Role of Lactic Acid Bacteria (LAB) in Displacing Biofilms in the Food Industry

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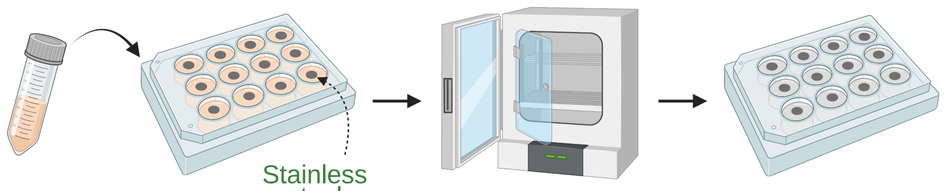
INTRODUCTION & AIM

Food contact surfaces are especially prone to microbial adhesion and biofilm development owing to the availability of nutrient-rich food residues[1]. **Foodborne biofilms** compromise the proper functioning of equipment and impair food safety and quality, thus constituting a major concern[2]. The higher tolerance of biofilms to traditional cleaning treatments has emphasised the development of **new methodologies to control biofilms** in the food industry[1,3]. **Probiotics** and their products have shown great potential for preventing attachment and biofilm formation by a large spectrum of foodborne microorganisms[4].

This work aimed to evaluate the ability of four lactic acid bacteria (LAB) strains to displace pre-formed biofilms of *Escherichia coli*, a bacterium commonly found on biofilms developed in food contact surfaces.

MATERIALS & METHODS

E. coli biofilm formation

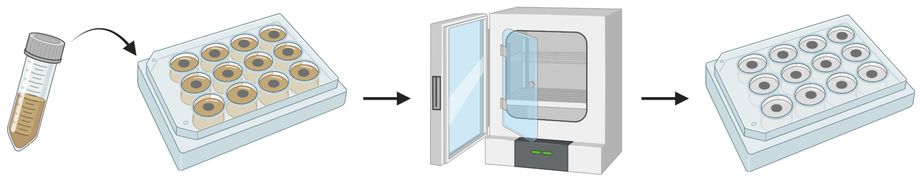


Model pathogen cell suspension
Escherichia coli SS2 in TSB medium

Static incubation of 12-well plates for **24 h** at **25 ºC**

Supernatant removal

Biofilm exposure to LAB



LAB suspensions
in MRS medium

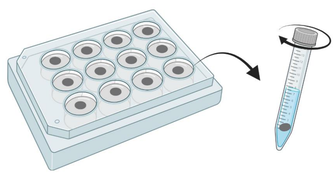
Static incubation of 12-well plates for another **24 h** at **25 ºC**

Supernatant removal

- ***Limosilactobacillus fermentum***
- ***Lacticaseibacillus paracasei***
- ***Lactiplantibacillus plantarum***
- ***Lactococcus lactis* subsp. *lactis***

The **control** consisted of adding fresh TSB to pre-formed pathogenic biofilms instead of a LAB suspension. Assays were performed in **two independent biological assays**, with **three technical replicates** each.

Quantification of biofilm cells



Stainless steel coupons dipped in NaCl and vortexed for 2 min

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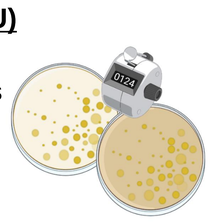
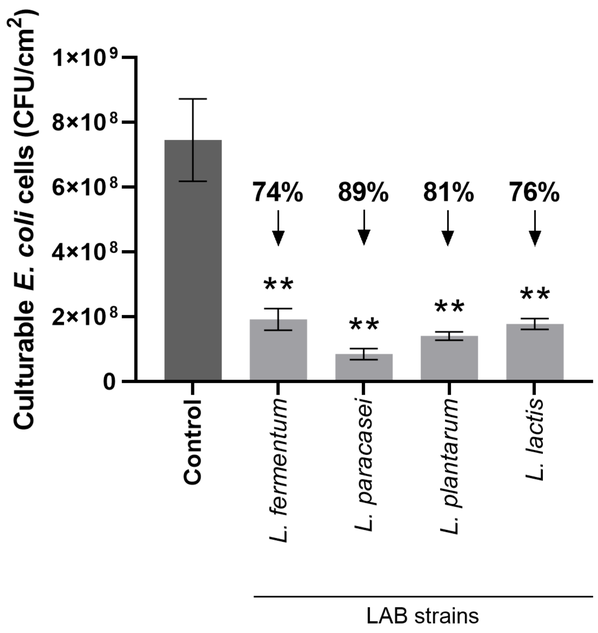
Biofilm cell suspensions

Colony-forming units (CFU)
Biofilm culturable cells
PCA + ampicillin – *E. coli* cells
MRSA – LAB cells



Flow cytometry
Biofilm total cells
E. coli and LAB cells gated based on Forward Scatter and Side Scatter signals

RESULTS & DISCUSSION



Antimicrobial effect

All tested LAB strains had a significant antimicrobial effect against ***E. coli***, with biofilm **culturability reduction of up to 89%**.

L. paracasei was the most promising LAB strain.

Figure 1. Culturable *E. coli* cells after 24 h of contact between the pre-formed biofilm and LAB suspensions. Data are presented as mean ± standard deviation. Asterisks denote significant differences between each treatment group and the control (** *p*-value < 0.001).

Antibiofilm effect

LAB could displace established biofilms of *E. coli;* the **number of pathogen cells decreased by 49–74%**.

L. paracasei was the most promising LAB strain.

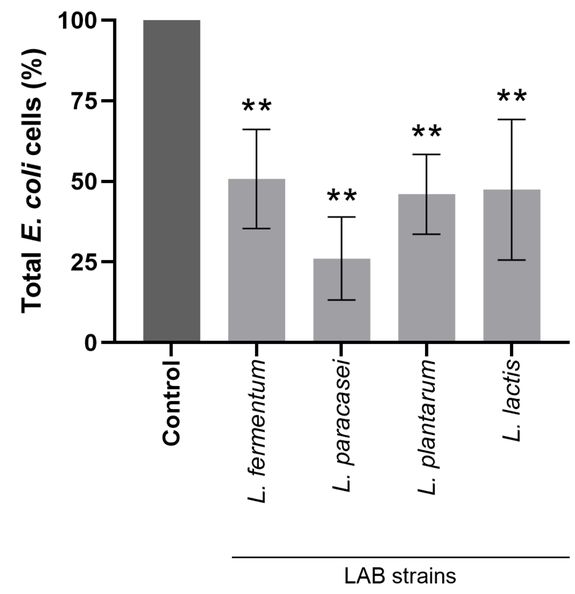
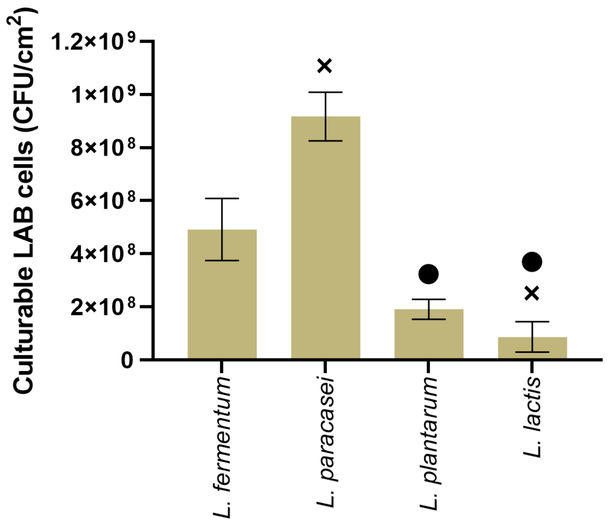


Figure 2. Percentage of total *E. coli* cells after 24 h of contact between the pre-formed biofilm and LAB suspensions. Data are presented as mean ± standard deviation. Asterisks denote significant differences between each treatment group and the control (** *p*-value < 0.001).

The antagonistic activity of LAB against *E. coli* biofilms may be due to **competition for nutrients and growth factors**, or to the **production and release of antimicrobial metabolites** (e.g., bacteriocins, biosurfactants, organic acids, hydrogen peroxide, exopolysaccharides and quorum-sensing inhibitors).



A direct relationship was observed between the **high culturability of *L. paracasei*** and **its superior antimicrobial activity against *E. coli* biofilms.**

Figure 3. Culturable LAB cells after 24 h of contact between the pre-formed biofilm and LAB suspensions. Data are presented as mean ± standard deviation. Significant differences are presented for *p*-value < 0.05 by ✕, ● and ■ when compared to *L. fermentum*, *L. paracasei* and *L. plantarum*, respectively.

CONCLUSIONS

- **The results showed the potential of *L. fermentum*, *L. paracasei*, *L. plantarum* and *L. lactis* against *E. coli* biofilms**. ***L. paracasei* was the most promising probiotic strain** considering both antimicrobial and antibiofilm activity**.**
- The application of probiotics in food production processes can reduce the occurrence of foodborne outbreaks and improve the public health in a **sustainable and environmentally friendly way**.

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