose, 0,5%), slow drying and storage in reduced water activity (a_W) conditions. nd . not determined

			Survival to desiccation processes			
Strains	**Identification**	**Description**	**freeze-rying**	**freeze-drying (w. trehalose)**	**slow drying**	**storage at low a_w.**
T902	*Rhodococcus erythropolis*	Isolated from desiccated soil, growth on diesel oil	26%	30%	66%	12%
TF1	*Acinetobacter johnsonii*	Isolated from desiccated soil, growth on diesel oil	6%	16%	10%	6%
TF7	*Micrococcus luteus*	Isolated from desiccated soil, growth on diesel oil	2%	8%	5%	4%
ATCC 13939	*Deinococcus radiodurans*	desiccation-tolerant reference	16%	nd	42%	40%
LB400	*Pseudomonas sp.*	Growth on PCB	0.1%	nd	0.3%	0.03%
LH168	*Acinetobacter calcoaceticus*	Growth on oil	0.2%	nd	0.3%	0.03%
A5.1.	*Alcaligenes eutrophus*	growth on 4-chlorobiphenyl	0.02%	0.1%	0.6%	<0.01%
SK15	*Arthrobacter sp.*	Growth on biphenyl	0.36%	nd	nd	nd
LB126	*Sphingomonas sp.*	Growth on fluorene	0.05%	nd	0.2%	<0.01%
LH240	*Pseudomonas sp.*	Growth on oil	<0.01%	nd	0.8%	0 8%
AEX5	*Alcaligenes eutrophus*	Growth on chlorobiphenyls and chlorobenzoates	0.1%	nd	0.9%	<0.01%
PaW1	*P. putida*	Growth on toluene	0.09%	nd	0.2%	0.1%
GpO1	*P. oleovorans*	Growth on oil	0.01%	nd	0.3%	0.03%

3. Targets of desiccation damages and the proposed mechanisms responsible for dessication tolerance

Desiccation-damage targets were identified : the phospholipid bilayer membranes, the nucleic acids and the proteins. This list includes all the major cell components.

3.1. MEMBRANES

Membrane damages have been identified as responsible for most of the loss of viability during desiccation. To monitor the loss of membrane specific permeability during dehydration-rehydration cycles, the level of lactate dehydrogenase (LDH) activity in the supernatant after rehydration of the desiccated cells was measured and compared to the level of activity of a completely lysed sample. It serves as an indication of the leakage of the content of the cells due to phospholipid bilayer disruption (Castro et al., 1997; Weekers et al., 1999a). The effect of the water activity of the culture medium used prior to desiccation-rehydration cycles was measured (table 2).

Table 2 : Level of membrane lysis (%) as measured by LDH activity in the supernatant after drying and after storage at low a_W of the cells grown in different a_W conditions.

	a_W = 0.98		a_W = 0.96		a_W = 0.94		a_W = 0.92	
	slow drying	storage at low a_W	slow drying	storage at low a_W	slow drying	storage at low a_W	slow drying	storage at low a_W
ATCC 13939	55	56	49	50	40	40	No growth	No growth
T902	25	28	23	25	24	23	19	20
TF1	92	92	88	88	79	80	76	74

The level of membrane lysis of *Deinococcus radiodurans* after slow drying (see table 2) was equivalent to the cell mortality during dehydration (see table 1). Membrane leakage would be the only cause of cell death in *Deinococcus radiodurans.* This result is related to the ability of *Deinococcaceae* to repair, upon rehydration, the DNA damages caused by the desiccation (Battista, 1997). In comparison, some desiccated-soil-strains have a level of membrane lysis that does not account for the total mortality fraction. Other mechanisms of desiccation damage are involved in cell death.

Membrane lysis measurements do not evolve with storage time. This type of damages occurs only during the drying (or rehydration) time. Cells grown in a medium with reduced a_W, undergo less membrane lysis (Chen and Alexander, 1973; Weekers et al., 1999a).

3.1.1. Membrane desiccation-damage mechanisms

Membranes are mainly composed of phospholipids with membrane-proteins held in association by hydrophobic forces. Even for purified phospholipids there are several possible structures. The lamellar bilayer, in gel or liquid crystalline phase, and the hexagonal phase are the more frequent. The organisation of the phospholipid-water system is mainly dependent on composition, temperature, hydration state of the bilayer and on ionic strength and pH of the surrounding medium.

In water and at physiological temperatures, the polar head groups of the phospholipids are hydrated (about 10 molecules of water per phospholipid head). The water molecules spatially separate the polar head groups. When water is removed, the head groups get closer together. The packing, in turn, increase the opportunity for the hydrocarbon chains to interact. As a result, the temperature at which the chain melts to form the liquid crystalline phase (T_m) increase (Crowe and Crowe, 1982; Crowe et al., 1993a; Crowe et al., 1993b).

Thus, when phospholipid bilayers are dried, their phase transition temperature, T_m, increases, which, in turn, makes them undergo a phase transition from liquid crystalline phase to gel phase even when kept at a constant room temperature (figure 1). The hexagonal phase is usually not reached during drying because it is localised in high temperature region (> 80°C).

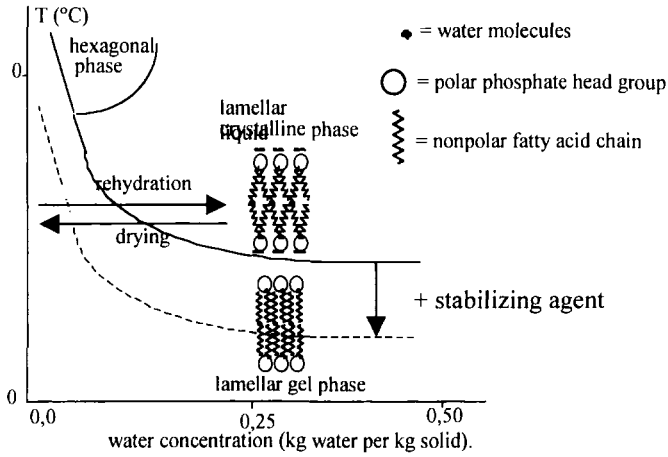

Figure 1 : Phase diagram of a simple phospholipid-water system. Variation of T_m of the bilayer with water content (adapted from Lievense and Van't Riet, 1993)

For example, palmitoyloleoyl phosphatidylcholine (POPC) has a T_m in water of -7°C. In the dry state, T_m reaches +60°C. Thus, when dried at room temperature, the phospholipid bilayer goes through a phase transition. Dry baker's yeast *(Saccharomyces cerevisiae)* packages always state that rehydration should be operated in warm water (about 40°C). It has been established that T_m for membrane phospholipids in these dry yeast cells is 35-40°C. In the hydrated cells, the T_m is about 10°C. Thus, if the dry cells are placed in water at temperatures below 40°C, their membranes undergo a phase transition during rehydration (Gelinas et al., 1989).

Unfortunately, phospholipid bilayers, as they undergo phase transitions, are known to become transiently leaky (Chapman, 1994; Crowe et al., 1989; Linders et al., 1997) In addition, biological membranes consist of a mixture of phospholipids. Each type enters the gel phase at a different temperature and hydration state thus at different times leading to segregation of the different phospholipids during drying. This separation is called 'lateral phase separation' and is considered an important mechanism in damaging biological membranes during dehydration. It becomes then important to prevent such phase transition during drying.

3.1.2. Role of disaccharides in membrane tolerance to desiccation

In the presence of disaccharides such as trehalose and sucrose the melt temperature of the phospholipidic bilayers is lowered (Crowe et al., 1987; Goodrich et al., 1991). This phenomenon enables the drying of biological membrane systems without going through a phase transition, avoiding, in turn, leakage of the content of the bilayer membrane system (Crowe et al., 1988; Crowe et al., 1985; Hoekstra et al., 1992; Strauss et al., 1986). The phospholipid bilayer is in liquid crystalline phase, even in the dry state and at room temperature.

The sugar molecules replace the water shell around the polar phosphate groups acting as 'spacers' (Crowe et al., 1993) (figure 2). To be effective at protecting the membranes, the disaccharides must be present on both sides of the lipidic bilayer (Eleutherio et al., 1993). That implies that if the cell produces trehalose, it must exit the cell to protect the outer side and if trehalose is added in a formulation of a dry starter culture to protect the cells of desiccation damage, it must enter the cells.

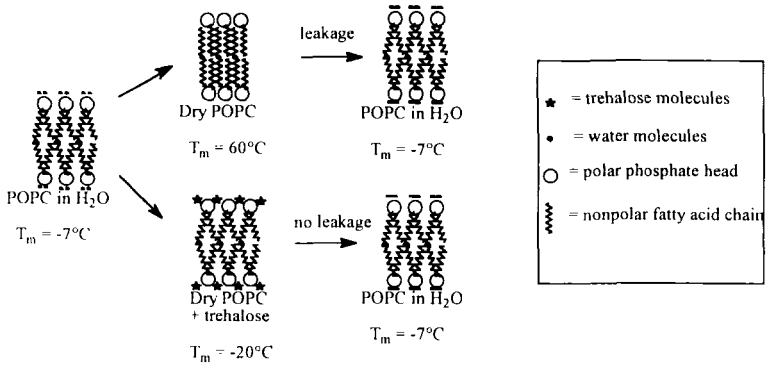

Figure 2 : Role of disaccharides in stabilisation of phospholipid bilayer during dehydration (adapted from Aguilera and Karel, 1997)

3.2. PROTEINS

In consideration of adaptation of microorganisms to extreme conditions (temperature, pH and pressure), it is generally assumed that the protein evolution is driven toward the achievement of optimum function rather than maximum stability. Adaptation to desiccation can be viewed quite differently for one main reason : a desiccated cell does not grow and the time the cell remains desiccated may represent the largest part of the 'life' of the cell and of its composing proteins. Unless desiccation-tolerant cells accumulate proteins that serve some structural or protective role (and no evidence for that has been forthcoming), the consideration of protein function in a desiccated cell is irrelevant. However, the question of optimal function might be critical at the time the cell emerge from desiccation. Since there is no evidence that proteins from desiccation-tolerant bacteria are more stable than the equivalent proteins of their desiccation-sensitive counterparts, we must take into account other mechanisms of proteins stabilisation.

3.2.1. The anhydrobiotic cell and a water replacement hypothesis

According to the *preferential exclusion hypothesis,* when some cells are submitted to an osmotic stress they are know to produce 'compatible solutes' which act as stabilisers for the proteins by being preferentially excluded from their direct vicinity. Such an exclusion is thermodynamically not favourable, but if the proteins were 'unfolded' from

their native configuration, they would expose an even greater surface to the solute what would be even more unfavourable (Levine and Slade, 1992). As a consequence, the presence of these solutes stabilises the proteins. However, the anhydrobiotic cell is characterised by a far lower water content than a cell submitted to an osmotic stress or than a cryotolerant cell in presence of extracellular ice. The preferential exclusion hypothesis does not hold at these low moisture contents, but only in intermediate moisture systems (Crowe et al., 1993).

Some desiccation-tolerant prokaryotes accumulate large amounts (up to 20% of the dry weight) of either or both of the disaccharides trehalose and sucrose. The observations led to the conclusions that they were efficient at protecting enzymes during both freeze-drying and air-drying. However, as the preferential exclusion hypothesis does not hold, they do not act as compatible solutes.

A *water replacement hypothesis* was developed to account for the protective effect of these polyhydroxyl compounds in the desiccated systems (Clegg et al., 1982; Crowe et al., 1993a; Crowe et al., 1993b). Essentially, the hypothesis is that the compounds, such as trehalose, replace the shell of water around the macromolecules, circumventing damaging effect during drying. The expression 'water replacement' may also be applied to the role of trehalose in stabilising the lipidic bilayer systems of the membranes (Leslie et al., 1995).

3.2.2. Vitrification of the cytoplasm as mechanism of tolerance to desiccation

There is some controversy whether the exclusion hypothesis is the explanation of the stabilising effect of the disaccharides on the cells or not. Some groups of researchers involved in the food industry claim that the most important property of saccharides relevant for the protection of anhydrobiotic cells is their ability to form a vitreous (glassy) phase (Crowe et al., 1997; Slade and Levine, 1991).

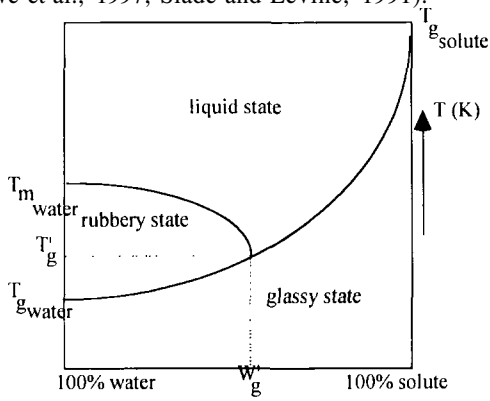

Figure 3 . 'Dynamic' phase diagram of a glass forming solute in water.

In intermediate moisture systems, such as bacterial cells, most physical and chemical processes (with the exception of free radical reactions) are under kinetic control, i.e., they are diffusion limited. The living organisms may be in a stationary state, but not in

thermodynamic equilibrium. In a simple system composed of two components, a solute in water, a continuum of hydration states can be achieved from a pure solute to a solution of infinite dilution. Each hydration state has a characteristic temperature that defines the point of kinetic change in physical state : the glass transition temperature. This transition occurs between a metastable glassy solid that is capable of supporting its own weight against gravity to a rubbery viscous fluid that can flow in real time. At temperatures below this glass transition temperature, $T<T_g$, diffusion-limited processes are inhibited by an extremely high viscosity and water is virtually unavailable (figure 3).

Water acts as a plasticiser : the net effect of increasing the water content, W, is equivalent to the net effect of increasing the temperature. The viscosity of the system decreases. T'_g is an invariant point on the continuum curve of T_g and represents the state-specific subzero T_g of the maximally freeze-concentrated, amorphous solute/unfrozen water matrix surrounding the ice-crystals in a frozen solution. T'_g corresponds to, and is determined by, the intersection of the glass curve and the non-equilibrium extension of the equilibrium liquidus curve for the T_m of ice. This solute-specific point defines the composition of the glass that contains the maximum practical amount of plasticising moisture (W'_g) (Levine and Slade, 1992).

Several solutes may be accumulated by anhydrobiotic organisms causing the vitrification of the cytoplasm under physiological conditions. The vitrified cells are stabilised during conservation in the dry state. A water system alone cannot be in the vitrified phase under physiological conditions, the glass transition temperature of pure water being -137°C. When compounds such as trehalose, sucrose or polyhydroxy-compounds are added to water, they raise the T_g of the system, which stabilises the vitrified phase under 'normal' conservation conditions (figure 4). Sucrose has a T_g of 67°C and trehalose has a T_g of 79°C.

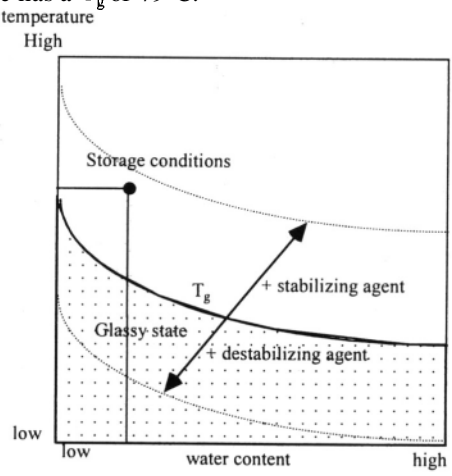

Figure 4 : Effect of (de)stabilising solutes on T_g of a desiccated product (adapted from Lievense and Van't Riet, 1993)

Vitrification stabilises anhydrobiotic systems by virtually stopping the rate of all chemical reactions, which were under diffusion limitations (including the reactions of degradation of biological materials during low-moisture preservation). Vitrification also inhibits water loss by reducing diffusion rates to the free surface, prevents fusion of vesicles during dehydration and stops solute leakage during rehydration.

Trehalose permits water content as high as 2 water molecules per glucose ring while still in the glassy state (i.e. up to 17 weight % of water) at ambient conditions.

3.3. NUCLEIC ACIDS

Both DNA and RNA are targets of desiccation damages. In large parts, the damages reflect the accumulation of mutations during the time there is no cell growth i.e. desiccation (Potts, 1994). The mechanisms of repair are unlikely to operate in air-dried cells and these damages become manifest only upon rehydration. Damage to DNA in the dry form may arise through chemical modifications (alkylation, oxidation), cross-linking (between protein and DNA), base removal such as depurination, or ionising or non-ionising radiations. As opposed to the damage to the membranes, DNA damages continuously accumulate during the time of dry storage. The control of the conditions of storage are essential for the preservation of dry starter cultures.

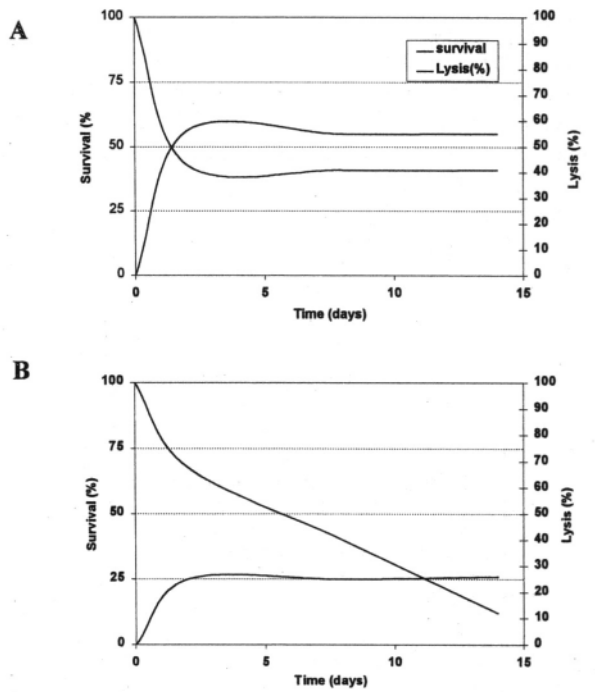

Figure 5 : Evolution of survival (%) and membrane lysis (%) during storage of D. radiodurans (A) and Rhodococcus erythropolis (B)

The survival value of *Deinococcus radiodurans* is stable during storage, because the major damage (i.e. membrane alterations) occurs during the drying process itself and later does not evolve anymore (figure 5A). *Deinococcus radiodurans* accumulates single- and double-strand breaks during the time of desiccation, but *Deinococcaceae* can tolerate massive DNA damages because, upon rehydration, they have the ability to efficiently repair them. As opposed to *Deinococcus,* the storage in the dry form of the drought-tolerant *Rhodococcus* strain results in a continuous decrease of the survival although the membrane injuries are not evolving anymore (figure 5B). *Rhodococcus* strains have not been shown to be able to erase all DNA damages upon rehydration. The accumulation of DNA damages are probably responsible for the steady decrease of survival although in the experiments with *Rhodococcus,* damages to the DNA are not dissociated from the damages to the proteins.

3.3.1. Mechanisms of tolerance to DNA damages during desiccation

Deinococcus radiodurans has a unique mechanism of tolerance to nucleic acid damages occurring during desiccation or irradiation as discussed before. For a good review about it see Battista's paper (Battista, 1997).

Some terrestrial cyanobacteria utilise another technique to prevent DNA damages : the accumulation of photoprotective pigments with broad UV absorption spectrum. They play a role in the radiation tolerance of the dry cells.

Vitrification may play an important role in slowing down the rates of destruction of the nucleic acids, by impeding the diffusion of the reactive species (excepted, as noted before, for the free-radical reactions).

3.3.2. UV irradiation as a tool for the selection of drought-tolerant bacteria

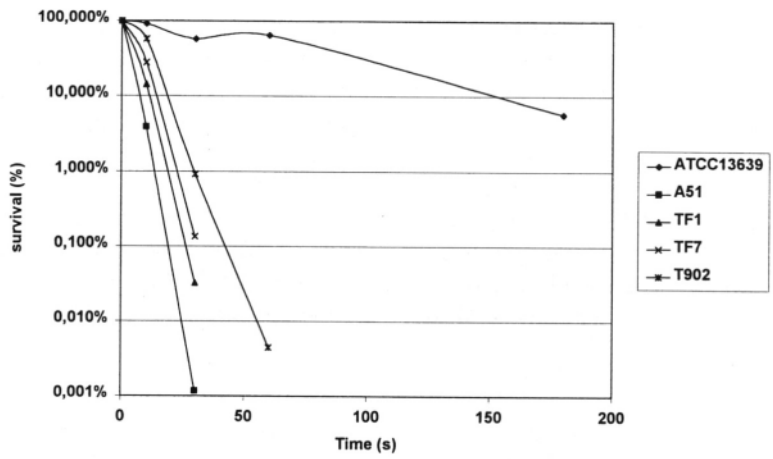

Figure 6 : Log of the percentage of survival of the strains (see table1) vs. duration of UV radiation (254 nm)

Desiccation-tolerance is viewed as an important factor for the use of living microorganisms in technological processes. Therefore quick selection procedures for drought-tolerant bacteria were set-up (Weekers et al., 1999a). As functions necessary to survive ionising radiation are also necessary to survive desiccation, UV radiation tolerance measured by exposure to UV radiation (254 nm) served as a tool to quantify the drought-tolerance of a strain. This measurement only accounts for the DNA damages and not for the damages to the membranes.

As DNA damages are the main consequence of irradiation, the *Deinococcus* strain is notably more resistant than the other strains. The behaviour of the drought-tolerant group of strains is different from the sensitive ones modelled by A_{51}. The survival of the former is up to 1000 folds higher than the latter (figure 6) after 30 seconds of irradiation.

4. Factors influencing survival

When used as a preservation technique, desiccation may be optimised toward a higher level of survival of the final dry product. Several factors influence the final survival and each of these factors may be optimised.

4.1. BACTERIAL SPECIES

Bacterial species cannot usually be chosen for a given use. The nature of the microorganism is dictated by the properties required by the technological applications one will make of it. However, when selecting bacteria according to their desiccation tolerance as a first criteria before looking for biodegradation properties (or any other activity) among the drought-tolerant selected strains, the selection is made on the species basis. The required activity may be transferred to the strain in a second step with genes borne on a plasmid such as catabolic plasmid. With this strategy, the selection is made according to the bacterial species. As a rule of thumb, one can roughly distinguish between sensitive and less sensitive vegetative bacterial cells, by distinguishing between Gram-negative and Gram-positive bacteria, respectively. With the method used for the selection of drought-tolerant strains from a desiccated soil, 20 strains out of 26 collected were gram-positive (77%). This ratio is in agreement with the general tendency of higher resistance for the gram-positive bacteria. The difference in membrane behaviour during desiccation is mainly responsible for the observed difference in desiccation-tolerance between Gram positive and Gram negative bacteria.

4.2. GROWTH CONDITIONS

The growth conditions and growth phase of the bacterial cell is important for their survival (Gelinas et al., 1989; Labuza et al., 1972; Linders et al., 1997; Siegele and Kolter, 1992).

- The growth conditions will influence the membrane composition, which in turn, influences the membrane phase transition, as discussed before.

- The degree of saturation of the phospholipids of the membrane (influenced by the aeration of the culture) is related to the stability of the membrane. Higher degree of saturation would correlate to a higher survival.
- Generally, the highest dehydration resistance is found for bacteria harvested in the stationary growth phase.
- Most protective compounds are produced during the stationary phase, as yeasts producing trehalose. It is better to wait until that time to collect and dry them.

4.3. PROTECTIVE ADDITIVES

Research on the protective effect of various additives is abundant (Combes et al., 1990; De Cordt et al., 1994; De Valdez et al., 1985; De Winder et al., 1989; Gianfreda et al., 1991; Graber and Combes, 1989; Izutsu et al., 1994; Roser, 1991). It is probably because -and it has been acknowledged- the use of additives is the most fruitful strategy for obtaining optimal survival after drying. Positive effect has been reported for sugars, polyalcohols, glycerol, carboxylic acids, milk, skim milk, culture medium, proteins, amino acids, polymers, metallic cations and salts. The effective protective effect of each additive is species-specific.

The interactions of some of these additives with membranes were discussed earlier and the hypothesis of the water replacement or the glass formation theory were explained. Some additives may also act as anti-oxidant or encapsulating agents isolating the cells from the lethal effect of oxygen and oxygen related species.

4.4. CELL CONCENTRATION

It is generally reported that higher concentrations in the suspension to be dried give higher survival. An explanation to this effect could be the release of intracellular compounds of damaged cells that could protect other cells.

4.5. DRYING GAS, RATE AND EXTEND

When oxidation of cellular compounds plays an important role in reducing survival, nitrogen can advantageously replace air in air-drying processes.

Drying rate, or the rate of water activity reduction, has also an effect on the survival (Antheunisse and Arkesteijn-Dijksman, 1979; Gervais et al., 1992; Poirier et al., 1996). If the rate of drying is too rapid, there is no time for adaptation mechanisms to take place (such as accumulation of protective compounds) and the survival is low. On the other hand, if the drying rate is too slow, the cells are submitted to an environment of unfavourable water activity for longer periods of time, which is also unfavourable. There is an optimal intermediate drying rate, which balances these two opposite effects. The fluidised bed drying technique with warm air (45°C) allows drying rates that respect best this balance. Drying rate effect in given conditions can be predicted by the reduction of a_w in solution by addition of glycerol or any other solute reducing the water activity of the solution.

The survival of bacteria is undoubtedly related to the final water concentration, due to the dehydration inactivation. A low water concentration is necessary to obtain storage

stability and, therefore, an optimum has to be found between survival after drying and stability during storage. The use of protective additives also influences this effect. With desiccation-tolerant soil strains, the long-term conservation is the same with a residual a_w of 0.29 and 0.17. It means that once the stability of the dry product is guaranteed, it is not necessary to decrease a_w to lower values, because survival at low water activity is smaller than survival at higher values.

4.6. REHYDRATION

Rehydration conditions, such as temperature, composition, osmolality, rehydration medium or rate, can significantly influence survival. Some authors regard rehydration as the most important step since the damages to membranes happen more during rehydration than dehydration. The rehydration temperature directly correlate with the membrane phase transition theory.

4.7. STABILITY DURING STORAGE

In the preservation of commercial bacterial starter cultures, low inactivation rate during storage is as important as high viability after drying. High survival yields must be obtained but they are not sufficient. To enter the commercial chain, the half-life of the dry product must be at least equivalent to the time necessary for the product to reach the final user. Storage stability is increased by decreasing temperature. The presence of oxygen in the storage atmosphere is detrimental (Mary et al., 1993). The inactivation during storage was related to the formation of radicals in the presence of oxygen. As possible radical reaction, fatty acid oxidation and DNA damage have been reported. Light is also expected to be detrimental and storage in the dark is recommended. The glass formation theory is also relevant for the storage of biological products. Many authors report a maximum water concentration below which the cells have to be stored (Lievense and Van't Riet, 1994; Scott, 1956). Since the diffusion coefficient in the glassy state are very small, diffusion-limited reactions become undetectable. To reach the glassy state, low temperature and low moisture content are usually necessary, but one can achieve the glass state by adding compounds that bring the glass transition curve to higher temperature closer to ambient. Starch hydrolysis products such as maltodextrins are efficient at stabilising products by their glass forming properties. Radical reactions are not diffusion limited and will thus not be reduced by the glassy state. However, the rate of diffusion of oxygen into the product will be slowed and this will decrease the rate of production of radicals.

5. Conclusions

The study of desiccation tolerance of cells requires the application of a judicious mix of biophysics, structural biochemistry, and molecular ecology to the study of the whole cells and their purified components. The membrane lysis is responsible for most of the mortality during desiccation. It does not evolve during storage of the desiccated product. It is possible to reduce or prevent the phase transition of the membranes with

protecting compounds and growth conditions affect the desiccation-tolerance of the microorganisms. The conditions of storage of desiccated biological materials must be controlled because the nucleic acid damages accumulate during the time of desiccation.

The complexity of the desiccation-tolerance phenomena is related to the complexity of a cell and to the multiplicity of its components. There is not a universal additive that will protect all cells from all desiccation damages, nor there are techniques and conditions that will allow best survival and storage preservation in any case. Each species is a different case.

Quick selection techniques such as resistance to UV radiation exposure can be used to select desiccation-tolerant strains for their technological application.

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