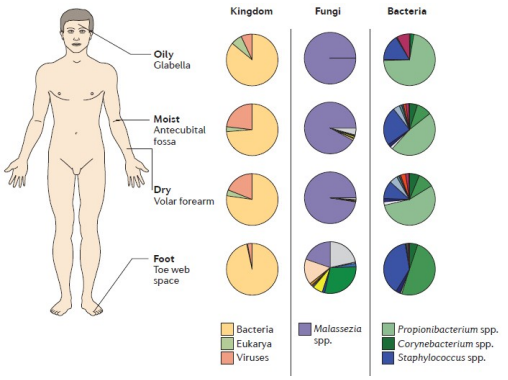
# Microbiota co-culture models for cosmetics ingredients evaluation: Metabolism and skin adhesion studies

Benoît ROUBINET, Mateja SENICAR, Lisa RUBIO and Ludovic LANDEMARRE

## Introduction

The cutaneous microbiota is composed of numerous microorganisms that are distributed in well-defined balance which depends on their environments (*e.g.* dry, sebaceous, and moist) and localizations on the skin. The symbiosis of micro-organisms with skin cells is the key for ensuring functionality of biological skin barrier thus preventing pathogen colonization, microbiota equilibrium and appropriate immune response to maintain healthy skin.[1,2]

Here we present two complementary methods allowing to study the action of cosmetics ingredients on microbiota: **co-culture microbiota metabolism and modulation of the microbiote-skin adhesion.**

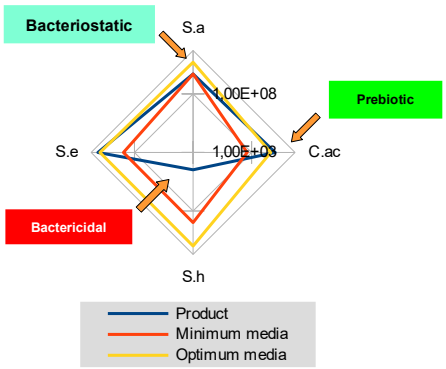



## Microbiota metabolism

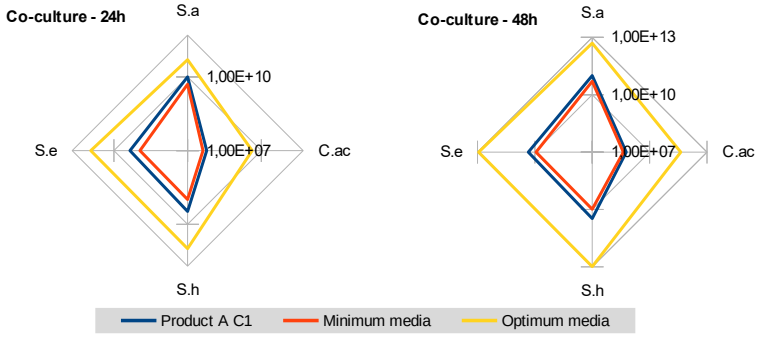
Models of microbiota in co-culture (standardized or customized), with 4 to 5 different strains selected according to the skin area composition (see above), were developed **to study the activity of a product on the growth and or the decrease of each strain living together in the same conditions**.

**Method:**

- Microorganisms cultivated in a **same liquid culture media based on minimum nutriments**, that allows to keep a slow growth of each strain in desired proportions and culture conditions (pH, temperature, humidity, oxygen).
- Microorganisms **from collections or skin wild type strains** (collected from healthy skin donors – GLYcoDiag bank).
- Proportions mimicking eubiosis equilibrium or dysbiosis related to specific cutaneous diseases.
- Standard counting performed at 24h and 48h by plating on selective agar to evaluate the residual microbial population.
- Data analysis: Residual population of each strains obtained with product are compared with the optimum & minimum media in absence of product to conclude on the product effect: prebiotic, bacteriostatic or bactericidal.




**Results:** Evaluation of product A on standard model (*S.aureus, S.epidermidis, S.hominis, C.acnes*) at 24h and 48h.




Product A have the same growth than minimal co-culture media *(No real decrease or growth of bacterial populations at 24/48 h).*
**Product A has a "Microbiote Friendly" effect profile.**

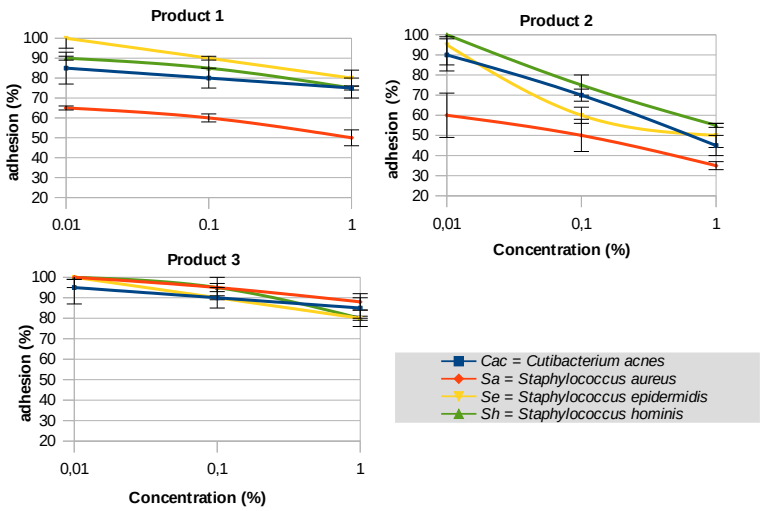
## Microbiota-skin adhesion

The surface of all cells (eukaryotes and prokaryotes) is composed of different glycans structures and conversely glycans binding proteins which interact each others. These glycobiological interactions initiate the installation of a microbial population on the skin and enable the identification of pathogen by immune system. Based on these considerations, we developed *in vitro* **models with one to four different strains to study the effect of product on microbiota skin cells adhesions.**

**Method:**


- Cells: corneocytes obtained after collection from the arm of volunteers with D-squams® disks, or keratinocytes obtained after primary cells culture.
- Products effect on adhesion are measured as follow:
  1. Incubation of products on cutaneous cells,
  2. Washing of cells to remove product excess,
  3. Incubation with selected strain (labeled with fluorescent dye)
  4. Removal of non adherent microbial cells
  5. Measurement with microplate reader.
- Data analysis: The fluorescent values obtained with product are compared with the absence of product and translated in % of adhesion.

**Results:** Examples of results obtained with polysaccharides




**Product 1 :** Specific decrease of Sa adhesion (around 40%)
**Product 2 :** All strains show a decrease of adhesion (over 40 % at 1%)
**Product 3 :** No influence on corneocytes microbial adhesions

## Conclusions

**Microbiota co-culture models are totally dedicated to the research of prebiotics, "microbiote friendly" or antimicrobial products, allowing to measure the effects of cosmetics ingredients on the differential growth or decrease of mixed bacterial strains.**

**Microbiota-skin cells adhesion is complementary and devoted to highlight differential strains adhesion after application of cosmetics ingredients.**

Bibliography:
1. Byrd, A. L., Belkaid, Y., Segre J. A. *The human skin microbiome, Nat. Rev.* **2018**, *16*, 143-155.
2. Grice, E. A., Segre J. A. *The skin microbiome, Nat. Rev.* **2011**, *9*, 244-253.

**GLYcoDiag**
Orléans – France
Phone: +33 (0) 9 72 50 13 36
e-mail : contact@glycodiag.com
web: www.glycodiag.com