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Deodorants and antiperspirants affect the axillary bacterial community

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Abstract The use of underarm cosmetics is common practice in the Western society to obtain better body odor and/or to prevent excessive sweating. A survey indicated that 95 % of the young adult Belgians generally use an underarm deodorant or antiperspirant. The effect of deodorants and antiperspirants on the axillary bacterial community was examined on nine healthy subjects, who were restrained from using deodorant/antiperspirant for 1 month. Denaturing gradient gel electrophoresis was used to investigate the individual microbial dynamics. The microbial profiles were unique for every person. A stable bacterial community was seen when underarm cosmetics were applied on a daily basis and when no underarm cosmetics were applied. A distinct community difference was seen when the habits were changed from daily use to no use of deodorant/antiperspirant and vice versa. The richness was higher when deodorants and antiperspirants were applied. Especially when antiperspirants were applied, the microbiome showed an increase in diversity. Antiperspirant usage led toward an increase of *Actinobacteria*, which is an unfavorable situation with respect to body odor development. These initial results show that axillary cosmetics modify the microbial community and can stimulate odor-producing bacteria.

Keywords Underarm · Human microbiome · *Corynebacterium* · Skin · Cosmetics

Introduction

In our society, people are concerned about their personal hygiene and are less tolerant toward bad body odor [19]. The use of underarm cosmetics aims to control sweating and malodor formation. These products are important for social confidence and improve the quality of life of many people worldwide [27]. Deodorants and antiperspirants are utilized by over 90 % of US consumers, generating sales of over $1 billion per year [20]. Deodorants are substances applied on the human skin to enhance the pleasantness of the skin odor and counteract the odor caused by bacterial breakdown of skin secretions in the axillae, feet and other areas of the body. Antiperspirants are a subgroup of deodorants that affect odor as well as prevent sweating by blocking the sweat glands. They are classified as an over-the-counter drug by the US Food and Drug Administration and must undergo rigorous testing. Deodorants are classified as cosmetics, intended to make the skin smell better by deodorizing and masking the body odor. While most antiperspirants function as a deodorant, deodorants do not necessarily act as an antiperspirant [11]. According to the European legislation, both deodorants and antiperspirants are categorized as cosmetics [7].

Important bacterial groups colonizing the axillary region are *Staphylococcus*, *Corynebacterium*, *Propionibacterium* and *Micrococcus* spp. [12]. Each bacterial species in the community has its own enzymes, converting non-odorous sweat into volatile compounds [30]. Aerobic lipophilic *Corynebacterium* spp. (*Actinobacteria* phylum) are

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considered to be responsible for a strong axillary malodor, while *Staphylococcus* spp. (*Firmicutes* phylum) and other axillary species only reveal low levels of odor [16, 21]. Research has shown that major odor-causing compounds in human sweat include short volatile branched-chain fatty acids, steroid derivatives and sulfanylalkanols. It has been hypothesized that *Corynebacterium* spp. are involved in all three processes [2].

A range of antimicrobials are added to deodorants (Deo) and antiperspirants (AP) to decrease the density of the skin bacterial community. Propylene glycol, triclosan, benzalkonium chloride and metal salts are regularly used in Deos/APs and possess antimicrobial and antifungal properties [3]. Certain flavoring agents, such as cinnamaldehyde, eugenol and geraniol possess antimicrobial properties [1, 17]. These ingredients help to decrease the abundance density of skin microbiota, although complete elimination cannot be obtained [9]. Some people indicate that the use of Deos or APs leads toward a less pleasant axillary odor. Little is known how the use of Deos and APs affect the underarm bacterial community. It is unknown whether the ingredients have a broad-spectrum effect, or rather target specific odor-causing microbiota. This study investigated how the axillary community changed when the AP or Deo use is discontinued and resumed. The objective of this study was to identify whether deodorant/antiperspirant usage could affect the autochthonous microbial community structure, diversity and dynamics.

Materials and methods

Study design

An online consumer survey was executed to identify the exact presence of deodorants and antiperspirants in our daily life. Nine healthy subjects were selected for the experiment. Eight subjects, who normally used a deodorant (Deo) or antiperspirant (AP), were asked to discontinue using Deo or AP for 1 month. Four subjects (subjects E, F, G, H) normally used a Deo and the four other subjects (subjects A, B, C, D) normally used an AP. Subject I normally did not use a Deo/AP and was asked to use a Deo for 1 month. A period of 1 month was chosen, as the total epidermis turnover time is 28 days [14, 15]. Axillary samples were taken before, during and after the discontinuation of AP/Deo use. The full contents of the Deos/APs are represented in online resource Table S1. The DNA was extracted and the microbial community was investigated by denaturing gradient gel electrophoresis (DGGE). Descriptive diversity and dynamics analysis was performed on the results.

Consumer survey analysis

To obtain a current view of the underarm cosmetics usage in the daily life of active people, a consumer survey analysis was performed. Cross-sectional consumer data were collected through an online survey with 314 Belgian consumers in October–November 2011. From this sample, four cases were excluded from the analysis because of inconsistent responses or missing observations in one or more of the variables of interest, thus yielding a final valid sample of 310 cases (online resource Table S2). The sample involved 36 % males and 64 % females. The respondents had a wide variety of socio-demographic backgrounds. The highest obtained diploma of the respondents was in 1 % of the cases primary education, in 30 % of the cases secondary education and 69 % of the cases higher education. 2 % of the respondents were between 12 and 18 years old, 59 % between 19 and 30 years old, 26 % between 31 and 50 years old and 13 % were older than 50 years. A large part of the sample reflected the young adult population. 67 % of the respondents actively participated in sports, while 33 % did not play any sports.

Sampling

Triplicate specimens were obtained from the left and right axilla of nine healthy subjects with no history of dermatological disorders or other chronic medical disorders and with no current skin infections. Samples were collected between autumn and spring from subjects working in the area of Ghent (Belgium) with a temperate maritime climate, by the North Sea and Atlantic Ocean. Six individuals were Belgians (five males and one female) and three individuals were not from Belgium (two males and one female) (online resource Table S3). The mean age was 26.7 years (range 23–29 years). All were in good health and had not received any antibiotics for at least 2 months. No attempts were made to control the subjects' diet or hygiene habits. All subjects (except one) generally applied deodorants/antiperspirants and were asked to discontinue using a Deo/AP during 1 month, whereas axillary samples were taken before, during and after this period. An indication of a 2 s spray or three roll-ons was made to the participants. A moistened sterile cotton swab (Biolab) was thoroughly swabbed for 15 s in the axillary region to detach and absorb the microorganisms, and it was vigorously rotated for 15 s in a sterilized reaction tube filled with 1.0 ml of sterile physiological water to transfer the bacteria [10]. The bacterial samples were immediately pelletized and frozen at −20 °C until further DNA extraction. To minimize the effect of washing and deodorant use, the samples were taken on the same hour of the day (online resource Table S3).

DNA extraction, PCR and DGGE

Total DNA extraction was performed as previously described [4]. The 16S rRNA gene regions were amplified by PCR using 338F and 518R primers targeting the V3 region [24, 26]. A GC clamp of 40 bp [24, 26] was added to the forward primer. The PCR program consisted of 10 min at 95 °C; 35 cycles of 1 min at 94 °C, 1 min at 53 °C, 2 min at 72 °C; and a final elongation for 10 min at 72 °C. The amplification products were analyzed by electrophoresis in 1.5 % (wt/vol) agarose gels stained with ethidium bromide. DGGE was performed as previously described [4]. An internal marker was developed in-house and used to align different DGGE gels and identify bacterial species. The normalization and analysis of DGGE gel patterns was done with the BioNumerics software 5.10 (Applied Maths, Belgium). In this process, the different lanes were defined, background was subtracted, differences in the intensity of the lanes were compensated during normalization and bands and band classes were detected.

Diversity analysis

The community richness (R) was analyzed by means of quantification of the total number of bands present on the DGGE gel. This gives an indication of the richness and genetic diversity of species within the bacterial community. The community evenness was analyzed by means of the Gini coefficient [28]. Pareto–Lorenz (PL) evenness distribution curves for community organization was constructed based on the DGGE profiles as previously described [22, 32]. The community dynamics (Dy) was studied computing the moving window analysis plot of consecutive DGGE profiles of the same subject. The microbial community rate of change (dynamics) was conducted using the UPGMA and distance matrices of each DGGE based on the Pearson correlation similarity coefficient to cluster the samples using BioNumerics software (Applied Maths, Belgium).

Statistical analysis

Statistical analysis was performed in SPSS (SAS institute, North-Carolina, USA) and significant cutoff values were set at the 95 % confidence level. The presented values are the average from triplicate measurements. The DGGE patterns were examined by means of clustering, principal component analysis (PCA) and analysis of the intensities of bacterial groups. The information from the different tracks was analyzed for one type of group-specific fragments by calculating a distance matrix of all the possible gel tracks within the DGGE patterns by using Pearson correlation. Based on the values of the resulting matrix, a cluster analysis was performed and the samples were visualized in dendrograms, using the unweighted pair group with mathematical averages (UPGMA) dendrogram method. Relevant and non-relevant clusters were separated by the statistical cluster cutoff method (BioNumerics Manual 5.10). Secondly, PCA was applied to explain the DGGE results, by putting the data directly in a binary band-matching table. This technique produces two-dimensional plots in which the entries were spread according to their relatedness. Thirdly, the bacterial profile was divided into subgroups of *Firmicutes* (having a low GC content and forming bands on the left side of the DGGE gel) and *Actinobacteria* (having a high GC content and forming bands on the right side of the DGGE gel). The weights (intensities) of both phyla were analyzed next to each other, as they can have a dissimilar impact on body odor production. The intensities of the bacterial groups were analyzed throughout the time, to give an idea about the evolution.

Band identification

The bands were identified by comparing the DGGE patterns with internal markers. The bands of the internal marker were identified by means of colony isolation and identification. The isolates were obtained from plating on blood agar plates. Identification was done by means of Sanger sequencing and comparing the sequences with the NCBI database. The closest match of each isolate was identified and submitted to NCBI with accession numbers KC107217–KC107221. Other internal markers were sequenced by means of 454 pyrosequencing and contained high amounts of *Corynebacterium* spp. with diverse bands in the lower DGGE gel part (consequently, bands with a high GC % content). A fastq file of the 454 pyrosequencing data was created and submitted to NCBI with SRA study accession: SRP023149 [4]. For the sake of simplicity, the strains were grouped in the *Firmicutes* and the *Actinobacteria* phyla.

Ethics statement

The study was approved by the Ghent University Ethical Committee with approval number B670201112035. All participants gave their written consent to participate in this study as well as for the case details to be published.

Results

Survey

In a survey (online resource Table S2) conducted on 310 Belgians, with a major part reflecting the young adult

population (19–30 years old), it was indicated that 95 % of the respondents utilized a deodorant or antiperspirant. According to the data, 57 % of the respondents used a Deo/AP on a daily basis, 20 % used it more than once a day, 13 % used it more than once per week but not every day, 2 % used it solely after sports, 3 % used it on occasion and 5 % never used it. A spray was applied in 81 % of the cases, a roll-on in 29 % and a stick in 5 % of the cases (more than one option was possible in this case). It was found that 29 % of the respondents permanently used the same brand and type of Deo/AP; 30 % of the respondents permanently used the same brand, but frequently used another type of the same brand and 42 % of the respondents frequently changed the brand.

Interpersonal axillary diversity

Denaturing gradient gel electrophoresis fingerprinting analyses of all bacteria indicated a diverse microbial community between nine individuals. The results reflected the unique character of the interindividual axillary microbiota. Generally, two important groups in the upper and lower gel fragment were distinguished. Bands in the upper gel fragment (situated left in Fig. 1 for person A) were identified as staphylococci (*Firmicutes*—having a low GC content) and bands in the lower gel fragment (situated right in Fig. 1) were identified as corynebacteria (*Actinobacteria*—having a high GC content). Three subjects (subjects A, D, E) mostly had *Staphylococcus* spp. and six subjects (subjects B, C, F, G, H, I) had populations of both *Staphylococcus* and *Corynebacterium* spp. (shown in online resource Figure S1, S2, S3, S4, S5, S6, S7, S8 and S9). Generally, the microbiome of the left axilla was similar to that of the right; however, for two subjects large differences were seen (subject F and I—online resource Figure S6 and S9).

Shift to *Actinobacteria* when antiperspirants were applied

The abundance of the dominant bands changed considerably when AP was applied. For all four subjects who applied an AP (subject A, B, C and D), the abundance of *Firmicutes* increased and that of *Actinobacteria* decreased on discontinuing AP usage (Fig. 2a). When AP use was resumed after 1 month, a clear enrichment of *Actinobacteria* occurred, while the abundance of *Firmicutes*-related bands strongly decreased (Fig. 1). When no AP was used, the *Actinobacteria*-related bands were not present, or not detectable by means of DGGE. Several *Actinobacteria*-

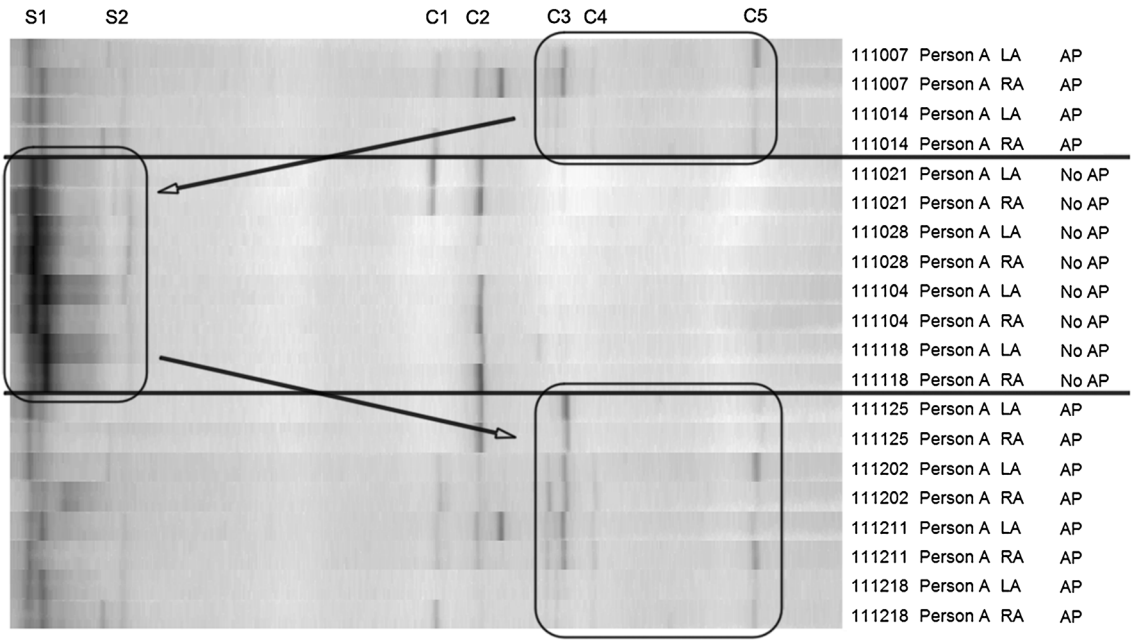

Fig. 1 Temporal DGGE profile of the axillary samples of person A. *Above* identified bands: bands S1 were identified as *Staphylococcus epidermidis* (100 % identity), bands S2 were identified as *Staphylococcus* spp. (99 % identity), bands C2 and C3 were identified as *Corynebacterium* spp. (99 % identity) and bands C1, C4 and C5 were identified as *Corynebacterium* spp. (from pyrosequencing). *Right* sample names indicate: Date (yymmdd) | Person | Left axilla (LA) of right axilla (RA) | Antiperspirant use (AP) or no antiperspirant use (No AP). When no AP was applied, the *Actinobacteria*-related bands decreased in abundance, while the *Firmicutes*-related bands strongly increased. When AP was again applied by the subject, *Firmicutes* decreased, while *Actinobacteria* increased in abundance (*differences are marked on the figure*)

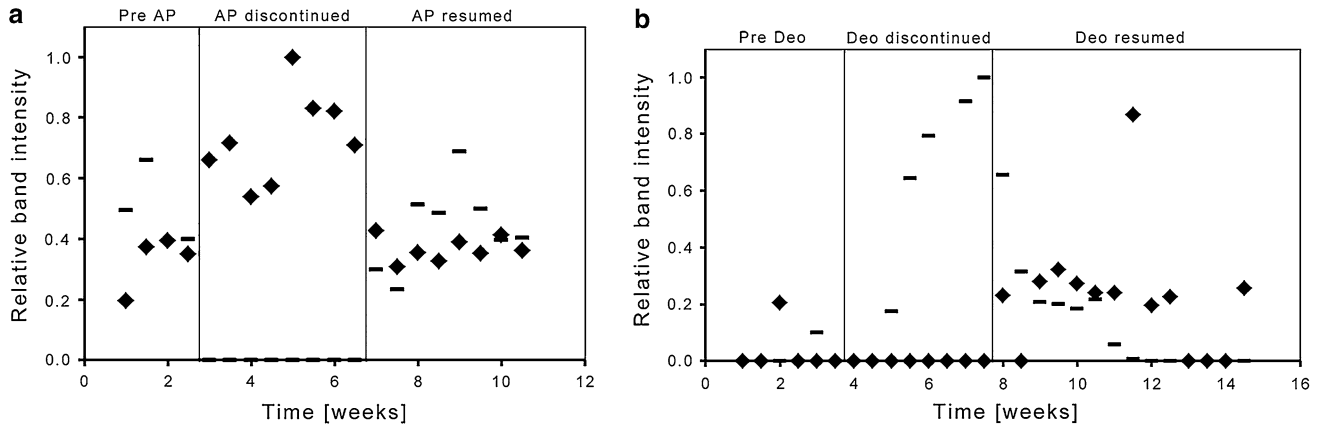

Fig. 2 Evolution of the DGGE band intensities of the *Firmicutes* (*filled diamond*) and *Actinobacteria* (*hyphen*) of subject A (**a**) and subject F (**b**). *Subject A* representing the AP-using subjects; *subject F* representing the Deo-using subjects

related bands appeared immediately when AP use was resumed. This was coupled with a substantial intensity decrease of the *Firmicutes*-related bands. For subject B, the *Firmicutes*-related bands were able to gain dominance when AP use was discontinued, while the *Actinobacteria*-related bands did not grow into abundant quantities (online resource Figure S2). Once AP use was resumed, an increase in band intensity was observed for the *Actinobacteria*-related bands, coupled with an intensity decrease of the *Firmicutes*-related bands. For subject C, several *Actinobacteria*-related bands appeared after 3 weeks when AP use was discontinued (online resource Figure S3). When AP use was resumed, the intensity weight of the different *Actinobacteria*-related bands altered and enhanced into abundant quantities. The band intensity of the autochthonous axillary community decreased clearly. The initial intensity increase in *Firmicutes*-related bands after resuming the AP was abrogated after 1/2 weeks. Subject D initially had a very low concentration of one *Actinobacterium*-related band (online resource Figure S4). When AP use was stopped, the intensity of the *Firmicutes*-related bands strongly increased, while the *Actinobacterium*-related band decreased. When AP use was resumed, the *Firmicutes*-related bands were vigorously affected, while the intensity of the *Actinobacterium*-related band (and some other *Actinobacterium*-related bands) slightly increased. As indicated by the four subjects, AP usage resulted in a shift of the microbial community toward more *Actinobacteria*.

No common trend was found when deodorants were applied

Clear axillary community changes were seen for the subjects using a Deo; however, the results were less pronounced compared to the changes caused by an AP. The effects of a Deo on the microbial community were somewhat different for every individual. In three cases (subject F, G, I), the intensity of the *Actinobacteria* slightly increased when no Deo was applied, the effect being opposite to that of AP usage (Fig. 2b; online resource Figure S6, S7 and S9). When Deo was applied, a decrease in abundance was frequently noticed, while other transient bacteria found the opportunity to grow. The Deo content of subject E, I, G and F was very similar (online resource Table S1); however, the effect on the axillary microbiota was totally dissimilar. An opposite effect was occasionally detected between the left and right axilla when Deo was applied (subject I—online resource Figure S9). A single participant changed the Deo brand during the study (subject F—online resource Figure S6). The effects on the axillary microbiome were minimal, with only minor changes in bacterial community and structure. The change to another deodorant did not cause a shift in the microbiome.

Temporal diversity in response to antiperspirant usage

The temporal diversity (dynamics) was analyzed by means of moving window analysis, where the dissimilarities (changes) of two successive sampling points were positioned throughout time. During the period of AP application or no AP application, the community was relatively stable. The highest dissimilarities were noticed during the transition periods. When AP usage was discontinued or resumed, a noteworthy dissimilarity was detected (resulting in a peak; see online resource Figure S10). Clearly separated clusters were obtained when clustering the DGGE patterns according to Pearson correlation (Fig. 3). The axillary samples where no AP was applied had an average similarity of 89 ± 6 %. They clustered distinctly from the axillary samples where AP was applied, having an average

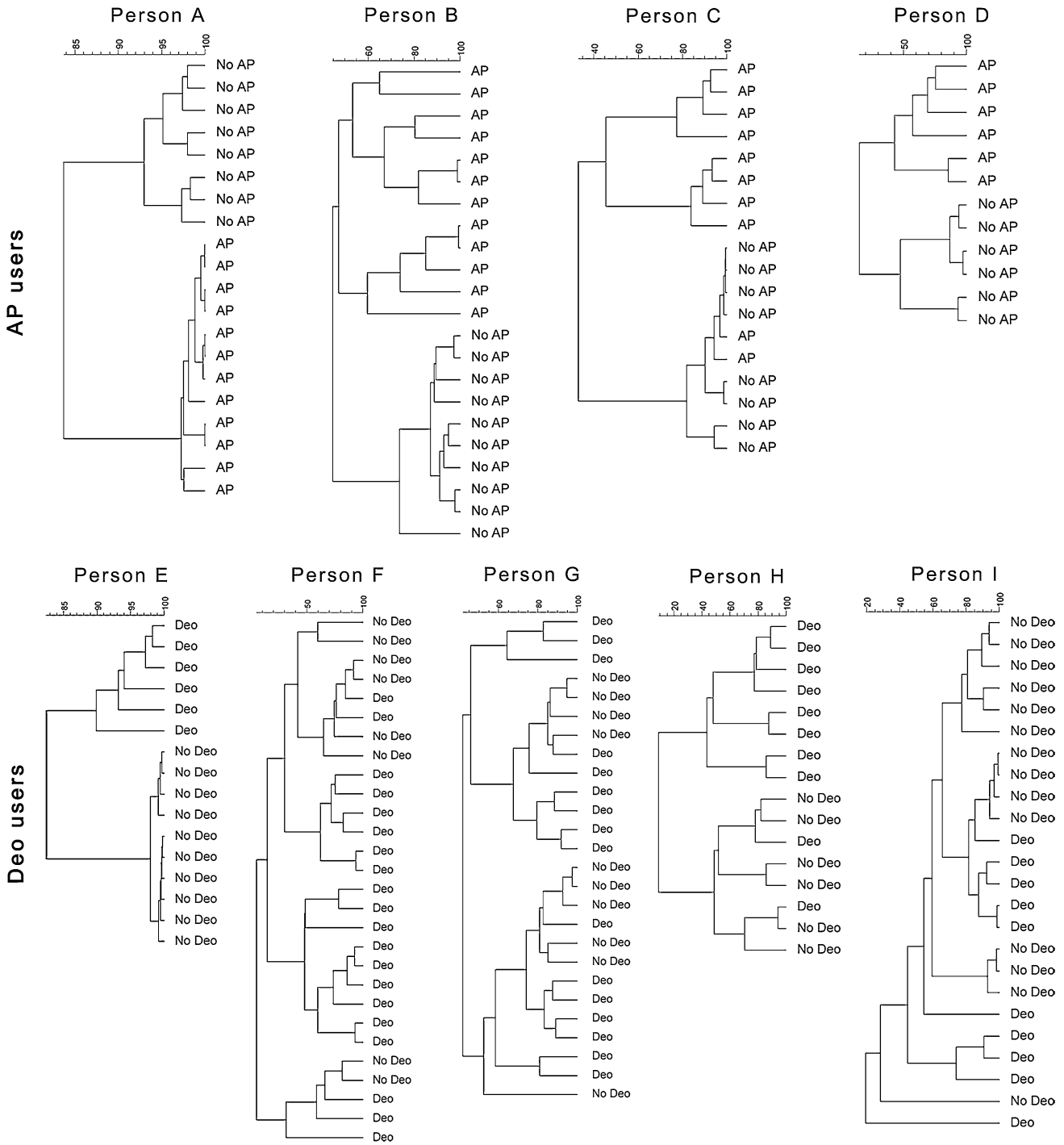

Fig. 3 DGGE clustering of the individual axillary samples during the experiment. Deo users generated more inconsistent microbiome changes compared to AP users

similarity of 87 ± 8 %. The average similarity of the samples during the transition period (AP to non-AP usage or vice versa) was 58 ± 21 %, which was significantly lower ($p < 0.01$) than the similarities when AP or no AP was used. PCA plots were constructed to graphically represent relationships between the samples in two-dimensional space. The non-AP samples clustered separately from the samples when AP was used (Fig. 4a). When AP use was resumed, the samples clustered together with the samples obtained before AP discontinuation. Such clear sample separation was achieved for all the subjects using an AP (online resource Figure S2, S3, S4).

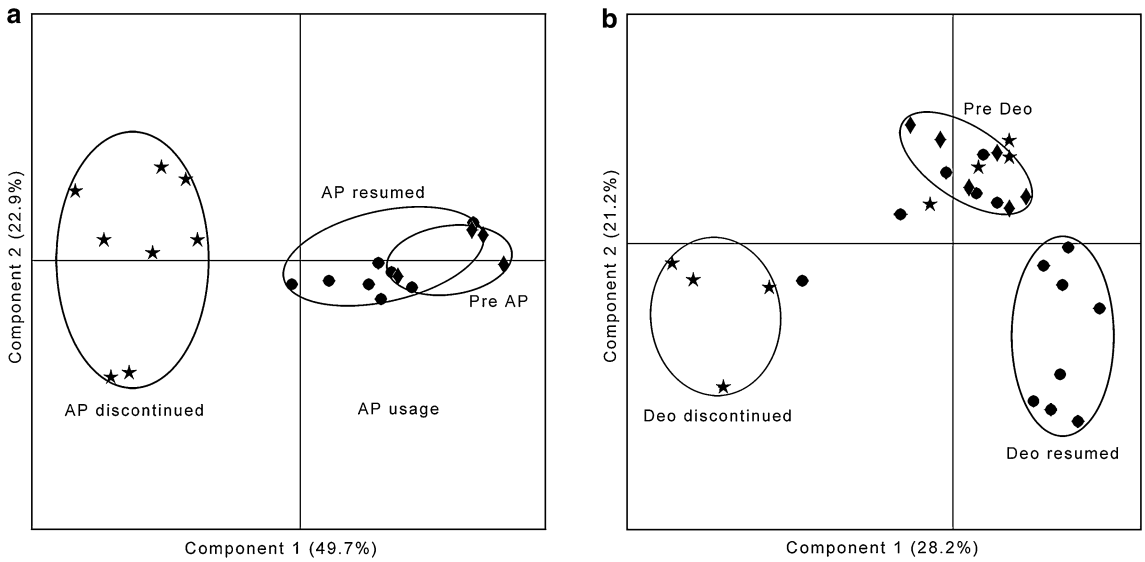

Fig. 4 PCA analysis on the DGGE fingerprinting composite data set. *Subject A* (**a**) represents the AP-using subjects; *subject F* (**b**) represents the Deo-using subjects. The samples before AP/Deo discontinuation (*filled diamond*) generally clustered separately from the samples when no AP/Deo was applied (*filled star*). When AP/Deo use was resumed (*filled circle*), the samples recurrently clustered in a different manner

Temporal diversity in response to deodorant usage

The effect of discontinuing and resuming Deo usage was less considerable than discontinuing and resuming an AP. The dissimilarity in the transition periods was often not as apparent as the dissimilarity when AP usage was discontinued or resumed (online resource Figure S10). In the case of subject F and G, a change was perceived after 1 and 2 weeks, respectively. A lag effect was noticed. For subject F, only after 2 weeks of no Deo use, apparent differences were observed. The same happened when Deo was again used, as the differences were only perceptible after 2 weeks of Deo usage. In the case of subjects E and I, clearly similar decreases were detected at the first moment Deo use was discontinued or resumed. In the case of subject H, no real similar changes were observed when Deo use was discontinued or resumed. The non-Deo samples generally clustered separately from the Deo samples; however complete separation between the two was rarely achieved (Fig. 3). The PCA plots of the subjects using a Deo gave sufficient clustering (Fig. 4b). Slight microbiome changes were frequently encountered 1 month after Deo discontinuation; however a close match was recurrently obtained for all Deo samples. The average similarity of the non-Deo and Deo samples was 73 ± 18 % and 69 ± 13 %, respectively (Fig. 3). The average similarity of the samples during the transition period (Deo to non-Deo usage or vice versa) was lower with a value of 60 ± 27 %. The results of subject I acted as a control, as this person normally did not use Deo. The results of the 1-month daily Deo usage were comparable with those of the others subjects when using Deo.

A higher community richness after deodorant and antiperspirant usage

DGGE fingerprinting analyses exhibited considerable differences when deodorant or antiperspirant usage was discontinued or resumed. Table 1 represents the average number of bands observed in the axillary samples over the period of sampling for eight individuals. Generally, before the experiment, a significant higher band richness was perceived compared to when no AP/Deo was applied (significance level of $p < 0.01$ for AP and $p < 0.05$ for Deo-using subjects). The number of visible bands declined when AP/Deo use was discontinued. When AP/Deo use was resumed, an immediate increase in band richness was seen, with some bands being present at very low abundances. The results were more distinctive for AP-using subjects.

A higher community evenness after antiperspirant usage

Table 1 represents the evolution of the Gini coefficient of the AP/Deo samples. An increase in evenness (reduction of the Gini value) was detected when the subject was using an AP/Deo. Only in the case of AP use, significant differences were found ($p < 0.01$). In the case of subject A, when AP use was resumed, a large increase in bacterial bands was observed, while strong abundant bands were no longer detected. This resulted in a much more even axillary community than compared to the community when no AP was applied. A strong dominance in *Firmicutes* was noticed when AP use was discontinued, while other

Table 1 Average richness and evenness before, during and after AP/Deo discontinuation

	Richness			Evenness		
	Pre	Discontinued	Resumed	Pre	Discontinued	Resumed
AP	7.0 ± 3.7	5.8 ± 1.8	9.1 ± 1.7	0.61 ± 0.21	0.71 ± 0.10	0.43 ± 0.18
Deo	9.0 ± 4.1	7.1 ± 3.8	10.6 ± 4.5	0.50 ± 0.14	0.68 ± 0.27	0.60 ± 0.27

bacteria were suppressed or were undetectable by DGGE analysis. For the other three AP-using subjects, similar results were observed, while the evenness differences between AP samples and non-AP samples were less distinctive. Overall, the use of AP led toward a lower dominance of the abundant bands, and, as such, a higher community evenness. Deodorant usage had a diverse effect toward the bacterial community structure for each volunteer. The results between Deo and non-Deo samples were not significantly different ($p = 0.31$). Deo did not persistently cause abundance declines of certain bacterial bands.

Discussion

In the past decades, the use of axillary deodorants and antiperspirants has gained significant popularity. Our survey, with a major part reflecting the young adult Belgian population, revealed that 94.8 % of the consumers generally used an underarm deodorant or antiperspirant. 76.7 % of the respondents used axillary cosmetics one or more times per day. This study describes the effect of commonly used deodorants and antiperspirants on the axillary bacterial community of nine subjects. Although conducted on a small subset of participants, it was found that APs and Deos strongly affected the bacterial community when applied in the axillary region. A 1-month AP/Deo discontinuation was imposed on people who utilized the underarm product on a daily basis. During this period, the entire epidermis is replaced, as the epidermis turnover time equals 28 days [14, 15]. The results indicate that the AP/Deo samples and the non-AP/Deo samples clustered separately from each other. During the 1-month AP/Deo discontinuation, the axillary community altered considerably for some subjects. Molecular analysis on the axillary samples exhibited significant differences, before, during and after a 1-month AP/Deo discontinuation (Figs. 3, 4). A dissimilarity of the abundance of the microbial bands was additionally seen (Fig. 1). When no AP/Deo was applied, the abundance of certain bands was higher. This is due to the antimicrobial compounds used in Deos/APs, such as ethanol, triclosan, quaternary ammonium compounds and fatty acid esters of glycerol [27]. These ingredients cause a decrease in microbial density and impose stress on the community, resulting in an altered microbial community. The autochthonous community was generally able to recover completely after the changed situation. This suggests that the community of the deeper layers of the skin returns when the situation is again favorable. Research has shown that bacteria are present in the dermis and the dermal adipose of the human skin [25]. These findings support that the bacteria in the sweat glands, around the hair roots and deeper in the epidermis are important as indigenous host microbiome [33].

The axillary microbial community showed an apparent interpersonal diversity. Each of the axillary bacterial fingerprints of the nine subjects depicted unique microbial patterns. This was in correlation with previous findings in the armpit [4] and on the skin [13, 33]. Intrapersonal diversity, seen as left–right asymmetry, was occasionally observed, as previously described [4, 8]. The temporal diversity was relatively stable when no AP/Deo was applied. Also, when the subjects were asked to use an AP/Deo on a daily basis, the microbial pattern was relatively stable. The pattern was, nevertheless, strongly altered when AP was suddenly used or discontinued. A microbial disturbance was likewise seen when a Deo was applied, but the effect was less pronounced. It seems that stable hygiene and cosmetic habits result in a stable microbial profile. The results imply that the bacterial community gets adapted to AP/Deo usage. An increase in diversity was observed when deodorants, and especially antiperspirants, were applied in the axillary region. A richness in increase was noticed for both Deo and AP usage. An apparent increase in evenness was solely observed when an AP was applied. The use of makeup was likewise associated with a remarkable increase in bacterial diversity [31]. Similar diversity increases were found in the axillae, when applying more deodorant [4]. It is yet unknown whether the diversity increase can lead toward an altered axillary odor. Further research should focus on the relationship with body odor. This research showed that the use of underarm cosmetics strongly interfered with the composition and structure of the bacterial communities.

Dissimilar effects were found for an AP versus a Deo. The usage of AP considerably shifted the microbial community in the axillary region. Distinct clusters were obtained within the same subject for the same armpit when AP was applied (Fig. 3). A noteworthy dissimilarity was noticed the moment AP usage was discontinued or resumed

(online resource Figure S10). Substantially higher community diversity (richness and evenness) was observed when AP was applied (Table 1). These results were uniform for all subjects using APs. Aluminum is added in relatively large amounts to APs as a sweat gland-blocking agent and was considered to be the main difference between the used Deos and APs (online resource Table S1). It can be held responsible for the larger effects on the microbial community compared to the Deo results. The community was disturbed, with a lower dominance of the abundant bacteria, while other bacteria were able to gain abundance. It is known that aluminum is toxic to soil bacteria and interacts with the DNA of bacteria [29]. The aluminum salts had a dissimilar effect on the *Firmicutes* and the *Actinobacteria*, two of the most abundant species in the axillary region [4, 12]. The *Actinobacteria* phylum was less prone to this substance as compared to the *Firmicutes* phylum. In some cases, the *Actinobacteria* phylum was able to gain more dominance. *Corynebacterium* spp. forms an important share of this phylum. An enhancement of *Corynebacterium* spp. in the axillary region can lead toward the development of more body odor [21]. It is suggested, based on this research that the long-term use of APs in the axillary region induces changes in the skin microbiome and can lead toward altered odor production of the armpit. Many subjects suffering malodorous axillae tried different underarm cosmetics and reported a worsened situation after use of several of these products. The reason is likely to be found in the altered axillary microbiome. Further research and a better understanding of these changes are essential so that the effects can be mitigated.

The application of Deo in the axillary region implied fewer changes in the bacterial community as compared to AP usage. The results indicated an increase in richness (Table 1), leading toward a higher diversity. Nevertheless, no significant increase in community evenness was achieved. Similar results were found for the subject who did not use a Deo regularly, but applied a Deo for 1 month (subject I). A substantial dissimilarity was observed when discontinuing or resuming Deo use, but generally included a lag phase of 1 or 2 weeks (online resource Figure S10). The effects on the microbial community were different for every individual, although some subjects used a similar deodorant. The effect of the ingredients on the existing bacterial community seemed to be strain dependent. Although the Deo brand was changed for one subject, only minor changes were noticed in the microbial community and structure. It is suggested that the microbial effect likewise depended on the amount of Deo that was utilized, the individual sweat rate, sweat content and skin pH. The results indicated that the application of a Deo implied stress on the bacterial community leading toward a higher richness. Preferential growth and specific inhibition were frequently encountered, but in contrast with AP usage no common trend was identified for all Deo-using subjects.

This research indicated that cosmetic underarm products can shift ecological balances. When these products were used in a consistent way, the ecological balance was shifted in a stable manner. As long as these controlling factors were present on the axillary skin, the community was stable. The use of makeup on the forehead likewise caused far-reaching community alterations [31]. Other environmental factors are known to have a significant role on the skin microbial communities, such as sweating, pH, temperature and ultraviolet light [13]. The application of antibiotics was reported to create a long-lasting alteration of the microbiome [18]. A microbial community change on the skin was observed after contact sports [23]. Human-to-human and human-to-surface contacts have been acknowledged as important factors for microbial exchange [5, 6]. The understanding of these shifts, their influences and their impact on odor production and disease susceptibility needs to be further investigated, as very little is known about these.

This study is a step forward in the understanding of the effects of antiperspirants and deodorants on the axillary microbiome. The introduction of new compounds, including antimicrobials, salts, fragrances, alcohol and/or propellants, has a sustained impact on the existing microbiota. The ingredients seem to open the skin niche, leading toward a higher diversity, especially in the case of antiperspirants. Further investigations should focus on the specific effect of aluminum on the axillary microbiota. With the current knowledge, deodorant and antiperspirant manufacturers should focus on using antimicrobials specific toward the odor-causing microbiota, rather than broad-spectrum antimicrobials. Promoting the growth of good bacteria and inhibiting the growth of odor-causing bacteria could greatly improve the deodorant/antiperspirant efficiency.

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