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Cell proliferation and cell migration are two essential processes during wound healing and development. These cellular functions are regulated by phosphoinositide 3-kinase (PI3K), and PI3K activity is modulated by the G-protein Rac GTPase. Rac GTPase signaling activates AKT, leading to a cascade of protein interactions with downstream effects that activate mTOR. Thus, PI3K activity ultimately downregulates apoptosis, and upregulates the cell cycle to increase proliferation (Rafalski and Brunet, 2011; Peltier et al, 2007). However, the role of PI3K in cell motility is not as clearly defined. What is known is that an interaction between Ras and PI3K increases cell motility, as preventing Ras binding to PI3K results in upregulation of E-cadherin expression, due to an increase in expression of the protein Reelin. This increase in focal adhesion protein decreases cell motility and migration (Castellano et al, 2016; Shujun et al, 2015). Additional data has shown that PI3K also regulates the actin cytoskeleton through regulation of the protein cofilin, thus providing an additional link between PI3K and motility machinery (Kolsh et al, 2008).

To study the effects of PI3K inhibition on cell migration and proliferation, we used cell tracking reagents, proliferation assays, and live cell kinetic assays, to perform a quantitative analysis of wound healing and closure using dermal fibroblast and epidermal keratinocyte cell models. Using these tools, we reveal that pharmacological inhibition of PI3K activity results in a dose-dependent decrease in cell migration and proliferation, thus adversely affecting wound healing rates of both cellular models.

Microbiology and Aquatic Sciences (B262)

491/B262

Flow Cytometry: A Relevant Tool to Assess the Integrity, Polarization, and Fluidity of Bacterial Cytoplasmic Membrane

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The cytoplasmic membrane is a key target for injury induced by chemical compounds during bacterial cultures. A constant intracellular environment must be kept to achieve vital cell functions, and for that, the membrane functionalities must be preserved. When the cytoplasmic membrane cannot adapt by changing its lipidic composition, bacterial cells die consequently.

Lignocellulosic biomass is an alternative to beet and wheat to produce carbohydrates for the fermentation medium. But the pre-treatment to fractionate lignocellulosic biomass into cellulose, hemicellulose, and lignin, generates phenolic compounds such as vanillin and ferulic acid, carboxylic acids, and furans, all toxic for bacterial growth. In a previous work, we proposed a method to eliminate carboxylic acids and furans, but the phenols remained within the medium. The aim of this work is to assess how phenolic compounds act on the bacterial cytoplasmic membrane.

Materials and Methods: *Strain and culture:* *Bacillus subtilis* 168 was inoculated in MBS medium (xylose 10g/L, Yeast extract 2.5g/L, Bacto Yeast nitrogen base 3.35g/L, with or without, vanillin (3.3mM) or ferulic acid (10.3mM).

Flow cytometry analysis: Bacterial cells were analyzed by flow cytometry (CyFlow Space, SYSMEX) after staining for measurements of viability/mortality (cFDA/PI), membrane polarization (DiBAC), and membrane fluidity (DPH). This last measurement was performed by fluorescence anisotropy. For that, the flow cytometer was equipped with an UV laser, vertical and horizontal polarizers, a half-wave retarder plate, and two photomultipliers for I_v and I_H.(1)

Results: With vanillin, the lag phase was extended by 2 hours, compared to the reference, but when growth began, it was similar to the reference. During the lag phase, the cytoplasmic membrane was depolarized, but the membrane polarization increased along the growth, and at last, the membrane was more polarized than the reference at the end of the culture. The membrane fluidity changed like the reference, with rigidification increasing slightly between lag phase and stationary phase.

With ferulic acid, growth began at the same time as the reference, but after 6 hours of culture the viability decreased, at 8 hours, 90% of cells had died (loss of membrane integrity), and all the cells were strongly depolarized. The membrane was more and more rigid. The fluorescence anisotropy of viable cells changed from 0.19 (4h) to 0.23 (7h), while that of dead cells was 0.25. In presence of a mix of vanillin and ferulic acid, a synergic effect on membrane fluidity was observed.

Conclusion: Vanillin and ferulic acid are known to have antimicrobial effects, and can be used as food preservatives. But mechanisms of action on bacterial cells are not known. This study intended to bring a contribution to this understanding.

Reference:

1. Bouix M., Ghorbal S. 2017. Assessment of bacterial membrane fluidity by flow cytometry. *Journal of Microbiological Methods*, 143, 50–57.

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Variation and Dynamics of the Salivary Microbiota Revealed by a Cytometric Approach

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Microbial flow cytometry is an established fast and economic technique for complex ecosystem studies and enables visualization of rapidly changing community structures, by measuring characteristics of single microbial cells. Cytometric evaluation routines are available, such as flowCyBar, which are useful for automatic data processing.

In this study, a cytometric workflow was established, which allows routine analysis of salivary microbiomes, as with this example of 10 oral healthy subjects. First, saliva was collected within a 3-month period, cytometrically analyzed, and the evolution of the microbiomes followed, as well as the calculation of their intra- and inter-subject similarity. Second, the respective microbiomes were stressed by exposition to high sugar or acid concentrations, and immediate changes were recorded. Third, bactericide solutions were tested on their impact on the microbiomes.

In all three set ups huge intra-individual variations in cytometric community structures were found to be largely absent, even under stress, while inter-individual diversity was obvious. The bacterial cell counts of saliva samples were found to vary between 3.0x10⁷ and 6.2x10⁸ cells per sample, and subject in undisturbed environments. The application of the 2 bactericides did not cause noteworthy diversity changes, but the loss in cell numbers by about 50% was high after treatment. Illumina® sequencing of whole microbiomes, or sorted sub-microbiomes, revealed typical phyla such as *Firmicutes*, *Proteobacteria*, *Actinobacteria*, *Bacteroidetes*, and *Fusobacteria*.

This approach is useful for fast monitoring of individual salivary microbiomes, and automatic calculation of intra- and inter-individual dynamic changes and variability, and opens insight into ecological principles leading to their sustainment in their individual environment.