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Physiological modulations of the pathogen *Enterococcus faecalis* MMH594 by epinephrine

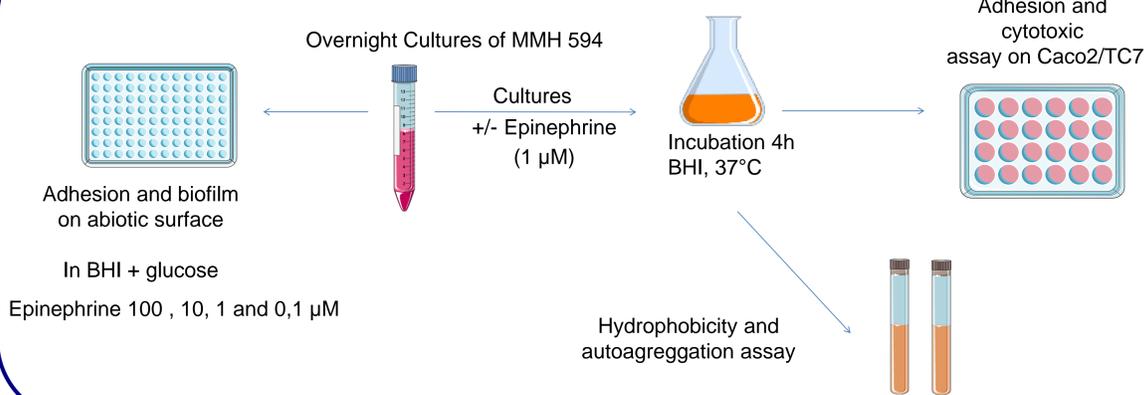
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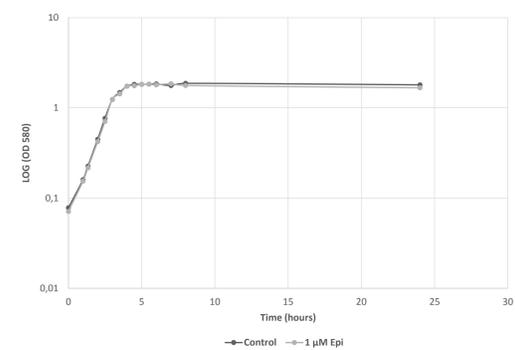
Introduction

Nowadays, hospital-acquired infections are a huge problem of public health in the world. According to the last InVS report, several micro-organisms can induce these infections as *Escherichia coli* (for 23.6% of the nosocomial cases), *Staphylococcus aureus* (13.8%), *Enterococcus faecalis* (6.5%), *Pseudomonas aeruginosa* (6.3%), and *Klebsiella pneumoniae* (5.6%). *E. faecalis* has been incriminated in endocarditis, bacteremia and urinary tract infection. It is also a bacterium which belongs to the commensal human gut microbiota, where a multitude of molecules are secreted and needed for the proper functioning of the body. Bacteria are constantly in contact with these substances, some of them can respond to these signals, by adapting their physiology and pathogenicity. This is the case of the bacteria *Escherichia coli* O157:H7, which is able to modulate its capacity to form biofilm and its mobilities as well as *Vibrio harveyi*, under exposure to epinephrine and/or norepinephrine. Bacteria are able to respond to these molecules thanks to two component systems, like QseC/QseB in *E. coli* O157:H7 or the BasS/BasR system in *Salmonella Typhimurium*. Here, we study the effect of epinephrine on growth, biofilm formation, adhesion on abiotic and biotic surfaces, hydrophobicity, autoaggregation and cytotoxicity of *E. faecalis* MMH 594.

1 Methods

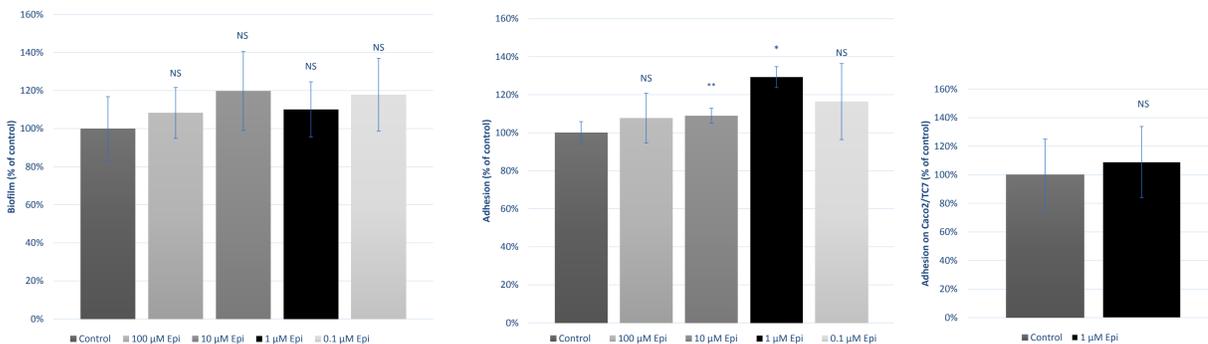


2 Bacterial growth assay



-> Exposure to 1 µM of epinephrine does not modify the growth curve of the strain *E. faecalis* MMH 594.

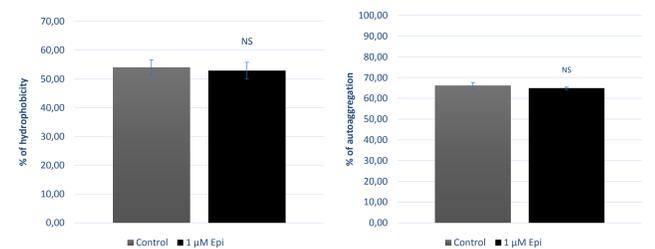
3 Biofilm and adhesion on abiotic and biotic surfaces



-> The capacity of MMH 594 to adhere on abiotic surface is increased upon exposure to 10 µM and 1 µM of epinephrine. Epinephrine does not modify its capacity to form biofilm or its adhesion towards the intestinal Caco2/TC7 cell line.

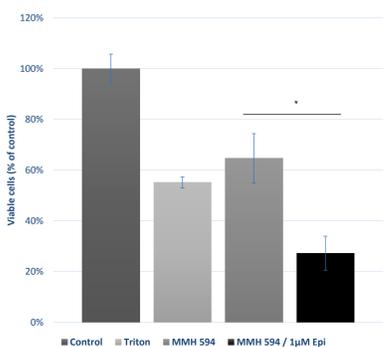
4 Hydrophobicity and autoaggregation assay

Parameters related to the capacity of adhesion of *E. faecalis*.



-> No impact of 1 µM of epinephrine on these parameters.

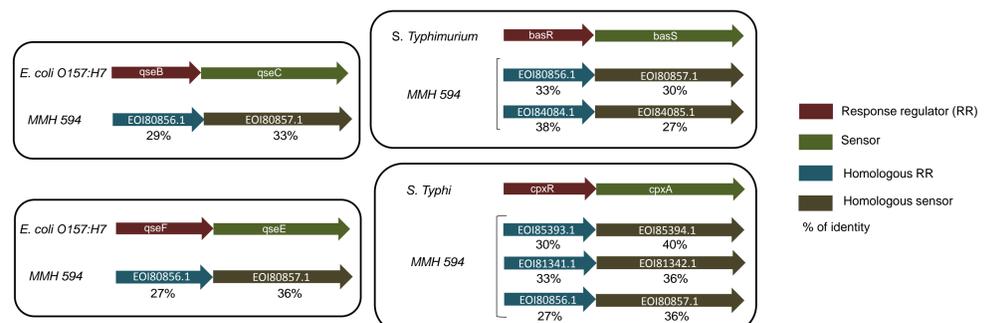
5 Cytotoxic activity/viability assay on Caco2/TC7 cells



-> The pre-treatment of MMH 594 with 1 µM of epinephrine leads to an increase of its cytotoxicity (reduction of the viable Caco2/TC7 cells)

6 Putative two component systems (TCS) homologous to epinephrine's sensor

Each sequence of TCS known to play a role in the recognition of epinephrine by three specific bacteria were blasted against the genome of the strain MMH 594. For each system known, one or more putative TCS from MMH 594 are represented.



-> The two component system EOI80856.1/EOI80857.1 is homologous to the four two component systems known to sense epinephrine in three different bacteria.

Conclusion

This study showed that epinephrine can impact some aspects of *Enterococcus faecalis* MMH 594. One micromolar of epinephrine does not modify its growth curve but can modulate its capacity to adhere on abiotic surface and its cytotoxicity towards the intestinal cell line Caco2/TC7. Epinephrine can also, at a concentration of 10 µM, increase its adhesion to abiotic surface. However, the biofilm is not impacted upon exposure to various concentrations of epinephrine as well as hydrophobicity and autoaggregation. Complementary studies like biofilm including an adhesion step are necessary, along with qRT-PCR of genes involved in adhesion. The strain MMH 594 contains many putative two component systems (TCS) that may be interesting candidates to identify the sensor able to perceive epinephrine. Molecular docking and mutants construction could help to find the sensor of epinephrine in this particular strain.

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