Sporulation - freeze - encapsulation: What impact on the stability and activity of the cells?

To compare the three treatments (*ie* sporulation, lyophilization and encapsulation), it is useful to detail the principle of each of them.

sporulation

Sporulation is **natural adaptive phenomenon** few bacteria are capable of doing. This ability derives from a complex genetic program that is activated when the bacteria are facing extreme circumstances of their environment (eg, lack of nutrients, dehydration, temperature ...). Compared with parent cells, spores are smaller, have a thicker shell and their metabolism is virtually inactive. **These features allow spores to be extremely resistant to the assaults of the environment and therefore remain stable over very long periods of time.** The spores may, for example, withstand the extreme conditions of the vacuum of space. Fig. 1 illustrates the various stages of sporulation. Although they are metabolically very active, spores are able to compete with other microorganisms for adhesion to surfaces (Ghelardi *et al.,* 2015). When environmental become more favorable conditions, spores again become vegetative cells **able to grow** via the reverse of sporulation, called germination.

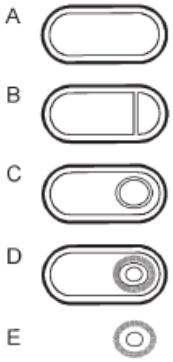

Figure 1: Stages of sporulation. The mother cell (A) undergoes a section (B) forming a nascent spore (C). It is surrounded by a thick membrane (D) before being released into the environment (E) (Dirks, 2002)

Freeze-drying

Freeze-drying is a physical process of removing water from a product by sublimation. This method allows to convey the water from the solid state (ice) in the gaseous state (steam) while maintaining negative temperatures. This has the advantage of avoiding the use of high temperatures that would affect cell viability. This industrial technique is very **common although quite expensive.**

This method could therefore not be used in liquid products as this would cause immediate rehydration of bacteria. In addition, in the case of freeze-drying of bacteria (or spores) **the art can adversely impact the viability of microorganisms and can denature the surface proteins** bacteria involved in their adhesion to the surfaces. To counter these side effects, it is necessary to use specific protection agents that appear as additional ingredients in the finished product (Gotor Vila *et al.,* 2016). Another drawback associated with freeze drying is the possibility of causing genetic changes uncontrolled into the genome of the bacterium (Fairhead *et al.,* 1994). This last point should definitely be avoided to maintain the known characteristics of the bacteria in the finished products.

encapsulation

The encapsulation technique allows to collect the microorganisms of interest in an enclosure formed by a suitable material. **This process is very useful to protect living cells in complex environments (** eg food, digestive system ...) **and increase their lifespan.**
Furthermore, this technique allows **make specific site of action** probiotics. This is particularly interesting for Probiotics in food. Indeed, their development should take place in the digestive system and not in the food, otherwise modify the sensory characteristics specific to the food. In the context of detergents, sites of action are extremely variable so that encapsulation is not an option.

Comparison of techniques and findings

Detergent-based probiotics requires optimization of conditions of stability of bacteria. Indeed, non-stabilized vegetative bacteria could survive long term in the finished products in particular because of the lack of nutrients. To ensure sufficient stability of finished products, three options can be considered: sporulation, lyophilization and encapsulation. **Sporulation appears as the most suitable option in the world of detergency.**

This technique provides resistance of the spores to environmental adverse conditions while leaving them available to act (as opposed to encapsulation, which is more specific). Secondly, unlike lyophilisation which may impact the adhesion of cells to surfaces capabilities, spores are capable of colonizing the field. Another advantage of sporulation face freeze drying is the best preservation of the viability of spores can germinate and become active more efficiently.

Finally, the stability of bacteria in probiotic-based detergents is a key feature that determines the effectiveness of the finished product. To ensure product quality, Probiotic Group uses the technique of **sporulation to stabilize the bacteria of interest and allow them to remain active throughout the product preservation.**

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