



L'ingénierie des actifs naturels

Quand le métagénomique révèle un déséquilibre du microbiote cutané au cours du vieillissement.

Mme Christine Grimaldi, PhD, Responsable du laboratoire Microbiologie Référent, SILAB



Le microbiote cutané

- Sa composition et sa distribution sont influencées par la génétique et le mode de vie
- Le microbiote est responsable de l'immunité, de la nutrition et de la défense de la peau



OBJECTIF SILAB

Comparer la répartition du microbiote entre deux groupes d'âge différents de femmes Caucasiennes



Principalement étudié dans un contexte pathologique

Très peu de données sur une peau saine

1



Prélèvement sur le visage

- *Jeunes* : 17 volontaires Caucasiennes en bonne santé, âge moyen 28 ± 3 ans
- *Âgés* : 17 volontaires Caucasiennes en bonne santé, âge moyen 62 ± 5 ans

2



Analyse des communautés bactériennes

- Amplification des régions V3-V4 de l'ADNr 16S
- Séquençage des amplicons sur Illumina MiSeq

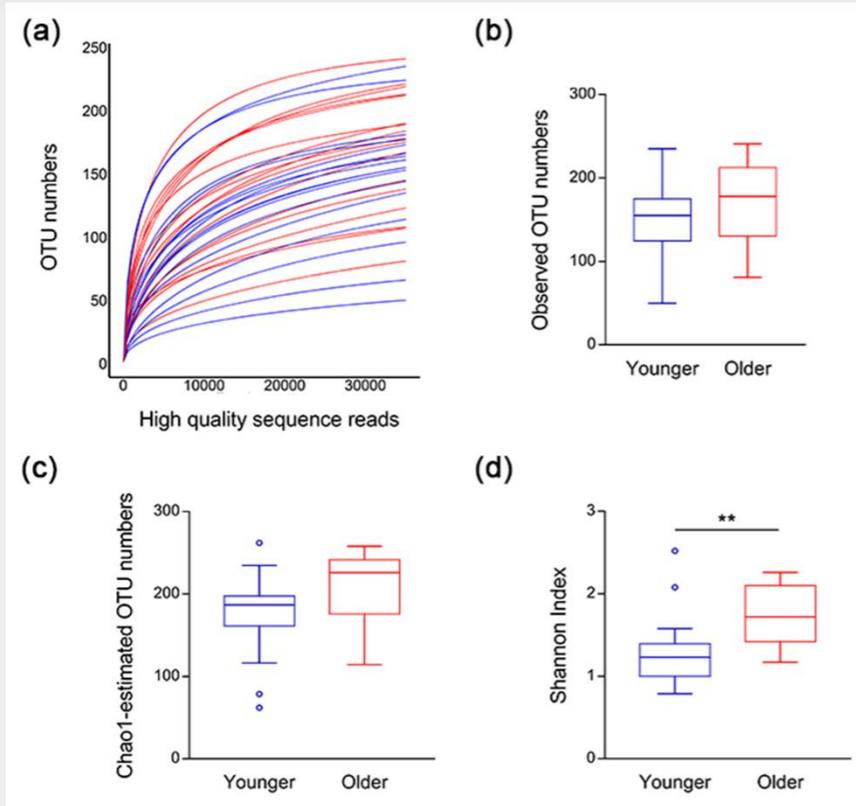
3



Analyses bioinformatiques

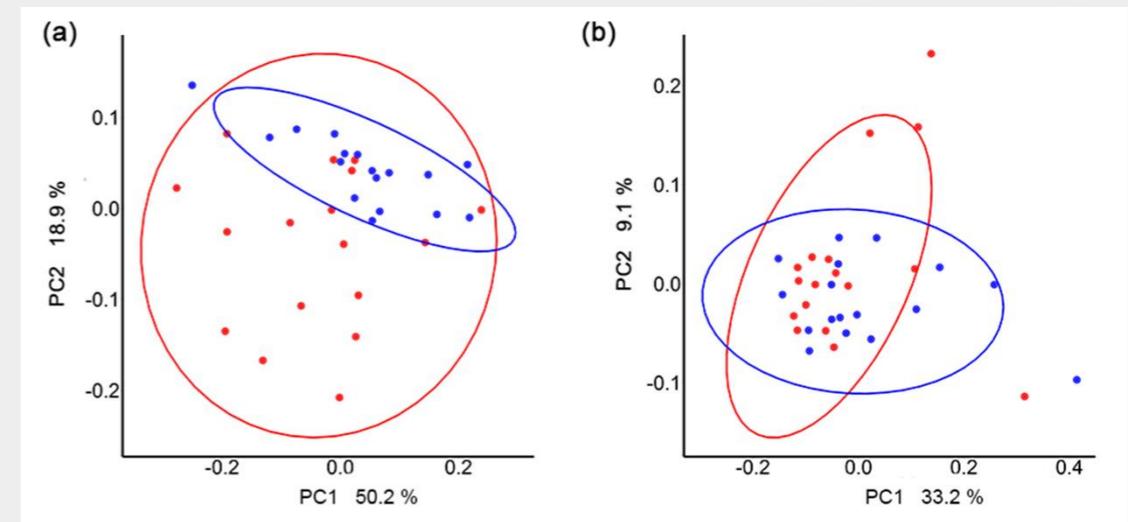
- Traitement des données
- Validation de la méthode d'analyse
- Analyse des OTUs et classification taxonomique (FROGS)
- Analyse de l' α -diversité et de la β -diversité (sous R)

Alpha diversité



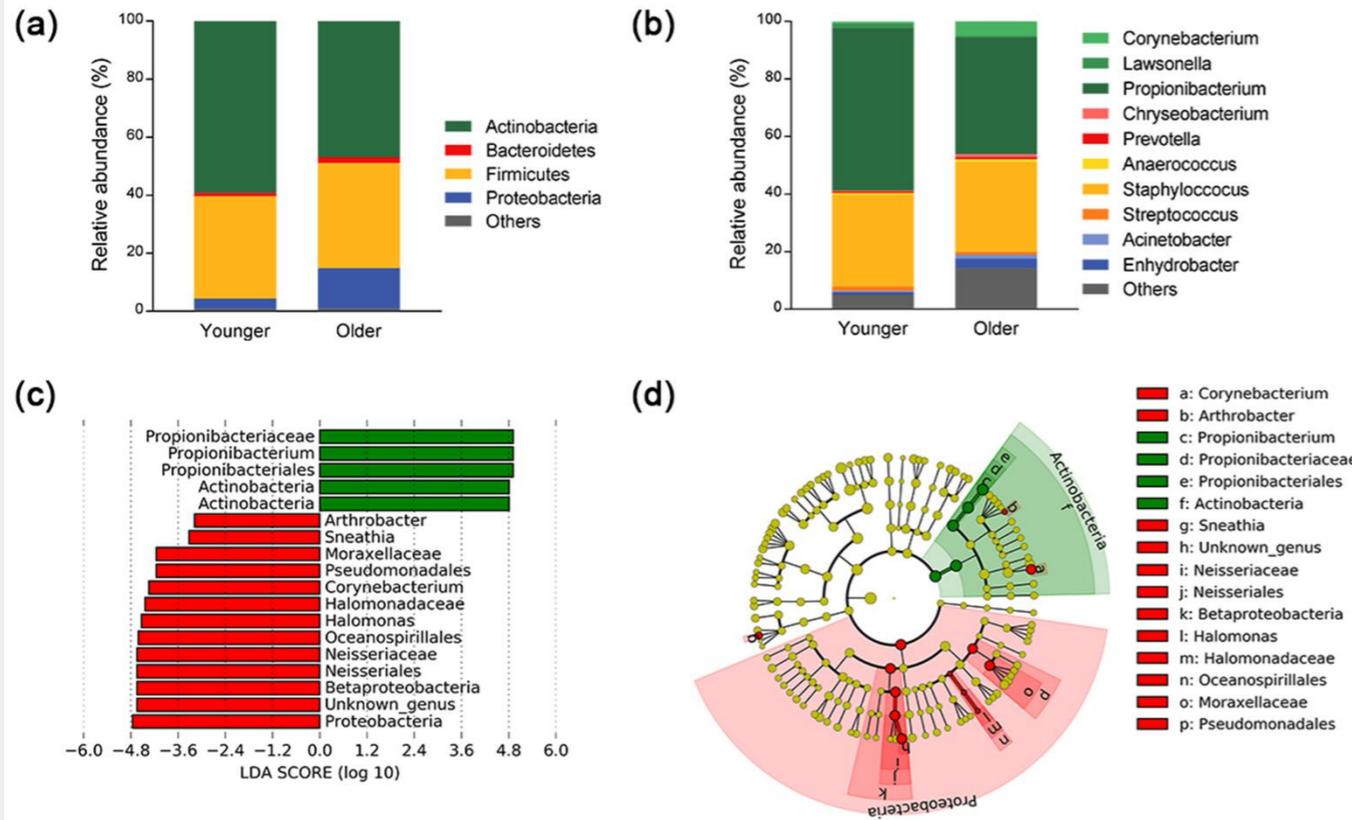
➔ Impact du vieillissement sur la répartition mais pas sur la richesse du microbiote cutané

Beta diversité

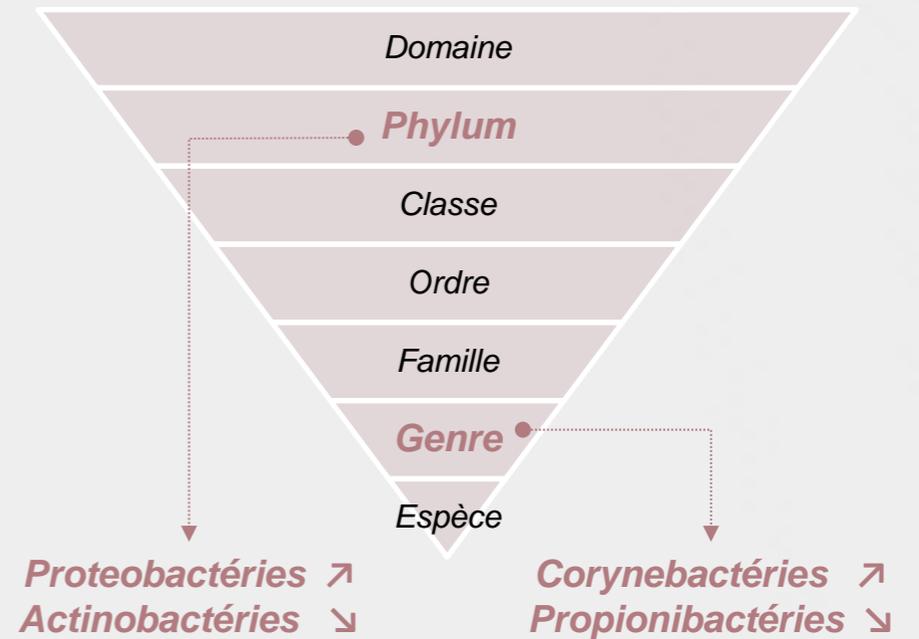


➔ Impact du vieillissement sur l'abondance mais pas sur les membres du microbiote cutané

Classification taxonomique



Etude taxonomique (abondance relative)



➔ Impact du vieillissement sur la classification taxonomique, au niveau du phylum et du genre, avec une augmentation de l'abondance relative des bactéries opportunistes

WHEN METASEQUENCING REVEALED A MICROBIOTA IMBALANCE ACROSS AGING



C. Grimaldi, R. Jugé, P. Rouaud-Tinguely, D. Boudier, S. Bordes and B. Closs.

R&D Department, SILAB, Drive-Is-Gaillard, France
 email: cgrimaldi@silab.fr

INTRODUCTION

The skin hosts a real ecosystem composed of various microorganisms that are harmless and even beneficial! Among the diversity of skin microbes, the bacterial flora represents the most studied community!

Inter-personal variations of skin microbiota have been largely reported. Indeed, the composition of human skin microbiota is influenced by multiple factors such as gender, environment, lifestyle, hygiene practices and aging¹.

While few studies give new insights in microbial diversity variations with aging, analyses are restricted to Asian skin².

To our knowledge, no study has identified potential age-related changes in cutaneous microbiota for Caucasian ethnicity.

AIM

In this context, the aim of our study was to compare the skin microbiota between two different age groups of Caucasian women.

METHODS

Swabbing from the forehead

- Young group: 17 healthy female Caucasian volunteers, mean age 29 ± 3 years
- Old group: healthy female Caucasian volunteers, mean age 62 ± 5 years



Bacterial communities analysis

- Amplification of 16S rDNA V3-V4 regions
- Sequencing of amplicons on Illumina MiSeq[®]



Bioinformatics analysis

- Data processing
- Validation of our analysis pipeline
- OTU analysis and Taxonomic classification through QIIME2 pipeline³
- Analyses through R softwares:
 - Bacterial richness: alpha diversity
 - Structural difference according to the phylogenetic distance: beta diversity



RESULTS

1. Impact of aging on alpha diversity

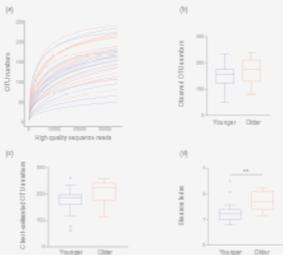


Figure 1. Comparison of bacterial alpha diversity between younger and older age groups. (a) Rarefaction curves obtained on samples from younger (blue) and older (red) subjects. Box and whiskers plots showing observed (b) as well as chao1-estimated (c) richness and Shannon diversity index (d) for each group, calculated at the OTU level. Circle indicate the outliers. ** P < 0.01 using Wilcoxon's test. Sign test.

- Rarefaction curves: tended to reach a plateau.
- Sufficient number of OTUs analyzed for each sample to exhibit the full species diversity.
- Observed and chao1-estimated OTU numbers: no variation between two age groups.
- No impact of aging on the richness of cutaneous microbiota.
- Shannon alpha diversity index: significant increase in older age group.
- Impact of aging only on the evenness of cutaneous microbiota.

2. Impact of aging on beta diversity

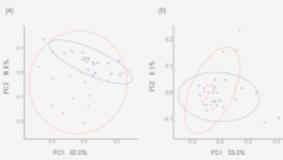


Figure 2. Comparison of the bacterial community beta diversity between younger and older age groups. Principal coordinate analysis (PCoA) of bacterial communities from forehead samples at the OTU level using weighted UniFrac (a) and unweighted UniFrac (b) UniFrac distance according to younger (blue) and older (red) age groups. The percentage of the variation attributed to an axis is indicated.

- Weighted UniFrac: distinct clustering of both age groups.
- Change in abundance of bacterial communities across aging.
- Unweighted UniFrac: no clustering.
- No change in membership of bacterial communities with age.

3. Impact of aging on taxonomic classification

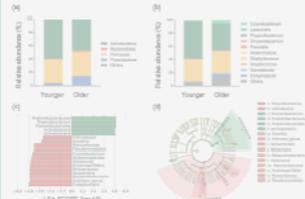


Figure 3. Taxonomic analysis of forehead microbiota in younger and older subjects group. Stacked bar charts showing the relative abundance of the four most prevalent bacterial phyla (a) and the 10 most prevalent genera (b) in the forehead microbiota samples. Other phyla and unknown genera or genera with < 0.5% mean relative abundance are grouped into the category "other". (c) Linear discriminant analysis effect size (LDA) between older (red) and younger (blue) age group (P < 0.05, LDA score > 2.5). (d) Cladogram plotted from LDA analysis. Nodes represent phylogenetic levels from domain to genus (from inside out). Each node diameter is proportional to the taxon abundance and is colored by age group (red for older and green for younger age group) for which it is significantly more abundant.

- At phylum level, aging significantly decreased Proteobacteria and Firmicutes.
- At genus level, aging significantly decreased *Staphylococcus* and *Corynebacterium*.
- Impact of aging on taxonomic classification

CONCLUSION

- This study represents the first identification of skin microbiota shift during aging of Caucasian women.
- The modifications in pH level, sebum secretion or immune defenses that are characteristic of aged skin could lead to a lower selective pressure of commensal bacteria. Hence, opportunistic such as *Proteo-bacteria* and *Corynebacterium* can colonize the skin, leading to the different distribution of bacterial communities observed between young and aged volunteers.
- We hypothesize that the state of skin microbiota could become a readout predicting the progression of aging on skin.
- In the future, this study will support new approaches in order to rebalance the skin microbiota and prevent age-related skin disorders.

ACKNOWLEDGEMENTS

We thank all the volunteers for skin samples collection.

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➔ 1^{ère} démonstration d'une modification du microbiote cutané au cours du vieillissement chez la femme Caucasiennne

➔ Dû à une diminution des barrières mécaniques et immunes locales au cours du vieillissement ?

➔ 1^{ère} étape visant à développer des approches pour

MERCI POUR VOTRE ATTENTION ! rééquilibrer le microbiote cutané et prévenir les désordres cutanés liés à l'âge.

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Shift in skin microbiota of Western European women across aging.
 Jugé R¹, Rouaud-Tinguely P¹, Breugnot J¹, Servaes K¹, Grimaldi C¹, Roth MP², Coppin H², Closs B¹.