# Probiotic adhesion to skin keratinocytes and underlying mechanisms

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## Abstract

The beneficial effects of probiotics on the digestive system are well known, however, several probiotic benefits resulting from their topical application have recently been investigated. Improvements in different skin disorders such as atopic dermatitis, acne, eczema and psoriasis have been reported related to their topical use. One of the mechanisms through which such benefits are documented is by inhibiting colonization by skin pathogens.

Invasion and adhesion studies have been carried out using keratinocytes showed that the pathogenic bacterium *Escherichia coli* is not able to invade skin keratinocytes, but adhered to them. *Lactobacillus rhamnosus* and *Propioniferax innocua* decreased the viable counting of pathogenic bacteria *E. coli*, *Pseudomonas aeruginosa*, and *Staphylococcus aureus*. *L. rhamnosus* inhibited *S. aureus* adhesion significantly when compared to the control ($P < 0.01$). On the other hand, the probiotic *L. delbrueckii* also revealed the best results for *S. aureus*, however, with no significant differences in relation to control ($P > 0.05$). Contrarily, *P. innocua* did not inhibit pathogenic bacteria adhesion, but when added simultaneously with *S. aureus* (competition assay) a significant adhesion reduction ($1.12 \pm 0.14$ $log_{10}$CFU/mL) was observed. Probiotic bacteria seem to adhere to the keratinocytes through carbohydrates, while *S. aureus* uses proteins to adhere to keratinocytes.

*L. rhamnosus* showed promising results in pathogen inhibition both *in vitro* and *ex-vivo* experiments and can potentially be used as a co-adjuvant in the treatment of skin dysbiosis.

## Introduction

The main function of the skin is to act as a physical barrier for the protection of the body against pathogenic organisms or toxic substances (Chiller, Selkin and Murakawa, 2001). Being constituted by three major layers: the dermis, the epidermis and the hypodermis, the biggest organ in the human body, plays a crucial role in protecting against external damage.

The skin is an ecosystem that supports the growth of indigenous microbiota that can be influenced by diverse host factors, such as skin site, sex, immune status and skin disease. Besides that factors, it is clear that genetics influence the presence of microorganisms on the skin (Egert and Simmering, 2016; Grogan *et al.*, 2019).

Although over time, various definitions for probiotics have appeared the most accepted definition nowadays appeared in 2001, from FAO-WHO, that defined probiotics as "live microorganisms, which when administrated in adequate amounts confer a health benefit in the host", not restricting the application of the term only to oral probiotics with results at the gut level (World Health Organization Food and Agriculture Organization and Nations, 2006; Cinque *et al.*, 2017).

Probiotics can be live bacteria or even yeasts; among the most used are LAB species (*Lactococcus*, *Lactobacillus*, *Streptococcus* and *Enterococcus*) and *Bifidobacterium* (Tsiouris and Tsiouri, 2017; Silva *et al.*, 2020).

These microorganisms can provide beneficial effects to healthiness through regulation of the microbiome and performing biological functions while colonizing the host (Silva *et al.*, 2020).

Some clinical trials suggest that probiotics do not exert their beneficial effects only by the gastrointestinal route but also through topical applications. This administration route shows a direct effect on the application site through the induction of natural defense mechanisms (Al-Ghazzewi & Tester, 2014). Revealing promising results in the treatment of various skin diseases as atopic dermatitis, wound healing, acne, reactive skin and aging skin.

## Methodology

The probiotic strains utilized in this study were, *Lactobacillus delbrueckii* subsp. *bulgaricus* 20081, *Lactobacillus rhamnosus* 20021 and *Propioniferax innocua* 8251 and the pathogenic bacteria used were *Pseudomonas aeruginosa*, *Staphylococcus aureus* and *Escherichia coli*. The probiotic strains have the same origin, DSMZ (Deutsche Sammlung von Mikroorganismen und Zellkulturen, Braunschweig, Germany), the pathogenic strains of *E. coli* and *P. aeruginosa* are originated from the international collection of CINATE (Centre for Innovation and Technological Support), and *S. aureus* from CBQF (Centro de Biotecnologia e Química Fina) collection.

**Probiotics adaptation to skin conditions**

- pH (3, 4, 5, 6 and 7)
- Temperature (18, 25, 37 and 45°C)
- Lipids (palmitic acid and linoleic acid)
- NaCl (10, 20, 40, 60 and 80mM)

**Cell Culture assays (HaCaT)**

- UV-radiation
- Invasion
- Adhesion (displacement, competition and exclusion)

**Human skin equivalents with *S. aureus* and *L. rhamnosus***

- Bacterial counting
- Macroscopic monitorization

## Results

In invasion assays with HaCaT cells the pathogenic *Escherichia coli* was not able to invade skin keratinocytes.

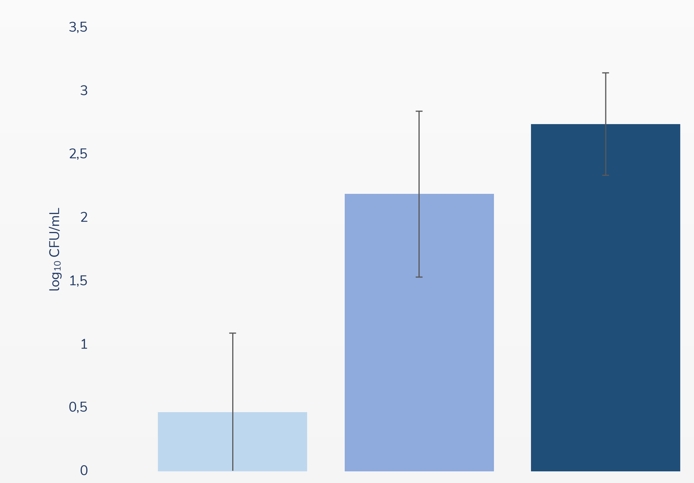

**Figure 1-** Pathogenic bacteria invasion of HaCat cells; *Escherichia coli* (■), *Pseudomonas aeruginosa* (■), and *Staphylococcus aureus* (■). Error bars are ± standard deviation.

In adhesion assays with HaCaT cells *Lactobacillus rhamnosus* and *Propioniferax innocua* decreased the viable counting of pathogenic bacteria *Escherichia coli*, *Pseudomonas aeruginosa*, and *Staphylococcus aureus*

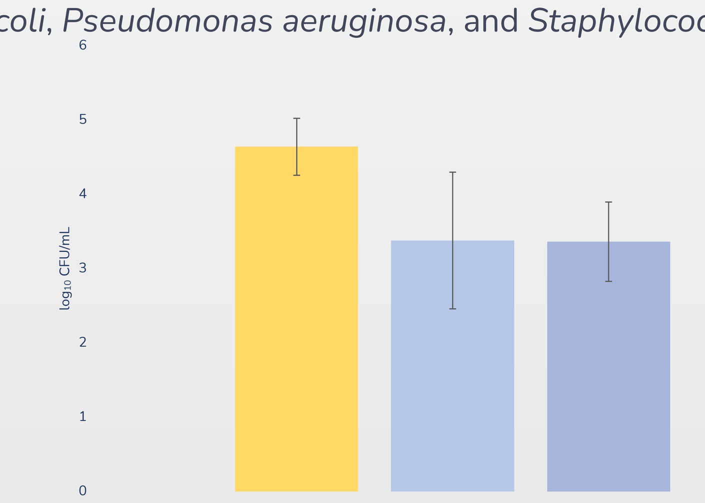

**Figure 2-** HaCaT cell adhesion by *Pseudomonas aeruginosa* in the presence of probiotics. *P. aeruginosa* adhesion control (■), competition assays with *L. rhamnosus* (■) and competition assays with *P. innocua* (■). Error bars are ± standard deviation.

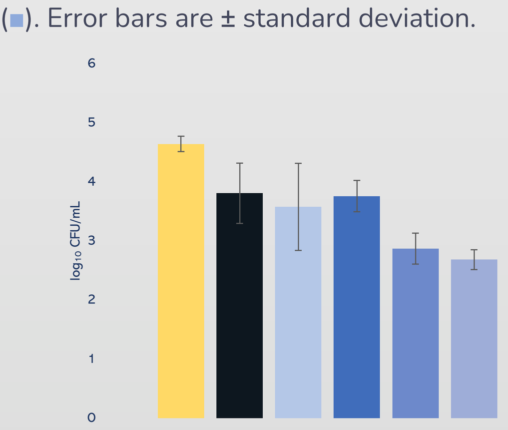

**Figure 3-** HaCaT cell adhesion by *Escherichia coli* in the presence of probiotics. *E. coli* adhesion control (■), displacement assays with *L. rhamnosus* (■), displacement assays with *L. bulgaricus* (■), displacement assays with *P. innocua* (■), competition assays with *L. rhamnosus* (■) and competition assays with *P. innocua* (■) . Error bars are ± standard deviation.

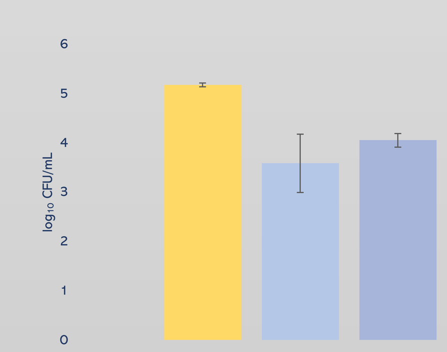

**Figure 4-** HaCaT cell adhesion by *Staphylococcus aureus* in the presence of probiotics. *S. aureus* adhesion control (■), displacement assays with *L. rhamnosus* (■) and competition assays with *P. innocua* (■). Error bars are ± standard deviation.

*L. rhamnosus* showed promising results in pathogen inhibition both *in vitro* and *ex-vivo* experiments.


**Figure 5-** Macroscopic photo images of the human skin equivalents during the experiment, following the wound healing process in the skin models.

## Conclusion

The probiotics *Lactobacillus rhamnosus*, *Lactobacillus delbrueckii* subsp. *bulgaricus* and *Propioniferax innocua* were able to successfully grow in skin-like conditions;

Probiotics from the *Lactobacillus* genus proved capable to invade the keratinocytes more effectively than all the pathogenic bacteria tested;

Probiotics can decrease pathogenic adhesion in some circumstances and pathogen also can affect the adhesion of probiotics to keratinocytes;

The mechanisms behind bacterial adhesion to keratinocytes were explored, concluding that probiotics could adhere through carbohydrates and the pathogen *S. aureus* utilizes proteins, generally named as adhesins;

In the *ex-vivo* assay, significant differences were detected between the skin model infected only with the pathogen and the model infected with the pathogen and the probiotic;

The findings described in this research show that the studied probiotics could be used topically with relevant pathogen inhibition, representing an important adjuvant in the clinical approach to treat patients with skin dysbiosis such as, atopic dermatitis, acne or conditions leading to cutaneous infections, as complicated wounds.

References:

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