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ORIGINAL ARTICLE

Material-dependent growth of human skin bacteria on textiles investigated using challenge tests and DNA genotyping

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Abstract

Aims: To investigate the influence of different fibre materials on the colonization of textiles by skin bacteria present in human sweat.
Methods and Results: The total bacterial content of axillary sweat samples was determined using DNA quantification, and the diversity of bacteria present was investigated. Fabrics made of different fibres were then challenged with these sweat samples; the bacterial DNA was quantified, and the bacterial taxa present were determined. We found differences in the overall colonization, with polyester and polyamide showing the highest bacterial mass. Also, significant differences in the various taxa of bacteria present on the different materials were found. In general, synthetic materials showed a selective growth of bacterial taxa underrepresented in sweat. In contrast, a cellulose-based material showed only very few taxa, identically with those predominant in sweat.
Conclusions: Our investigations demonstrated that besides the bacterial content of sweat itself, the type of material has a strong impact on the bacterial colonization of textiles.
Significance and Impact of the Study: Odour generation is one of several effects resulting from an interaction of skin bacteria with textiles, and it is a common experience that there are differences in odour generation by different materials. Our investigations suggest that a selective growth of potentially odour-producing bacteria may account for this.

Introduction

Clothing textiles are in close permanent contact with skin and thus provide an ideal basis for the attachment of bacteria transferred from human skin either by direct contact or sweat. Recent investigations have demonstrated that the microbial ecology of human superficial skin is highly complex and that the bacterial species present on skin vary highly between individuals (Gao *et al.* 2007). It is affected by environmental factors, such as temperature and humidity, and host factors, such as gender, immune status, and use of cosmetics (Roth and James 1988).

Skin bacteria pose neither odour problems nor problems with the loss of performance of textile materials under normal conditions. But under favourable growth conditions, bacteria rapidly multiply and cause, in particular, odour generation (Höfer 2006; McQueen *et al.* 2007; Obendorf *et al.* 2007), loss of performance or discolouration of textiles (Szostak-Kotowa 2004). High humidity represents a favourable growth condition for bacteria. Thus, sweat is an ideal bacterial breeding ground. Many of the characteristic malodours associated with the human body are because of the presence of large populations of micro-organisms (Leyden *et al.* 1981; Rennie *et al.* 1991), the associated malodours being the result of micro-organisms

digesting nutrients present in sweat and releasing volatile pungent waste products (Rennie *et al.* 1990; Austin and Ellis 2003; James *et al.* 2004a,b).

Textiles have a strong effect on sweating and consequently have much influence on odour development. Enhanced retainment of sweat by the textile leads to a persistent increase of the metabolic activity of skin bacteria and an accumulation of odourous products of the bacterial metabolism (Höfer 2006).

Until relatively recently, odour development was not considered as a real major problem in textile industry. However, the greater use of synthetic fibres and blends has 'accelerated' this problem (Williams and Cho 2005). The humidity transport characteristics of fabrics from these synthetic fibres and blends tend to cause a greater degree of 'perspiration wetness' than those of natural fibres (Redford 1973), which might result in increased bacterial growth and odour production. Hygienic problems arise as when textiles impair the antimicrobial defence of skin by causing mechanical influence to the barrier function, but also by increased humidity caused by clothing and shoes. The problem of 'athlete's foot' (tinea pedis) is known to be triggered by humid socks and sport shoes (Adams 2002). To address these problems caused by microbial growth on textiles, a range of antimicrobial substances have been either applied to textile products as treatments on the finished textiles or built into the structure of man-made textile fibres. Some of the commonly used bioactive substances are triclosan, silver ions in various chemical compositions, quaternary ammonium salts and chitosan (Takai *et al.* 2002; Gao and Cranston 2008). Especially, the chlorinated organic substances are viewed critically (Kalyon and Olgun 2001), whereas silver is promoted for its low toxicity to humans and reported effective odour reduction (Obendorf *et al.* 2007). But there is also a general critical view that development of microbial resistance is a serious problem to consider (Wollina *et al.* 2006) and can be evoked by the widespread use of effective antimicrobials under poorly controlled conditions, as the use of everyday textiles (Silver 2003). Influences on the delicate balance of the skin microbial flora by antimicrobial textiles should be carefully considered (Wollina *et al.* 2006). Therefore, there is considerable interest from a health and safety aspect in alternative approaches to reduce odour and prevent hygienic problems in textiles.

Material-specific influence on bacterial growth as determined by quantitative analysis was recently reported. A trend for a lower overall growth on natural polymer materials compared to synthetic materials under certain conditions of humidity was observed (Teufel and Redl 2006). This effect was supposed to be because of a reduction in free water content by the water-absorbent natural polymer materials (Schuster *et al.* 2006).

In the present study, we used an approach, which also includes qualitative analysis, to investigate in greater detail the interaction of skin bacteria with textiles and to evaluate various materials in their ability to selectively enhance or reduce growth of different *taxa* of skin bacteria present in human sweat. These investigations were undertaken by challenging different materials with human sweat samples and determining bacterial *taxa* present on them after the challenge compared to those in the inoculum.

Materials and methods

Sweat samples

Subaxillary sweat was collected from ten subjects (five women and five men) during a stay (50 min) in an infrared cabin using sterile 1·5-ml tubes. The mean age of the subjects was 40·5 years (range 32–53 years); all were in good health and had not consumed any antibiotics for at least 1 month immediately preceding the study. They were told not to use deodorants for at least three days before the sweat sampling. The pH values of sweat were determined using pH indicator strips with 0·5 graduations.

Textiles

Materials used for the challenge tests were knitted fabrics from 100% polyester (PES), 100% polyamide (PA), 100% polypropylene (PP), from TENCEL® (TE), a man-made cellulosic material of the generic fibre called lyocell and 100% cotton (CO). Polyester, polyamide and TENCEL® fabrics were raw white, cotton fabric was bleached, polypropylene fabric was of blue spun-dyed fibres, containing phthalocyanine pigments (Pigment Blue 15, C.I. 74160 B).

To have fabrics representing the intrinsic material properties of the textile fibres and to avoid any interference from textile finishing, the fabrics were prepared from commercial yarns in the textile pilot plant at Lenzing AG, Austria. After knitting, the cotton fabric was scoured and bleached. No chemical textile finishing was applied to any of the fabrics. All fabrics were laundered four times at 70°C with commercial washing powder to remove remainders of spin finish and waxes used in yarn spinning, rinsed extensively and handled under sterile conditions thereafter. Textiles were not autoclaved to avoid fibre destruction. However, no growth of bacteria was detected when the prewashed pieces of textiles were routinely incubated on nutrient agar plates. The fabrics were of single jersey construction with area weights between 145 and 175 g m^{-2} after washing. Table 1 gives the fabric properties.

Table 1 Properties of the textiles used. All textiles were knitted (heavy single), with no finishing added. Thickness was determined according to EN ISO 5084:1996

Fabric code	Fibre material	Area mass after washing (g m^{-2})	Thickness at 0·5 kPa (mm)	Treatment
PES	Polyester	159	0·49	–
PA	Polyamide	147	0·49	–
TE	TENCEL® (Lyocell)	145	0·50	–
CO	Cotton	175	0·68	Scoured, bleached
PP	Polypropylene	177	1·04	–

In vitro challenge tests

In vitro challenge tests using different textiles and sweat samples from volunteers were performed according to Teufel *et al.* (2008) with the following modified procedure. Prewashed pieces (2 × 2 cm, weighing between 0·06 and 0·07 g) of textiles were challenged with 100 μl of sweat from men and women. The resulting humidity was about 150% water added to 100% dry textile. The pieces of textiles were then incubated for 24 h at 37°C in a 'wet chamber' at near 100% air humidity. Thereafter, the bacteria present on different materials were lysed, and the bacterial DNA was extracted. Assays were performed in triplicates.

DNA extraction

DNA from bacteria present in sweat samples and on textiles was extracted by a two-step procedure developed recently (Teufel *et al.* 2008). In the first step, the sweat-incubated pieces of textiles (2 × 2 cm) or sweat samples (100 μl) were incubated in 0·5 ml cm^{-2} of TNE buffer (10 mmol l^{-1} Tris–HCl pH 8·0, 10 mmol l^{-1} NaCl, 10 mmol l^{-1} EDTA) containing 0·1% Triton X-100 (Serva, Heidelberg, Germany) and 25% (v/v) 5 mg ml^{-1} of lysozyme (Roche, Mannheim, Germany) for 1 h at 37°C on a head-over-head shaker with vigorous shaking. In the second step, 0·01% SDS and 0·5% (v/v) 20 mg ml^{-1} proteinase K (Roche) were added with subsequent incubation for 2 h at 55°C, again with vigorous shaking.

DNA quantification by PicoGreen®

Measurements were observed in black 96-well microplates (Greiner GmbH) as described previously (Batchelor *et al.* 2003). Briefly, 100 μl of DNA extracts and 100 μl of a 1 : 200 fold solution of PicoGreen® (Molecular Probes, Carlsbad, CA) diluted with TNE buffer, 0·1% Triton X-100 and 0·01% SDS were mixed and incubated in the dark for 10 min prior to the assay (Teufel *et al.* 2008). Fluorescent measurements were taken in the Fluoroskan II fluorescent microplate reader (GMI). DNA standards were prepared from lambda DNA stocks (Molecular Probes). The linear concentration range for DNA quantification extended from 25 pg ml^{-1} to 1 μg ml^{-1}, with a single dye concentration. DNA yield was calculated as ng DNA cm^{-2} of textile.

Statistical data treatment

The measured values for overall growth of sweat bacteria on textiles were analysed by analysis of variance, using the Statgraphics Centurion XV software (StatPoint Inc., Warrenton, VA 20182, USA). The algorithm according to Kruskal–Wallis applies a multiple comparison procedure to determine which means are significantly different from others. The graph produced shows the means and the 95% confidence intervals. When the intervals do not overlap, the difference between means is statistically significant at the 95% confidence level. Homogenous groups can be identified for samples that form a group of means within which there are no statistically significant differences. The method currently used to discriminate among the means is Fisher's least significant difference (LSD) procedure. With this method, there is a 5.0% risk of calling each pair of means significantly different when the actual difference is 0.

16S rRNA amplification, cloning procedure and sequence analysis

PCR amplification was performed with primers specific for conserved bacterial 16S rRNA sequences (Felske *et al.* 1996; Heuer *et al.* 1997). PCR with primers F 968: 5′-AACGCGAAGAACCTTAC-3′ and R 1401: 5′-CGGTGTGTACAAGGCCCG-3′ amplified a bacterial 16S rRNA fragment from nucleotide positions 967 to 1400 (*Escherichia coli* GenBank accession number J01859). PCR amplification was performed using the following conditions (final concentrations): 50 mmol l^{-1} KCl, 10 mmol l^{-1} Tris–HCl (pH 9·0), 0·01% gelatine, 0·1% Triton X-100, 3 mmol l^{-1} $MgCl_2$, 0·2 mmol l^{-1} dNTPs, 50 pmol of each primer and 2·5 U GoTaq® Polymerase (Promega, Madison, WI) in 50 μl. Generally, 27 cycles were performed. Each of them entailed denaturation at 95°C for 60 s, annealing at 48°C (primers F967 and

R1400) for 60 s and primer extension at 72°C for 60 s. PCR products were analysed on 1·5% agarose gels, stained with ethidium bromide. The PCR fragments were gel eluted, ligated with the pGEM®-T vector (Promega) and transformed into *E. coli* DH5α competent cells. After sequencing, an analysis of closest relatives was carried out by comparison with sequences available in the Ribosomal Database Project (RDP) II (release 9·39) and GenBank (http://www.ncbi.nlm.gov) databases, by using the standard nucleotide–nucleotide BLAST program.

Phylogenetic analysis

All sequences were examined for chimerism using Greengenes (DeSantis *et al.* 2006a). No chimeras were detected. Phylogenetic trees were constructed with sequences obtained in this study (16S rRNA nucleotide positions 967–1400) and the nearest neighbouring sequences from NCBI. The sequences were aligned with NAST at Greengenes (DeSantis *et al.* 2006b), and the phylogenetic trees were generated using MEGA 4.1 (Tamura *et al.* 2007).

Results

Characterization of sweat samples

All sweat samples were characterized by measuring the pH values and the amount of total DNA. Table 2 shows that there was a significant difference in the pH values of female and male sweat, collected under the same external condition. Whereas the sweat samples from men showed values between pH 5 and pH 6, the sweat from women was more basic with values between pH 7 and pH 8.

To estimate the overall bacterial content of the sweat samples, the amount of total DNA was quantified by the PicoGreen® method (Singer *et al.* 1997). Table 2 shows that the amount of DNA varied between 20·4 ng and 161·2 ng DNA per millilitre of sweat. Assuming a mean amount of 5 fg DNA per bacterial cell (Jeffrey *et al.* 1996; Button and Robertson 2001), a total amount of 4×10^6–$3{\cdot}2 \times 10^7$ bacteria per millilitre of sweat can be calculated (Table 2). In general, the variation in the overall bacterial content per millilitre was much more pronounced in men, reflecting marked differences in the sweating rate of males.

Genotyping of bacterial *taxa* present in sweat

Overall, 922 clones containing amplified 16S rRNA derived from the ten different sweat samples were analysed (Table 3). Figure 1 shows that 49 bacterial taxa, characterized down to genus or family level, were detected, and they belonged to six bacterial phyla: *Actinobacteria, Proteobacteria, Firmicutes, Bacteroidetes, Cyanobacteria* and *Deinococcus-Thermus*. Because of DNA sequence failure, 35 clones could not be analysed. As expected, there was a great variation between individual sweat samples. The number of taxa that were detected in each sweat sample ranged from 5 to 27. Nevertheless, two taxa *Staphylococcus* sp. and *Enterobacteriaceae* accounted for more than 55% of the total numbers of bacteria found. Both taxa were found in all subjects investigated, but there was a significant difference in their prevalence in samples from different individuals. *Staphylococcus* sp. represented 14–64·5%, and *Enterobacteriaceae* represented 6·3–53·5%. Interestingly, *Halomonas* sp. was found in eight of the ten samples. *Corynebacterium* sp. was detected in seven of ten sweat samples, and in one sample (M3) this taxon was the most prevalent with 32·5%. *Pseudomonas* sp. and *Bacillus* sp. were detected in six of ten samples, while *Propionibacterium* sp. was present in five of ten samples. Of the 49 taxa, 25 were found only in one sample, thus demonstrating the high variation between individuals (Table 3). A detailed phylogenetic tree of all bacteria found in this study is given as digital supporting information (Fig. S1).

Table 2 pH values of sweat, total amount of DNA in sweat samples and calculated number of bacteria in sweat samples

Sweat sample	pH	Amount of DNA ml^{-1}*(ng)	Calculated number of bacteria ml^{-1}†
F1	7·5	114·0 (±22·4)	$2{\cdot}3 \times 10^7$
F2	8	161·2 (±15·6)	$3{\cdot}2 \times 10^7$
F3	8	102·1 (±12·0)	$2{\cdot}0 \times 10^7$
F4	7·5	106·4 (±5·6)	$2{\cdot}1 \times 10^7$
F5	7·5	88·8 (±4·4)	$1{\cdot}7 \times 10^7$
M1	5	22·4 (±1·2)	$4{\cdot}4 \times 10^6$
M2	6	20·4 (±1·6)	$4{\cdot}0 \times 10^6$
M3	5·5	120·8 (±11·6)	$2{\cdot}4 \times 10^7$
M4	5	56·4 (±6·0)	$1{\cdot}1 \times 10^7$
M5	5·5	138·4 (±7·6)	$2{\cdot}7 \times 10^7$

*Mean amounts of three parallel measurements.
†Number of bacteria was calculated using a mean value of 5 fg DNA/bacterial cells.

Overall growth of sweat bacteria on textiles made of different fibre types

To investigate the influence of fibre type on the overall colonization of textiles by sweat bacteria, five different fabrics made of TENCEL® (lyocell), cotton, polyamide, polyester and polypropylene were used. TENCEL® is a rather recent type of a regenerated cellulosic fibre, which is available since 1984 (White 2001; Schuster *et al.* 2006).

Table 3 Percentage of phylotypes in sweat samples

	% clones									
	Male sweat samples					Female sweat samples				
Bacterial genera or family	M1	M2	M3	M4	M5	F1	F2	F3	F4	F5
Actinomyces sp.	–	–	–	–	–	–	–	–	1·07	–
Alcanivorax sp.	–	–	–	–	–	–	1·07	–	1·07	–
Anaerococcus sp.	–	–	2·10	–	1·15	–	–	–	–	1·04
Anoxybacillus sp.	–	–	–	–	–	–	–	–	1·07	–
Arthrobacter sp.	–	–	–	–	–	2·50	–	–	–	–
Bacillus sp.	–	1·05	1·05	3·19	–	–	–	1·07	1·07	1·04
Bradyrhizobiaceae	–	–	5·26	–	–	–	–	–	–	–
Caldicellulosiruptor sp.	–	–	–	–	–	–	–	–	1·07	–
Chryseobacterium sp.	–	–	–	–	–	–	1·07	–	–	–
Corynebacterium sp.	–	32·63	7·37	7·45	8·04	2·50	–	12·90	3·22	–
Crenotrichaceae	–	–	–	1·06	–	–	–	–	–	–
Deinococcus sp.	–	2·10	–	–	–	–	–	1·07	–	–
Enterobacteriaceae	38·54	16·84	24·21	51·06	10·34	6·25	12·90	20·43	18·28	53·12
Gemella sp.	–	–	–	–	–	–	–	–	1·07	–
Geobacillus sp.	–	–	1·05	–	–	–	–	–	1·07	–
Haemophilus sp.	–	–	1·05	–	–	–	–	–	2·15	–
Halomonas sp.	4·17	–	4·21	13·83	–	–	1·07	4·30	7·53	1·04
Hyphomicrobiaceae	–	–	1·05	–	–	–	–	–	–	–
Idiomarina sp.	2·08	–	3·16	2·13	–	–	–	–	–	–
Kocuria sp.	–	–	–	–	2·30	–	–	–	–	–
Kytococcus sp.	–	–	–	–	–	–	1·07	–	–	–
Lactobacillus sp.	–	–	–	–	–	–	7·53	–	–	–
Massilia sp.	–	–	–	1·06	–	–	–	–	–	–
Methylobacterium sp.	1·04	7·37	–	–	–	–	1·07	2·15	–	–
Micrococcus sp.	–	–	1·05	–	–	–	–	–	1·07	–
Moraxella sp.	–	–	–	–	–	–	1·07	–	–	–
Mycobacterium sp.	–	–	–	–	–	–	–	1·07	–	–
Neisseria sp.	–	–	–	–	1·15	–	–	–	1·07	–
Neisseriaceae	–	–	1·05	–	–	–	–	–	–	–
Nesterenkonia sp.	–	–	–	1·06	–	–	–	–	–	–
Nostoc sp.	–	–	–	–	–	–	–	–	1·07	–
Ochrobactrum sp.	–	2·10	–	–	–	–	–	–	–	–
Paracoccus sp.	–	–	1·05	–	–	–	–	–	–	–
Peptoniphilus sp.	–	–	2·10	–	–	–	–	–	1·07	–
Peptostreptococcus sp.	–	–	–	–	1·15	–	1·07	–	1·07	–
Porphyromonas sp.	–	–	–	–	–	–	–	–	–	–
Prevotella sp.	–	–	–	–	–	–	–	–	1·07	–
Propionibacterium sp.	–	–	3·16	1·06	13·79	–	–	4·30	1·07	–
Pseudomonas sp.	1·04	–	–	–	–	42·50	1·07	1·07	–	–
Psychrobacter sp.	–	–	–	1·06	–	–	1·07	–	5·38	–
Ralstonia sp.	–	2·10	7·37	–	–	–	–	–	–	–
Rhodococcus sp.	–	–	–	–	–	–	–	1·07	–	–
Rothia sp.	–	–	–	–	1·15	–	–	–	–	–
Staphylococcus sp.	50·00	31·58	32·63	13·83	52·87	46·25	64·52	46·24	39·86	35·42
Streptococcus sp.	–	–	–	2·13	1·15	–	1·07	–	1·07	–
Varibaculum sp.	–	1·05	–	–	–	–	–	–	–	–
Variovorax sp.	–	–	–	–	–	–	–	–	1·07	–
Veillonella sp.	–	–	–	–	–	–	1·07	–	–	–
Vibrio sp.	1·04	–	–	–	–	–	–	–	–	–
Sequence failure	2·08	3·16	1·05	1·06	6·87	–	3·20	4·30	6·45	8·33

The prewashed pieces of textiles were challenged with 100 μl of sweat and incubated for 24 h at 37°C at near 100% of humidity. Thereafter, the textiles were extracted, and the total amount of bacterial DNA present was

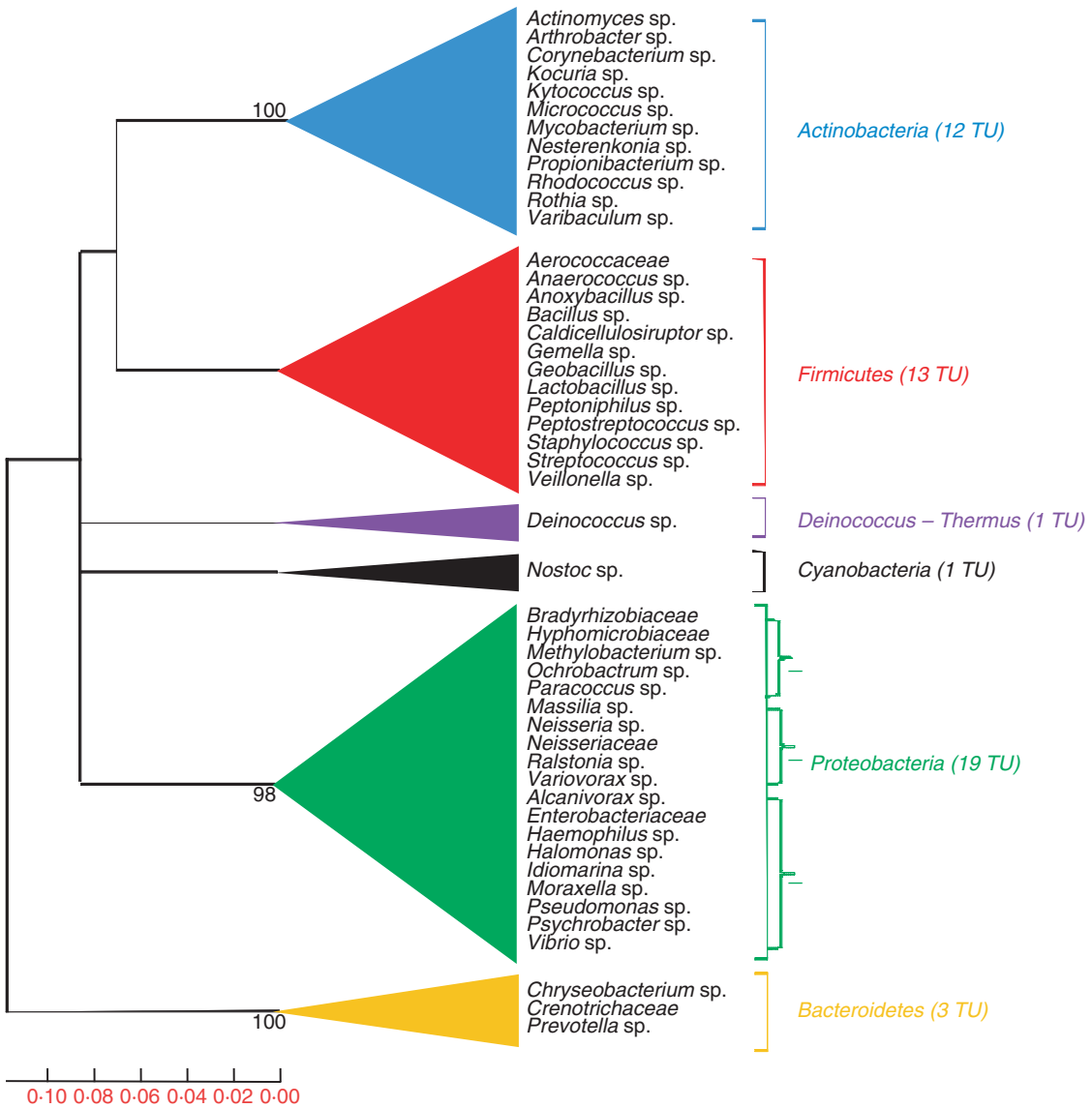

Figure 1 Phylogenetic analysis of bacterial 16S rRNA sequences detected in sweat samples from ten subjects. Sequences of about 922 clones were analysed, representing six bacterial phyla and 49 genera/families. The evolutionary history was inferred using the neighbour-joining method (Saitou and Nei 1987). The optimal tree with the sum of branch length 2·50329652 is shown. The percentage of replicate trees in which the associated taxa clustered together in the bootstrap test (500 replicates) are shown next to the branches (Felsenstein 1985). Evolutionary distances were computed using the Jukes–Cantor method (Jukes and Cantor 1969) and are in the units of the number of base substitutions per site.

determined and used as a parameter for overall growth/colonization of textiles (Teufel *et al.* 2008). There was significant bacterial growth on all types of materials and with almost all sweat samples (Fig. 2). For an objective evaluation of the differences between materials and individual sweat samples, a nonparametric analysis of variance (ANOVA) was performed separately for the experiments with male and the female sweat. Fig. 2 shows the result of the Kruskal–Wallis test and the Fisher's LSD procedure, drawn as means and 95% confidence intervals. The means for which the confidence intervals do not overlap are those with significant difference. With the male sweat samples (Fig. 2a), only PP showed significantly lower growth than the other materials. There is a trend of higher growth on CO, PA and PES when compared to TE. With the female sweat samples (Fig. 2b), two homogenous groups are identified. PA and PES showed a significantly higher colonization than CO, TE and PP. The overall trend is that PA and PES exhibited the highest overall growth of skin bacteria.

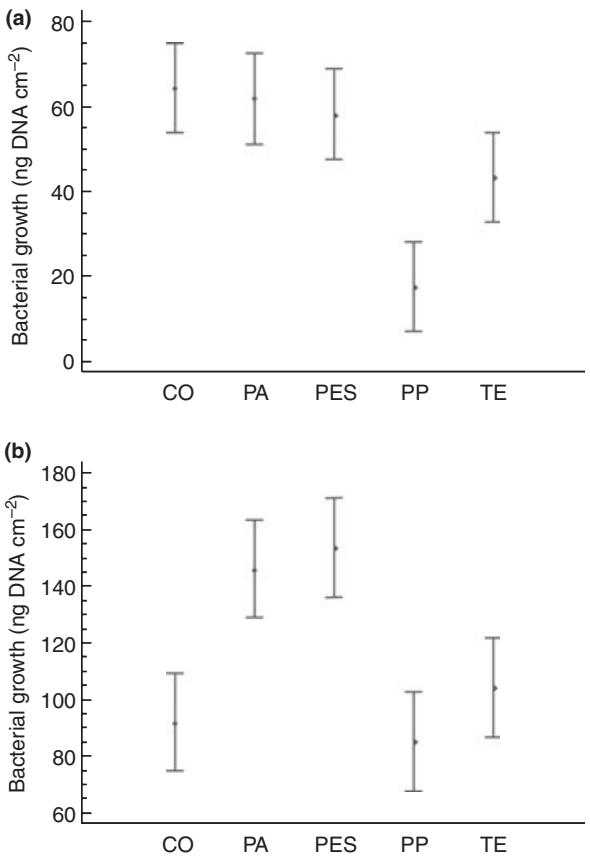

Figure 2 Overall bacterial growth on various materials. Sweat samples were taken from male (a) and female (b) test persons. Colonization is expressed as nanogram bacterial DNA cm^{-2} of textile. The means and the 95% confidence intervals according to Fisher's least significant difference procedure are shown. Initial values were between 0·5 and 4·0 ng bacterial DNA cm^{-2} of textile (Table 2). $n = 15$ for (a), $n = 12$ for (b). Note that PP was spun-dyed with a copper-containing pigment and was of higher thickness than the other materials (Table 1).

Genotyping of bacterial taxa present on textiles after the challenge tests

In order to find out whether different fibre types may selectively reduce or enhance the growth of specific *taxa* of skin bacteria present in sweat, we determined the bacterial taxa found on these materials after challenging them with the sweat samples. When comparing the bacterial taxa on the different materials after the challenge with those found in the original sweat samples, a marked reduction was seen in the diversity of taxa. Of the 49 different taxa found in sweat, only 26 were also found on textiles (Fig. 3). We cannot rule out the possibility that some reduction is caused by the lower numbers of clones analysed from textile samples than from sweat samples. However, there are some taxa that were higher on textiles than in sweat, which argues against a general reduction solely because of the lower numbers of clones investigated. In Fig. 4, an overview of the prevalent bacterial taxa found on the textiles after the challenge is shown. The most pronounced reduction in bacterial diversity was seen on TE, even after challenging with sweat samples with the broadest spectrum of different bacteria. The predominant taxa found on TE were identical with the predominant types present in sweat, which was *Staphylococcus* sp. in nine of ten samples (Fig. 4). No enrichment of bacterial taxa underrepresented in sweat was found on TE. On CO, the reduction in bacterial taxa was not as highly pronounced as on TE, and in general, a pattern more similar to that found in sweat was obtained with this material.

With the synthetic materials PP, PA and PES, we made the most striking observation of a selective enrichment of bacteria underrepresented in sweat. Enrichment with *Bacillus* sp. and *Pseudomonas* sp. was conspicuous on these materials (Fig. 4).

A detailed description of the results of the challenge tests with individual sweat samples is given in the digital supporting information (Tables SF1–5 and SM1–5). There was a considerable enrichment of *Bacillus* sp. on PP, PA or PES with four of the five male sweat samples (Tables SM1–4) and with three of the five female sweat samples (Tables SF1, SF4 and SF5). A selective enrichment of *Pseudomonas* sp. was observed on PP, PA or PES with sweat from individuals M1–M4, F1 and F2 (Tables SM1–4, SF1 and SF2). In some cases, enrichment of *Bacillus* sp. and *Pseudomonas* sp. was also observed on CO (Tables SM2–5, SF1, SF2 and SF5). Besides *Bacillus* sp. and *Pseudomonas* sp., some other taxa underrepresented or not detected in sweat, including *Micobacterium* sp., *Acinetobacter* sp., *Delftia* sp., *Geobacillus* sp., *Halomonas* sp., *Moraxella* sp., *Psychrobacter* sp., *Wautersiella* sp., *Paenibacillus* sp., *Dermabacter* sp., *Marinomonas* sp., *Ralstonia* sp. and *Exiguobacterium* sp., were found on the synthetic materials (Tables SM1, SM2, SM4, SM5, SF1 and SF3–5).

Discussion

Systematic investigations concerning the interaction between the bacterial skin flora and textiles are hindered by the overwhelming diversity of the skin flora and the lack of classical microbiological methods to estimate the overall colonization of textiles and to characterize bacterial populations on them. In addition, manufacture of fabrics that differ only in fibre content is extremely difficult, also contributing to the dearth of systematic studies. In the present study, we have used molecular biological methods to overcome the limitations of classical

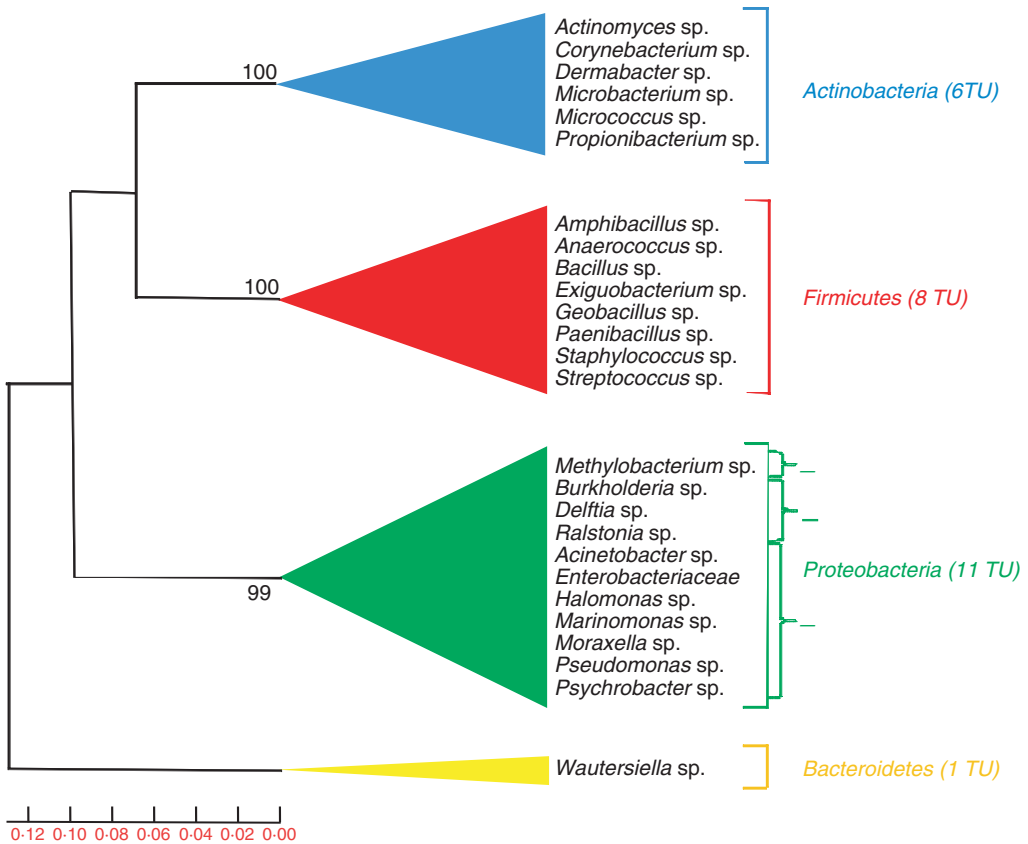

Figure 3 Phylogenetic analysis of bacterial 16S rRNA sequences found on textiles after the challenge test with sweat samples. Sequences of 2432 clones were analysed, representing four bacterial phyla and 26 genera/families. The evolutionary history was inferred using the neighbor-joining method (Saitou and Nei 1987). The optimal tree with the sum of branch length = 1·53983204 is shown. The percentage of replicate trees in which the associated taxa clustered together in the bootstrap test (500 replicates) are shown next to the branches (Felsenstein 1985). Evolutionary distances were computed using the Jukes–Cantor method (Jukes and Cantor 1969) and are in the units of the number of base substitutions per site.

microbiological methods (Hamlyn 1995; Teufel *et al.* 2008), which enabled us to perform experiments using a mixed bacterial population of skin bacteria present in native sweat samples instead of using selected model micro-organisms. For this purpose, using a molecular approach, we analysed the characterization of bacterial *taxa* present in the axillary sweat from ten individuals. When comparing our data from bacterial genotyping with a recent study on the bacterial forearm skin flora by Gao *et al.* (2007), the number of taxa found in sweat is less compared to that on skin. In our study, we could identify 49 different *taxa*, whereas on the skin 182 *taxa* were found (Gao *et al.* 2007). Nevertheless, all bacteria found in sweat belong to the same six phyla as identified on skin. Of the 49 taxa found in sweat, 18 taxa, including *Alcanivorax* sp., *Anoxybacillus* sp., *Bacillus* sp., *Caldicellulosiruptor* sp., *Chryseobacterium* sp., *Geobacillus* sp., *Halomonas* sp., *Idiomarina* sp., *Kytococcus* sp., *Massilia* sp., *Moraxella* sp., *Nesterenkonia* sp., *Ochrobactrum* sp., *Psychrobacter* sp., *Ralstonia* sp., *Varibaculum* sp., *Variovorax* sp. and *Vibrio* sp., have not been described on the forearm skin. This indicates that the bacterial skin flora is even more complex and supports the idea of high individual and local variations.

Interestingly, all samples from women showed pH level between 7 and 8, whereas samples from men were in the pH range 5–6. It has been reported that the pH of sweat is sweat rate dependent, being acidic at low sweat rates and alkaline at higher ones (Emmrich *et al.* 1968). However, in our experiment, the sweat rate of the male test persons was clearly higher than that of the females. This is also supported by the fact that the sweat samples from men were more diluted, containing less bacteria per millilitre compared to that of women.

The interaction of skin or sweat bacteria with textiles may have several consequences, including loss of

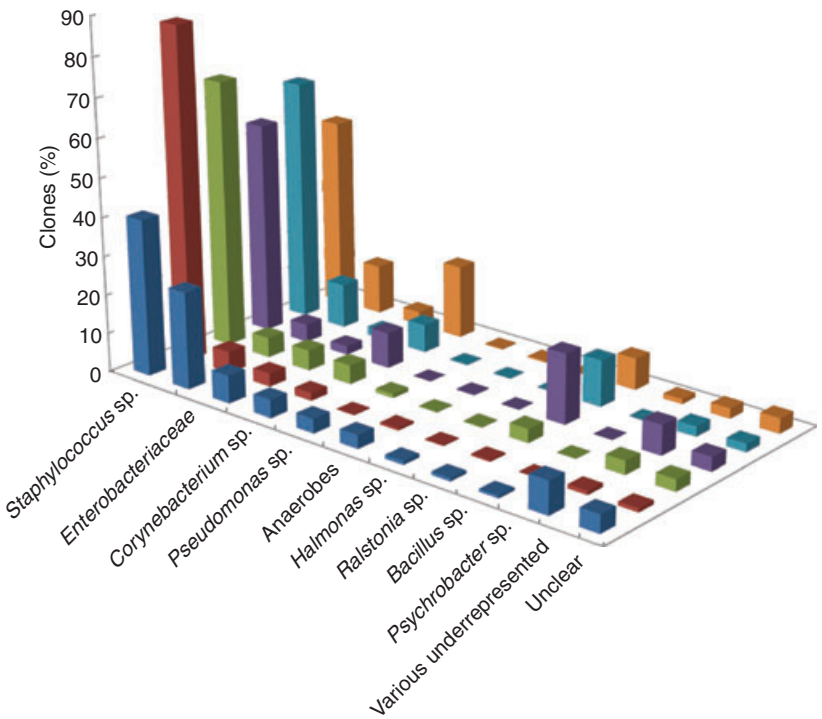

Figure 4 Overview of the prevalent bacterial *taxa* present on different textiles after a 24-h challenge test with human sweat samples. The results of the ten individual sweat samples (five women and five men) investigated were summarized. (■, Native sweat; ■, Tencel®; ■, cotton; ■, Polyamide; ■, Polyester and ■, Polypropylene).

performance, discolouration of fabrics and enhanced production of malodours. Therefore, the control of undesirable effects of microbes on textiles is becoming an important issue in textile industry (Mao and Murphy 2001; Höfer 2006). Currently, there is much interest in hygienic fabrics that offer an advantage in terms of cleanliness and odour prevention as a result of their antimicrobial properties or inherent properties to reduce bacterial growth (Payne and Kudner 1996; Purwar and Joshi 2004; Williams and Cho 2005; Gao and Cranston 2008).

We compared the overall colonization of five different materials by the bacteria present in sweat. With sweat samples from female test persons, the highest numbers of bacteria were found on PA and PES, consistent with the results of previous *in vivo* wearing trials (Teufel *et al.* 2008). TE, CO and PP showed an overall lower bacterial density. However, in previous *in vitro* testing with *Staphylococcus aureus* using a challenge test modified after standard JIS-1902 (Teufel and Redl 2006), PP materials were found to show higher growth than polyester. As opposed to that earlier testing, the PP material used here contained phthalocyanine pigments with complexed copper ions, and the possibility of a weak antibacterial effect of this pigments cannot be excluded. As well, the somewhat heavier weight and higher thickness of the PP knit may play a role. With the sweat from male persons, only the PP material showed a statistically significant lower growth than the other material, and differences between the other materials can be seen as a trend. On CO, bacterial colonization was different between male and female sweat. With female sweat, CO was in the group with a low overall bacterial colonization, whereas with male sweat it was in the group with a higher overall growth. Although there is no general rule, our results suggest that materials like TE might indeed have inherent properties to reduce the overall growth of sweat bacteria under certain conditions.

The involvement of textiles in odour production was already realized in the 1950s, when textiles were suggested to contribute to odour development with odour potentially more intense in the fabric substrate than in the adjacent axilla (Shelley *et al.* 1953). However, most studies about body odour development and skin bacteria have been performed without considering the influence of textiles. Leyden *et al.* (1981) described that the axillary flora is a mixture of bacterial taxa, predominantly *Micrococcaceae*, coryneforms and *Propionibacterium* sp. and that a high number of bacteria can be recovered from the axillary of persons with pungent axillary odour. This typical 'pungent/biting' type of odour was related to the activity of aerobic coryneforms. In contrast, *Micrococcaceae* tended to give rise to a weaker 'acidic/sweaty' type of odour attributed to isovaleric acid. However, it has to be mentioned that, at that time, *Staphylococcus* sp. was a member of the *Micrococcaceae* and that the coryneforms were not well defined. Malodourous androstenes seem to

be produced by bacteria in the axilla (Nixon *et al.* 1988), and the pathways leading to these biotransformations by axillary coryneforms have been characterized (Austin and Ellis 2003). Short-chain fatty acids and isovaleric acids were also found to be the main odour-creating compounds of foot sweat (Kanda *et al.* 1990). According to this line of evidence, a clinical study showed that the reduction of malodour correlates with a reduction of both *Micrococcaceae* and coryneforms (Guilett *et al.* 2000).

However, these investigations have been limited to body odour development. A recent investigation into textiles showed that odour intensity was strongly associated with the fibre type, with PES fabrics rating high in odour intensity and CO and wool mid-to-low odour intensity (McQueen *et al.* 2007). This relationship between fibre type and odour production was not influenced by the bacterial numbers present, and there was no enrichment of *Corynebacteria* on the more odourous material. In our studies, we have not directly determined the relationship between odour production and fibre type. Nevertheless, consistent with the study of McQueen *et al.* (2007), we did not find an enrichment of *Corynebacteria* on the textiles. A statistically significant association of axillary malodour and microbial counts was also reported for *Micrococci*, although these bacteria were not a predominant *taxon* on axillary skin (Taylor *et al.* 2003). In our study, *Micrococcus* sp. did not appear as a major *taxon* present in human sweat, becasue we found it only in one sweat sample. Nevertheless, we cannot rule out a role of *Micrococcus* in odour development becasue with one sample a considerable enrichment of *Micrococcus* sp. was seen on PES.

The most surprising result was the fact that, on the cellulose-based TE, only very limited numbers of taxa were found after the challenge, which on all materials tested corresponded to *Staphylococcus* sp., the most prevalent taxon present in sweat. In contrast, a greater variety of taxa were detected on synthetic materials. Interestingly, most of the synthetic materials showed an enrichment of bacteria underrepresented in the sweat flora. In several cases, a selective growth of *Bacillus* sp. and *Pseudomonas* sp. and a number of gram-negative *Proteobacteria* were observed. On CO, growth of underrepresented taxa was less pronounced.

More intensive studies on relationship between bacterial species and odour production were triggered with the investigations into the development of oral malodour. These studies have shown that most of the odour production is the result of Gram-negative bacterial metabolism, with the Gram-positive bacteria contributing very little to oral odour (McNamara *et al.* 1972; Goldberg *et al.* 1997). On the other hand, *Bacilli* were detected in larger numbers on the planar skin of subjects with strong foot odour, and this group of bacteria was shown to be closely associated with increased odour (Ara *et al.* 2006). Nevertheless, further studies have to clarify whether a selective growth of bacteria identified in our study on different materials, especially synthetic materials, contributes essentially to the odour production on textiles.

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Supporting Information

Additional Supporting Information may be found in the online version of this article:

Figure S1 Evolutionary relationships of 58 taxa of human skin-derived bacteria found in this work.

Table SF1 Prevalent bacterial genera/families in native sweat of female sample 1 compared to the bacteria present on sweat-inoculated textiles.

Table SF2 Prevalent bacterial genera/families in native sweat of female sample 2 compared to the bacteria present on sweat-inoculated textiles.

Table SF3 Prevalent bacterial genera/families in native sweat of female sample 3 compared to the bacteria present on sweat-inoculated textiles.

Table SF4 Prevalent bacterial genera/families in native sweat of female sample 4 compared to the bacteria present on sweat-inoculated textiles.

Table SF5 Prevalent bacterial genera/families in native sweat of female sample 5 compared to the bacteria present on sweat-inoculated textiles.

Table SM1 Prevalent bacterial genera/families in native sweat of male sample 1 compared to the bacteria present on sweat-inoculated textiles.

Table SM2 Prevalent bacterial genera/families in native sweat of male sample 2 compared to the bacteria present on sweat-inoculated textiles.

Table SM3 Prevalent bacterial genera/families in native sweat of male sample 3 compared to the bacteria present on sweat-inoculated textiles.

Table SM4 Prevalent bacterial genera/families in native sweat of male sample 4 compared to the bacteria present on sweat-inoculated textiles.

Table SM5 Prevalent bacterial genera/families in native sweat of male sample 5 compared to the bacteria present on sweat-inoculated textiles.

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