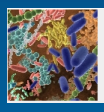
Probiotic adherence to HaCat cells *'in vitro'* and competition with pathogens

Â. Rêgo[1*], D. Moreira[1], A. Cardelle-Cobas[1], P. Gullón[1], B. Gullón[1], E. Keating[1,2], F. K. Tavaria[1]

[1]CBQF – Centro de Biotecnologia e Química Fina – Laboratório Associado, Escola Superior de Biotecnologia, Universidade Católica Portuguesa/Porto, Rua Arquiteto Lobão Vital, Apartado 2511, 4202-401 Porto, Portugal
[2]FMUP - Department of Biochemistry, Faculty of Medicine of University of Porto, Alameda Prof. Hernani Monteiro, 4200-319 Porto, Portugal
* Presenting author



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Introduction

The beneficial effects of probiotics are well studied when these are ingested. However, recently, other aspects have focused upon incorporation of probiotics into matrices that are not nutritional in nature, as for example, the skin. When the skin's barrier function is disturbed, several skin-associated disorders do occur. To date, management of most skin conditions comprises repair and protection of the skin barrier with proper hydration and topical therapy, which includes the use of moisturizers and anti-inflammatory and corticosteroid medications. However, the negative side effects associated with these treatments, have stimulated the search for a targeted and multifactorial treatment approach such as the topical application of probiotic bacteria, which has been scarcely investigated.

The aim of this reasearch work was to explore the potential topical use of probiotics to manage the proliferation of unwanted bacteria present in skin disorders. For that purpose, a human keratinocyte cell line (HaCaT cells) was used to study the adherence capabilities of two probiotic strains (*Bifidobacterium lactis* and *L. paracasei*) to keratinocytes, as well as simultaneously evaluate their ability to inhibit adhesion, by displacing, excluding or competing with pathogens (*E. coli*, *S. aureus*, *P. aeruginosa* and *P. acnes*) in cell culture.

Methods

Preparation of bacterial inocula

First pre-inocula (PI) were prepared from the culture stored at – 80 ^{0}C in cryovials, for all the bacteria (probiotic and pathogens) and put to grow at 37 ^{0}C.
From this PI, the inoculum was prepared and allowed to grow overnight also at 37^{0}C.
After this, bacterial cells were centrifuged at 5500 rpm for 10 minutes, and washed with Ringer solution. The pellets were afterwards ressuspended in half of the volume.

Adhesion assays

The HaCaT cell line (keratinocytes) was grown in a 24 well plate until confluence with DMEM medium supplemented with 10% fetal bovine serum (FBS) and 1% of Antibiotic/ antimycotic (AB/AM).
To prepare the adhesion assay, the medium had to be substituted for one without antibiotic for one hour and left in contact to clean the antibiotic influence. Cells were then washed with 200µL PBS+0.05% EDTA (twice).
Depending on the technique (Competition, Displacement or Exclusion) the order of adding probiotic or pathogen to the wells was different: for competition, 100µL of probiotic and 100µL of one pathogen were added at the same time to the well and allowed to adhere for 2 hours; for exclusion, first 100µL of probiotic was added to the well and left to adhere for 1 hour. After this, the non-adherent cells were removed and the well was washed with 200µL of PBS+0.05% EDTA. After this, 100µL of pathogen was added and one more hour was allowed to promote their adhesion to cells; for displacement, the method is similar to the exclusion technique, but here the pathogen is added first, allowed to adhere for one hour, the non-adherent bacteria were removed, the cells washed with PBS+0.05% EDTA, and then the probiotic was added and one hour allowed for adherence. After adherence is promoted for all techniques, the non adherent bacteria were removed, the wells washed with PBS +0.05% EDTA, and 1% of Triton X-100 was added at each well for 5 minutes. After this, cells were harvested and plate-counted after decimal serial dilutions.

Results

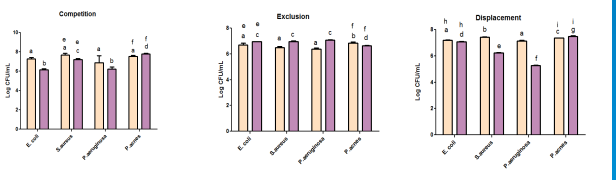

Figure 1 - Probiotic adhesion to HaCaT cells , against four skin pathogens (*E.coli, S.aureus, P. aeruginosa* and *P. acnes*), by three different techniques (Competition, Exclusion and Displacement) *Lactobacillus paracasei (L-26)* Pathogens.

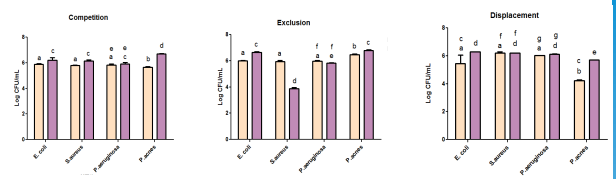

Figure 2 - Probiotic adhesion to HaCaT cells , against four skin pathogens (*E.coli, S.aureus, P. aeruginosa* and *P. acnes*), by three different techniques (Competition, Exclusion and Displacement) *Bifidobacterium lactis* (Bb12) Pathogens.

Discussion and Conclusions

Comparing the results obtained for the three techniques (percent log reduction), it is visible that the probiotic bacteria, adhere best to keratinocytes by displacement and competition, since the probiotic adhesion was higher with these two techniques. But, when looking at the three techniques individually, it is quite apparent that the probiotics act differently with each pathogen. For example, *L. paracasei* L-26, in the competition technique competes for adhesion sites with the three pathogens, with *P. acnes* being the only one that showed a little more adherence than the probiotic (7.4 vs.16.0 Log reduction, respectively). In the exclusion technique, the probiotic was excluded from their binding sites by the pathogens, but with non-significant differences. In the case of displacement, the probiotic L-26 exceeded pathogen adhesion with higher values for *S. aureus* (32.1) and for *P. aeruginosa* (42.9). This is a very important result these are considered major pathogens involved in skin infections.

For *B. lactis* Bb12 in the competition technique, probiotic and pathogen had almost the same adhesion capability to the cells. But for the exclusion technique, in the case of *S. aureus,* it is visible that the probiotic showed a considerably higher adhesion to cells than the pathogen (32.9 vs. 24.7). Again, since *S. aureus* is often associated with a lot of skin infections it is important to detect bacteria that are capable of decreasing their adherence and/or infection and pathogenic action, like *B. lactis*. For displacement, it is visible that both probiotic and pathogens showed the same kind of adhesion, probably because probiotic bacteria were able to displace some of the pathogens by competing for the binding sites. It is also interesting to note that for *B. lactis* (with all techniques), *P. acnes* always displayed lower adhesion values than the probiotic (for competition 20.4 vs. 36.4) ; for exclusion 19.2 vs. 27.2; and for displacement 32.1 vs. 52.6).

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