Enhancing bacterial viability after drying by pulsed electric field pre-treatment

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Background

Probiotic bacteria are gaining more and more interest in both medical and nutritional applications. They are often supplied to the customer in dried formulation in order to increase product shelf-life. Nowadays living bacteria are usually dried using the energy consuming freeze drying process. Spray drying is another type of drying in which hot air is used to dry the material; a process commonly applied in food industry and less energy consuming than freeze-drying. However, bacterial viability after spray drying is very low because of the higher temperatures involved. A pre-treatment step, which makes the bacteria more robust towards the harsher conditions, could improve the viability after spray drying.

Objective

The aim of this project is to develop a novel method, employing pulsed electric field treatment, in order to increase the viability of probiotic bacteria after spray drying (Fig. 1).

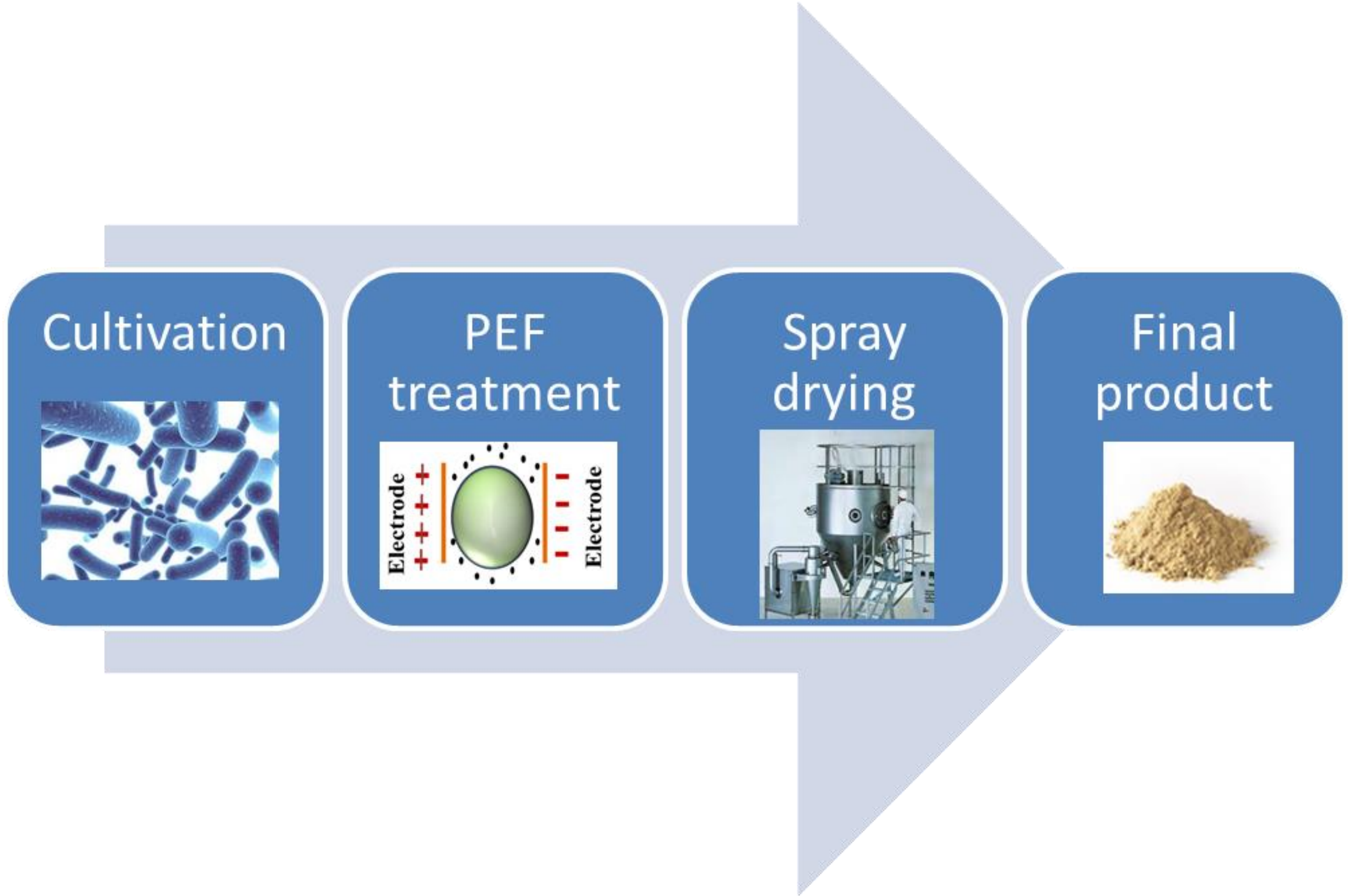


Figure 1. Schematic overview of the proposed production process for dry bacterial formulations.

Approach

Pulsed electric field

Pulsed electric field (PEF) is a method to create pores in a cell membrane by the application of high voltage pulses. Pore formation in the membrane can be reversible or irreversible. In this project we aim at reversible pore formation in order to facilitate transport of protective solutes across the cell membrane to enhance heat robustness and retain cell viability (Fig. 2).

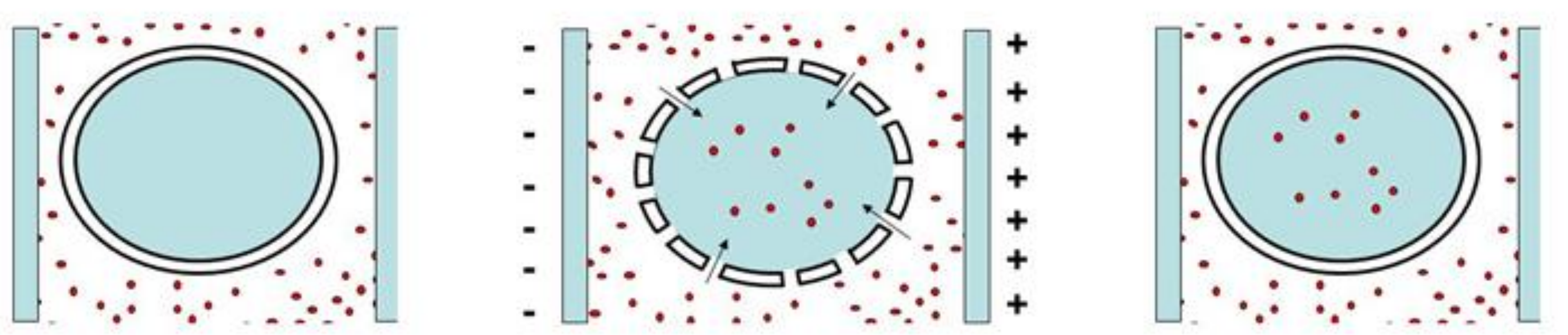


Figure 2. The principle of pulsed electric field treatment for increasing intracellular solutes concentration: When an electric field is present pores in the membrane can be formed and solutes can diffuse across the membrane. Afterwards, the pores in the membrane can reseal.

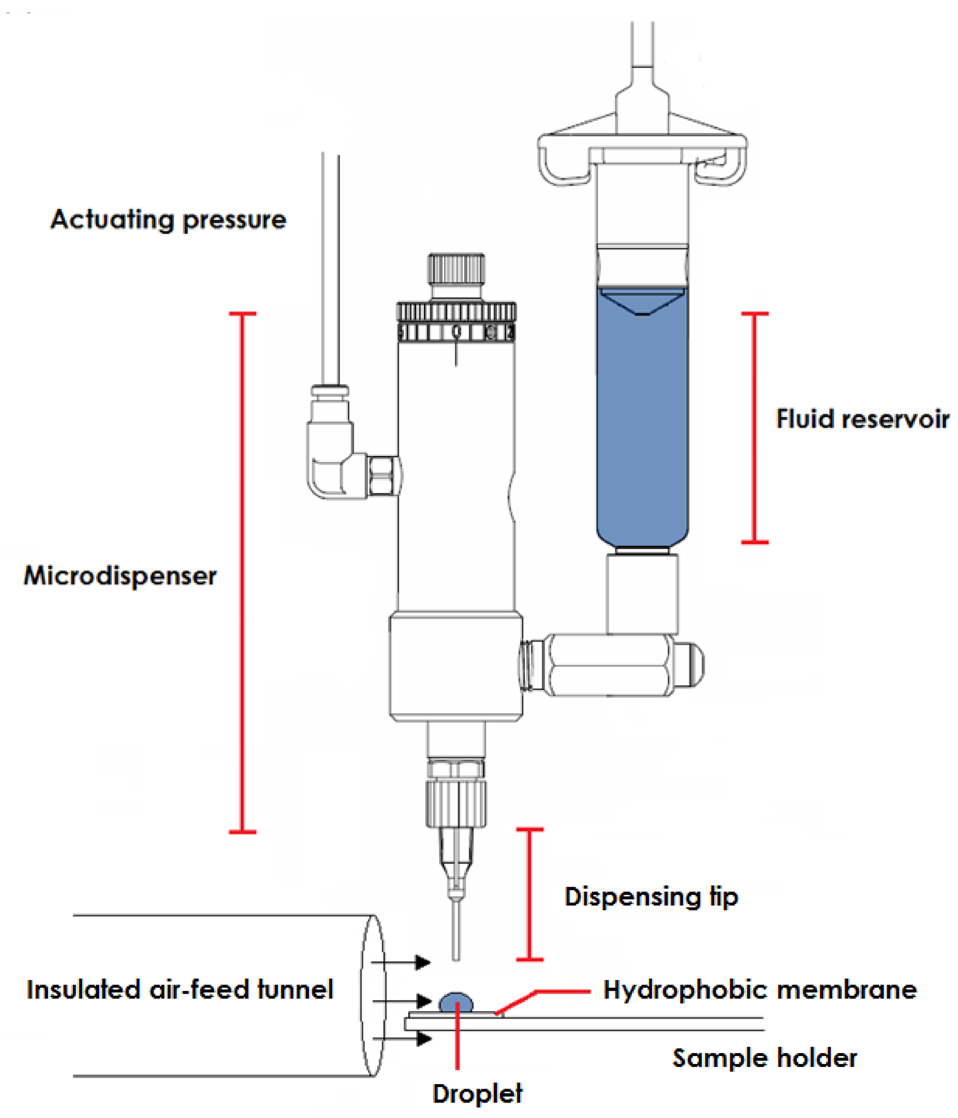
Many parameters are expected to influence the outcome of the PEF treatment (Table 1). A systematic experimental investigation will be carried out to establish the optimal conditions that lead to high intracellular solute concentration while retaining cell viability.

Table 1. Overview of different factors influencing bacterial viability and intracellular solute concentration after PEF treatment

Process parameters	Microbial characteristics	Treatment medium characteristics
Electric field strength	Gram positive/negative	Composition
Treatment time	Species and strain	Conductivity
Pulse width	Growth conditions: -Temperature -Medium -Growth phase -History	pH
Specific energy input		Water activity
Pulse frequency		Recovery conditions: -Medium -Temperature -Recovery time
Temperature		
Pulse shape		

Single droplet drying

Spray drying can be mimicked by using a single droplet drying approach (Fig. 4). This method requires only small sample size and can be used for fast screening of different drying parameters like temperature. The viability after single droplet drying will be assessed by LIVE/DEAD staining of the bacteria followed by fluorescent microscopy (Fig. 3) and/or conventional plate counting.



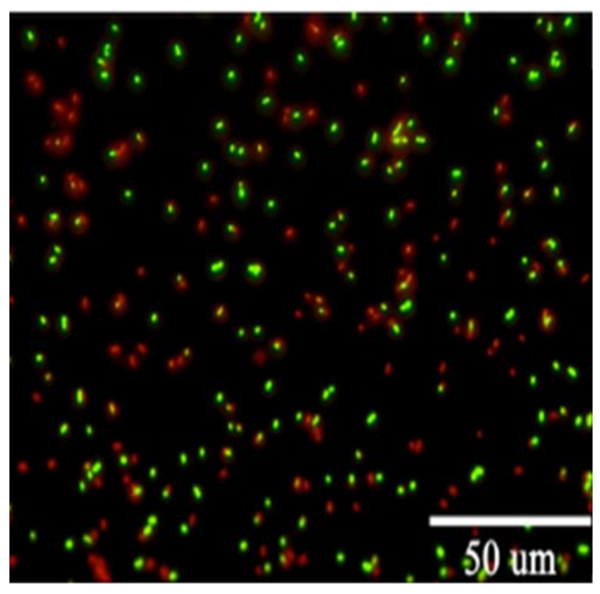


Figure 3. Fluorescent microscope picture of bacteria (*L. plantarum* WCFS 1) stained with LIVE/DEAD kit. Green cells are alive, red cells are dead.

Figure 4. Schematic representation of the single droplet drying equipment.

Final remarks

The novel approach introduced in this poster could lead to more applications of probiotics in the food industry. For example a full formulation infant formula, containing probiotics, could be dried. Besides that, it could be that the pulsed electric field pre-treatment also positively influences the gastric acid resistance of the probiotics. This could make the product more efficient since a smaller dose could be sufficient to gain the same beneficial health effect.