Models to study optimized combinations of physiological biotics for acne-prone skin

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Introduction:

Skin microbiota is involved in skin homeostasis and protects the skin against colonization by pathogenic microorganisms [1].
However, under certain conditions, perturbation of the skin microbial balance could occur and may lead to harmful conditions like **acne disorders**.
In acneic prone skin, ***Cutibacterium acnes* virulent** strains increase and cause **inflammation and redness**.
However, some skin "protectors" such as *Staphylococcus epidermidis* can protect the skin against the opportunistic pathogens [2]. This bacterium can notably use fermentation initiators (FIs) to produce short chain fatty acids (SCFAs) reducing the growth of *C. acnes* [3].
To better study and optimize this specific potential of *S. epidermidis* against *C. acnes*, **a bacterial and 3D microbiotic skin model** was developed to evaluate the **bacterial interactions and the effect of biotic ingredients**. To carry out these models, two bacteria were chosen: *S. epidermidis* strain ATCC 12228, a reference commensal strain [4] and *C. acnes* ATCC 6919, a bacterium isolated from acne lesions on human facial skin [5].

Materials & Methods:

- **Bacterial preparation**

S. epidermidis strain ATCC 12228 was inoculated in Tryptic Soy Broth medium (TSB) at 37 °C for 24h under continuous stirring at 150 rpm in aerobic condition. *C. acnes* ATCC 6919 was inoculated in Schaedler medium at 37 °C for 72h without stirring in anaerobic condition.

- **Bacterial growth in monoculture model**

Bacterial suspensions were prepared by adjusting the concentration of *S. epidermidis* at OD =0.05 at 600nm and at OD =0.3 for *C. acnes* in fresh medium diluted halfway with Phosphate Buffer Saline (PBS) in 96 well plates, with or without biotic ingredients (FI + SCFA) at 0.2 % and 2%. The bacterial growth was measured by densitometry (OD with a spectrophotometer) after 24H in aerobic condition for *S. epidermidis* or 72h in anaerobic condition for *C. acnes* (n=4).

- **Bacterial growth in coculture model**

Mixt bacterial suspensions were prepared by adjusting the concentration of both bacteria at 1.10^6 in TSB medium. The coculture was incubated in 37°C for 72h without stirring in anaerobic condition, with or without biotic ingredients in 2% (FI + SCFA). To count *S. epidermidis*, serial dilutions were spread in TSB agar plates and incubated 24h in aerobic condition; To count *C. acnes*, serial dilutions were spread on TSB agar plates supplemented by furazolidone and incubated 72h in anaerobic condition (n=3).

- **Bacterial growth in coculture in 3D microbiotic skin model (RHE)**

Keratinocytes (15 yo donor) were grown on the top of an insert for 4 days of submerged culture in DMEM/HAM-F12 medium with hydrocortisone 0.4µg/mL, insulin 4.16µg/mL, ascorbic acid 50µg/mL, EGF 10ng/mL and 5% of Bovine Calf Serum (BCS) before elevation at the air-liquid interface for 7 days in the same medium except for BCS at 1% and without EGF.
Reconstructed Human Epidermis (RHE) were infected by *S. epidermidis* or *C. acnes* separately or combined at 10^5 *and* 10^7 respectively. After 1 hour, samples were rinsed with PBS and then cultured up to 3 days post-infection. The efficiency of bacterial adhesion with biotic ingredient at 2% (FI + SCFA) was estimated using the colony forming unit assay at 1 hour post infection to evaluate adhesion and 72 hours post-infection for growth (n=3).

Results & Discussion:

Monoculture

S. epidermidis + **FI + SCFA at 2%** ⇨ Dose-dependent prebiotic effect **(+41%)**

C. acnes + **FI + SCFA at 2%** ⇨ No direct inhibitory effect

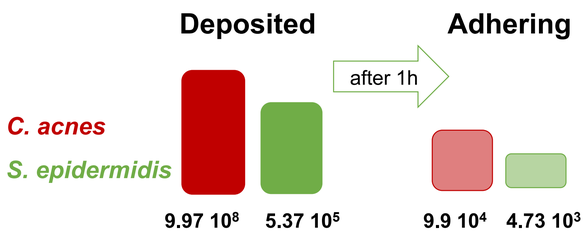
Coculture

S. epidermidis + **FI + SCFA at 2%** ➔ Increase production of succinic acid (**x2.3**)

S. epidermidis + *C. acnes* ➔ Moderately reduced *C. acnes* growth

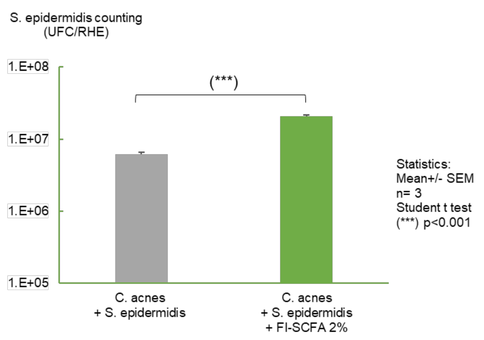
S. epidermidis + *C. acnes* + **FI + SCFA at 2%** ➔ Efficiently decreased *C. acnes* growth by **-65%**

3D microbiotic epidermal model



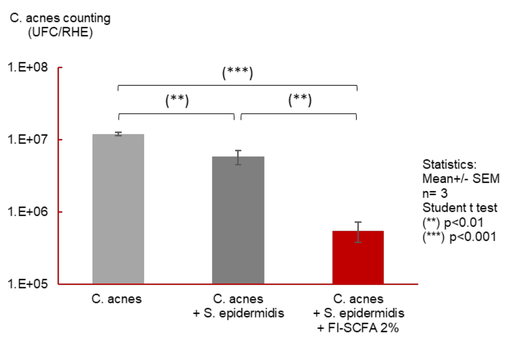
Final ratio
➔ Higher proportion of *C. acnes* compared to the *S. epidermidis* ($10.\ 10^4/5.\ 10^3$)
➔ Mimicking the ratio of acneic prone skin

S. epidermidis growth



With FI + SCFA
➔ Increase of *S. epidermidis growth*
➔ Prebiotic effect on *S. epidermidis*

C. acnes growth



With FI + SCFA
➔ Decrease of *C. acnes growth*

Conclusions:

From a simple model in monoculture to a more complex model of a co-culture of bacteria infecting a RHE, the **natural protective effect of *S. epidermidis* inhibiting *C. acnes* growth has been demonstrated**. All these models have also allowed to **select and develop biotic ingredients** improving those beneficial effects.
Studying the interactions between commensals and opportunistic pathogens helped us to design actives **inspired by the natural ecology of the skin microbiota** to promote the natural mechanisms of skin defenses.

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References:

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