Deciphering the biological mechanisms behind unpleasant axillary odors

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Abstract

The enzymatic degradation of sweat odorless molecules by cutaneous bacteria produces malodorous volatile compounds such as thiols, known to be responsible for particularly unpleasant odors. The aim of this study was to explain the odor intensity variation within a group of volunteers from the same ethnicity with standardized hygienic and alimentary practices. For this purpose, the axillary odor was evaluated by qualified experts on the t-shirts of a group of volunteers that underwent a physical activity. A malodorous and a non-odorous groups were identified with significantly different odor intensities. To go further, the axillary microbiota of each volunteer was sampled. The bacterial relative abundance was investigated by DNA sequencing, revealing an increase of *S. hominis* relative abundance in the malodorous group. Very interestingly, the assay of the enzymatic activity responsible for thiol release reveals that this one is significantly higher in the *S. hominis* sampled on volunteers from the malodorous group. These new scientific insights pave the way to the development of innovative deodorant active ingredient targeting the biological origin of unpleasant axillary odors.

Keywords: Bromhidrosis; unpleasant odors; axillary; *Staphylococcus hominis*; C-S lyase

Introduction.

From a physiological point of view, perspiration is a natural process that regulates body temperature *via* the secretion of sweat [1]. It is produced by sweat glands, epidermal structures that can exist as two types: eccrine and apocrine. Eccrine glands are found over the entire body at birth and produce sweat composed primarily of water and ions [2]. Apocrine glands on the other hand are associated with hair follicles and develop at the time of puberty in certain specific zones such as the underarms, mammary areolas or genital regions. In addition to water and ions, the sweat released by these glands also contains organic compounds (proteins, sugars, lipids). Underarm apocrine glands are therefore an important source of nutrients for the cutaneous microbiota, thereby promoting bacterial proliferation.

Sweat is odorless when it is secreted because its constituent molecules themselves are also. Among the broad diversity of bacteria composing the cutaneous microbiota, some species stand out for their ability to transform odorless secretions into volatile odor products [3, 4]. This is the case of *Staphylococcus hominis.* This odor-generating property results from its ability to metabolize sweat compounds thanks to specific enzymatic activities such as the C-S lyase's one.

Three main strategies have been used for several decades to prevent odors arising from perspiration. They involve preventing the secretion of sweat, masking odors or eliminating bacteria of the axillary microbiota [5]. Nevertheless, these strategies are currently contested [6]. The antiperspirant strategy is not physiological since it prevents sweat from being released onto the surface of the skin. It also often requires the use of aluminum salts that are controversial ingredients [7]. The fragrance compounds used to hide perspiration odors are a source of irritation for the underarm skin because of their alcohol content. Finally, the antiseptic strategy uses broad-spectrum antimicrobial agents and does not respect the balance of the axillary cutaneous microbiota. In addition, antiseptic products used are also irritant for the skin,

allergenic or suspected of being endocrine disruptors [5;6]. These different issues explain the need to develop new anti-odor strategies that are risk-free, natural and that specifically target the biological origin of unpleasant axillary odors.

In this context and to develop an active ingredient specifically targeting the mechanisms involved in unpleasant odor generation, a modeling study was implemented by SILAB, based on its expertise in sensorial analysis and its understanding of the microbiota, to determine if the production of malodorous molecules was the result of a quantitative modification of the microbiota (dysbiosis) and/or a qualitative modification (modified enzymatic activity of the microbiota).

Materials and Methods.

Study design

Samples were obtained from the underarm of 24 non-smoking volunteers, 18 women and 6 men, with an average age of 46. All volunteers live in Brive-la-Gaillarde (Corrèze, France) or nearby and gave a written informed consent for their participation in this study. Before starting, all subjects were asked to stop using anti-perspirants 3 weeks before, the use of a provided deodorant was allowed during the 2 first weeks. All the volunteers also used the same shower gel and shampoo provided by the company, during the 3 weeks preceding the study to prevent any effect linked to the use of a personal care product. Wearing perfume, body lotion, eating spicy or strong-smelling foods and drinking alcohol were prohibited 3 days before the physical activity session. The volunteers underwent a 30-minute session of indoor cycling under the surveillance of a sports instructor in a fitness center. During this session, each volunteer was instructed to wear a provided white 100% cotton T-shirt, newly bought from the same manufacturer with a standardized machine washing. After the cycling session, subjects waited

an hour in a temperate room in controlled conditions, still wearing the T-shirts so that odors could form.

Unpleasant axillary odor rating

Rating sessions took place in a well-ventilated room and were conducted by a panel of experts qualified for sensorial measurement and trained to note the presence and intensity of odors of a sample. Each expert smelled the T-shirt sample in a plastic bag and noted the perspiration odor intensity on a scale from 0 to 4. The evaluation was conducted on the same day as the physical activity. The odor score used was the mean of the scores of all the evaluators.

Study of axillary microbiota

Cutaneous microbiota samples were taken from the underarms. Genomic DNA was extracted from swabs and the V1–V2 hypervariable region of the 16S rRNA gene was amplified using polymerase chain reaction (PCR) with specific primers. After sequencing, sequences were demultiplexed, and the generated FASTQ were used for downstream data analysis. Sequences were clustered into operational taxonomic units (OTUs) using SWARM clustering method (v3.0.0) and Chimeric OTUs were removed. Only OTUs that made up to 0.005% or more of the total sequences were considered [8]. A representative sequence for each OTU was used to assign taxonomy using BLAST (v2.10) against a curated version of the SILVA 16S rRNA database (release 138 with sequences displaying a Pintail value of 100) with a minimum identity and coverage threshold of 96% and 99% respectively. Annotated OTUs were used to determine the relative abundance at genus level. Among them, some OTUs are assigned for species such as *S. epidermidis* or *S. hominis*. Analyses of alpha diversity was performed by calculating Shannon index using R language (v4.1.3) with the phyloseq package in R studio.

Study of C-S lyase enzymatic activity

The 24 cutaneous microbiota samples collected by swabbing on each volunteer 1h after the indoor cycling session were cultured on selective nutrient agar, a custom-made culture medium adapted to isolate some *S. hominis* clones, according to a culturomics' derived methodology [9]. After a 48h incubation at 37°C, the plates were analyzed and more than 66 colonies with a *S. hominis*-like-phenotype were picked up. Each isolate was then identified by Internal Transcribed Spacer-PCR [10] and 28 of them were confirmed as *S. hominis* strains. Crude extracts were obtained and the amount of protein in the *S. hominis* extracts was determined by colorimetric assay. The C-S lyase activity in crude extracts of *S. hominis* isolates was determined thanks to a dedicated protocol [21]. Thiols released by the action of C-S lyase induce fluorescence that can be quantified. The specific activity of C-S lyase was calculated by dividing this fluorescence increase (ΔF) by the quantity of proteins (mg) in the protein extract and is expressed as $\Delta F\ s^{-1}\ mg^{-1}$.

Statistical analysis

Comparison between the two groups was made with the Student's t test when the data followed a normal law (Shapiro-Wilk test of normal distribution more than 5%). In the opposite case, the non-parametric Mann-Whitney test was used. In each case, the test was two-tailed with a risk threshold set at 5%. P values of less than 0.05 were considered significant. ns: not significant, *: $P < 0.05$, **: $P < 0.01$, ***: $P < 0.001$. This statistical processing was done on Excel with the "Real Statistics" library (release 7.2, Copyright (2013-2020) Charles Zaiontz).

Results.

Odor intensity variation in volunteers after a sport activity

Among the volunteers, results of body odor investigation by a panel of experts reveal that after 30 minutes of indoor cycling and 1 hour of break, volunteers split into two groups. The non-odorous group is composed of 13 volunteers (11 women and 2 men, mean age 47 ± 6 years old) having odor scores of 0. The second group, named malodorous, is composed of 11 volunteers (7 women and 4 men, mean age 45 ± 10 years old) with odor scores ranging from 1 to 3.43 (mean score 2.68 ± 0.71) (Figure 1).

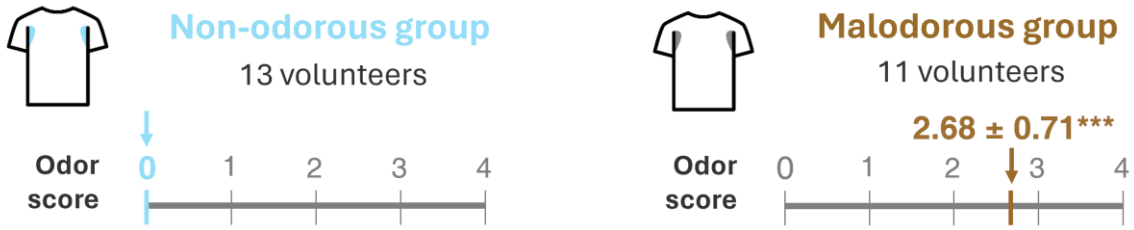

Figure 1. Identification of two groups of volunteers based on their unpleasant axillary odor rating by a panel of experts. Result(s)/non-odorous group with ***: P<0.001.

Bacteria relative abundance variation between non-odorous and malodorous volunteers

To decipher the malodor generation, axillary microbiota was sampled from the underarms of all the volunteers. The whole cutaneous microbiota was analyzed by 16S rRNA gene metasequencing and the α-diversity and the relative abundance of bacteria within the two groups was investigated. The Shannon index describes the diversity within a sample. Results presented in the figure 2a reveal that the diversity of bacterial communities of the axillary microbiota is not significantly modified between non-odorous and malodorous volunteers. Moreover, the relative abundance of the top 10 genus level reveals no significant differences in the genus from the samples originating from the malodorous and the non-odorous groups (Figure 2b).

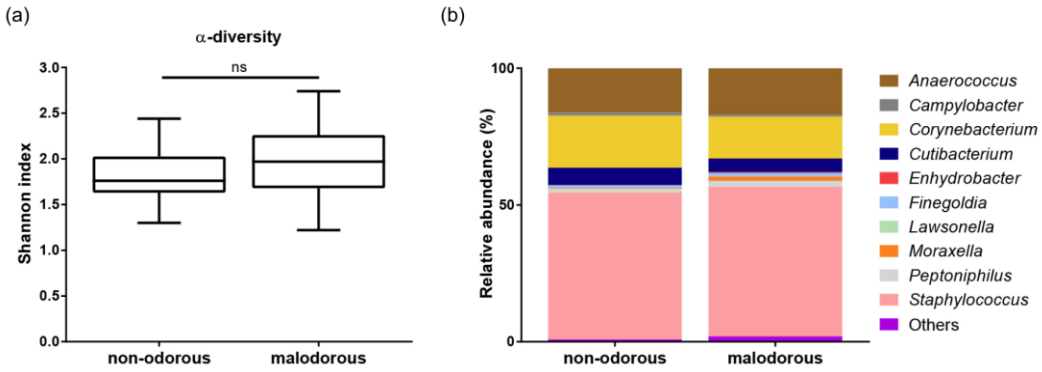

Figure 2. Study of the α-diversity of bacterial communities (a) and of the relative abundance of the top 10 genus level (b) in non-odorous and malodorous volunteers. Result(s)/non-odorous group with ^{ns}: non-significant.

As *S. hominis* is one of the species involved in axillary unpleasant odor generation, we investigate its relative abundance within the microbiota. Our results highlight that the relative abundance of this species is significantly increased in the samples originating from the malodorous group compared to samples from the non-odorous one ($P < 0.05$) (Figure 3a) while the relative abundance of *S. epidermidis*, the main species among the *Staphylococcus* in the axillary area, is not significantly modified (Figure 3b). The other species quantifiable in this study and related to odor generation did not vary significantly between the two conditions (data not shown).

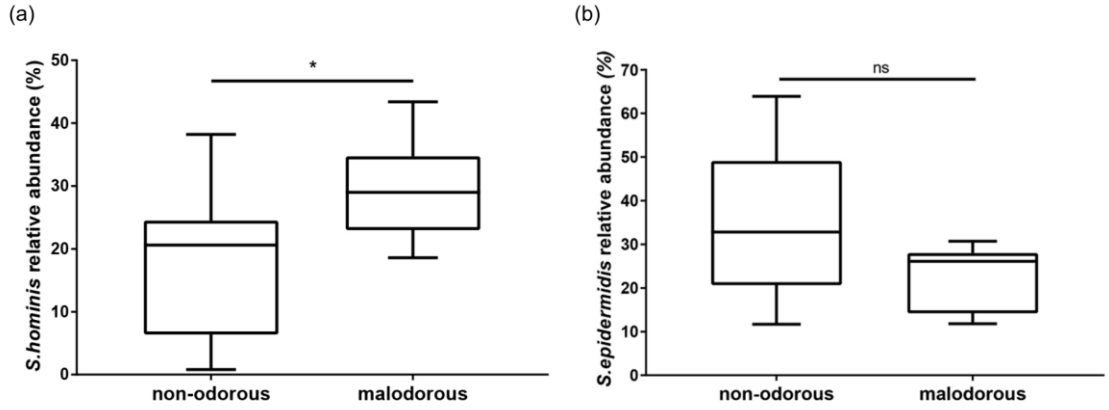

Figure 3. Relative abundance of *S. hominis* (a) and *S. epidermidis* (b) within the whole microbiota sampled from non-odorous and malodorous volunteers. Result(s)/non-odorous group with *:P<0.05 --- ns: non-significant.

Variation of C-S lyase activity of S. hominis sampled from non-odorous and malodorous volunteers.

Odor generation is due to the conversion of a non-odorous precursor into odorous 3M3SH thanks to the C-S lyase enzyme. To unravel the mechanisms behind the odor generation in the malodorous group, the enzymatic activity of 28 *S. hominis* isolates obtained on volunteers of each group was investigated. Very interestingly, our results presented in the figure 4 reveal that at equal protein content, the C-S lyase activity is significantly higher in crude extracts of *S. hominis* isolated from the underarms of malodorous volunteers compared to that of bacteria sampled from non-odorous volunteers.

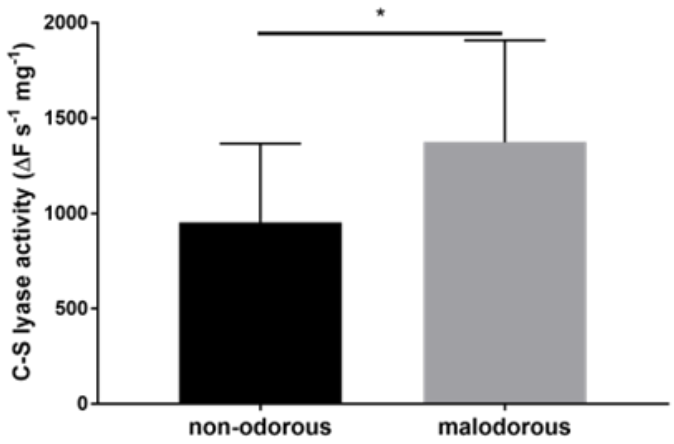

Figure 4. Measurement of C-S lyase activity in *S. hominis* isolates obtained from microbiota sampled from non-odorous and malodorous volunteers. Result(s)/non-odorous group with *:P<0.05.

Discussion.

This study demonstrates that in a group of volunteers from the same ethnicity, the *S. hominis* relative abundance is increased in volunteers with unpleasant axillary odors. For the first time, our results highlight that this bacterial strain sampled on volunteers with unpleasant odor has a higher C-S lyase activity. Hence, beyond a larger bacterial population, the extended enzymatic activity of these bacteria participates to the release of malodorous molecules (thiols). These new scientific insights pave the way to the development of innovative deodorant active ingredient targeting the biological origin of unpleasant axillary odors.

Conclusion.

Strong of these discoveries on unpleasant axillary odor generation, the company relied on its expertise and proposes a natural active ingredient capable of controlling perspiration odors at the same time as conserving the diversity and balance of the cutaneous axillary microbiota thanks to its action on both the abundance of S*. hominis* and the enzymatic activity of this bacterial species. It also soothes underarm skin irritations by reducing inflammation and strengthening the barrier function.

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Conflict of Interest Statement.

NONE.

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