FIRST EVIDENCE OF THE POTENTIAL ROLE OF MICROBIOME IN SKIN AGING ?

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1 INTRODUCTION

Extrinsic skin aging is a consequence of exposure to different external aggressors, and in particular to environmental oxidative stress [1-3]. Recent published data have shown that the diversity of the skin microbiota changes with the age of the subject [4] and that this diversity is also altered when exposed to various external aggressors, such as air pollution [5]. In this context, it is still **unclear whether there is a link between skin aging, oxidative stress and skin microbiome**.

For this purpose, we isolated skin microbiota on the skin of subjects with different ages and evaluated its response to oxidative stress. We chose ***Cutibacterium acnes*** as the representative species of the skin microbiome, since it is the dominant skin bacteria on sebaceous areas and that ownd a unique antioxidant potential thanks to the enzyme RoxP [6,7].

Here we analyzed their antioxidant potential by comparative genomic analysis, measurement of resistance to oxidative stress and gene expression analysis.

2 MATERIALS AND METHODS

STRAINS & GENOMES

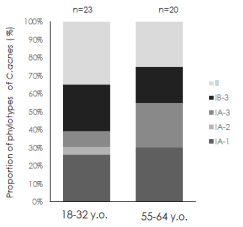

Figure 1: Repartition of C. acnes strains in the two age groups. Most of type IA-3 strains were isolated from aged skins (70% of the IA-3 isolates).

A bank of 43 C. *acnes* isolated from the face of healthy subjects of two age groups, young (18-32 yo; n= 23) and elderly (55-64 yo; n=19) was used; including various phylotypes (I-A1; I-A2; I-A3; I-B3; II).

Genomes were obtained after sequencing on an Illumina Hiseq2000 system. Sequences were analyzed and assembled to contigs with the Ray assembler with standard settings and annotated using RAST. Genomes phylotyping was assessed as previously described [8] using a set of 60 additional (publicly available) *C. acnes* genomes.

RTQPCR EXPRESSION

The different strains of *C. acnes* were prepared from 2 mL of a 24-hour culture. RNAs were extracted and purified using the RNeasy Mini Kit (Qiagen, 74104) according to the supplier's instruction. The relative abundance of the 3 genes (roxP, catalase, sod) and gapdh transcripts was determined by a two steps RT-qPCR. Results were interpreted via double delta Cq analysis, using strain 46 as a reference. The experiment was run in biological and technological duplicates.

Primers	Sequence	Reference
roxP_for_1	GCATCTAGCCCTCTCACCAT	[7]
roxP_rev_1	CTGAGAGTCCGGTAGGTGGT	
catalase_for_1	CTCATGGGTTAACGCCGAAG	This study
catalase_rev_1	TGAACCGGTAATCCTTGGCT	
sod_for_1	CTCAACACCATGCAGCTGTT	This study
sod_rev_1	CCTTGACGTTCTGGTACTGC	
gapdh_for_1	GCATCATGACTACCGTCCAC	[7]
gapdh_rev_1	CGGTGGTCTCCTTAGAGGTC	

Table 1: Sets of primers used in the study.

H_2O_2 SUSCEPTIBILITY

Susceptibility to H_2O_2 were determined by exposing bacteria to different concentrations of H_2O_2 (5, 10 et 20 mM; Sigma Aldrich) in 12 well plates, at 37°C, 65 rpm anaerobically. Absorbance (OD 600 nm) has been measured after 20 h using a spectrophotometer (SpectraMax M2). The experiment was run in biological and technological duplicates.

3 RESULTS & DISCUSSION

Presence of AOX genes in *C. acnes* strains

roxP, sod and catalase genes are present in all the 43 strains of *C. acnes*, and homologs of these genes exist within the phylotypes (Fig. 2). Phylotypes I (IA-1, IA-2, IA-3, IB-3) exhibit genetic differences compared to phylotypes II for the roxP and catalase genes, while only phylotype IB- 3 stands out from other phylotypes for the sod gene. These results confirm existing reports by Lood *et al.* on strains from acne skin type and expand their finding to a larger set of *C. acnes* strains isolated from healthy skin.

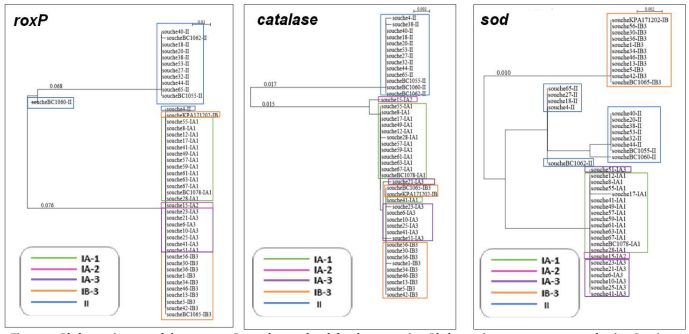

Figure 2: Phylogenetic trees of the genes roxP, catalase and sod for the 44 strains. Phylogenetic trees were generated using Seaview (http://doua.prabi.fr/software/seaview) with default options of Distance J-C; Bootstraps (n=100). Branch length were estimated with the option Br.

Gene expression & Strains susceptibility to H2O2

At the gene expression level, the phylotype IA-3 (and I-B3) showed significantly lower expression of roxP, catalase and sod compared to IA-1/I-A2. Phylotype II exhibited the lowest expression of roxP and catalase and similar expression levels were obtained for *sod* (Fig. 3). *In vitro*, the type I-A3 strains tested were less resistant to oxidative stress than other phylotypes (Fig.4).

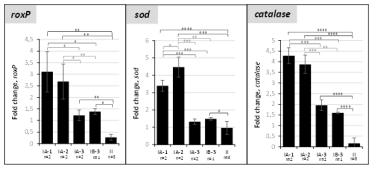

Figure 3: Expression of roxP, sod and catalase per group of strains. RT-QPCR were conducted on individual strains and averaged for each phylotype.

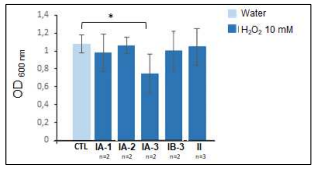

Figure 4: Sensitivity of C. acnes phylotypes to H2O2.

Here, from a collection of strains from healthy skin, we have shown that strains of C. *acnes* have different antioxidant potential at the genomic, gene expression and *in vitro* levels. More particularly, phylotype I-A3, which was mostly isolated on aged subjects presented a modest antioxidant potential.

4 CONCLUSIONS

The present data suggest a first link between skin age, oxidative stress and skin microbiome. Further, *in vitro* studies of larger strains bank and various oxidative stressors as well as other microbial species of the skin microbiome and a more comprehensive *in vivo* analysis of the stains inhabiting aged and photo-aged skins will be needed to better understand this relationship and its consequences on skin aging. However, this constitutes a major step in the understanding of the complex ecosystem at the skin surface and its potential implication in skin aging and opens a new field of investigation to develop innovative antiaging cosmetic approaches.

References:

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Aknowlegements :

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