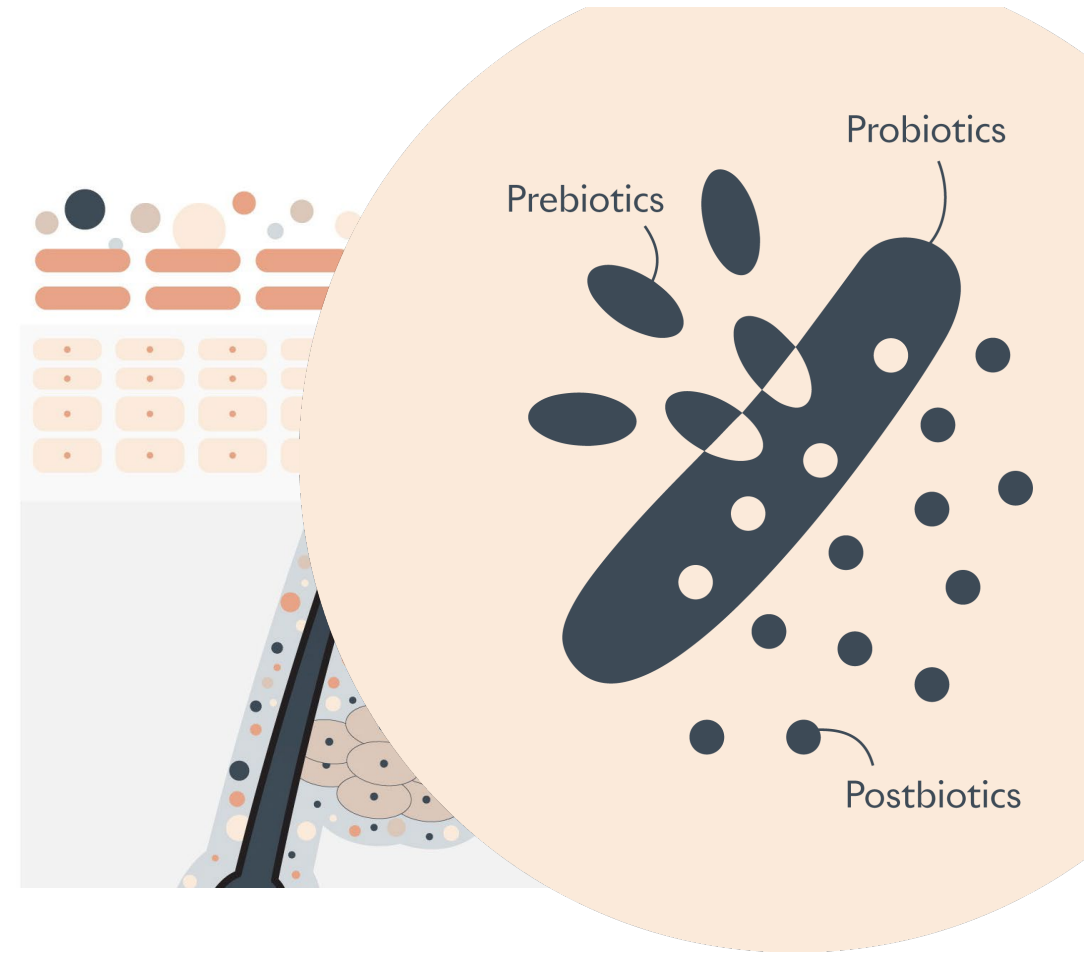


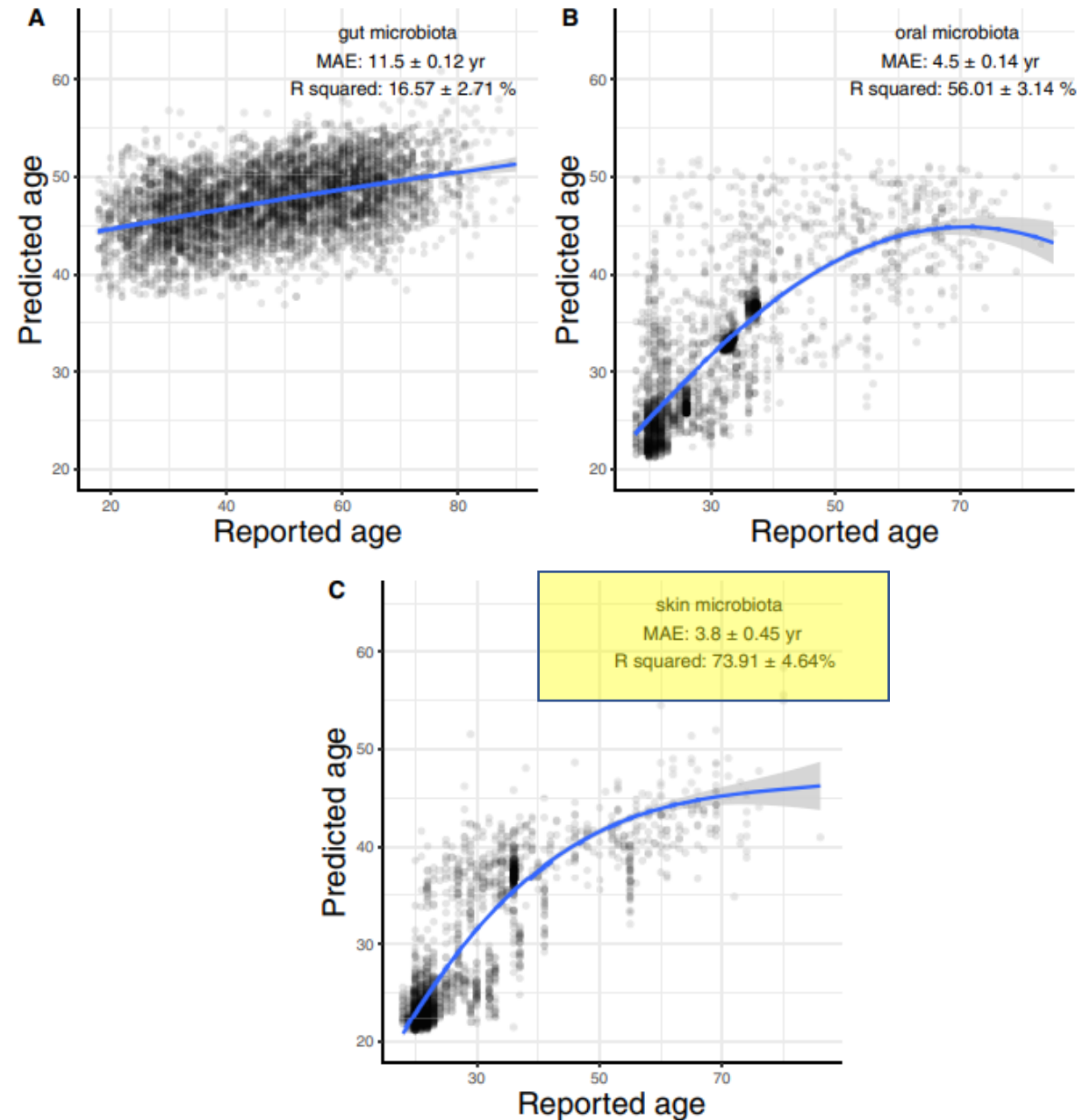
The future of antioxidant technologies: Probiotics and selective Postbiotics



Major contributions of *Cutibacterium acnes* to skin-ageing:
from clinical observations to in vivo modulation

Skin microbiome is fundamentally connected to skin ageing

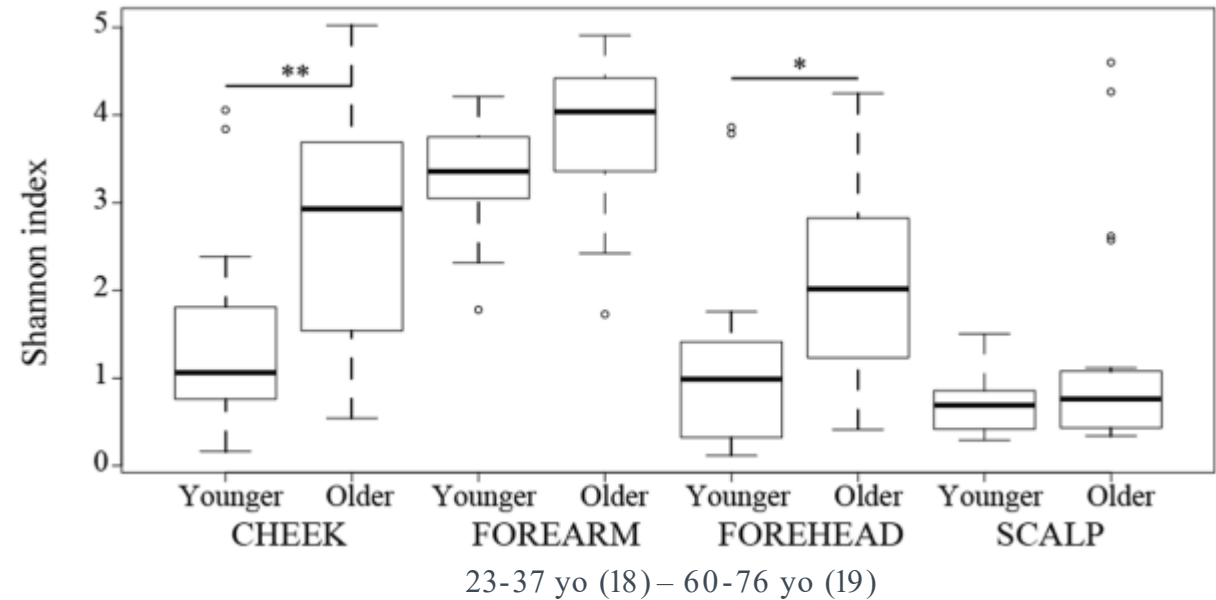
The skin microbiome is the best predictor of chronological age vs. gut and oral microbiomes



Skin microbiome composition changes with chronological age

Alpha diversity (species richness) between young and old is different.
Microbial diversity increases with age.

Japanese women (Shibagaki N., 2017)¹



Korean women (Kim M., 2020)²

Alpha-diversity / Forehead

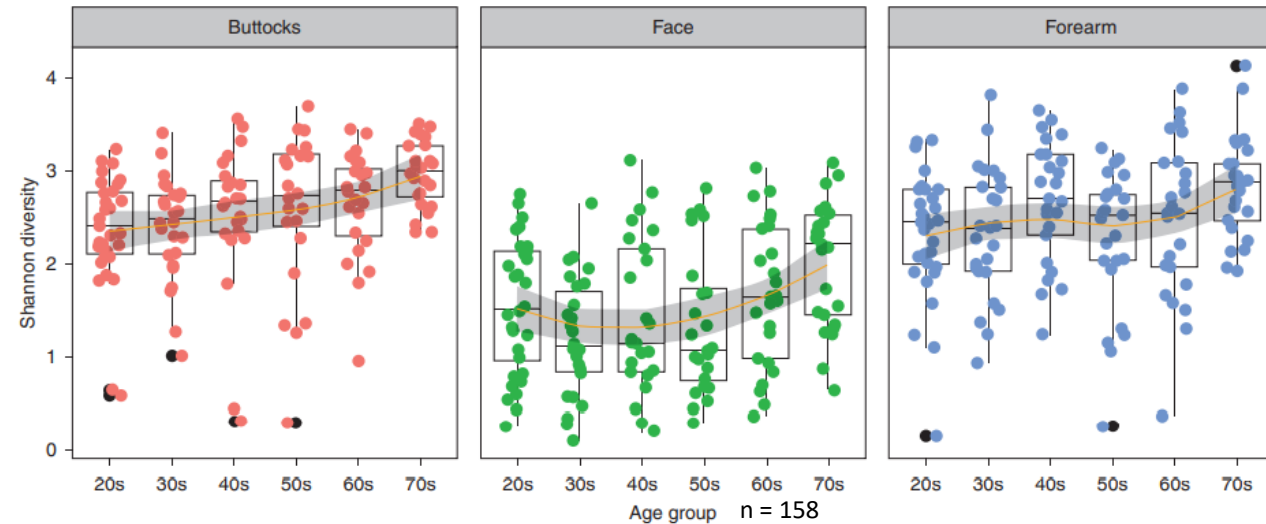
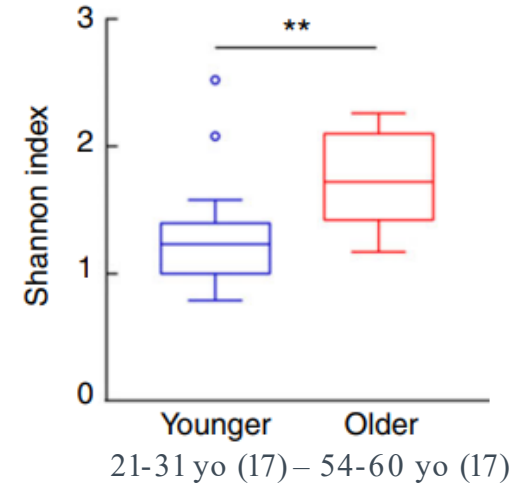
n	Age Group	Observed ASVs	Chao1	Evenness	Faith's PD ¹	Shannon's Index
24	10–29 years	122 ^b	128 ^b	0.49 ^b	11.57 ^b	3.36 ^b
21	30–49 years	155 ^b	157 ^b	0.58 ^{ab}	14.80 ^{ab}	4.00 ^{ab}
28	50–79 years	247 ^a	253 ^a	0.61 ^a	19.8 ^a	4.78 ^a
	<i>p</i> -value	<0.0001	<0.0001	0.0127	0.0017	0.0012
	Linear	<0.0001	<0.0001	0.0043	0.0004	0.0003
	Quadratic	0.3877	0.3351	0.3932	0.8394	0.9865
	SEM	13.33	13.64	0.02	1.01	0.17

1/ Shibagaki N. et al. Aging-related changes in the diversity of women skin microbiomes associated with oral bacteria. Scientific Reports 7, 10567 (2017)

2/ Kim M. et al. Investigation of age-related changes in the skin microbiota of Korean women. Microorganisms, 14;8(10):1581 (2020)

Skin microbiome composition changes with chronological age

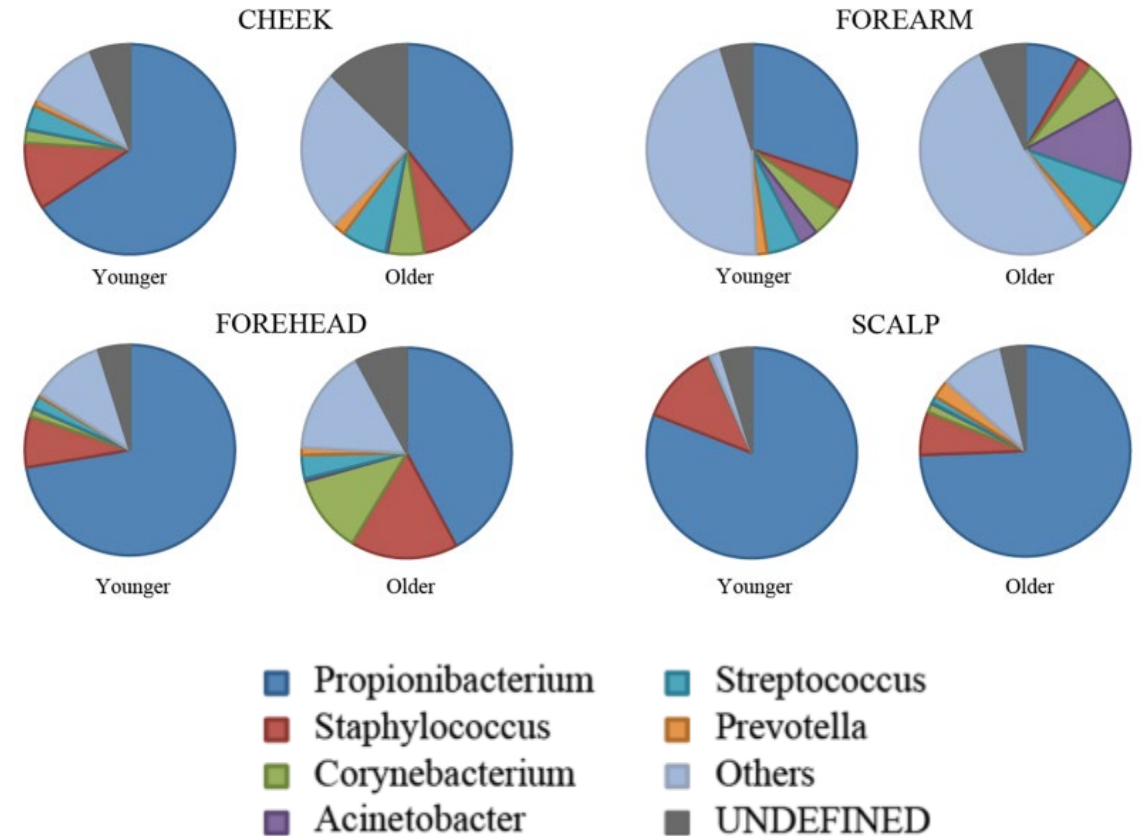
Microbial diversity increases with age.



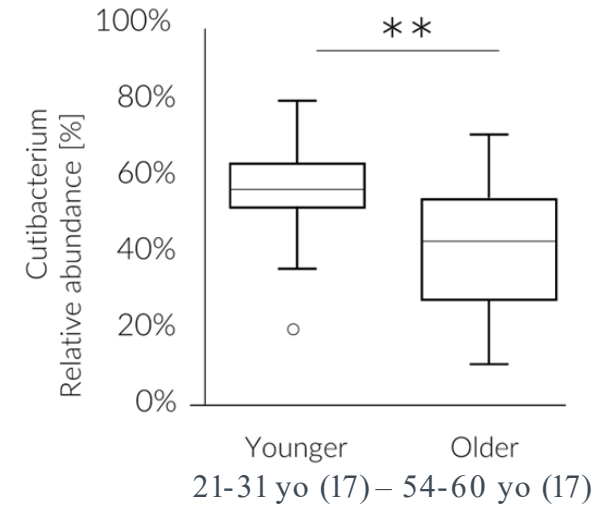
3/ Jugé R. et al. Shift in skin microbiota of Western European women across aging. *J Appl Microbiol* 125, 907–916 (2018)

4/ Howard B. et al. Aging-associated changes in the adult human skin microbiome and the host factors that affect skin microbiome composition. *J. of Invest. Dermatol.*, Vol.142, Issue 7: 1934-1946 (2022)

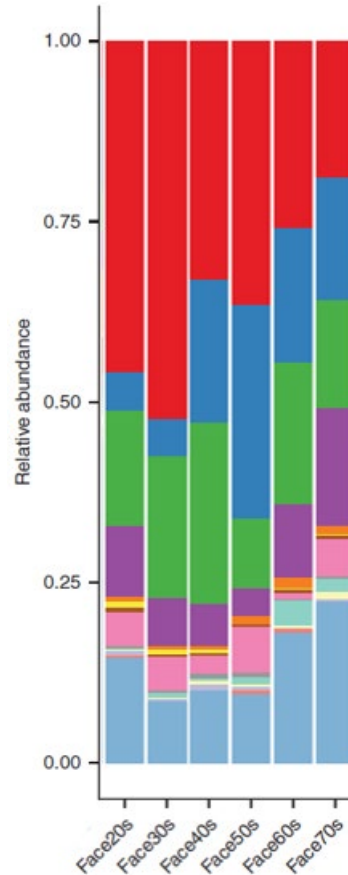
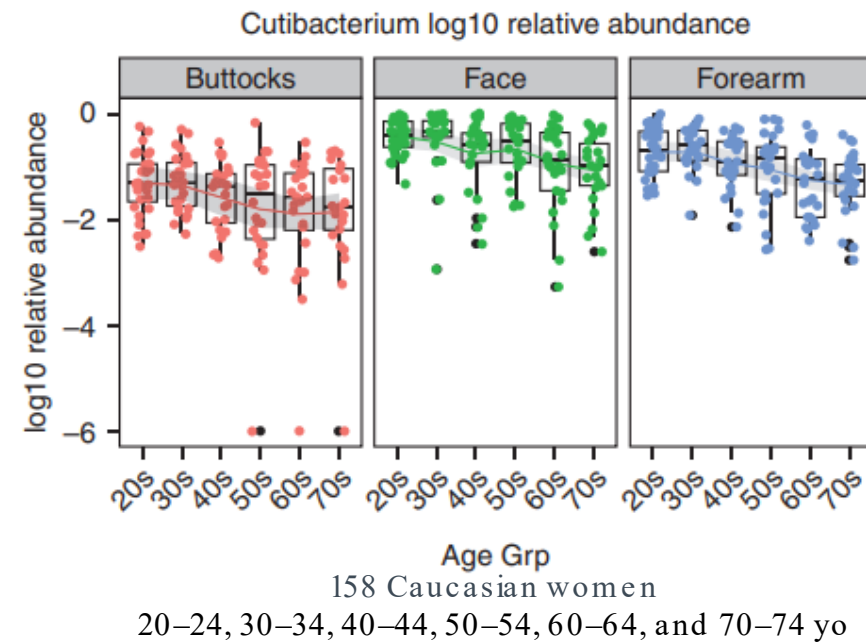
Cutibacterium acnes
relative abundance
decreases with
chronological age



Cutibacterium acnes relative abundance decreases with chronological age



Caucasian women (3,4)

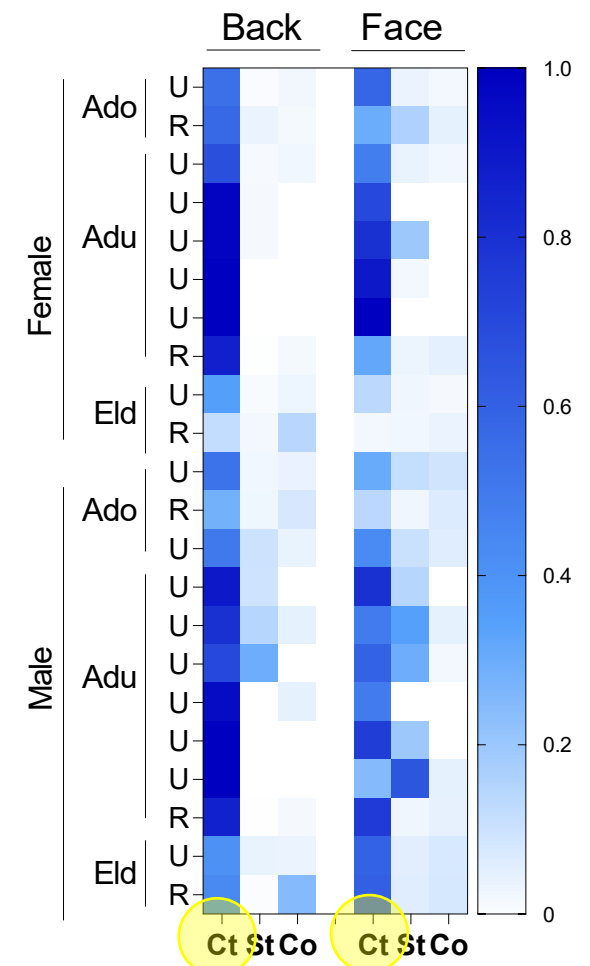
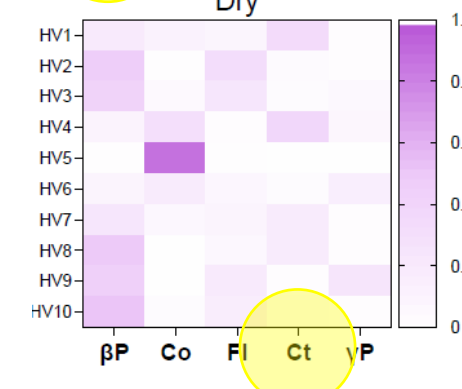
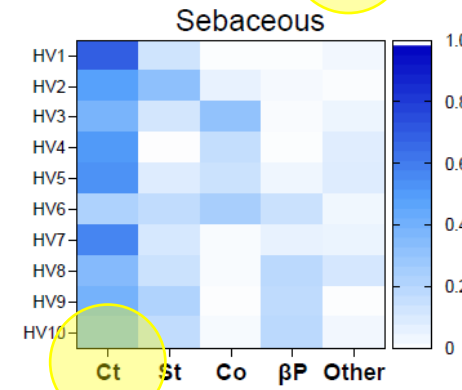
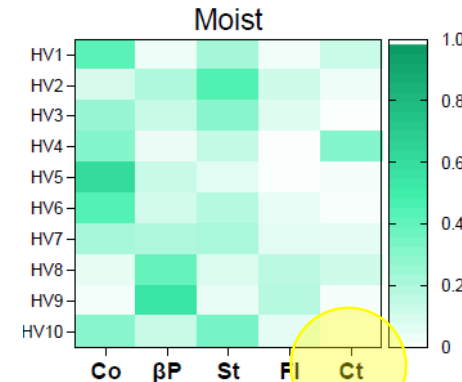


3/ Figure replotted for illustrative purposes from Jugé R. Et al. Shift in skin microbiota of Western European women across aging. *J Appl Microbiol* 125, 907–916 (2018)

4/ Howard B. et al. Aging-associated changes in the adult human skin microbiome and the host factors that affect skin microbiome composition. *J. of Invest. Dermatol.*, Vol.142, Issue 7: 1934-1946 (2022)

Cutibacterium acnes A keystone species of the skin microbiome

C. Acnes is highly represented in the skin microbiome, particularly in the pilosebaceous units
The predominant relative abundance of *Cutibacterium* in sebaceous sites is stable from different genders, ages, and locations.

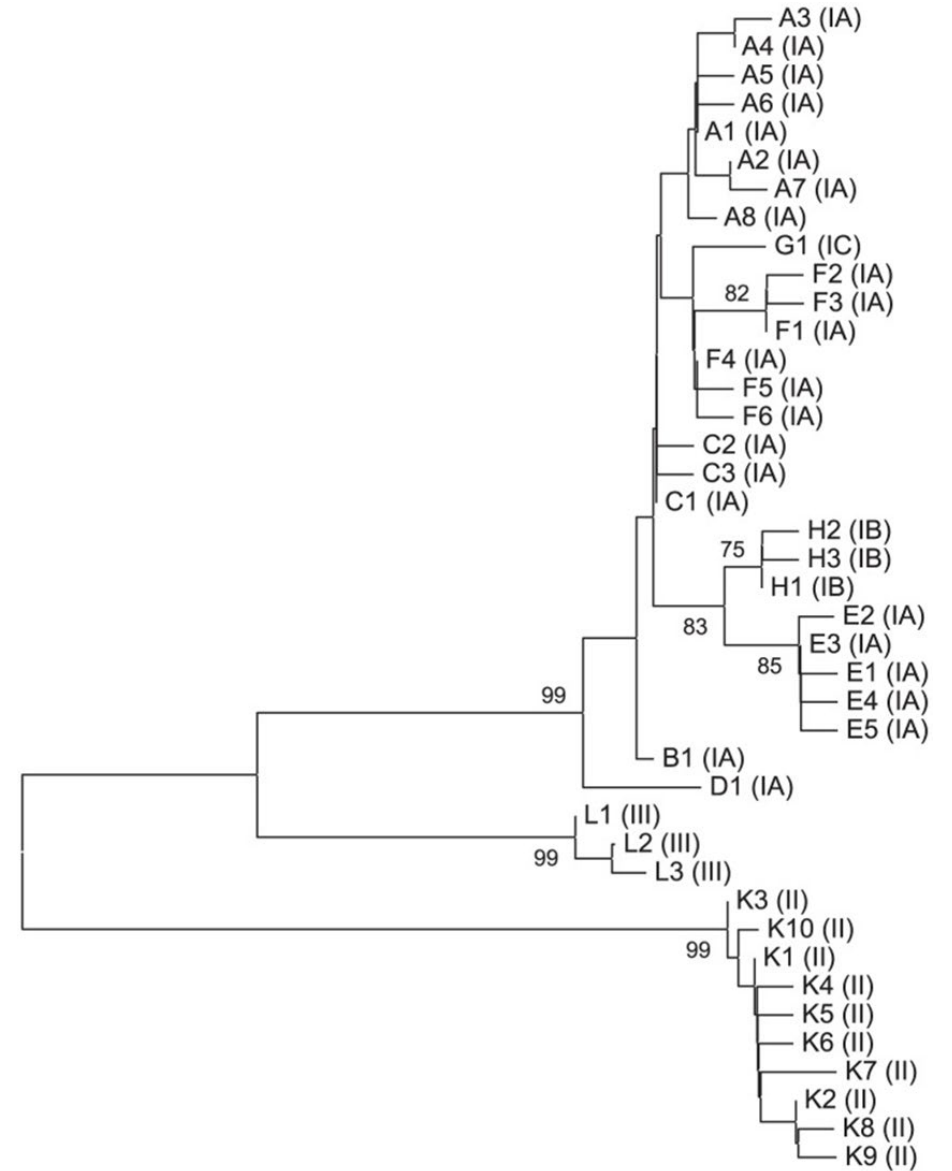


Ct: *Cutibacterium acnes*
 St: *Staph. epidermidis*
 Co: *Corynebacterium*
 βP: Betaproteobacteria
 Fl: Flavobacteria
 γP: Gammaproteobacteria

Ado: teenager,
 Adu: adult,
 Eld: elderly
 U: urban,
 R: rural

Cutibacterium acnes high strain diversity

Advances in deep sequencing technologies have revealed the high strain diversity of *C. acnes* with **three subspecies and six main phylotypes**.
To date, about 113 SLST types have been described.

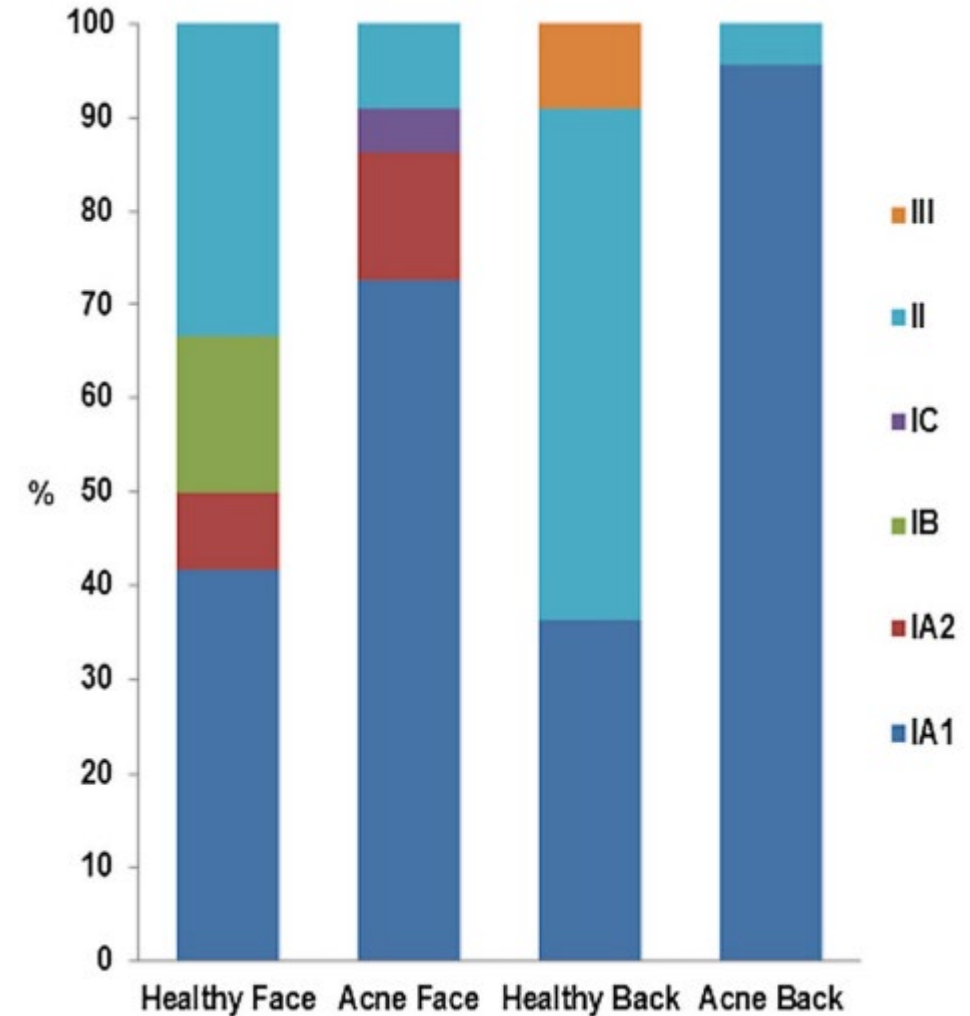


Cutibacterium acnes Paradigm shift

The phylogenetic cluster groups of *C. acnes* have revealed that **diversity plays a key role in maintaining skin homeostasis**. (Dreno et al., 2018)

“Contrary to what was previously thought, acne is not the result of a greater proliferation of all *C. acnes* strains, as patients with acne do not harbor more *C. acnes* in follicles than normal individuals. Rather than *C. acnes* hyperproliferation, it is the loss of balance between *C. acnes* phylotypes, together with a dysbiosis of the skin microbiome, which results in acne development.”

(Dreno B., 2018, 2020)

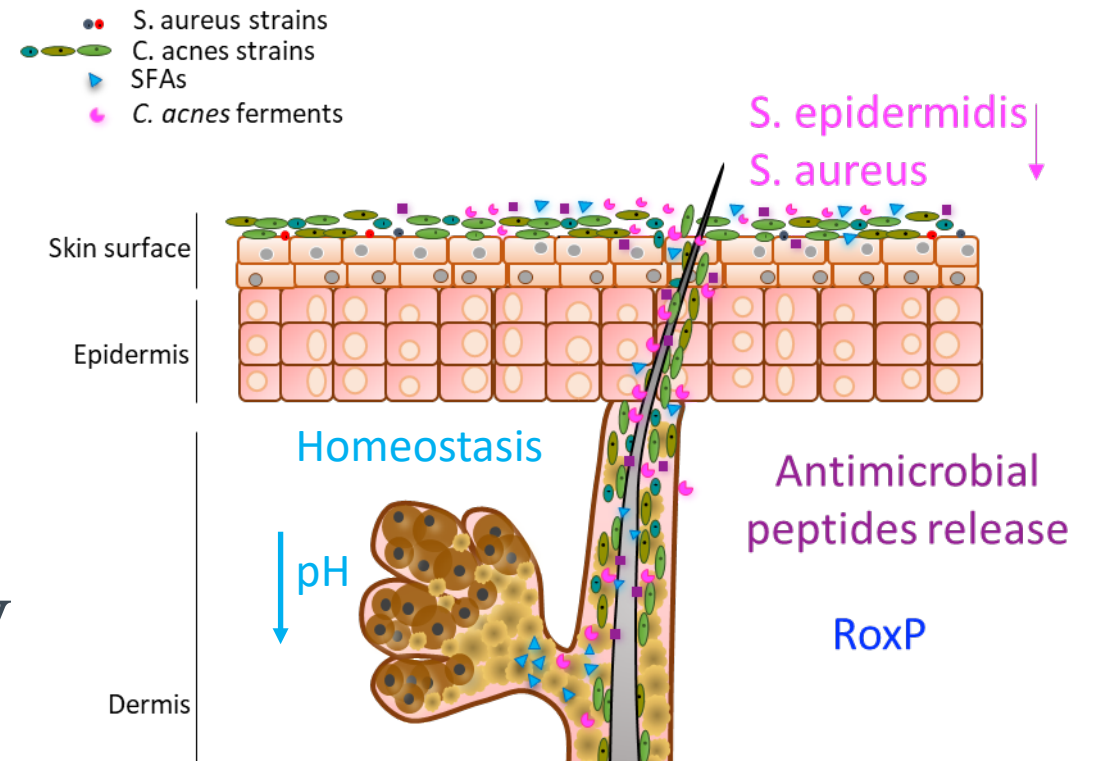


Dréno, B., et al. The Skin Microbiome: A New Actor in Inflammatory Acne. *Am J Clin Dermatol* 21 (Suppl 1), 18–24 (2020).

Cutibacterium acnes: a sentinel of the healthy skin microbiome

Advances in deep sequencing technologies have permitted the ID of *C. acnes* beneficial strains. These strains play a critical role as sentinel of the microbiome for skin health:

As we get older, our skin will miss the great substances produced by our “young” microbiome



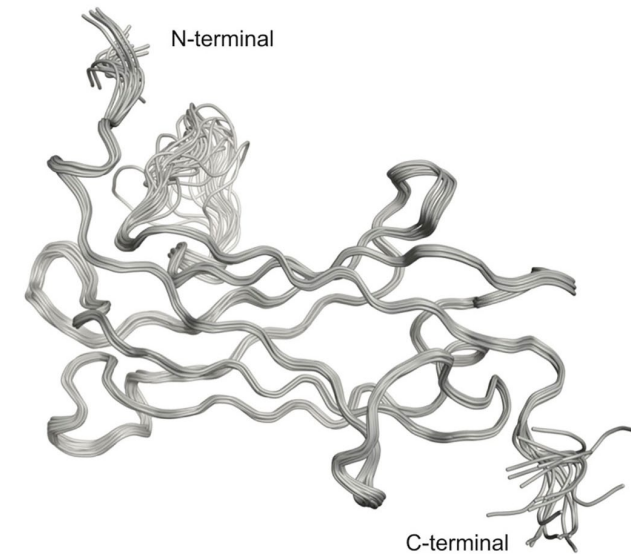
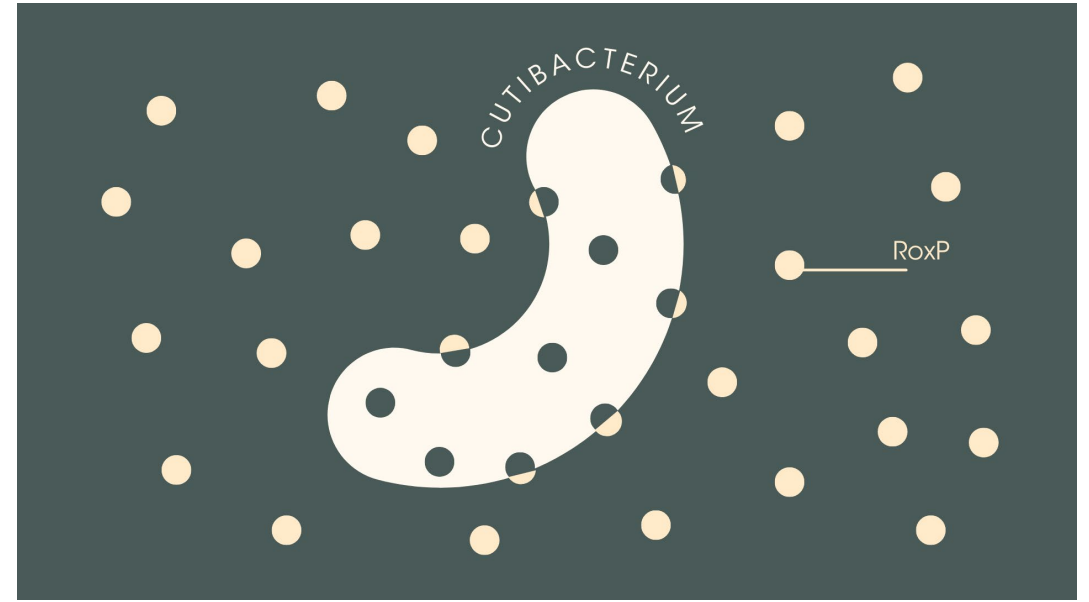
- colonization resistance: prevention colonization and invasion of pathogens via the hydrolysis of triglycerides in sebum and release of free fatty acids that are antimicrobial and contribute to an acidic pH of the skin surface,
- production of antimicrobial peptides,
- modulation of the immune system,
- production of beneficial metabolites.



Cutibacterium acnes uniquely and abundantly produces RoxP

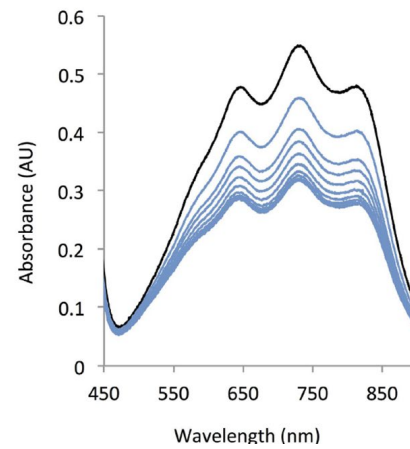
Radical oxygenase of *P. acnes*

RoxP adopts a compact, Ig-like domain,
which explains its pronounced stability



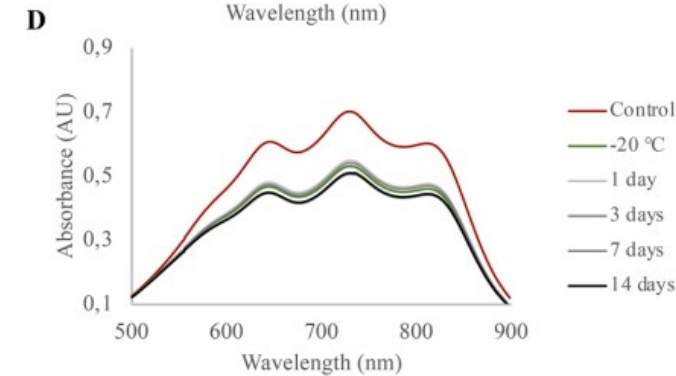
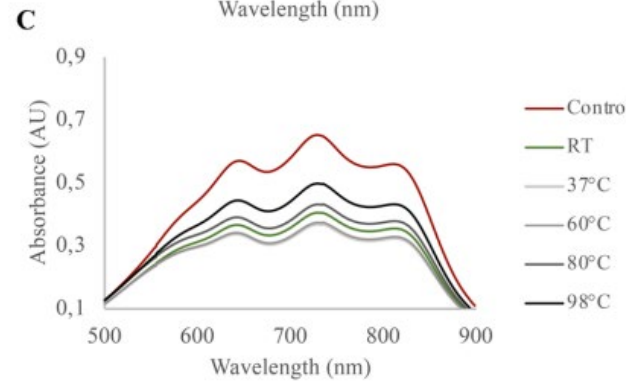
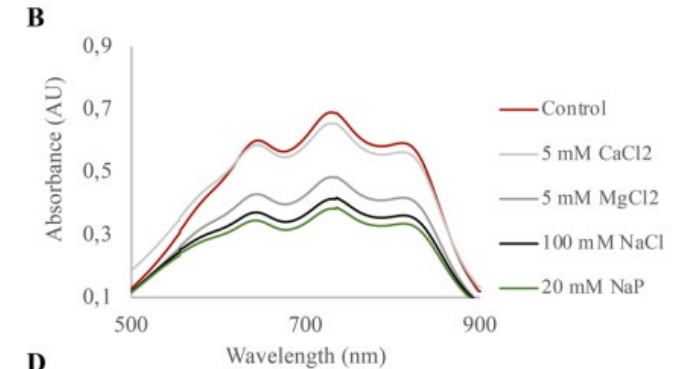
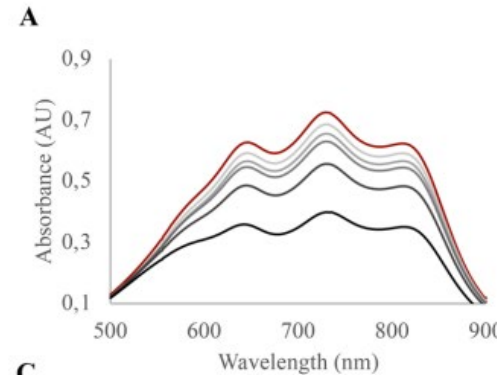
RoxP has strong antioxidant activity RoxP activity is highly resilient to adverse conditions

RoxP is able to endure temperatures of above 70C, as well as long-term storage in RT.



(min)	% inhibition
0	0,0%
2	16,4%
4	26,1%
6	29,8%
8	33,9%
10	37,0%
12	39,5%
14	41,0%
16	41,6%
18	42,3%

RoxP incubated with ABTS-radicals

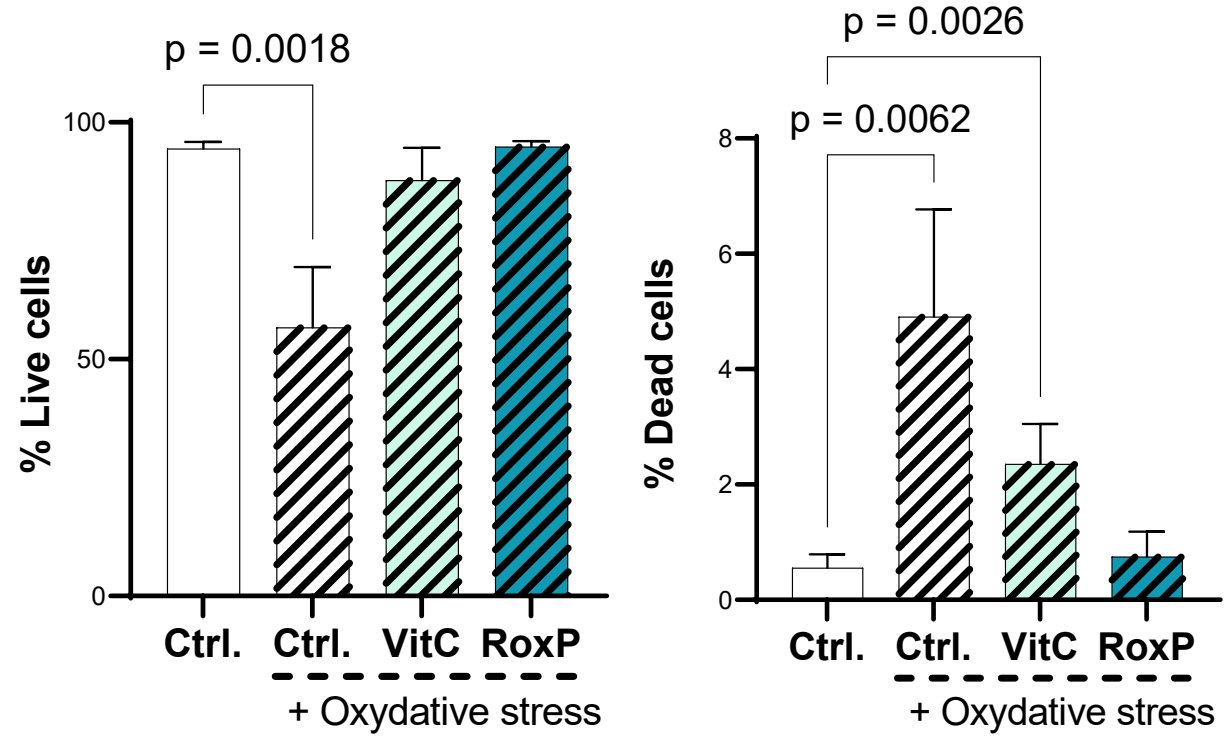


Allhorn, M., Arve, S., Brüggemann, H., and Lood, R. A novel enzyme with antioxidant capacity produced by the ubiquitous skin colonizer *Propionibacterium acnes*. *Sci Rep* 6. (2016)

Andersson, T., Ertürk Bergdahl, G., Saleh, K. et al. Common skin bacteria protect their host from oxidative stress through secreted antioxidant RoxP. *Sci Rep* 9, 3596 (2019)



RoxP protects skin from oxidative stress
 RoxP has a superior antioxidant activity



Primary keratinocytes cultured and pre-treated with vitamin C or RoxP, and later stressed with paraquat to generate ROS.
 Live/ dead protocols showed a **survival rate of 100%** in keratinocytes treated with RoxP, much better than Vit. C,

S-Biomedic Observational study Facial skin ageing

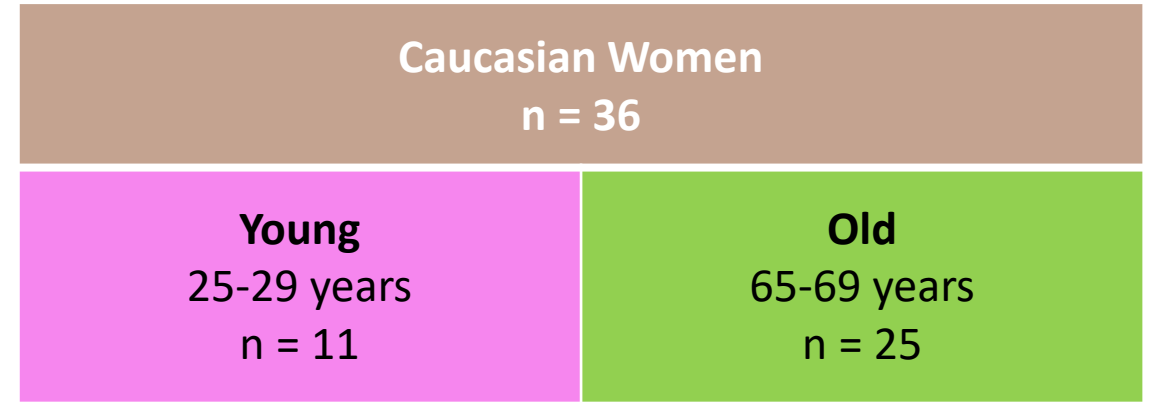
What differentiates a
chronological old “young looking” person?

Objective: determine whether **certain micro-organisms and the metabolites** they produce on the skin are related to **chronologically young skin and/ or old younger-looking skin**



S-Biomedic Observational study Facial skin ageing

1/ Panel selection



Inclusion criteria: Limited skin care routine, same hormonal status in each group.

Exclusion criteria: Smokers, heavy alcohol drinkers, heavy UV exposure, dermatological conditions, antimicrobials or cosmetic treatments, unhealthy diet, mask users.

S-Biomedic Observational study Facial skin ageing

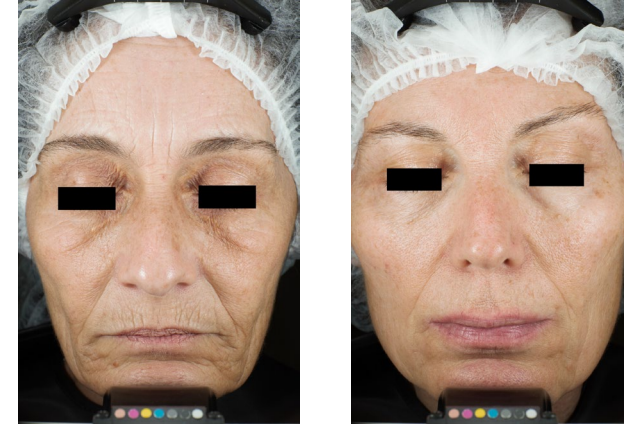
2/ Panel selection Old-Old & Old-Young

Selection on appearance:

Standardized images of 44 subjects
(*hair & eyes covered, Visia CR*)

Pair-wise selection “*who looks oldest*”

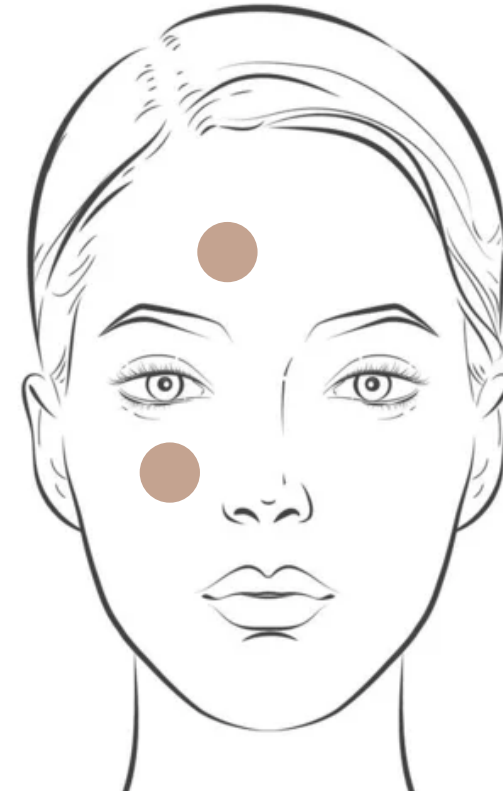
35 non-expert female panel (30-80 yo)



Caucasian Women N = 36			
Young YY 25-29 years N = 11	Old 65-69 years N = 25		
	Old-Young OY N = 12	Old-Old OO N = 13	

S-Biomedic Observational study Facial skin ageing

3/ Skin sample collection



Stratification pre-sampling routine:

- Washout phase 4 weeks (standardized cosmetics)
- 24 hours prior sampling no cosmetics use, no showering

Sampling:

- 2 swabs, forehead and cheek, pooled into 1 buffer (eNAT kit)

S-Biomedic Observ. study Facial skin ageing

4/ Data collection

Clinical data

- 24 features
- Wrinkles, radiance, TEWL, RoxP, pigmentation, etc.

Metagenomics (sequencing)

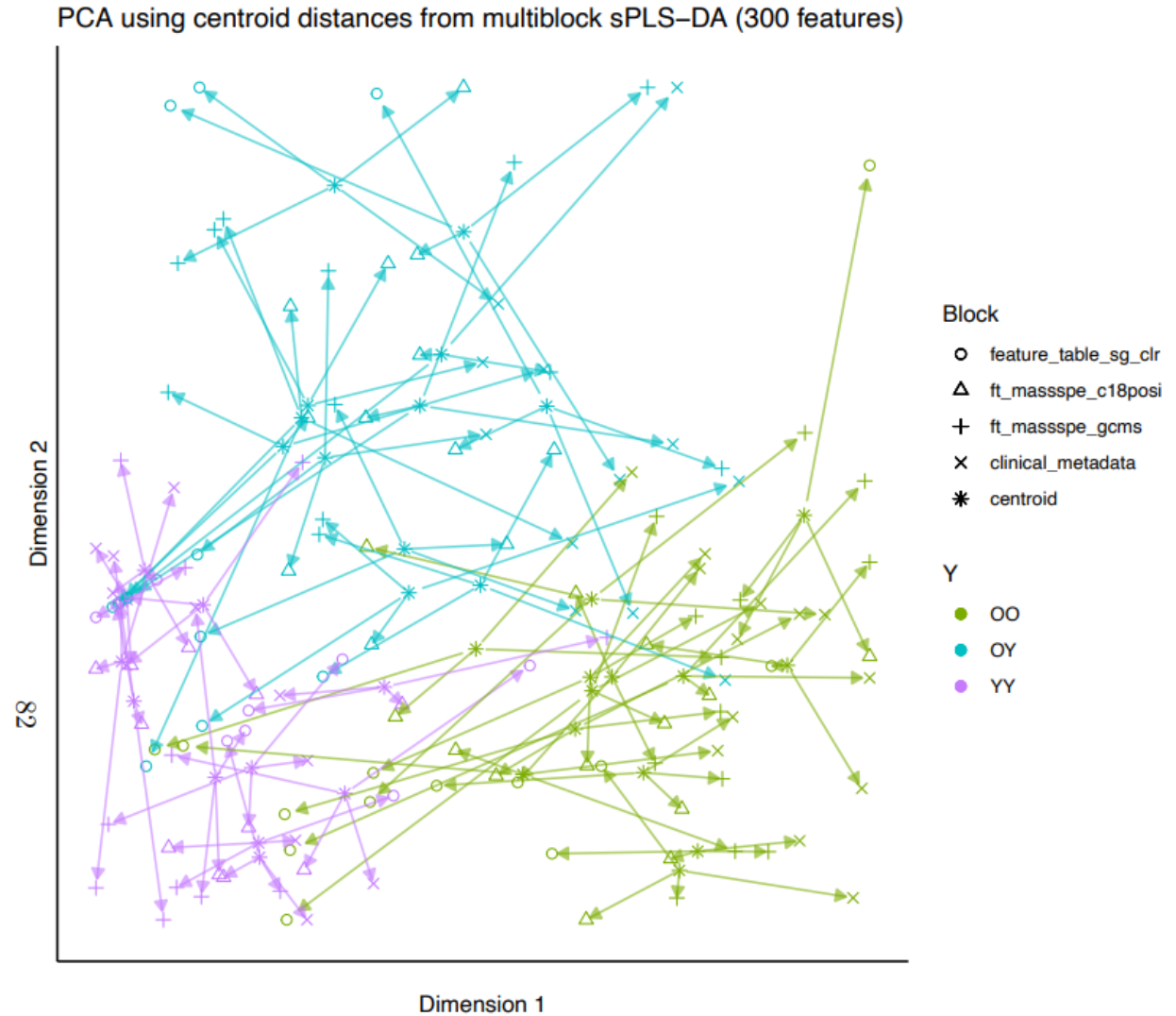
- At the strain level, incl. *C. acnes*
- 300 bacterial strains identified

Metabolomics

- GC-MS / LC-MS analyses
- 700 metabolites identified with GC/ MS
- 1400 metabolites identified with LC/ MS

OO, OY and YY test groups clustered separately

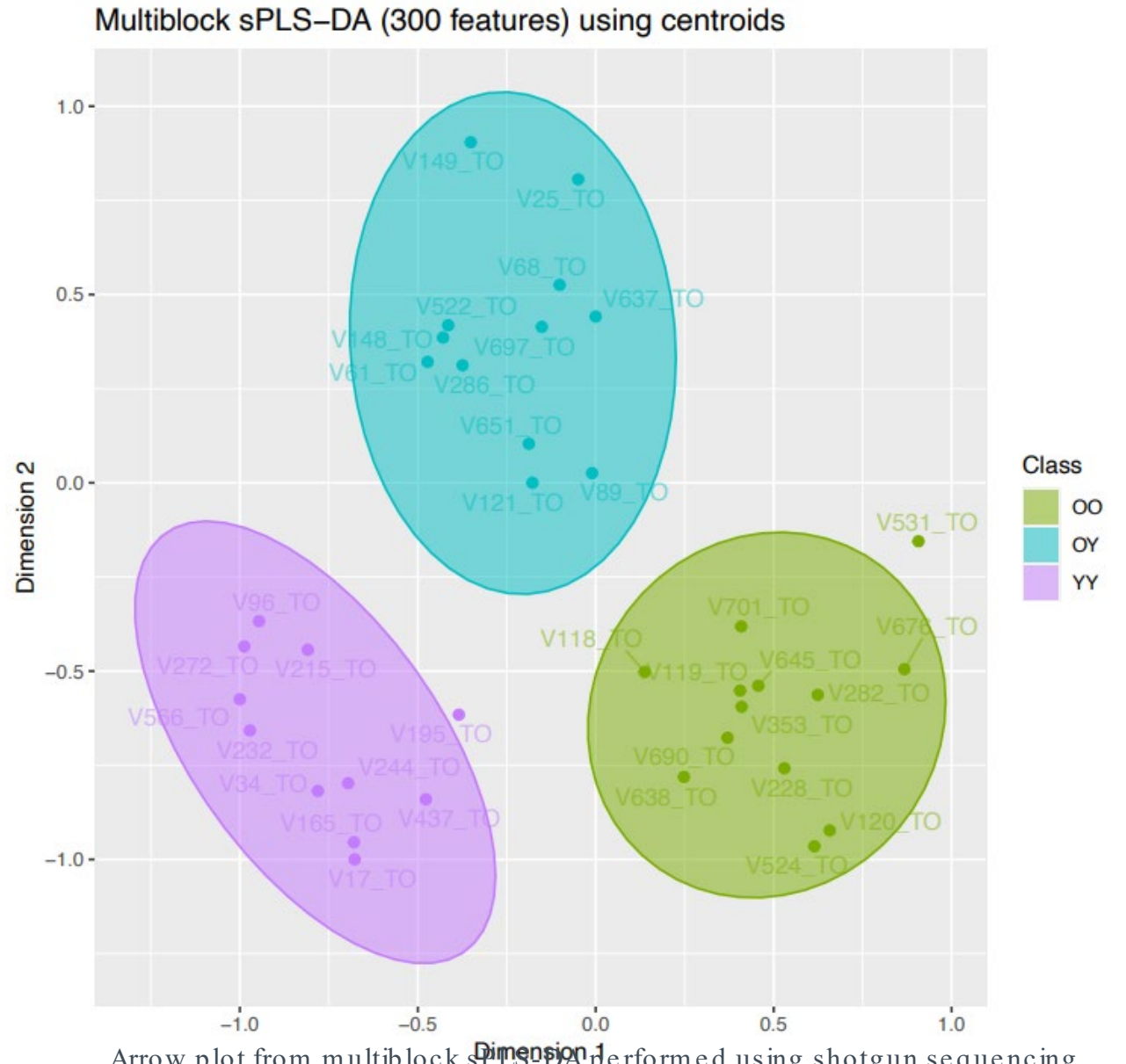
Sharp discrimination of OLD group into OO and OY based on multi-omics analysis.



Arrow plot from multiblock sPLS-DA performed using shotgun sequencing, LC-MS/ MS C18 Positive, GC-MS and clinical metadata data.

OO, OY and YY test groups clustered separately

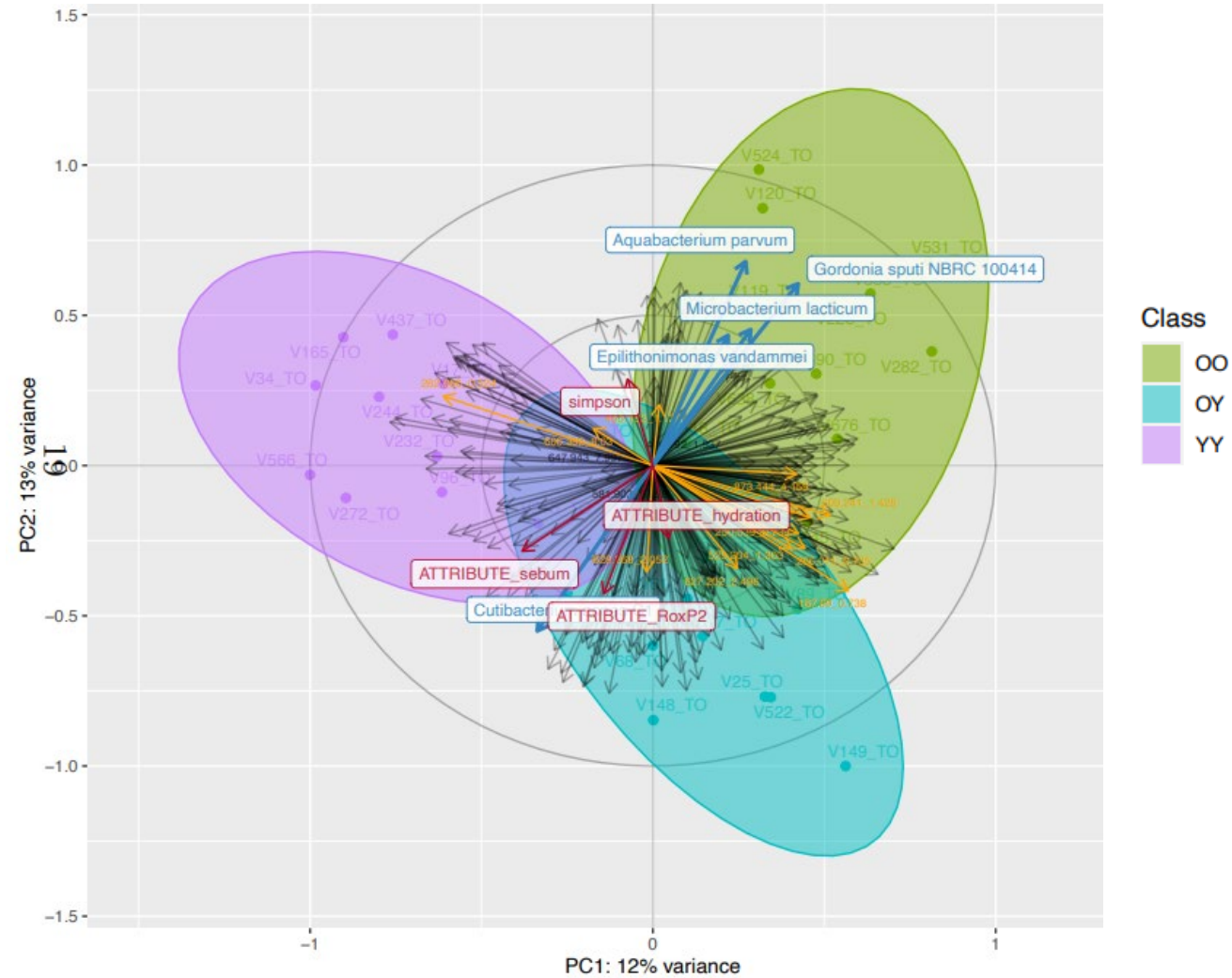
Sharp discrimination of OLD group into OO and OY based on multi-omics analysis.



Arrow plot from multiblock sPLS-DA performed using shotgun sequencing, LC-MS/MS C18 Positive, GC-MS and clinical metadata data.

Discriminant features separate OY and YY from OO

Using the most important discriminant features, “hydration”, “sebum” and “RoxP concentration” identified as clinical attributes correlating with young appearance, as well as with *C. acnes* abundance.

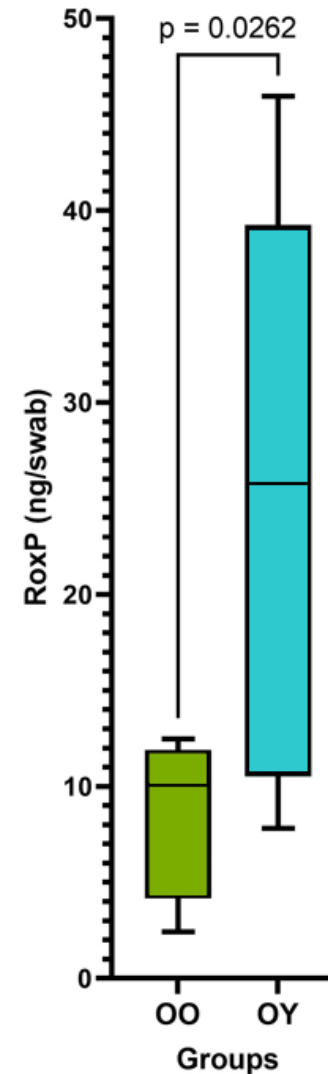


Selected significant Metabolome (sPLS-DA), microbiome (Pearson’s correlation) and clinical features (Pearson’s correlation).

A higher level of *RoxP* is associated with “younger look”

RoxP concentration is significantly higher in OY vs OO.
The clinical features of the young-looking old population correlate with *C. acnes* abundance and its secretome, RoxP concentrations for OY group.

***C. acnes* provides major contributions in skin aging.**



Concentration of RoxP (ng) at the surface of the skin of the 7 top patients of the OO and OY group.

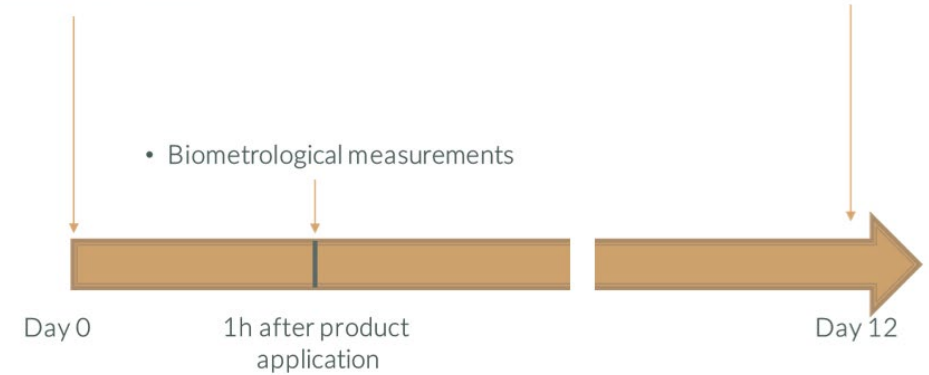
C. acnes selected strain and skin microbiome

Double blind randomized pilot study with a *C. acnes* strain selected to be a high RoxP producer (IP).



- Biometrological measurements
- Tape stripping
- Biometrological measurements
- Application of the products

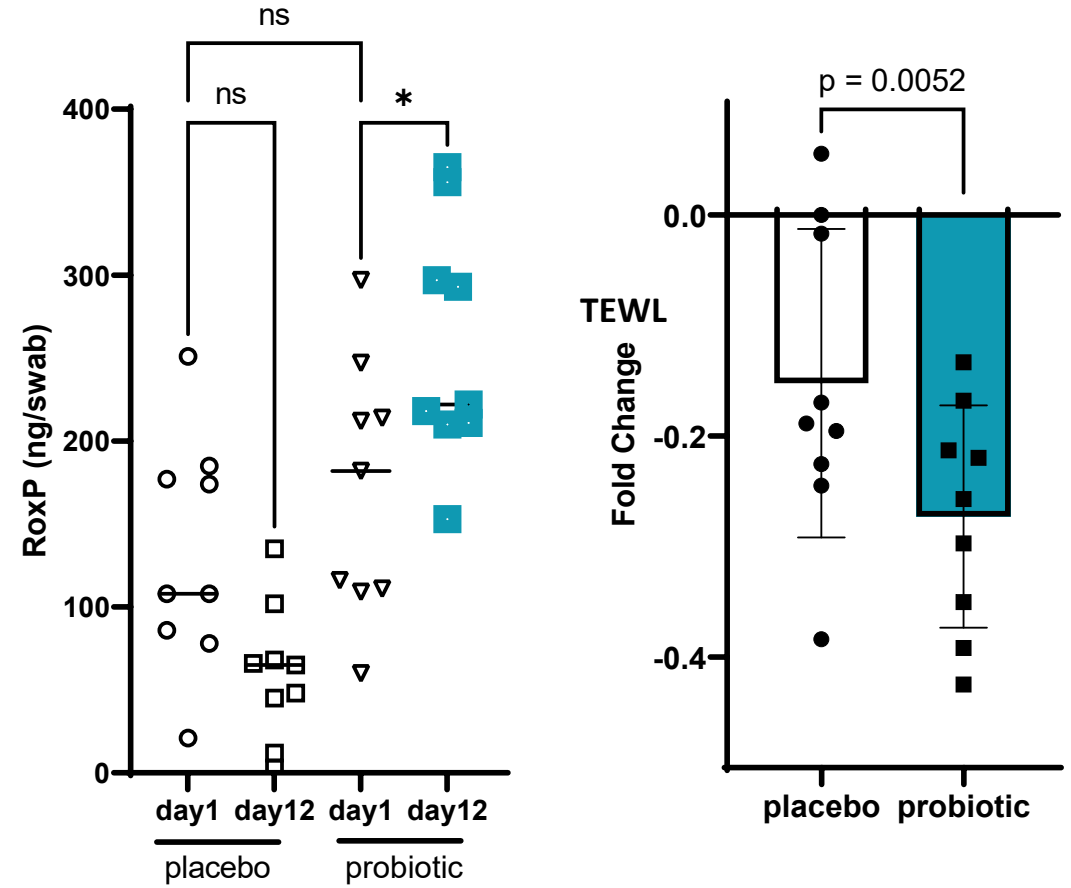
- Biometrological measurements
- Tape stripping
- Biometrological measurements
- Application of the products



Variation of RoxP (ng) between day 1 and day 12 at the surface of the areas treated with the placebo or the probiotic, on the forearm of 10 healthy woman. Skin functional capacity after acutely damaging the skin barrier through tape stripping, variation of TEWL, between placebo and probiotic after 11 days of products application.

C. acnes strain selected to be a high RoxP producer improves the skin barrier functions

C. acnes strain based probiotic is metabolically active when topically applied, delivers antioxidant, and improves skin barrier functions.

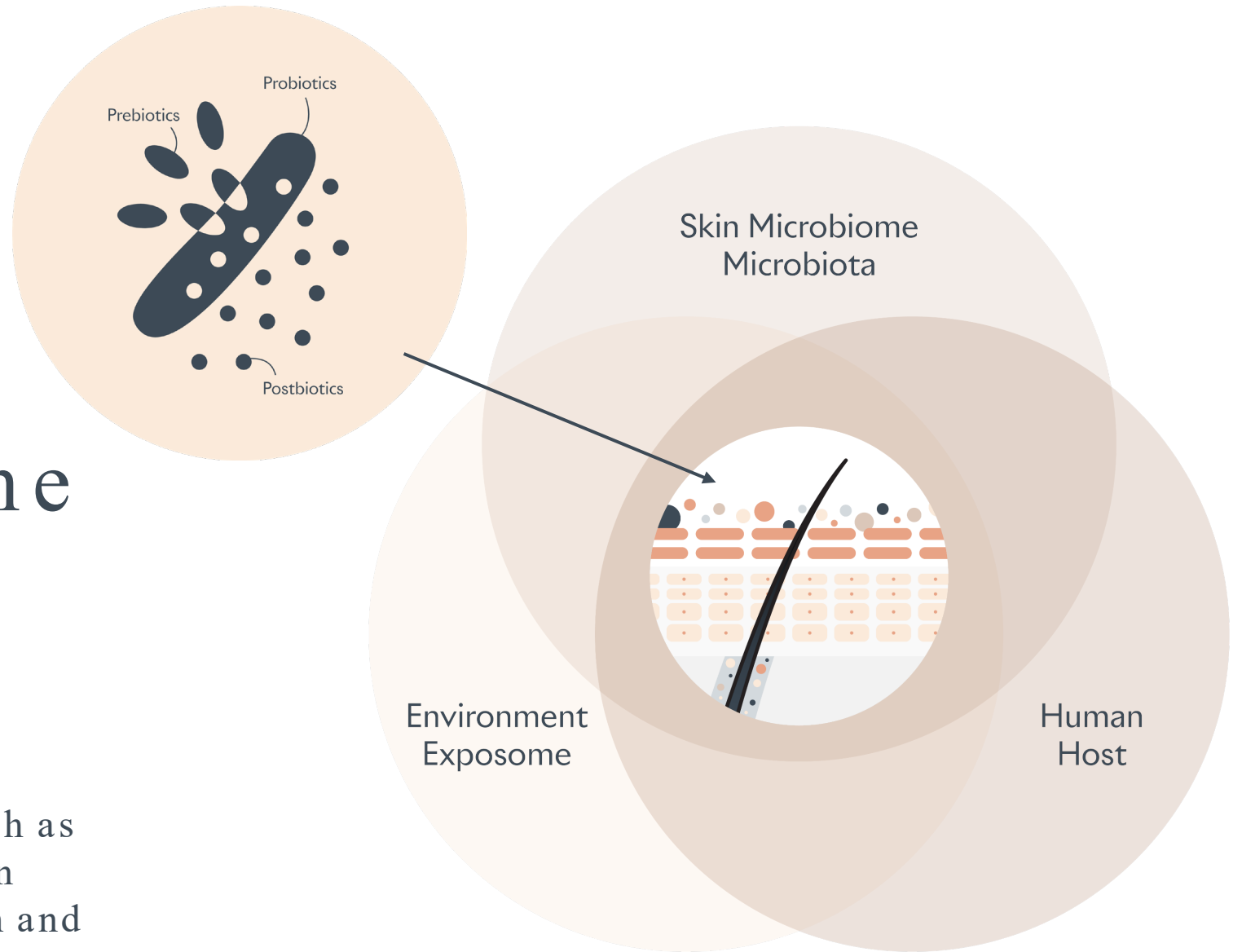


No statistical difference on RoxP quantity in the placebo group and a **significant increase** for the probiotic treated areas, indicating that the strain is metabolically active when applied.

This higher local concentration is **followed by a significant reduction in TEWL**.

Skin microbiome modulation

Skin microbiome modulators, such as probiotics and postbiotics offer an effective approach for skin health and healthy ageing.





s-biomedic

the skin microbiome company™

Paradigm shift

Unleash the full potential of the skin microbiome

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www.sbimed.com



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