Surface Conditioning Effects on Horizontal Gene Transfer

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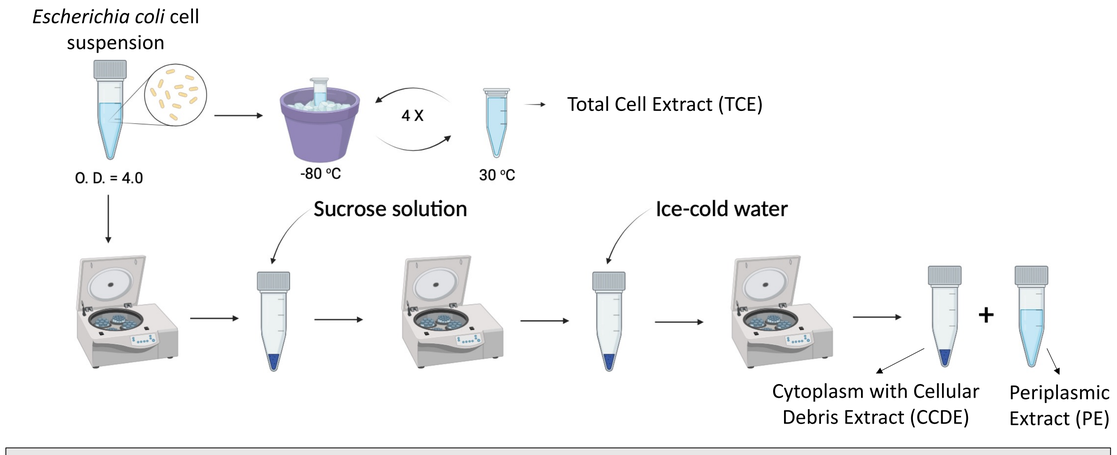
INTRODUCTION AND AIM

Biofilms are communities of microorganisms attached to surfaces held together by a self-produced extracellular polymeric matrix. They have been suggested as hot spots for **HORIZONTAL GENE TRANSFER (HGT)**, by which antibiotic resistance can be disseminated to potential foodborne pathogens[1]. HGT occurs because of physical cell-cell contact, the presence of a matrix that enhances these interactions, high cell density, and well-organised bacterial disposition within the biofilm[2]. Bacterial adhesion followed by biofilm formation starts with surface conditioning by components from culture medium or cell-derived compounds originating from cell lysis, such as individual molecules or cellular debris[3].

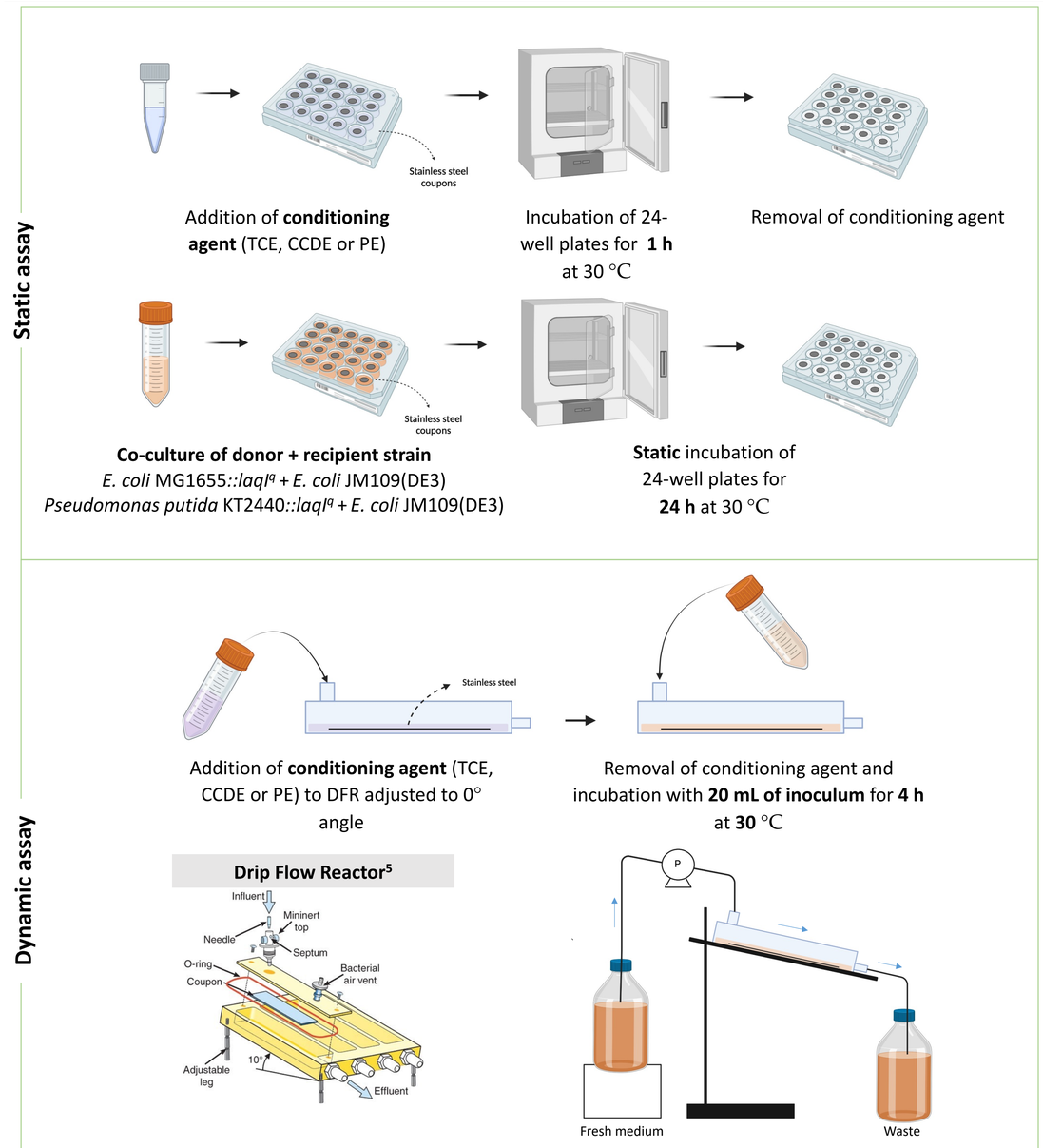
This study aims to investigate the link between HGT, preconditioned surfaces and multispecies biofilm formation.

MATERIAL AND METHODS

Preparation of surface conditioning agents[4]

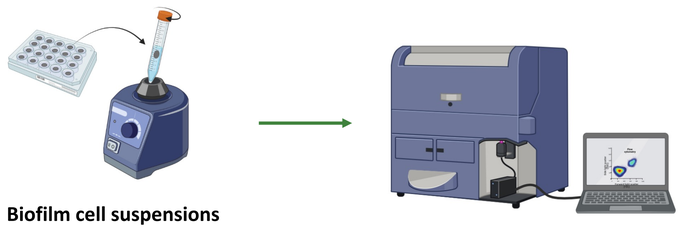


Biofilm formation



The DFR chambers were emptied, and the reactor was placed on a stand with a slight tilt of 10° to start the flow experiment. Fresh medium (1/2-strength LB) was dropped onto the slides at a constant flow rate of 0.4 mL min^{-1} per channel for 24 h at 30 °C.

Biofilm analysis by flow cytometry



Flow cytometry
Total biofilm cells
Green fluorescence detector (bandpass filter 530/30 nm) - transconjugants
Red fluorescence detector (bandpass filter 610/20 nm) - donors

RESULTS

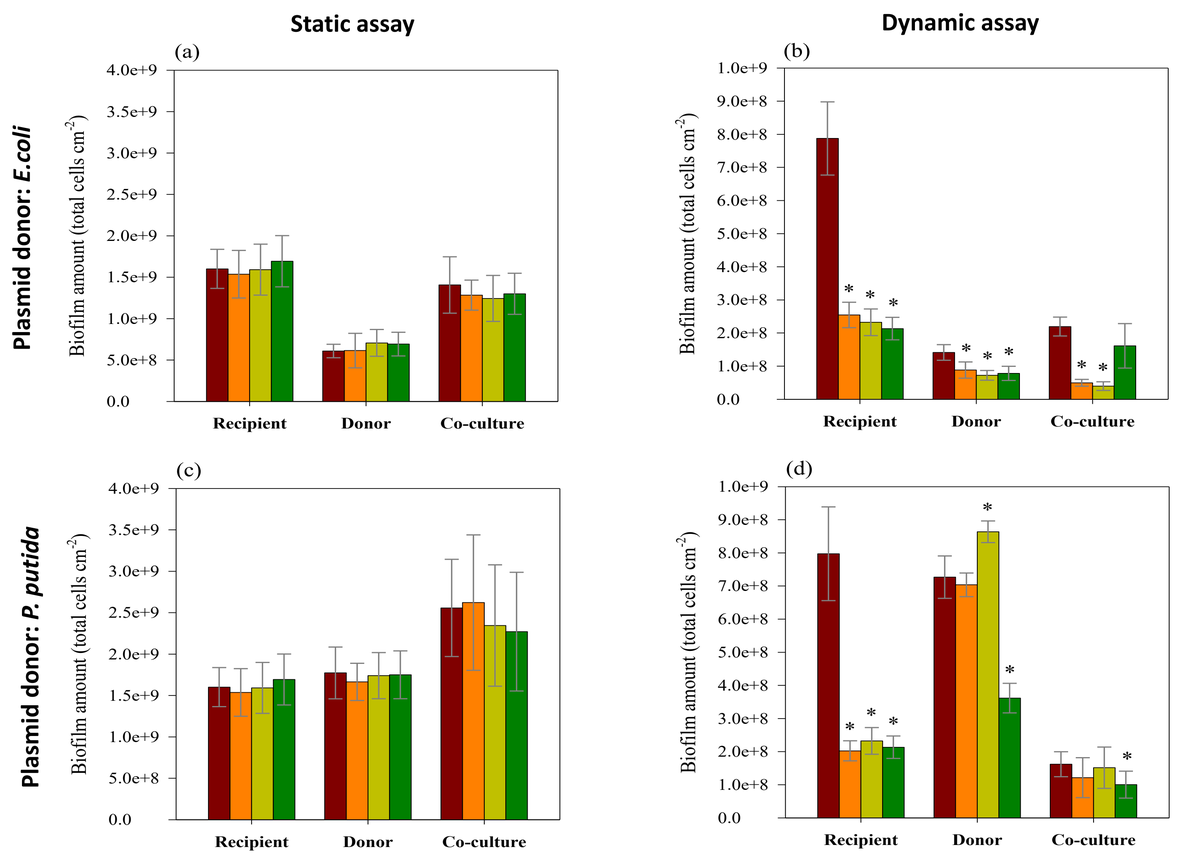


Figure 1. Cell density of single and dual species biofilms of recipient strain (*E. coli* JM109(DE3) and donor strains ((a) and (b) *E. coli* MG1655, or (c) and (d) *P. putida* KT2440) developed in24-well microplates ((a) and (c)) and drip flow reactor ((b) and (d)). The stainless-steel coupons inside both biofilm platforms were preconditioned with total cell extract (TCE,), cytoplasm with cellular debris (CCDE,) and periplasmic extract (PE,). Biofilms formed on unconditioned surfaces ( ) were used as control. (* *p*-value < 0.05).

Static conditions	The amount of biofilm formed on SST coupons was not affected by surface conditioning with cell extracts, regardless of the donor strain.
Dynamic conditions	The impact of surface conditioning was more evident. In mixed biofilms, the surfaces conditioned with TCE and CCDE reduced the number of adhered bacteria by up to 82% compared to the unconditioned surface (Figure 1b).

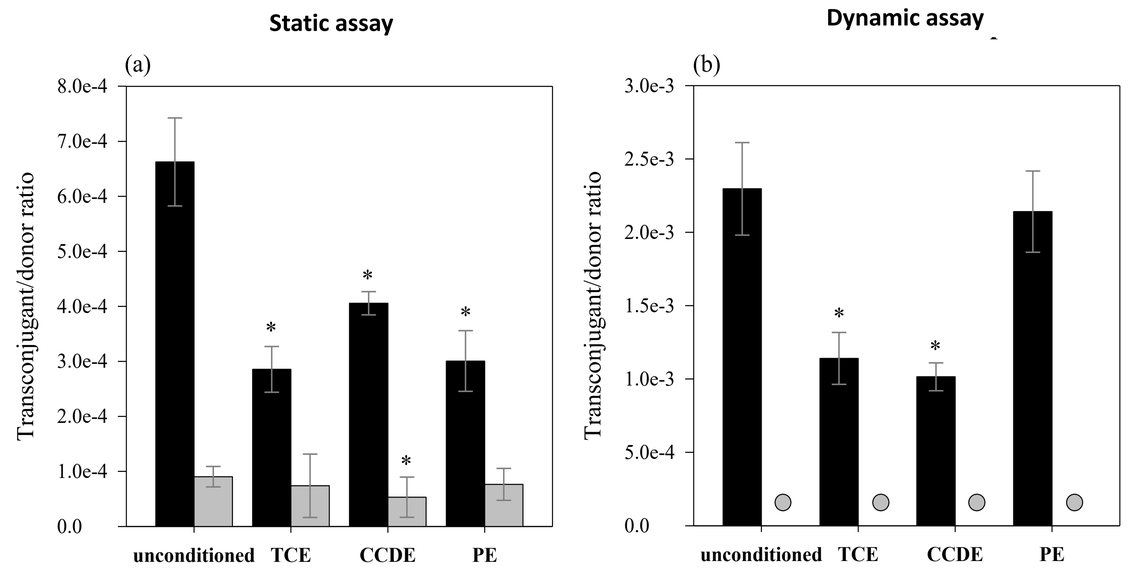


Figure 2. Transfer frequencies of plasmid pKJK5 by *E. coli* MG1655 ( ) or *P. putida* KT2440 ( ) (donor strain) to *E. coli* JM109(DE3) (recipient strain) within dual-species biofilms formed in 24-well microplates (a) and drip flow reactor (b). The stainless-steel coupons inside both biofilm platforms were preconditioned with total cell extract (TCE), cytoplasm with cellular debris (CCDE), and periplasmic extract (PE). (* *p*-value < 0.05). ● means no transconjugants were detected.

HGT	• The transconjugant/donor ratio was higher in flow than in static conditions. • No transfer of pKJK5 plasmid was detected in the drip flow system when *P. putida* was used as donor strain.

CONCLUSIONS

HGT events on food contact surfaces can lead to physiological changes in foodborne microorganisms, which can enhance their survival in an ecological niche, with consequent implications for food safety.

The safety implications of HGT in food systems need to be more thoroughly investigated, using approaches to better understand the bacterial species involved and the food ecosystems that favor the transfer.

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ACKNOWLEDGMENTS:

This work was 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); SurfSAFE project funded by the European Union's Horizon 2020 research and innovation programme under grant agreement No. 952471.