The importance of taking into account Cutibacterium acnes ribotype diversity in acne-prone skin

BI_381

Danoux, Louis*; Gault, Manon; Andre, Valerie
BASF Beauty Care Solutions France
* danoux.louis@basf.com

Introduction:

Recent data has established the presence of different phylogenic groups (phylotype I to III) among the *Cutibacterium acnes* population. Based on 16S rRNA gene analysis, *C. acnes* strains were divided into ribotypes (RTs), which could be used to differentiate between phylotypes I, II, and III, associated with healthy skin or acne [1].

In acne-prone skin it is now well established that the relative abundance of the *C. acnes* **phylotype I is increased while phylotype II is decreased** [2]. This modification of phylotype balance in *C. acnes* population could worsen cutaneous acneic lesions as a stronger interaction of phylotype I than phylotype II of *C. acnes* was demonstrated with human keratinocytes and sebocytes cultured *in vitro* [3].

Thus, we wanted to deeper investigate the difference between phylotype I and II of *C. acnes* by evaluating the release of virulence factors such as lipase and hyaluronidase, the activation of macrophages as well as the sensitivity of *C. acnes* to antimicrobial peptides (AMP).

Materials & Methods:

Bacterial strains used for all experiments
C. acnes ATCC6919 (phylotype IA-1) and ATCC11828 (phylotype II)

Release of virulence factors
C. Acnes were incubated within TSB-F broth at 37°C, for 3 days in anaerobic conditions. Then bacteria density was recorded by optical density (OD) at 600 nm, while enzymatic activities were evaluated in supernatant broth after its centrifugation for 10 minutes at 1500 g.
Lipase activity was evaluated by a fluorescent substrate while hyaluronidase activity was measured by determining the amount of degraded hyaluronic acid through a turbidimetric method.
Well established inhibitors were used in dose effect for these methods: respectively epigallocatechin gallate (EGCG) for lipase activity and glycyrrhizic acid for hyaluronidase activity.
Enzymatic activities are expressed referring to bacterial density.

Activation of Macrophages
Human macrophages (U937 cell line) were primarily activated by incubation with phorbol-myristate-acetate (PMA) for 2 days at 37°C and then were activated by *C. acnes* strains. In this aim, macrophages were incubated with a determined amount of heat-killed bacteria for 2 days at 37°C.
Finally, cell viability of macrophages was measured by MTT test while the released cytokines were evaluated by ELISA method (IL6 and IL8) on cell culture supernatants. Cortisol, as well-established inhibitor, was used in dose effect.

Sensitivity to AMP
C. acnes were incubated with a range of AMP concentrations diluted in TSB-F broth. The bacteria densities were recorded by measuring the optical density (OD) at 600 nm after an incubation at 37°C, for 3 days in anaerobic conditions.

Effect of active ingredients
Three distinct plant extracts were evaluated on bacterial density, lipase activity or macrophage activation.

Acknowledgments:

We would like to thanks the expert assistance of Mrs. Lydie Martin-Teixeira, Lucile Deposito and Aurélie Courtois.

Results & Discussion:

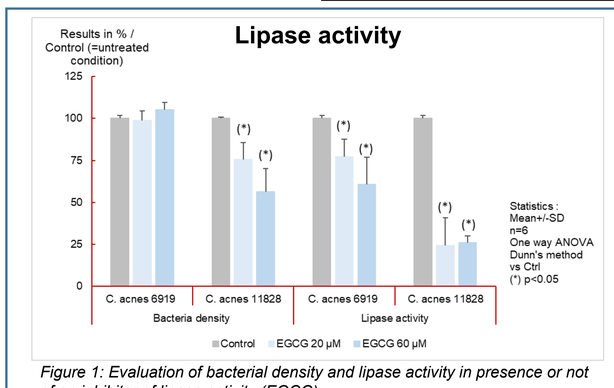

Figure 1: Evaluation of bacterial density and lipase activity in presence or not of an inhibitor of lipase activity (EGCG).

With EGCG:
- Higher growth decrease and lipase inhibition for ATCC11828

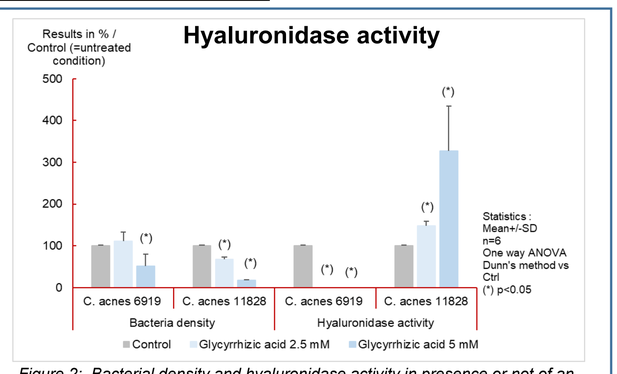

Figure 2: Bacterial density and hyaluronidase activity in presence or not of an inhibitor of hyaluronidase activity (glycyrrhizic acid).

With Glycyrrhizic acid:
- Higher growth decrease of ATCC11828
- Hyaluronidase inhibition on ATCC6919
- Hyaluronidase stimulation on ATCC11828

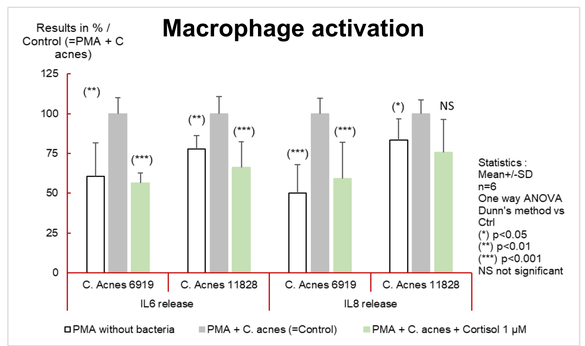

Figure 3: Evaluation of cytokine release in C. acnes-activated human macrophages in the presence or not of an inhibitor (Cortisol).

- IL6 & IL8 more increased by ATCC6919
- Higher inhibition of cortisol effect by ATCC6919

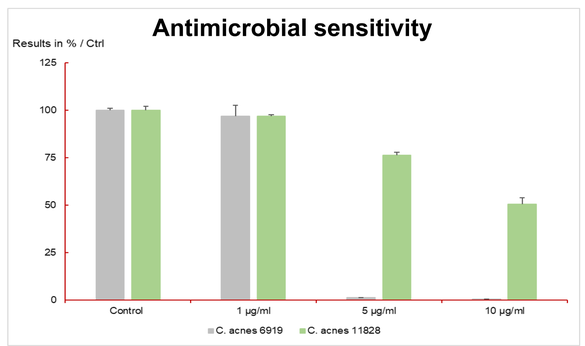

Figure 4: Effect of antimicrobial peptide (AMP) on C. acnes densities.

- Sensitivity to AMP of both strains
- Higher inhibition of ATCC6919

Evaluation of active ingredients

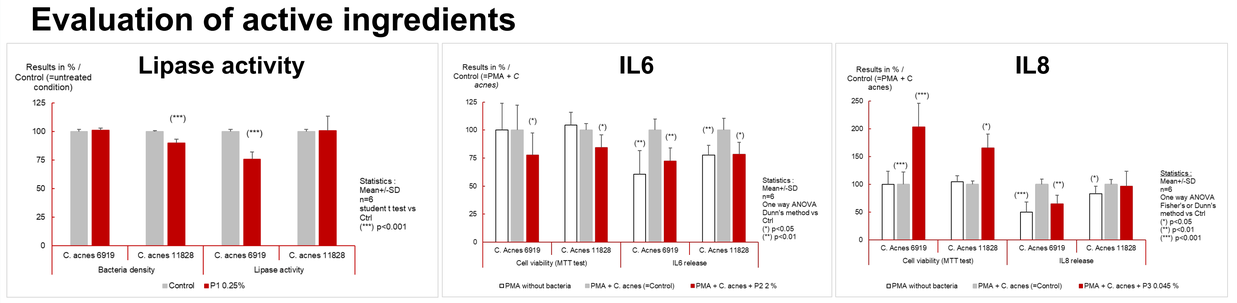

Figure 5: Effect of 3 plant extracts on bacterial growth, lipase activity and macrophage activation.

Summary

	P1		Activated macrophages			
			P2		P3	
	Density	Lipase	Viability	IL6	Viability	IL8
ATCC11128	-10% (***)	+1%	-22% (**)	-22% (*)	+65% (**)	-3% (*)
ATCC6919	+1%	-24% (***)	-39% (**)	-28% (**)	+103% (**)	-35% (***)

Figure 6: Comparison of plant extracts efficacies on the two C. acnes strains.

Three active ingredients have demonstrated their capacity to act differently with the two *C acnes* strains

Conclusions:

We have established that the *C. acnes* effects are dependent on the strain.
ATCC6919 *C. acnes* strain (phylotype IA-1 more prevalent in acne-prone skin) has induced at a higher level the hyaluronidase release and the macrophages activation but was more sensitive to AMP.
Moreover, the impacts of well-established antimicrobial molecules such as EGCG and Glycyrrhizic acid or AMP were also largely different referring to *C. acnes* strain.
To conclude, thanks to these experiments, we evidenced that to improve the signs of acne-prone skin, it seems crucial to evaluate the active ingredients on various strains of *C. acnes* and to target relevant virulence factors in order to have a more precise scope of their efficacy.

References:

1. Fitz-Gibbon S, et al.(2013) Propionibacterium acnes strain populations in the human skin microbiome associated with acne. J Invest Dermatol. 133:2152-60.
2. Dagnelie MA, Corvec S, Saint-Jean M, Bourdès V, Nguyen JM, Khammari A, Dréno B (2018) Decrease in Diversity of Propionibacterium acnes Phylotypes in Patients with Severe Acne on the Back. Acta Derm Venereol 98:262-267.
3. Spittaels KJ, et al (2020) Cutibacterium acnes Phylotype I and II Strains Interact Differently With Human Skin Cells. Front Cell Infect Microbiol. 16;10:575164.
4. Fournière M, et al (2020) Staphylococcus epidermidis and Cutibacterium acnes: Two major sentinels of skin microbiota and the influence of cosmetics. Microorganisms 8:1752.
5. Kistowska M, et al (2014) IL-1b Drives Inflammatory Responses to Propionibacterium acnes In Vitro and In Vivo. J Invest Dermatol 134:677–685.
6. Bagatin E, et al (2019) Adult female acne: a guide to clinical practice. An Bras Dermatol 94:62-75.
7. Contassot E, et al (2018) Propionibacterium acnes strains differentially regulate the fate of Th17 responses in the skin. J Invest Dermatol 138:251-253.