

# Two complementary methods to assess bacterial survival after single droplet drying



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## Background

Probiotics are gaining more and more interest in both medical and food applications because of their potential health benefits. They are often supplied to the customer in dried formulation to increase product shelf-life. One type of commonly used drying processes in food industry is spray drying. Spray drying is a continuous process in which hot air is used to dry the material. The drying process affects bacterial viability by dehydration and thermal inactivation. Single droplet drying is a method that can be used to study the effect of spray drying on bacterial survival at a smaller scale.

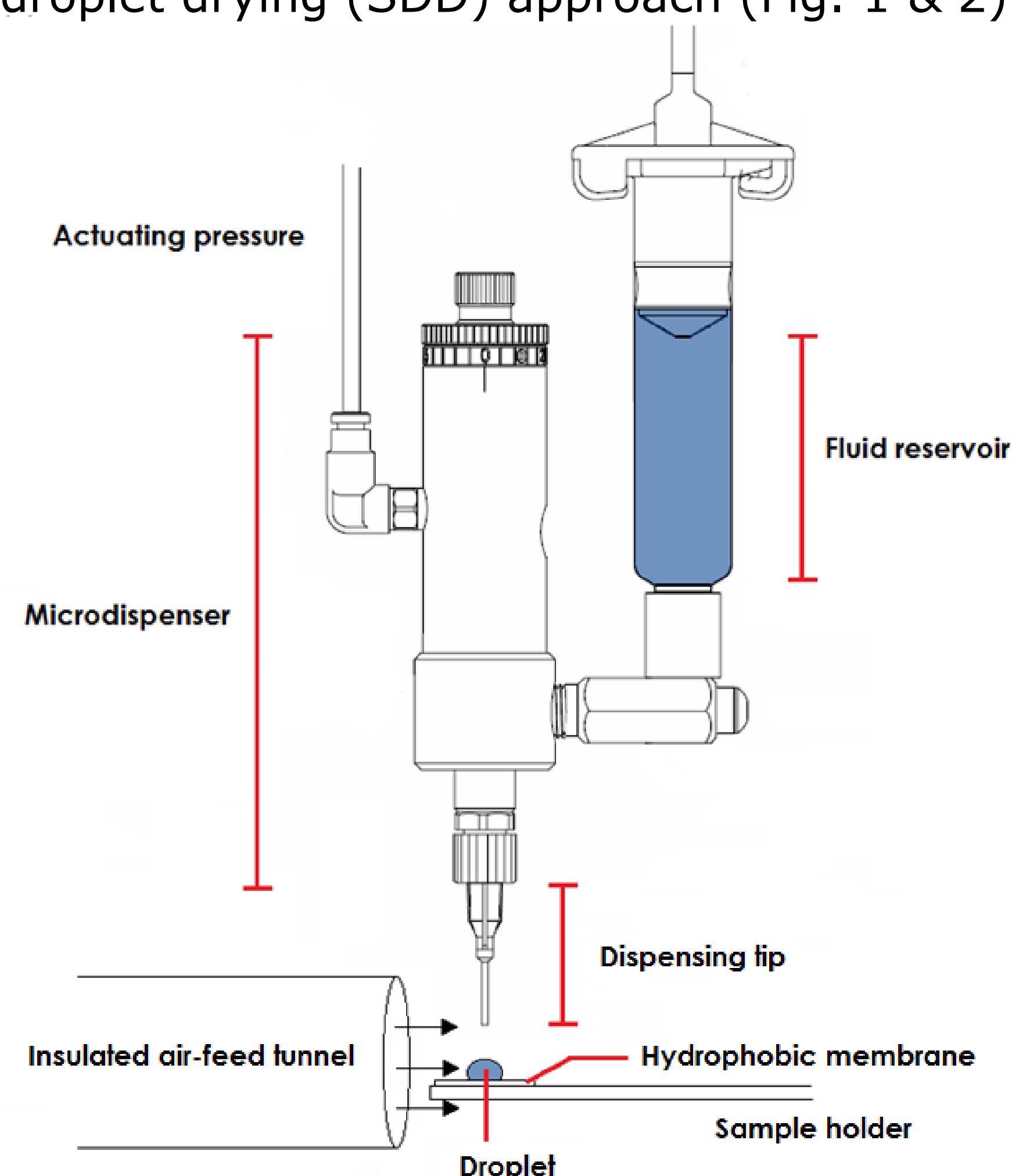
## Objective

The aim of this work was to develop a fast screening method for bacterial survival after spray drying by making use of sessile single droplet drying.

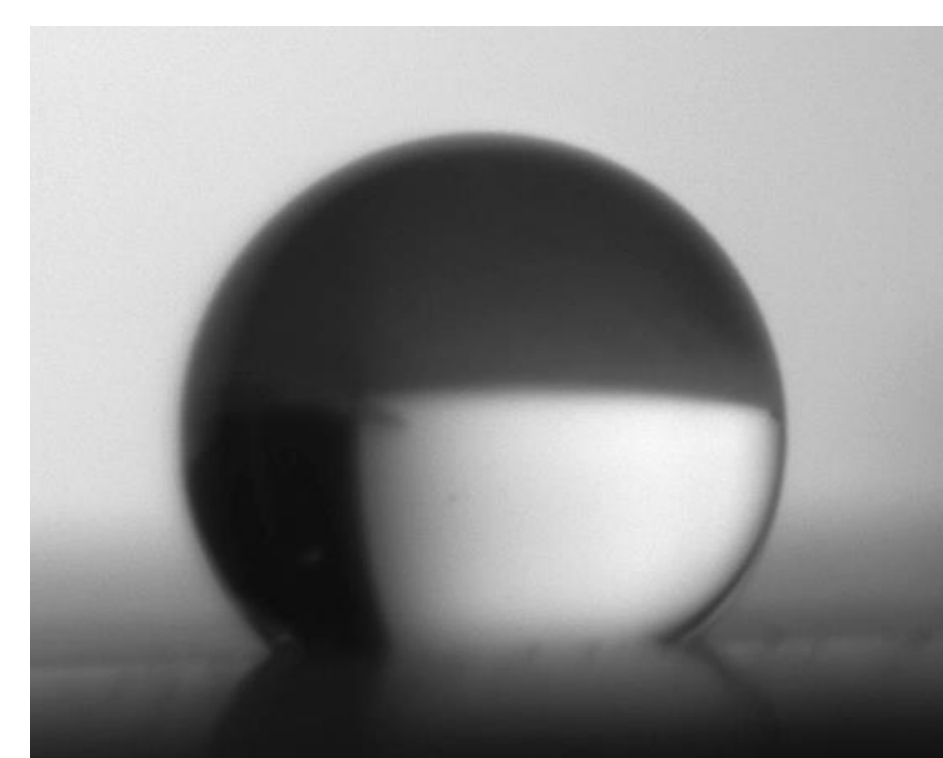
## Approach

### Single droplet drying

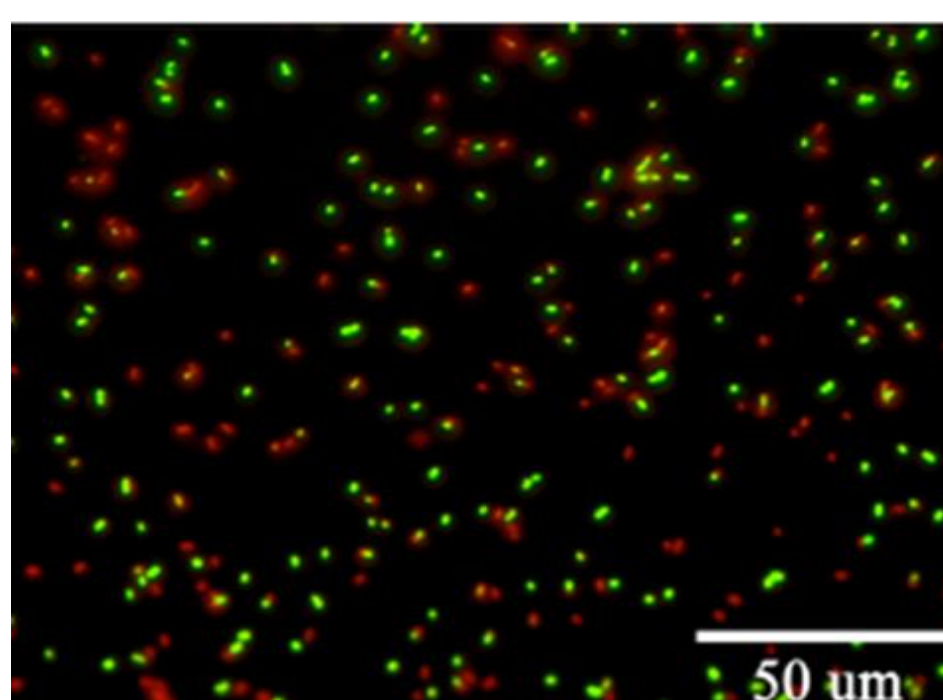
Spray drying of probiotics can be mimicked by using a sessile single droplet drying (SDD) approach (Fig. 1 & 2).



**Figure 1.** Schematic representation of the sessile single droplet drying equipment.



**Figure 2.** Single droplet deposited on a hydrophobic membrane.



**Figure 3.** Fluorescent microscope image of *L. plantarum* WCFS 1 stained with LIVE/DEAD fluorescent dyes. Red cells have a permeabilized cell membrane, green cells have an intact cell membrane.

### Experimental set-up:

- Two bacterial strains: *Lactobacillus plantarum* WCFS1 and *Lactococcus lactis* subsp *lactis* biovar *diacetylactis* FM03.
- Drying air temperatures: 45, 70 and 90 °C.
- Drying matrix with or without ~10% w/v trehalose.
- Viability assessment methods: LIVE/DEAD staining (SYTO9 and PI) followed by fluorescence microscopy and conventional plate counting.

### Survival assessment

LIVE/DEAD staining can be used to discriminate between bacteria that are alive or dead based on their membrane integrity. Propidium iodide (PI, red stain) can only enter cells with a damaged membrane, and SYTO9 (green stain) enters all cells. Based on fluorescent microscope images survival can be calculated (Fig. 3).

## Conclusions

Compared to conventional plate counting the LIVE/DEAD staining method ...

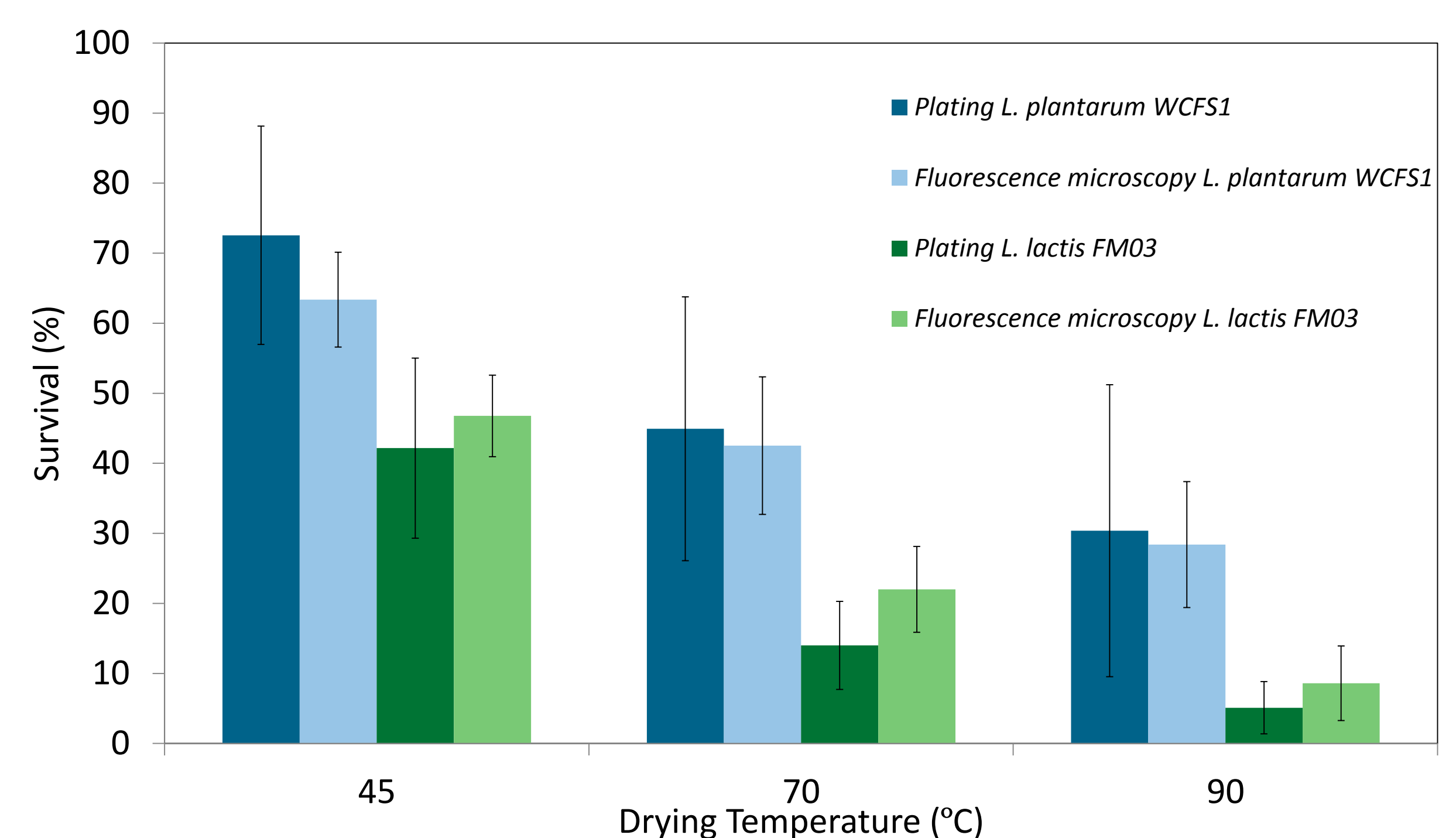
- was more time efficient
- gave similar survival percentages after single droplet drying
- resulted in smaller standard deviations between biological replicates

Bacterial survival after single droplet drying ...

- decreased with increasing drying air temperature
- increased by addition of ~10% w/v trehalose to the drying matrix
- was higher for *L. plantarum* WCFS1 than for *L. lactis* FM03

## Results

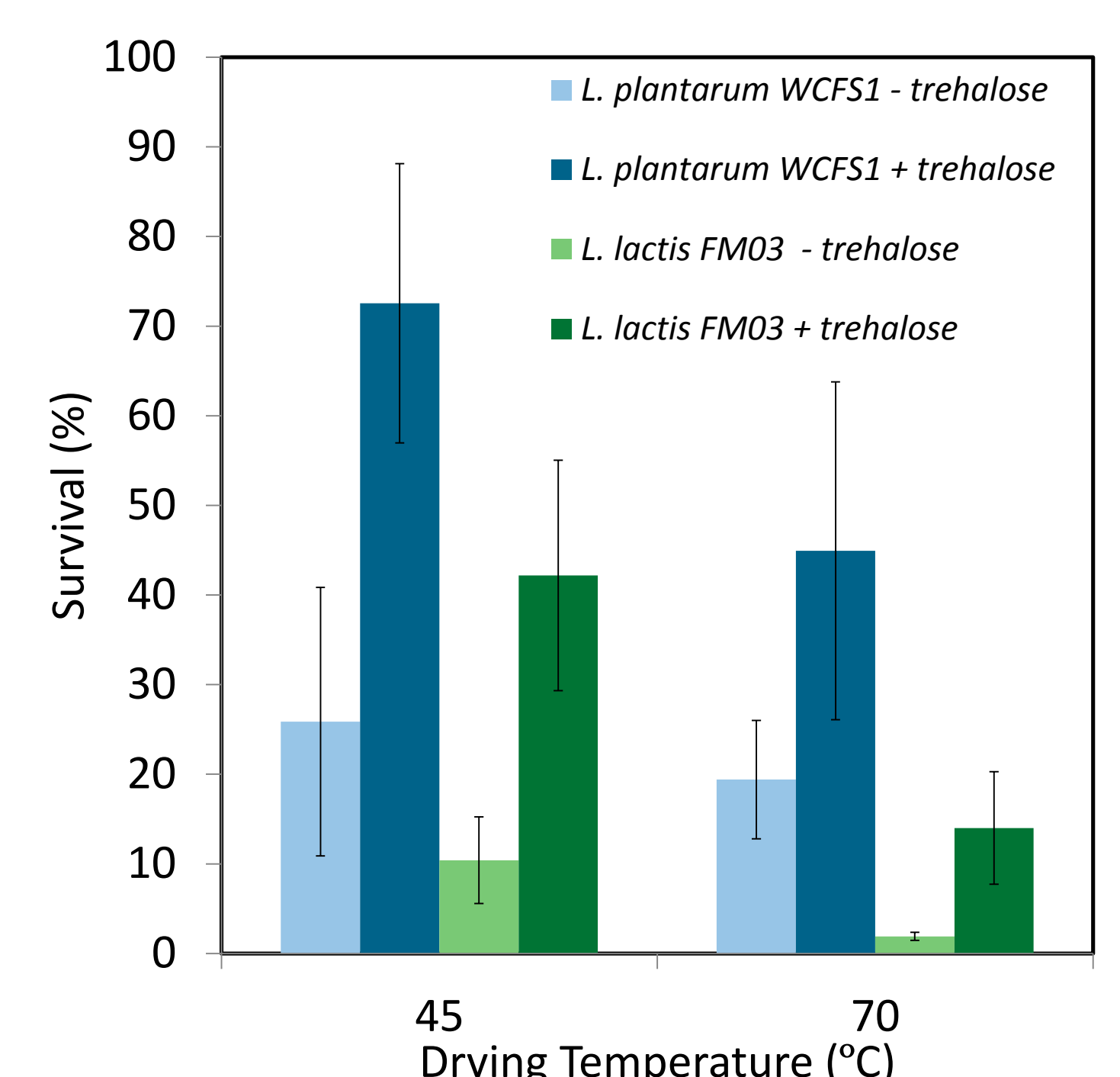
LIVE/DEAD staining followed by fluorescence microscopy is a fast method to study survival of bacteria after single droplet drying. Although this method is based on membrane integrity and conventional plate counting on cell proliferation, both methods gave similar survival percentages after drying (Fig. 4). In addition, the LIVE/DEAD staining method resulted in smaller standard deviations between biological replicates (Fig. 4).



**Figure 4.** Survival of *L. plantarum* WCFS1 and *L. lactis* FM03 after single droplet drying assessed by plate counting and LIVE/DEAD staining combined with fluorescence microscopy. All bacteria were dried with ~10% w/v trehalose in the drying matrix. Error bars indicate standard deviations of biological replicates, n≥3.

Our results also indicate that:

- Increasing drying temperature led to a decrease in survival for both *L. plantarum* WCFS1 and *L. lactis* FM03 (Fig. 4 & 5).
- *L. plantarum* WCFS1 was more robust towards drying than *L. lactis* FM03 (Fig. 4 & 5).
- Addition of ~10% w/v trehalose to the drying matrix increased survival of both *L. plantarum* WCFS1 and *L. lactis* FM03 (Fig. 5).



**Figure 5.** Survival of *L. plantarum* WCFS1 and *L. lactis* FM03 after single droplet drying with and without trehalose, assessed by plate counting. Error bars indicate standard deviations of biological replicates, n≥3.