Review

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Human Microbiome

Daily battle against body odor: towards the activity of the axillary microbiota

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The microbial community of the human axilla plays a key role in the formation of axillary odor by biotransformation of odorless natural secretions into volatile odorous molecules. Culture-based microbiological and biochemical studies have allowed the characterization of the axillary microbiota, but the advent of next-generation culture-independent DNA sequencing approaches has provided an unprecedented depth of data regarding the taxonomic composition of the axillary microbiota and intra- and interindividual variation. However, the physiological activity of the microbiota of an individual and its variation under different environmental conditions remains largely unknown. Thus, metatranscriptomics represents a promising technique to identify specific metabolic activities in the axillary microbiota linked to individual differences in body odor.

Characteristics of the human axilla as a microbial habitat

The human skin exhibits a diversity of ecological niches varying in moisture, the availability of nutrients, and the presence of host- and bacteria-derived antimicrobial peptides [1,2]. In general, skin regions can be classified as dry, sebaceous, or moist environments with specifically adapted organisms establishing a distinct microbial profile characteristic for each topographical region [3]. Comprehensive 16S rDNA profiling of the cutaneous microbiota (see Glossary) in the course of the Human Microbiome Project shed the first light on the community structure at different skin sites independent of microbial culture. In moist areas such as the human axilla, *Corynebacterium* spp. and *Staphylococcus* spp. dominate the resident flora, which was demonstrated earlier by culture-based approaches [4–6]. The axillary region is a specific habitat and differs significantly from other body parts because it harbors hair follicles with sebaceous glands and a high density of sweat glands [7]. In this occluded environment nutrients are readily available, which allows for dense bacterial colonization reaching up to 10^6 cells per cm^2 [6]. Different types of sweat glands are present including eccrine and apoeccrine glands which are responsible for thermoregulatory sweat secretion, releasing mainly H_2O and electrolytes (Na^+, K^+, Ca^{2+}, Mg^{2+}, Cl^-, HCO_3^-, lactate, urea and ammonium) to the skin surface [7,8]. Additionally, apocrine glands, so-called scent glands, contribute to the high density of nutrients and are highly characteristic for the axillae of human adults [9]. They secret a milky odorless fluid consisting of electrolytes, steroids, proteins, vitamins, and a variety of lipid compounds [6,7,10,11]. Despite the fact that the exact composition of apocrine sweat is currently unknown due to insufficient availability of pure samples [12], the microbial biotransformation of these secreted nutrients in the human axilla undoubtedly

Glossary

Anosmia: the inability to perceive odor.
Axilla: the hollow beneath the junction of the arm and shoulder (armpit).
Diversity: number and abundance distribution of distinct taxa of microorganisms.
Lipid-auxotrophic: used to describe corynebacteria lacking the ability to synthesize fatty acids *de novo* owing to a missing fatty acid synthase gene. These species are therefore also lipophilic.
Lipid-catabolizing: used to describe corynebacteria with the ability to degrade a variety of fatty acids, for instance via the β-oxidation pathway.
Lipophilic: used in corynebacterial taxonomy to describe the observation that growth of some *Corynebacterium* species is enhanced by the addition of 5% horse or sheep blood to synthetic media or by using mixtures of lipids as media supplements. These species generally grow poorly on standard laboratory media.
Metagenomics: direct culture-independent sequencing of total DNA obtained from a microbial community to describe the microbiome and to analyze the genomic potential of the microbiota.
Metatranscriptomics: sequencing of cDNA generated directly from RNA transcripts of a sample of the bacterial community. Thus, only the genes undergoing transcription are detected in a culture-independent manner to analyze the functional activity of a microbial community.
Microbiome: the collectivity of the genomes occurring in the microbiota.
Microbiota: the microorganisms that typically inhabit a bodily organ or part. The members of the human microbiota are estimated to outnumber the human cells by a factor of 10. The human skin microbiota consists of bacteria, fungi, and mites, and its composition is structured as a function of the skin subhabitat examined.
16S rDNA amplicon sequencing: the 16S rRNA gene is universally present in all bacterial species. Its suitability as a biomarker is characterized, on the one hand, by the presence of species-specific hypervariable regions allowing taxonomic classification with the aid of databases; for example, the Ribosomal Database Project (RDP). On the other hand, conserved regions enable the application of universal (or phylum-specific) PCR primers for amplicon generation. Importantly, this technique allows direct culture-independent description of microbial communities, which is further assisted by the development of high-throughput DNA sequencing techniques.

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leads to the development of characteristic and individual odor profiles [13–15]. A comprehensive set of studies have been conducted with detailed analysis and comparison of individual axillary sweat demonstrating the influence of genetic factors (gender and ethnicity) and behavioral patterns (diet) on the composition of human axillary odor and individual olfactory sensibility for particular odor compounds [14,16,17]. Also, perceptional sensitivity and pleasantness of specific odor compounds were reported to correlate with gender, sexual orientation, and the use of oral contraceptives [18,19].

It is of particular interest for the cosmetic industry to understand body-odor formation mechanisms to be able to design deodorants which specifically target and intervene with them. The classic functional mechanism of deodorants is the depletion of cutaneous bacteria employing unspecific antimicrobial agents. This, however, can cause skin irritations upon very frequent usage not only due to the direct topical action of alcoholic or organic substances. Also, the disruption of the integrity of the skin microbiota may have negative effects on the host in terms of health because symbiotic and commensal bacteria participate in immune defense against pathogens [20]. The following sections provide an overview of the biochemical origin of human axillary odor and the taxonomic composition of the axillary microbiota based on recent data generated with next-generation sequencing techniques. Additionally, the first culture-independent analysis of the activity of the axillary microbiota is described, followed by a proposed workflow to perform metatranscriptomics of human axillary samples to detect in detail active metabolic processes involved in body-odor formation.

Origin and composition of human axillary odor

In the past decade intensive research on axillary sweat and the resulting body odor was driven mainly by the cosmetic industry and led to the identification of a variety of characteristic odorous compounds (Figure 1). These include sulfanylalkanols, steroid derivatives, and volatile short-chain fatty acids, whose combination and ratios account for the intensity of human axillary odor [13,14]. The highest impact on axillary odor derives from volatile sulfur compounds, which exhibit a low olfactory threshold and account for a typical onion-like and musky malodor [21]. Amongst others, 3-methyl-3-sulfanylhexan-1-ol and its derivative 3-sulfanylhexan-1-ol (Figure 1A) are secreted as glycine–cysteine-conjugated precursor molecules by the apocrine sweat gland [22]. Upon cleavage by bacterial dipeptidases (TpdA) and C-S lyases (AecD) that cleave carbon-sulfur bonds the precursors are degraded and the odoriferous mercaptoalcohols are released [23]. The origins of the bacterial enzymes responsible for odor release have been controversial [24,25]. Initially, the C-S lyase activity was described in the *Corynebacterium striatum* isolate Ax20 and reported not to be present in staphylococci [24]. During incubation of the synthetic precursor *S*-benzyl-(S)-cysteine with several skin-resident staphylococci no cleavage activity was detected *in vitro*, whereas corynebacteria formed high levels of the product phenylmethanethiol. By contrast, it was demonstrated that an axillary isolate of *Staphylococcus haemolyticus* is able to generate a sulfuric odor from sterile human sweat secretions [21]. However, subsequent cleavage assays with a recombinant MetC-type C-S lyase from *S. haemolyticus* failed to detect an odor-associated precursor cleavage *in vitro* [25]. A recent analysis of C-S lyases by sequence alignment demonstrates a distinct grouping of these enzymes into MetC-type and MalY-type lyases [26]. Only MalY-type lyases, predominantly found in corynebacteria and named AecD, seem to be associated with precursor cleavage and sulfuric odor formation in the human axilla.

Another enzyme associated with corynebacteria is the N^{α}-acylglutamine aminoacylase AgaA which is responsible for the release of a broad variety of volatile short-, medium- and branched-chain fatty acids in the human axilla [13] (Figure 1B). Compounds such as 3-methyl-2-hexenoic acid (3M2H) evaporate from the skin surface after release from a glutamine residue by AgaA [27]. The origin of the glutamine-conjugated odor precursors is currently discussed in the literature because of different reports regarding the source of the glutamine residue. Initially it was demonstrated that the fatty acid 3M2H is carried to the skin surface bound to the lipocalin ApoD and that the expression of this carrier protein is specifically located in the apocrine glands [15,28,29]. However, Natsch *et al.* demonstrated the release of 3M2H from sweat secretions after incubation with the recombinant glutamine-specific AgaA enzyme, which points to the presence of a glutamine-conjugated form of 3M2H in human sweat [27]. To unite these findings it has been proposed that the Gln-conjugate derives from proteolytic degradation of ApoD bound to the odorous acid through its N-terminal glutamine residue [30]. However, it needs to be demonstrated whether equimolar levels of ApoD and volatile fatty acids are present in human sweat and axillary odor [26].

In the 1980s, generation of odoriferous steroids such as androstenol and androstenone (Figure 1C) by skin bacteria was associated with musk-like and urinous axillary malodors [31]. Later the underlying enzymatic processes were investigated and several enzymatic functions for the generation of malodorous steroids have been proposed [32]. Even though a distinct composition of corynebacteria is essential for the generation of steroid derivatives, so far it has been impossible to link this biotransformation to the expression of specific corynebacterial genes. Unlike sulfanylalkanols or volatile fatty acids, the biotransformation of steroids seems to be more complex, and requires the interaction between different corynebacterial species and ultimately various enzymatic functions (Figure 1C). Furthermore, the impact of androstenol and androstenone on axillary odor is currently in question because anosmia for these compounds is present in ~50% of the human population [26].

An additional highly discussed pathway for the generation of axillary odor is the degradation of skin lipids to volatile fatty acids (Figure 1D). The prominent ability of several lipid-catabolizing corynebacteria, sometimes erroneously named lipophilic, to degrade particular skin lipids incompletely has supported the hypothesis that intermediates and end-products of the β-oxidation pathway contribute to the bouquet of human axillary odor [33]. The generation of volatile fatty acids by β-oxidation requires

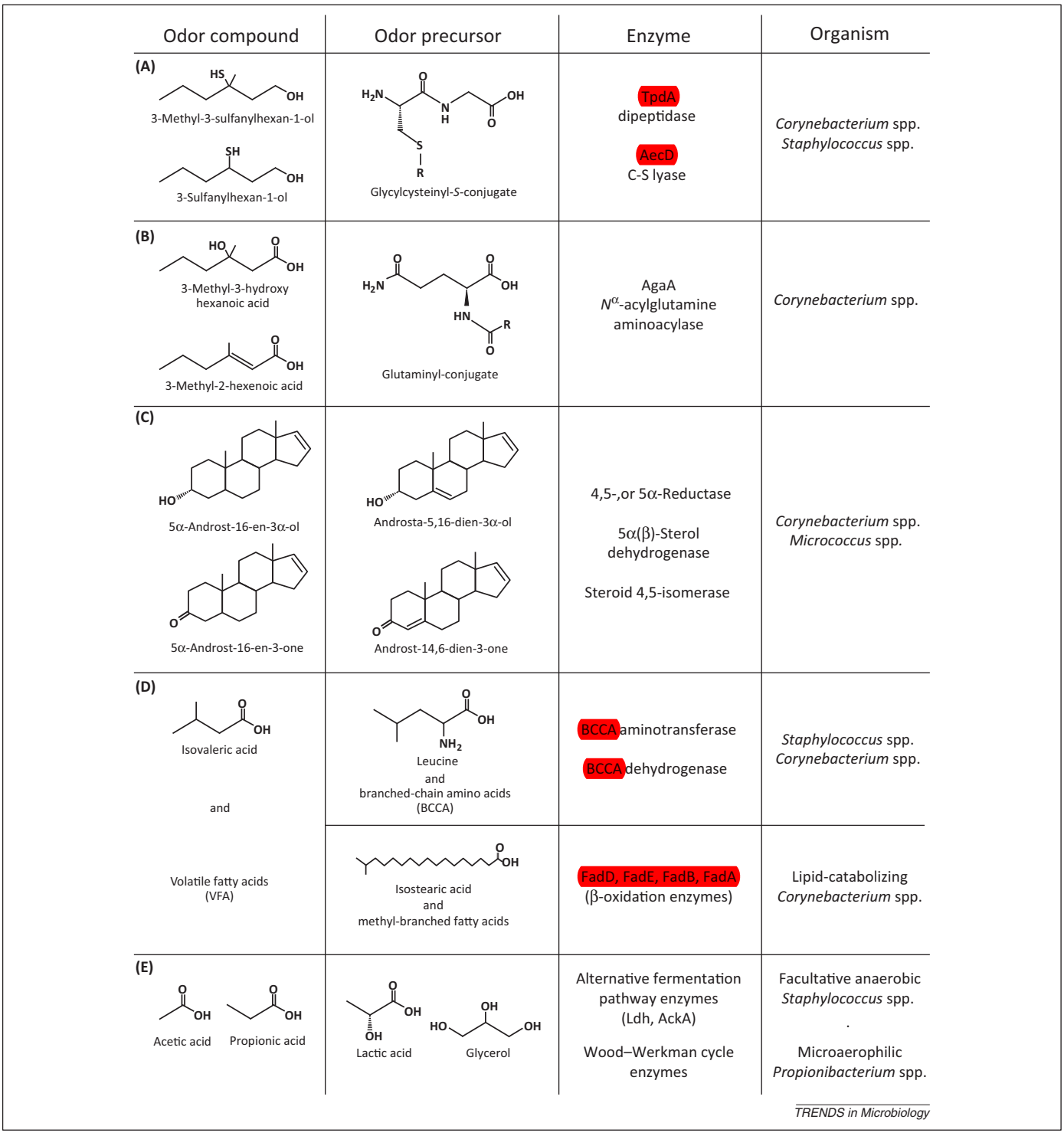

Figure 1. Representative odor compounds and their odorless precursors detected in axillary sweat. Prominent metabolites of the major chemical classes associated with axillary odor are shown by their structural formulas in panels **(A–E)**. Enzymes and microbes involved in the biotransformation of the precursor molecules are also listed. Abbreviations: *BCCA*, branched-chain amino acid.

the synergy of several enzymatic activities, similarly to steroid derivatives (Figure 1D). Furthermore, genome analysis of the lipid-auxotrophic axilla isolate *Corynebacterium jeikeium* K411 demonstrated that the relevant enzymatic activities are encoded by several paralogous genes, further complicating the elucidation of skin lipid degradation and its contribution to human axillary odor at the gene level [34,35]. The abundant malodor compound isovaleric acid (Figure 1D) is currently believed to originate mainly from the biotransformation of leucine by staphylococcal species, rather than from β-oxidation of skin lipids [26]. However, it has not been investigated whether the levels of leucine in human sweat samples correlate with the amount of volatile isovaleric acid in human axillary odor.

In addition, lactic acid and glycerol are highly abundant on the skin surface, the latter being released from

triacylglycerides by the action of bacterial lipases [33]. Their metabolism by staphylococcal species and propionibacteria leads to the formation of acetic and propionic acid (Figure 1E). Both carboxylic acids contribute to human axillary odor either directly by evaporation or by promoting bacterial growth in the human axilla. Propionibacteria can convert 3 moles of lactic acid into 2 moles of propionic acid, 1 mole of acetic acid, and 1 mole of each CO_2 and H_2O via the Wood–Werkman cycle [36]. The fermentation of 1 mole of glycerol leads to the generation of 1 mole of propionic acid and 1 mole of H_2O. In both cases pyruvate is the central pathway intermediate, therefore representing a key metabolic node in the biosynthesis of acetic and propionic acid. Facultative anaerobic staphylococci present on the human skin can generate pyruvate by alternative fermentation pathways involving enzymes such as lactate dehydrogenase (Ldh) or acetate kinase (AckA) [37].

All the reported enzymatic functions open up new opportunities in the design of deodorants by specifically targeting molecular processes leading to human axillary odor or by affecting the metabolism of odor-causing bacteria. In fact, they constitute potential targets for the daily fight against human body odor by means of inhibitory or competitive deodorant additives. Despite this tremendous progress in our understanding of the origin and composition of axillary odor at the microbiological and biochemical level, traditional culture-based methods and genetic studies with selected model organisms provide only a limited picture of the complex ecology in the human axilla. However, the very recent application of high-throughput DNA sequencing technologies has led to a massive increase and an unprecedented depth of data about the microbial communities on human skin sites, including the axilla, and has significantly broadened our view on the axillary microbiota.

Composition of the microbiota of the axillary skin

Investigations of the cutaneous microbiota at various skin sites were performed by employing high-throughput 16S ribosomal DNA (rDNA) amplicon sequencing [38,39]. These studies demonstrate that the composition of the skin microbiota correlates to the well-structured topographical landscape of the skin analogous to the several sub-habitats found in all naturally occurring ecosystems [40]. The most dominant four phyla were found to be *Actinobacteria*, *Firmicutes*, *Proteobacteria*, and *Bacteroidetes* [38,39]. Sebaceous areas of the skin, such as the forehead and the upper back, are the least diverse habitats, and are predominantly colonized by *Propionibacterium* spp. and *Staphylococcus* spp., whereas dry areas, such as the volar forearm, exhibit a mixed population in which *β-Proteobacteria* and *Flavobacteriales* are prevalent [4]. Moist skin sites including the groin region and the axilla were commonly found to be dominated by *Corynebacterium* spp. and *Staphylococcus* spp., and were reported to be intermediate in diversity as compared to other skin habitats. These results emphasize the determining role of the skin habitat in the composition of the cutaneous microbiota. It was also reported that (considering a single skin habitat) intraindividual similarity of community structure over time is generally higher than interindividual similarity of samples taken on the same day. Even though this effect seems to be site-specific and stronger in sebaceous sites than in dry sites [38,41], these findings are consistent with the general assumption that the composition of the skin microbiota is personalized and essentially determined by host factors and host genotype [40]. Temporal variation is generally reported to be site- and host-specific; in other words, after temporal changes, communities tend to be more similar to their earlier state when the same site and host is considered [38,39]. Even so, community structure can change significantly within weeks or months, indicating a temporal fluctuation, which might be inherent to skin communities due to endogenous or nutritional changes of the host as well as the influence of external factors such as weather, clothing, and cosmetics [42].

Recent analyses of composition and variation of the axillary microbiota have been performed in the context of the Human Microbiome Project employing 16S rDNA as a biomarker. In the study performed by Grice *et al*. [38], 16S rDNA data was obtained individually from axillary samples of nine healthy male and female volunteers. Probands were preconditioned not to apply topical antiseptics for 7 days and not to wash for 24 h before sampling. Nearly full-length 16S rDNA amplicons of ~1.4 kb in length were generated, cloned, and sequenced by Sanger technology. The sequences were assigned to species-level operational taxonomic units (OTUs) with a similarity cutoff of 99% and were classified employing the Ribosomal Database Project (RDP) database. The results demonstrate a high degree of interindividual variation (Figure 2A) and can roughly be subdivided into three types: four microbial communities characterized were found to be dominated by *Corynebacterium* spp. (HV1, 5, 6, and 10); three were dominated by *Staphylococcus* spp. (HV2, 4, and 7); and two were dominated by members of the taxon *β-Proteobacteria* (HV3 and 9). Further prominent bacterial taxa identified in this study include *Clostridiales*, *Lactobacillales*, *Propionibacterium*, and *Streptococcus* (Figure 2A). Although an experimental bias due to cloning of the 16S rDNA amplicons cannot be ruled out, to our knowledge, this study presents the first comprehensive insight into the taxonomic composition of the human axillary microbiota.

The microbial community of the human axilla depicted in the study performed by Costello *et al*. is represented by pooled samples collected from healthy male and female volunteers (the exact number remains unclear) [39]. Test persons were asked to bathe in the morning of the day of sampling without using deodorant or antiperspirant. rDNA amplicons of ~300 bp in length were directly sequenced on a 454 platform, OTU-clustered using a ≥97% sequence identity cut-off, and taxonomically assigned with the RDP classifier. This pool of axillary samples is apparently dominated by staphylococcal 16S rDNA sequences (Figure 2B). Corynebacteria and propionibacteria were also detected and constitute ~20% of the sequences, although their relative abundance might be higher in individual samples. The occurrence of 16S rDNA sequences assigned to the genus *Streptophyta* is considered to originate from plant pollen and, therefore, most likely represents a contamination rather than a resident member of the axillary microbiota.

In view of the semiquantitative nature of 16S rDNA amplicon sequencing data and potential sequencing bias,

Gao *et al*. applied a quantitative PCR approach to characterize the composition of the axillary microbiota of five male and female individuals without preconditioning [42]. rDNA amplicons of ~300 bp in length were generated and quantified using universal TaqMan MGB (minor groove-binding) probes in addition to specific probes for the genera *Corynebacterium*, *Propionibacterium*, *Staphylococcus*, and *Streptococcus*. These genera constituted the majority of the axillary community in four out of the five samples and accounted for interindividual variation (Figure 2C). In addition, significant temporal variation was observed at the intraindividual level for two subjects [42]. Moreover,

Figure 2. Composition and interpersonal variation of the axillary microbiota analyzed employing 16S rDNA and 16S rRNA as biomarkers in four different studies. Bars represent the relative abundance of bacterial taxa as determined by different methods. The experimental set-up of four studies is briefly indicated. **(A)** 16S rDNA amplicon sequencing of axillary samples from nine healthy volunteers [38]. **(B)** 16S rDNA amplicon sequencing of pooled axillary samples from healthy volunteers [39]. **(C)** Analysis of the abundance of the four bacterial genera *Propionibacterium, Staphylococcus, Streptococcus,* and *Corynebacterium* in the axillary communities of five individuals as performed by quantitative PCR [42]. **(D)** Analysis of the axillary communities of three male volunteers on the basis of 16S rDNA and 16S rRNA sequencing [49]. Data are adapted from [38,39,42,49].

pronounced left–right symmetry was reported regarding the four bacterial genera as well as yeasts of the taxon *Malassezia*. Despite the advantage of exact quantification, sources of experimental bias associated with PCR and probe specificity remain, in addition to the fact that no sequence data is generated for phylogenetic analysis or for the identification of additional members of the microbiota.

Altogether, the application of high-throughput 16S rDNA amplicon sequencing for the taxonomic characterization of the axillary microbiota has confirmed former findings based on culture-dependent microbiological techniques and has additionally led to the identification of further members of the microbial community as well as both intra- and interindividual variation [43,44]. Although it has proved to be a valuable technique, there are some issues which should be taken into account when considering 16S rDNA amplicon data. Despite the enhanced sequencing depth facilitated by next-generation sequencing techniques, the short read-lengths allow the sequencing of only a few hypervariable regions of the 16S rRNA gene, thereby restricting classification and potentially introducing bias because some variable regions are more suitable for the phylogenetic classification of certain species than others [45]. Furthermore, the co-occurrence of low-abundance OTUs within parallel samples was described in the course of the Human Microbiome Project, indicating a niche-specialization at the sub-genus level characteristic for each body habitat [46]. However, the classification of 16S rDNA amplicon data often is restricted to the class or genus level, with the consequence that variation at the species or sub-species level remains undisclosed.

Another important finding of the Human Microbiome Project is the stability of metabolic pathways despite changes in the composition of the microbiota, indicating the existence of a stable core microbiome rather than a core microbiota in healthy individuals [46]. Therefore, analysis of the axillary metagenome would allow the characterization of the total genomic potential including functional aspects of the microbial community. However, such an approach has not been pursued for axillary communities due to the lack of sufficient amounts of sample material that can usually be collected from skin swabs [3,38]. Furthermore, analysis of high-throughput metagenomic datasets consisting of relatively short reads is challenging from the computational perspective to reliably identify intraspecies and intragenus variability [47].

First clues of the active axillary microbiota

A major shortcoming of DNA-based 'omic' techniques such as 16S rDNA amplicon sequencing and metagenomics is the inability to discriminate between living microorganisms, representing the active resident fraction of the skin microbiota, and persisting bacteria transiently colonizing a habitat after contamination from a foreign source [3,46]. In the case of the axillary microbiota, it is well-established that microbial activity is responsible for body-odor formation and, therefore, the identification of metabolically active members of this microbial community should help to identify targets for cosmetic product development, such as deodorant additives. Because rRNA-based analyses directly target active members of a microbial community [48], Egert *et al*. compared rRNA- and rDNA-based community fingerprints to identify metabolically active bacterial members of the axillary microbiota [49]. Ten healthy male individuals were asked not to wash or apply deodorants for 48 h before sampling. The samples were used to generate 880 bp amplicons of 16S rDNA and reverse transcribed 16S rRNA covering the hypervariable regions V1–V5. Terminal restriction fragment length polymorphism (T-RFLP) fingerprinting identified higher intraindividual similarity (80%) than interindividual similarity (50%) for both rDNA- and rRNA-based T-RFLP peak profiles. In addition, comparison of rDNA and rRNA peak profiles demonstrated significant similarity, leading to the conclusion that the prominent members of the microbial communities tested were active. Cloning, sequencing, and phylogenetic analysis of 48 randomly chosen rDNA and rRNA amplicons from three individuals displayed an axillary microflora mainly consisting of corynebacteria, propionibacteria, staphylococci, anaerococci, and members of the genus *Peptoniphilus* (Figure 2D). Comparative analyses of the rRNA and rDNA profiles demonstrate a higher relative abundance of *Peptoniphilus* in the rRNA-based sample as compared to the rDNA-based profile of the right axillae, indicating a higher activity of *Peptoniphilus* spp. in this habitat, which might be associated with handedness [49]. This study represents the first culture-independent analysis of the activity of the axillary microbiota and identifies potential differences in the activity of the genus *Peptoniphilus*. However, the dataset considered for this finding is a small pool of sequences from only three test persons. A more focused experimental set-up could yield deeper insights not only into the diversity and variation of the axillary microbiota but also into the physiological functions, which are (differentially) expressed by the metabolically active members of the microbial community, in other words by using metatranscriptomics.

Future perspectives: towards a metatranscriptome of the axillary microbiota

The concept of a core microbiome with specific metabolic functions emphasizes the requirement to analyze the active microbial community of the human axilla at a functional level. To characterize unknown enzymatic functions as well as active metabolic processes involved in body-odor formation, metatranscriptomic analyses of the axillary microbiota are required in the future (Figure 3). However, such an approach has not been reported so far, which also might be due to the restricted amount of sample material that can be collected from the human axilla. Owing to the relatively small bacterial cell numbers present in the human axilla, yields of total RNA are usually in the single-digit nanogram range. Therefore, efficient enrichment of mRNAs, which is difficult for bacterial samples due to the lack of specific tags such as poly-A tails, is further restricted in the case of axillary samples. One possibility to overcome this limitation is to amplify cDNA generated from total RNA of a single axillary swab (Figure 3). Synthesis of sequencing libraries can generally be performed by means of ligation of platform-specific adaptors for high-throughput sequencing. Whereas 16S rRNA-derived sequences can be employed to extract phylogenetic information about the microbial community,

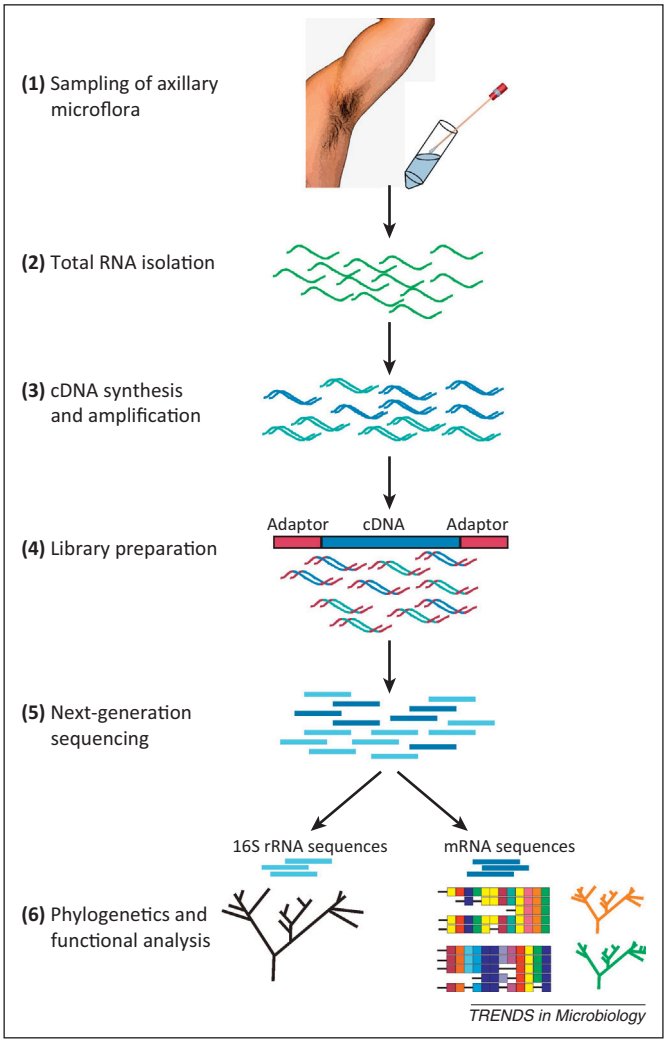

Figure 3. Schematic view of an experimental workflow to characterize the metatranscriptome of the axillary microflora. This diagram shows the process from collecting a sample from the human axilla to total cDNA sequencing and bioinformatic analysis. Data from 16S rRNA sequences and mRNAs are suitable for phylogenetic studies. Deduced amino acid sequences from mRNAs additionally provide information on the bacterial proteins and cellular functions expressed by the axillary microbiota.

translated sequences originating from mRNAs can be used to characterize specific enzymatic and metabolic functions of particular members of the axillary microbiota (Figure 3). In the case of axillary metatranscriptomics, it is necessary to analyze data from individuals in a comparative mode because the combination of host-specific endogenous characteristics, such as sex, age, handedness, ethnicity, and individual host factors, together with external factors including cosmetics, is unique for each person. Therefore, each of these factors might play a role in the individual body odor of each person, as demonstrated by former studies at the level of biochemical odorants and human perception. For instance, Fierer *et al*. identified significant effects of handedness, sex, and time since last washing on the composition of the microbial community of the hand surface [50]. Troccaz *et al*. identified different ratios of odorant precursors in men and women, which are potentially implicated in the gender-specific character of body odor [14]. Altogether, these studies indicate that individual differences in body odor are linked to specific metabolic activities which can be identified and analyzed in the context of metatranscriptomics (Box 1). Next-generation 'omic' techniques might therefore help to shift our current view from the static taxonomic description of the axillary microbiota towards the personalized activity of the microbial community and its dynamic variation in response to changing environmental conditions, for instance upon the application of candidate deodorant additives to the human axilla before sampling. Therefore, a metatranscriptomic investigation of the human armpit microbiota will yield valuable basic knowledge for the design of new deodorants specifically targeting individual malodor formation processes.

Box 1. Outstanding questions

- Is it possible to establish a standardized protocol for sampling and metatranscriptomics of the axillary microbiota as outlined in Figure 3?
- Which bacterial taxa are active in the human axilla? Which microbial activities can be correlated with the intensity of axillary malodor?
- Which bacterial taxa express homologs of TpdA, AecD, AgaA, and other enzymes responsible for the generation of axillary malodor?
- How do differences in the composition of the axillary microbiota (e.g., *Staphylococcus*- vs *Corynebacterium*-dominated) respond to the application of current and future deodorant additives?
- How does left–right asymmetry contribute to intraindividual variation and activity of the axillary microbiota?

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References

1 Fredricks, D.N. (2001) Microbial ecology of human skin in health and disease. *J. Investig. Dermatol. Symp. Proc.* 6, 167–169
2 Percival, S.L. *et al.* (2011) Microbiology of the skin and the role of biofilms in infection. *Int. Wound J.* 9, 14–32
3 Grice, E.A. and Segre, J.A. (2011) The skin microbiome. *Nat. Rev. Microbiol.* 9, 244–253
4 Grice, E.A. *et al.* (2009) Topographical and temporal diversity of the human skin microbiome. *Science* 324, 1190–1192
5 Taylor, D. *et al.* (2003) Characterization of the microflora of the human axilla. *Int. J. Cosmet. Sci.* 25, 137–145
6 Leyden, J.J. *et al.* (1981) The microbiology of the human axilla and its relationship to axillary odor. *J. Invest. Dermatol.* 77, 413–416
7 Noël, F. *et al.* (2012) Sweaty skin, background and assessments. *Int. J. Dermatol.* 51, 647–655
8 Harvey, C.J. *et al.* (2010) Formulation and stability of a novel artificial human sweat under conditions of storage and use. *Toxicol. In Vitro* 24, 1790–1796
9 Wilke, K. *et al.* (2007) A short history of sweat gland biology. *Int. J. Cosmet. Sci.* 29, 169–179
10 Labows, J.N. *et al.* (1979) Steroid analysis of human apocrine secretion. *Steroids* 34, 249–258
11 Stefaniak, A.B. *et al.* (2010) Formulation and stability of a novel artificial sebum under conditions of storage and use. *Int. J. Cosmet. Sci.* 32, 347–355
12 Kolarsick, P.A. *et al.* (2011) Anatomy and physiology of the skin. *J. Dermatol. Nurses' Assoc.* 3, 203–213
13 Natsch, A. *et al.* (2006) A broad diversity of volatile carboxylic acids, released by a bacterial aminoacylase from axilla secretions, as candidate molecules for the determination of human-body odor type. *Chem. Biodivers.* 3, 1–20
14 Troccaz, M. *et al.* (2009) Gender-specific differences between the concentrations of nonvolatile (*R*)/(*S*)-3-methyl-3-sulfanylhexan-1-ol and (*R*)/(*S*)-3-hydroxy-3-methyl-hexanoic acid odor precursors in axillary secretions. *Chem. Senses* 34, 203–210

15 Zeng, X-N. *et al.* (1991) Analysis of characteristic odors from human male axillae. *J. Chem. Ecol.* 17, 1469–1492
16 Kuhn, F. and Natsch, A. (2009) Body odour of monozygotic human twins: a common pattern of odorant carboxylic acids released by a bacterial aminoacylase from axilla secretions contributing to an inherited body odour type. *J. R. Soc. Interface* 6, 377–392
17 Martin, A. *et al.* (2009) A functional ABCC11 allele is essential in the biochemical formation of human axillary odor. *J. Invest. Dermatol.* 130, 529–540
18 Martins, Y. *et al.* (2005) Preference for human body odors is influenced by gender and sexual orientation. *Psychol. Sci.* 16, 694–701
19 Roberts, S.C. *et al.* (2008) MHC-correlated odour preferences in humans and the use of oral contraceptives. *Proc. Biol. Sci.* 275, 2715–2722
20 Cogen, A.L. *et al.* (2008) Skin microbiota: a source of disease or defense? *Br. J. Dermatol.* 158, 442–455
21 Troccaz, M. *et al.* (2004) 3-Methyl-3-sulfanylhexan-1-ol as a major descriptor for the human axilla-sweat odour profile. *Chem. Biodivers.* 1, 1022–1035
22 Starkenmann, C. *et al.* (2005) Identification of the precursor of (*S*)-3-methyl-3-sulfanylhexan-1-ol, the sulfury malodour of human axilla sweat. *Chem. Biodivers.* 2, 705–716
23 Emter, R. and Natsch, A. (2008) The sequential action of a dipeptidase and a β-lyase is required for the release of the human body odorant 3-methyl-3-sulfanylhexan-1-ol from a secreted Cys-Gly-(*S*) conjugate by *Corynebacteria*. *J. Biol. Chem.* 283, 20645–20652
24 Natsch, A. *et al.* (2004) Identification of odoriferous sulfanylalkanols in human axilla secretions and their formation through cleavage of cysteine precursors by a C-S lyase isolated from axilla bacteria. *Chem. Biodivers.* 1, 1058–1072
25 Troccaz, M. *et al.* (2008) Properties of recombinant *Staphylococcus haemolyticus* cystathionine β-lyase (*metC*) and its potential role in the generation of volatile thiols in axillary malodor. *Chem. Biodivers.* 5, 2372–2385
26 James, G.A. *et al.* (2013) Microbiological and biochemical origins of human axillary odour. *FEMS Microbiol. Ecol.* 83, 527–540
27 Natsch, A. *et al.* (2003) A specific bacterial aminoacylase cleaves odorant precursors secreted in the human axilla. *J. Biol. Chem.* 278, 5718–5727
28 Zeng, C. *et al.* (1996) A human axillary odorant is carried by apolipoprotein D. *Proc. Natl. Acad. Sci. U.S.A.* 93, 6626–6630
29 Spielman, A.I. *et al.* (1998) Identification and immunohistochemical localization of protein precursors to human axillary odors in apocrine glands and secretions. *Arch. Dermatol.* 134, 813–818
30 Preti, G. and Leyden, J.J. (2010) Genetic influences on human body odor: from genes to the axillae. *J. Invest. Dermatol.* 130, 344–346
31 Labows, J.N. *et al.* (1982) Perspectives on axillary odor. *J. Soc. Cosmet. Chem.* 34, 193–202
32 Decréau, R.A. *et al.* (2003) Production of malodorous steroids from androsta-5,16-dienes and androsta-4,16-dienes by Corynebacteria and other human axillary bacteria. *J. Steroid Biochem. Mol. Biol.* 87, 327–336
33 James, A.G. *et al.* (2004) Fatty acid metabolism by cutaneous bacteria and its role in axillary malodour. *World J. Microbiol. Biotechnol.* 20, 787–793
34 Tauch, A. *et al.* (2005) Complete genome sequence and analysis of the multiresistant nosocomial pathogen *Corynebacterium jeikeium* K411, a lipid-requiring bacterium of the human skin flora. *J. Bacteriol.* 187, 4671–4682
35 Barzantny, H. *et al.* (2012) Molecular basis of human body odour formation: insights deduced from corynebacterial genome sequences. *Int. J. Cosmet. Sci.* 34, 2–11
36 Liu, L. *et al.* (2012) Microbial production of propionic acid from propionibacteria: current state, challenges and perspectives. *Crit. Rev. Biotechnol.* 32, 374–381
37 James, A.G. *et al.* (2004) Generation of volatile fatty acid by axillary bacteria. *Int. J. Cosmet. Sci.* 26, 149–156
38 Grice, E.A. *et al.* (2009) A diversity profile of the human skin microbiota. *Genome Res.* 18, 1043–1050
39 Costello, E.K. *et al.* (2009) Bacterial community variation in human body habitats across space and time. *Science* 326, 1694–1697
40 Dethlefsen, L. *et al.* (2007) An ecological and evolutionary perspective on human–microbe mutualism and disease. *Nature* 449, 811–817
41 Gao, Z. *et al.* (2007) Molecular analysis of human forearm superficial skin bacterial biota. *Proc. Natl. Acad. Sci. U.S.A.* 104, 2927–2932
42 Gao, Z. *et al.* (2010) Quantitation of major human cutaneous bacterial and fungal populations. *J. Clin. Microbiol.* 48, 3575–3581
43 Kong, H.H. (2011) Skin microbiome: genomics-based insights into the diversity and role of skin microbes. *Trends Mol. Med.* 17, 320–328
44 Dekio, I. *et al.* (2005) Detection of potentially novel bacterial components of the human skin microbiota using culture-independent molecular profiling. *J. Med. Microbiol.* 54, 1231–1238
45 Conlan, S. *et al.* (2012) Species-level analysis of DNA sequence data from the NIH Human Microbiome Project. *PLoS ONE* 7, e47075
46 The Human Microbiome Project Consortium (2012) Structure, function and diversity of the healthy human microbiome. *Nature* 486, 207–213
47 Morales, S.E. and Holben, W.E. (2010) Linking bacterial identities and ecosystem processes: can 'omic' analyses be more than the sum of their parts? *FEMS Microbiol. Ecol.* 75, 2–16
48 Molin, S. and Givskov, M. (1999) Application of molecular tools for *in situ* monitoring of bacterial growth activity. *Environ. Microbiol.* 1, 383–391
49 Egert, M. *et al.* (2011) rRNA-based profiling of bacteria in the axilla of healthy males suggesting right-left asymmetry in bacterial activity. *FEMS Microbiol. Ecol.* 77, 146–153
50 Fierer, N. *et al.* (2008) The influence of sex, handedness, and washing on the diversity of hand surface bacteria. *Proc. Natl. Acad. Sci. U.S.A.* 105, 17994–17999