The Use of Probiotics to Control Biofilm Formation in the Food Industry

Andreia R. Tomé[1,2*], Rita T.-Santos[1,2], Mette Burmølle[3], Filipe J. M. Mergulhão[1,2] & Luciana C. Gomes[1,2]

1. LEPABE - Laboratory for Process Engineering, Environment, Biotechnology and Energy, Faculty of Engineering, University of Porto, Rua Dr. Roberto Frias, 4200-465 Porto, Portugal
2. ALiCE - Associate Laboratory in Chemical Engineering, Faculty of Engineering, University of Porto, Rua Dr. Roberto Frias, 4200-465 Porto, Portugal
3. Section of Microbiology, Department of Biology, University of Copenhagen, Universitetsparken 15, 2100 Copenhagen, Denmark

* atome@fe.up.pt

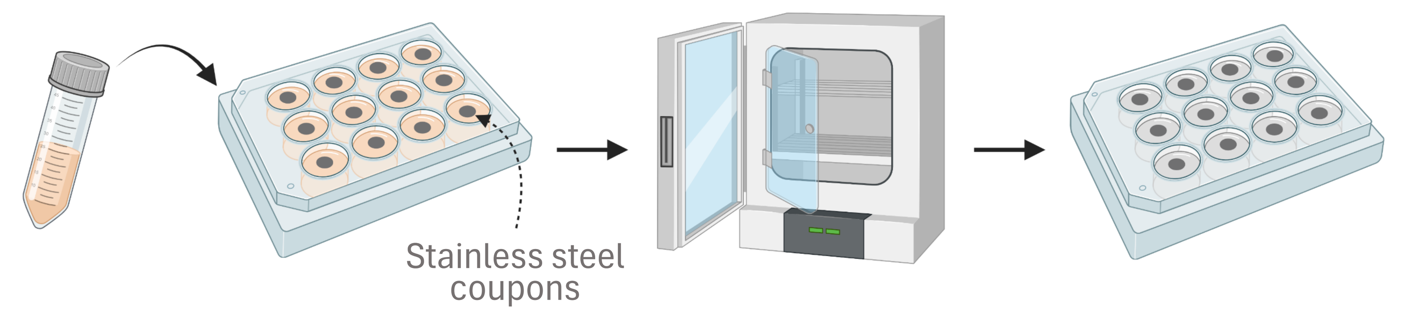
INTRODUCTION & AIM

Food contact surfaces are prone to biofilm development owing to the availability of nutrient-rich food residues[1]. **Foodborne biofilms** are sources of cross-contamination in food products, impairing food safety and quality, and can compromise the proper functioning of equipment[2]. The higher tolerance of biofilms to traditional cleaning treatments has prompted the development of **novel strategies to control biofilms** in food processing plants[1,3]. **Probiotics** and their metabolites have shown great potential to disrupt pre-formed biofilms of a large spectrum of foodborne microorganisms[2].

This work aimed to evaluate the ability of four lactic acid bacteria (LAB) strains to displace pre-formed biofilms of *Escherichia coli* and *Listeria monocytogenes*, which are Gram-negative and Gram-positive bacteria, respectively, commonly found in biofilms developed on food contact surfaces.

MATERIALS & METHODS

Foodborne biofilm formation

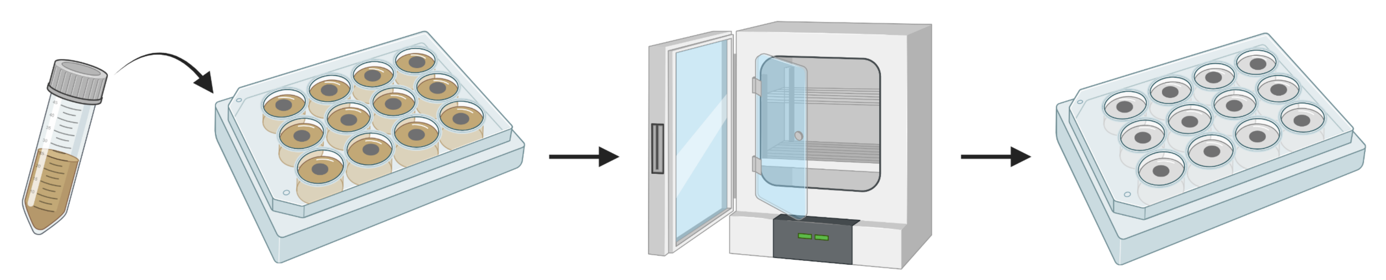


Foodborne suspension (Tryptic Soy Broth; 1×10⁸ CFU/mL) → *Escherichia coli* SS2 GFP, *Listeria monocytogenes* Scott A

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

Supernatant removal

Biofilm exposure to probiotics



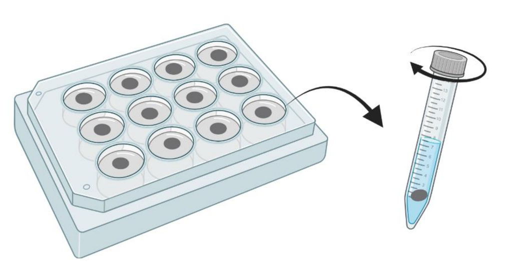
LAB suspension (Man, Rogosa, and Sharp Broth; 1×10⁸ CFU/mL) → *Limosilactobacillus fermentum*, *Lacticaseibacillus paracasei*, *Lactiplantibacillus plantarum*, *Lactococcus lactis* subsp. *lactis*

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

Supernatant removal

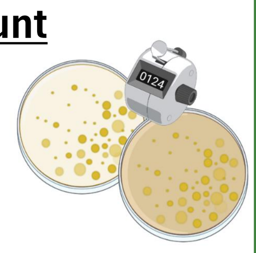
Negative controls consisted of adding fresh TSB medium to pre-formed foodborne biofilms instead of probiotics. Assays were performed in two independent biological assays, with three technical replicates each (**n=6**).

Biofilm cell quantification



Stainless steel coupons dipped in NaCl and vortexed for 2 min → **Biofilm cell suspensions**

Colony-forming units (CFU) count
Culturable biofilm cells
PCA – Foodborne cells
MRSA – LAB cells



Flow cytometry
Total biofilm cells
Foodborne and LAB cells gated based on Forward Scatter and Side Scatter signals

ACKNOWLEDGMENTS

This work was financially supported by LA/P/0045/2020 (ALiCE), UIDB/00511/2020 and UIDP/00511/2020 (LEPABE) and project 2022.05314.PTDC, funded by national funds through FCT/MCTES (PIDDAC); project SurfSAFE supported by the European Union's Horizon 2020 Research and Innovation Programme under grant agreement no. 952471. R.T.-S. acknowledges the receipt of a junior researcher fellowship from project PTDC/CTM-COM/4844/2020 (NanoCAT), supported by national funds through the FCT/MCTES (PIDDAC).

RESULTS & DISCUSSION

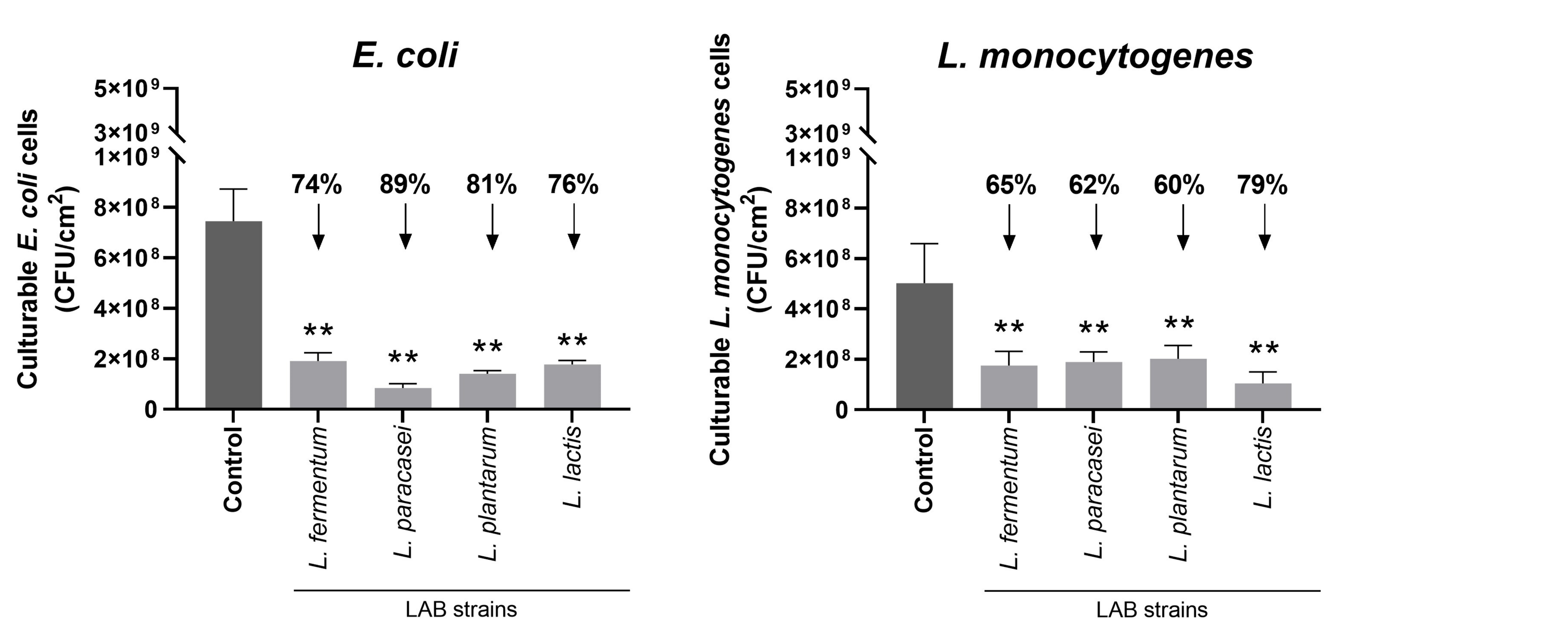


Figure 1. Culturable biofilm foodborne cells after 24 h of contact with the LAB suspensions. Data are presented as mean ± standard deviation. Asterisks denote significant differences between each treatment group and the control (** *p*-value < 0.001).

Antimicrobial effect	All tested LAB strains had a significant antimicrobial effect against ***E. coli*** and ***L. monocytogenes***, with biofilm **culturability reductions of up to 89%**. ***L. paracasei*** and ***L. lactis*** were the most promising LAB strains against *E. coli* and *L. monocytogenes*, respectively.

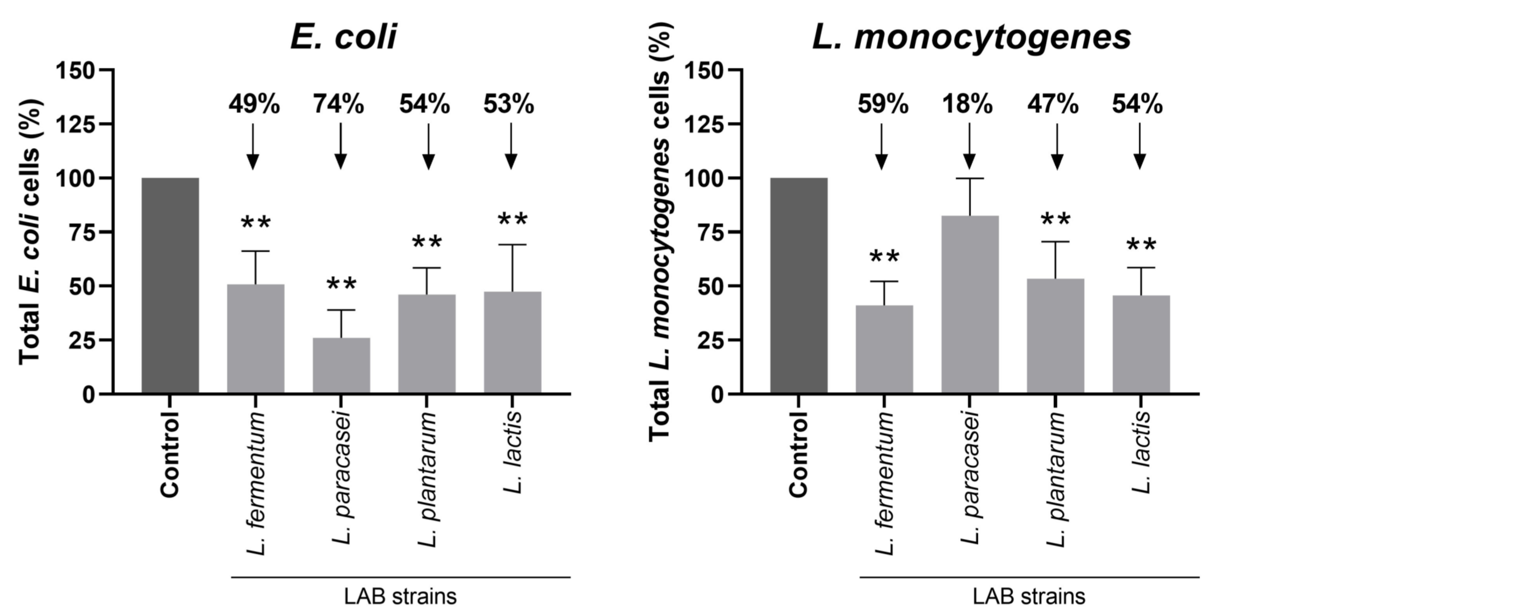


Figure 2. Percentage of total biofilm foodborne cells after 24 h of contact with the LAB suspensions. Data are presented as mean ± standard deviation. Asterisks denote significant differences between each treatment group and the control (** *p*-value < 0.001).

Antibiofilm effect	LAB displaced established biofilms; **percentage removals of 49–74%, and 18–59%** were obtained for *E. coli* and *L. monocytogenes*, respectively. ***L. paracasei*** was the most promising strain against *E. coli*; no particular LAB strain was found to be more active against *L. monocytogenes*.

LAB's antagonistic activity against pre-formed foodborne biofilms may be due to **competition for nutrients, cell-cell interactions**, and **production and release of antagonistic compounds** (such as bacteriocins, biosurfactants, organic acids, and hydrogen peroxide).

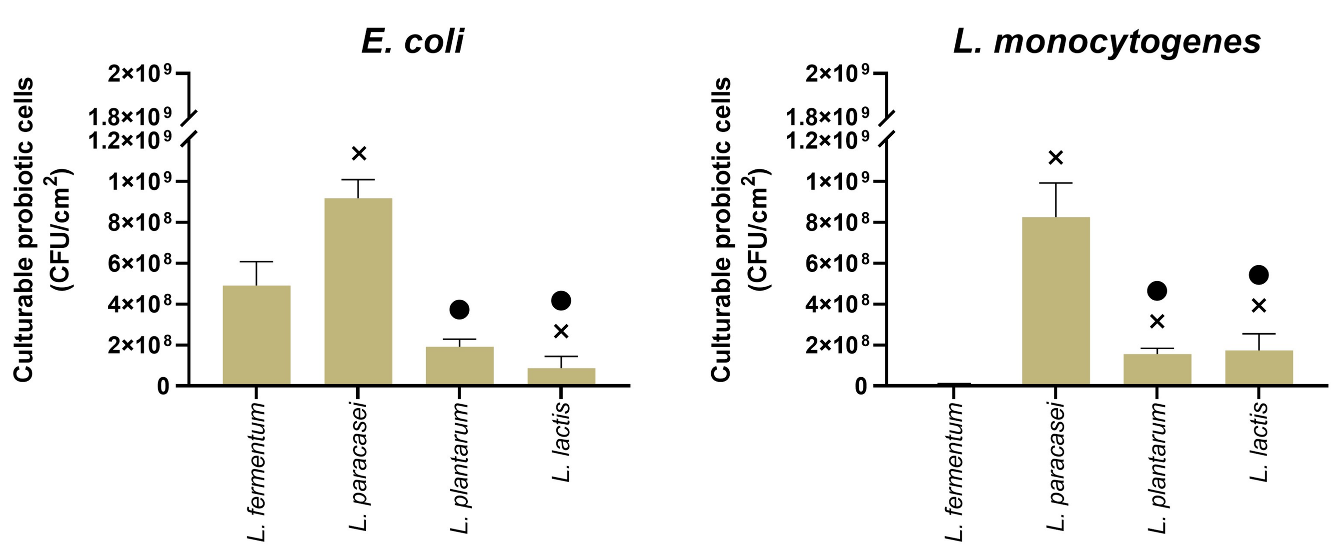


Figure 3. Culturable biofilm LAB cells after 24 h of contact with pre-formed biofilms. Data are presented as mean ± standard deviation. Significant differences are presented for *p*-value < 0.05 by ✕, ● and ■ when compared to *L. fermentum*, *L. paracasei* and *L. plantarum*, respectively.

L. paracasei showed **higher culturability**, but this probiotic displayed superior antimicrobial/antibiofilm activity only against *E. coli* ⇨ poor association between probiotics culturability and their antimicrobial/antibiofilm potential.

CONCLUSIONS

- **All probiotic strains showed antimicrobial and antibiofilm activity against *E. coli* and *L. monocytogenes* biofilms.** No particular probiotic showed a better performance under the tested conditions.
- The application of probiotics in food processing plants can reduce the occurrence of foodborne outbreaks and improve the public health in a **sustainable and environmentally friendly way**.

REFERENCES

1. Dhivya, R.; Rajakrishnapriya, V.C.; Sruthi, K.; Chidanand, D.V.; Sunil, C.K.; Rawson, A. Biofilm combating in the food industry: Overview, non-thermal approaches, and mechanisms. *J. Food Process. Preserv.* **2022**, *46*, e16282.
2. Tomé, A.R.; Carvalho, F.M.; Teixeira-Santos, R.; Burmølle, M.; Mergulhão, F.J.M.; Gomes, L.C. Use of Probiotics to Control Biofilm Formation in Food Industries. *Antibiotics* **2023**, 12, 754.
3. Merino, L.; Procura, F.; Trejo, F.M.; Bueno, D.J.; Golowczyc, M.A. Biofilm formation by *Salmonella* sp. in the poultry industry: Detection, control and eradication strategies. *Food Res. Int.* **2019**, *119*, 530-540.