SKIN METAPROTEOMICS: A KEY FUNCTIONAL APPROACH TO STUDY SKIN HEALTHINESS AND RESILIENCE

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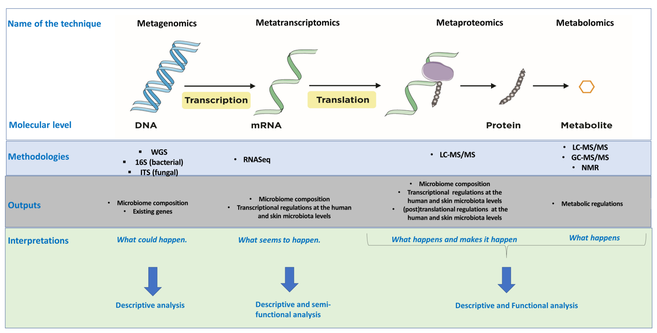
INTRODUCTION

Our skin health and its resilience against assaults (pollution, climatic conditions, pathogens) rests mainly on the epidermis and its microbiome. Indeed, the skin is colonized by commensal beneficial microorganisms called microbiome. This complex ecosystem is a shield for our skin ensuring protective functions while educating our immune system. Therefore, studying epidermis and its microbiome with adapted methodologies to clinical sampling might help to gain valuable insight during the development of solutions dedicated to skin health such as cosmetic ingredients.

BACKGROUND

Among the technologies available to study microbiome, we chose metaproteomics, which has only been used right now in gut microbiota field.

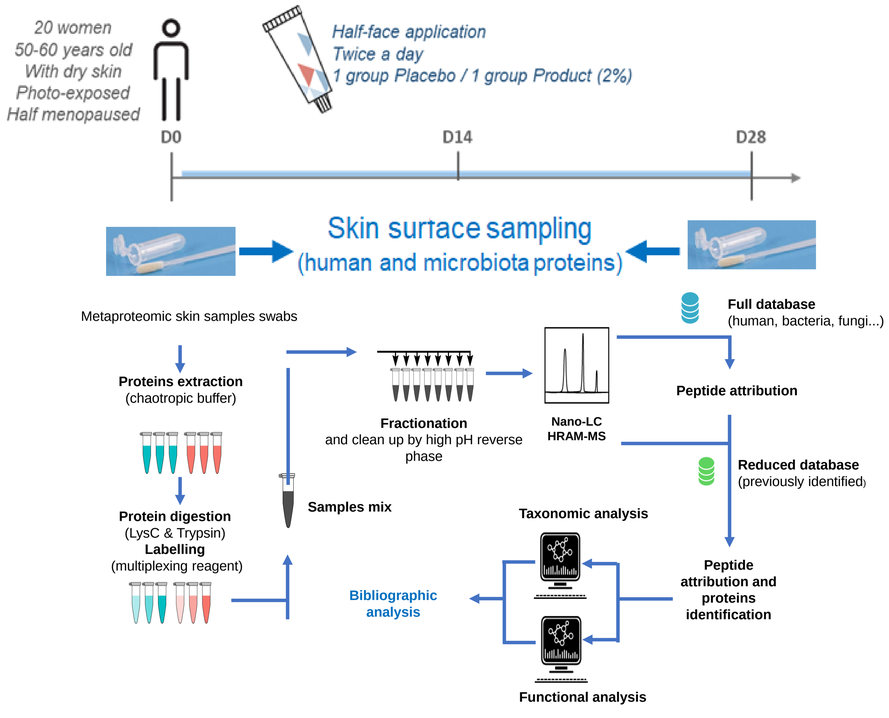


➔ **In contrast to metagenomics, the widely used technique to study skin microbiota, metaproteomics technique provides valuable descriptive and functional insights simultaneously on these two allies with complex interplay: the human skin epidermis and the microbiome.**

AIMS OF THIS WORK

The objectives of this clinical study using metaproteomics approach were:
1. Identification and taxonomic assignment of proteins expressed at the skin surface by human skin cells and sl microbiome.
2. Comparison of taxa abundance before and after 28 days of a cosmetic treatment (by "Galactinol Advanced" GA). Then comparison of taxa abundances between placebo and Galactinol Advanced group.
3. Evaluation of the treatment impact on microbiota: beta diversity analysis between placebo and GA group.
4. Identification and functional analysis of proteins significantly regulated GA a bioinspired biotech cosmetic active ingredient.
5. Demonstration of the beneficial effect of this active on skin healthiness based on biometrological assessment, pictures and questionnaire.

METHODS



Taxonomic analysis :

1. Taxonomic assignment : Peptides were assigned to the Lowest Common Ancestor by submitting their sequences to Unipept tool. Identified taxa were gathered in 3 different taxonomic groups: host, Bacteria and Fungi.

2. Taxa abundances : Calculated as the sum of associated proteins abundances.

3. Beta-diversity : Measure of inter-samples diversity and samples separation according to their microbiome composition. Distance metrics used in this analysis were Bray-Curtis (which take into account taxa abundances). Those distances were represented by hierarchical clustering and principal component analysis (PCoA). Statistical differences measured by PERMANOVA.

4. Functional analysis by HolXplore methodology (a Phylogene proprietary process):

Protein sequences (recovered from Uniprot and Uniparc) were submitted to EggNOG mapper to associate each protein to its closest annotated ortholog. Used functional terms were GO terms, COG category, COG and KOG terms and KEGG pathways, reactions and modules.

RESULTS

1. Number of peptides and proteins identified and their taxonomic assignments

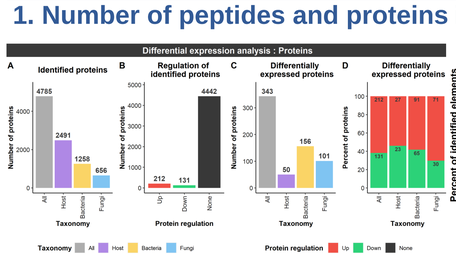


Figure 1. A. *Number of identified proteins and their taxonomic assignment (human, bacteria and fungi).* ***B.*** *Number of regulated proteins by GA.* ***C.*** *Number of regulated proteins in each studied taxonomic group.* ***D.*** *Number of proteins up and down regulated in each studied taxonomic group.*

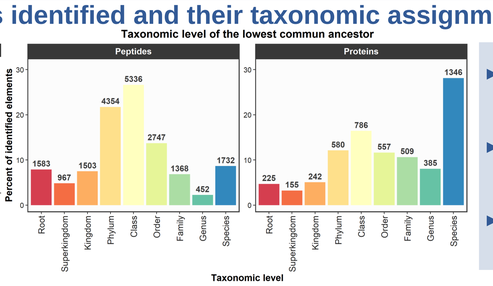


Figure 2. *Number of peptides and proteins assigned at each taxonomic level based on lowest common ancestors*

- **20042 peptides and 4785 proteins identified at the skin surface.**
- **Among all proteins:**
  - **40% assigned to the genus,**
  - **30% assigned to the species.**
- **GA regulates 343 proteins (212 up and 131 down).**

2. Taxa abundances

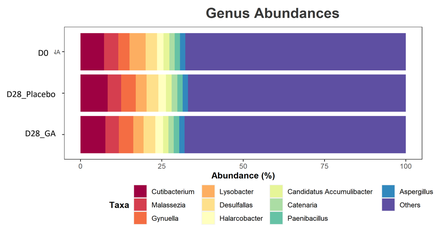


Figure 3. *Major microbiota taxa abundances proportions at the phylum and genus level before (D0) and after 28 days of application of placebo or GA formulated in a cream.*

3. Beta diversity

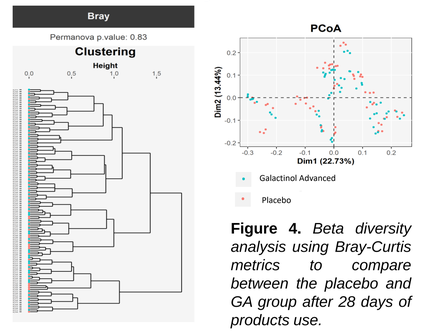


Figure 4. *Beta diversity analysis using Bray-Curtis metrics to compare between the placebo and GA group after 28 days of products use.*

- **The taxa abundance analysis did not reveal differences between the different groups.**
- **The analysis of beta diversity did not highlight a separation of samples according to treatment.**
- **GA can be considered as microbiota friendly.**

4. Identification of functional pathways regulated by GA in each taxa (human, bacteria and fungi)

4.1 Bioinformatic analysis allows the identification of mains functional pathways enriched by GA

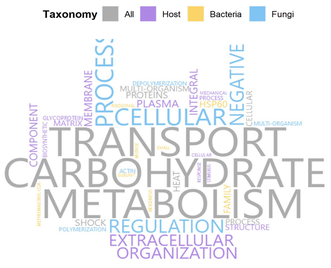


Figure 5. *WordCloud representation of main enriched functional pathways identified through HolXplore.*

➔ **Carbohydrate metabolism**
➔ **Proteins quality control**
➔ **Extracellular matrix organization**
➔ **Antioxidant**

4.2 Example of proteins belonging to enriched functional pathways stimulated by GA

Taxa Origin	Function	Protein name	Fold change	P Value
Microorganisms/ human skin cells	Metabolism (energy increase/glycolysis)	GAPDH (bacteria)	1.241	4,7. 10^{-4}
		GAPDH (fungi)	1.426	10^{-5}
		GAPDH (human)	1.125	10^{-5}
	Protein quality control	60 kDa chaperonin (bacterial)	2.677	10^{-15}
		HSP70 binding protein1 (HSPB1) human	1.381	3. 10^{-3}
Human skin cells	Anti-inflammatory	CD81	1.381	10^{-5}
		Complement 8B (lytic complex)	0.766	3. 10^{-5}
	Skin barrier function (stratum corneum/ waterproofing)	Late cornified envelope protein 3B	1.349	7. 10^{-4}
		Late cornified envelope protein 1B	1.245	5. 10^{-2}
	Mechanical properties & hydration (GAG synthesis)	UDP-glucuronic acid decarboxylase 1	1.209	3.10^{-2}
Microorganism	Antioxidant (ROS decrease/detoxification)	Super oxide dismutase (bacteria)	1.307	9. 10^{-4}
		Limonene 1,2-monooxygenase (bacteria)	1.239	10^{-7}
		Thioredoxin (fungi)	1,135	4. 10-3

Galactinol Advanced stimulates:

- **in all taxa the GAPDH (Glyceraldehyde-3-phosphate dehydrogenase), an enzyme that breaks down the glucose for energy production.**
- **in bacteria and human skin cells heat shock proteins crucial for the maintenance of proteins integrity.**
- **in bacteria and fungi, enzymes involved in detoxifications process.**
- **in human skin, cells proteins involved in skin barrier function, mechanical properties, detoxification processes and reduction of inflammation.**

5. Demonstration of skin beneficial effects of Galactinol Advanced on skin properties

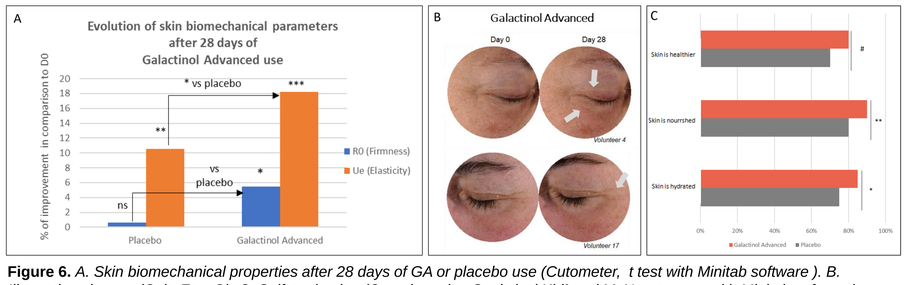


Figure 6. *A. Skin biomechanical properties after 28 days of GA or placebo use (Cutometer, t test with Minitab software). B. Illustrative pictures (ColorFace®). C. Self-evaluation (Questionnaire, Statistical Khi² and McNemar tests with Minitab software). *=p<0.05; **=p<0.01; ***=p<0.001; #=p<0.1.*

- **In photo-exposed facial skins, GA improves :**
  - **Skin mechanical properties: firmness and elasticity (A).**
  - **Skin appearance i.e. less visible wrinkles (B).**
- **Volunteers using GA found their skins healthier, nourished and hydrated (C).**

CONCLUSION

These clinical results highlight that metaproteomics is a powerful technology allowing to demonstrate that modulating proteins expression at keratinocytes and microbiome levels leads clearly to clinical and visible outcomes: improvement of skin wrinkles, mechanical properties and skin healthiness.

REFERENCES

Kunath *et al*. 2019 Metaproteomics: Sample Preparation and Methodological Considerations; Gonzalez *et al* 2020 High-Throughput Stool Metaproteomics: Method and Application to Human Specimens. Zhang 2016 MetaPro-IQ: a universal metaproteomic approach to studying human and mouse gut microbiota; Karaduta et al. 2021 Metaproteomics—An Advantageous Option in Studies of Host-Microbiota Interaction