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Note

Artificial sweat composition to grow and sustain a mixed human axillary microbiome

Chris Callewaert [a], Benjamin Buysschaert [a], Els Vossen [b], Veerle Fievez [b], Tom Van de Wiele [a], Nico Boon [a,*]

[a] Laboratory of Microbial Ecology and Technology (LabMET), Ghent University, Coupure Links 653, B-9000 Ghent, Belgium
[b] Laboratory for Animal Nutrition and Animal Product Quality (LaNuPro), Ghent University, Proefhoevestraat 10, B-9090 Melle, Belgium

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ABSTRACT

A novel artificial sweat composition, Skin Community Interaction simulation, designed to mimic the human axillary sweat, was compared to other artificial sweat compositions. Axillary microbiota grown in the novel composition closely resembled the original community. Volatile organic compound analysis showed good correlations with *in vivo* axillary (mal)odor components.

As most of the cosmetics are applied directly on the skin, it is necessary to test the possible side effects of cosmetics before release on the market. The risks associated with chemicals used in the industry or at home need to be tested to develop appropriate safety rules and guidelines (Leist et al., 2012). For this means, models have been sought and used throughout history. Attempts have been made to model and simulate the skin and the axillary malodor formation (Holland et al., 2008; James et al., 2004; Semlin et al., 2011). Few artificial sweat compositions are available (Kulthong et al., 2010) and their compatibility with the skin microbial community has rarely been investigated. This research focused on the development of a novel artificial sweat composition to sustain and grow the autochthonous mixed skin axillary microbiome.

The synthetic sweat was designed to be as similar as possible with *in vivo* human axillary sweat. The three main constituents of the synthetic sweat are amino acids, salts and fatty acids, dissolved in three different stock solutions. As a source of fatty acids, human abdominal subcutaneous fat was used obtained from liposuction plastic surgery operation. The fat was hydrolyzed by incubating for 6 h in a 1 M HCl solution at 50 °C. The hydrolyzed abdominal subcutaneous human fatty acids consisted of 23% palmitic acid (C16:0), 43% oleic acid (ω9C18:1), 8% linoleic acid (ω6C18:2), 5.6% palmitoleic acid (C16:1), 5% stearic acid (C18:0), 4% myristic acid (C14:0), among other lower-represented fatty acids. The subcutaneous fat was extracted, methylated and analyzed on GC as performed by Hostens et al. (2012). The artificial human axillary sweat contained 16.4 ml L^{-1} hydrolyzed human fatty acids. Subsequently, 2.0 ml L^{-1} squalene and 0.8 ml L^{-1} cholesterol (Nicolaides, 1974) was added to the solution. A range of amino acids, amino acid intermediates (Gitlitz et al., 1974), salts and other ingredients (Robinson and Robinson, 1954) were added, based on literature. The pH of the solution was set at 6.50 using 1 M NaOH. The complete composition of the SCIN artificial sweat is presented in Table 1. Bacterial inoculations of different male subjects were used for the simulations. The axilla was swabbed during 15 s with a moistened cotton swab (Biolab, Belgium) and immediately suspended in 1 ml of sterile physiological water (Evans and Stevens, 1976). The solution was diluted to 10^{-2} before inoculation. This suspension was used to dilute the three stock solutions until the correct concentrations were reached for the simulation. The study was approved by the Ghent University Ethical Comity with approval number B670201112035. The incubations were performed in glass petri dishes with agarose (10 g L^{-1}) as ground layer. A total of 100 μl of the artificial SCIN sweat with added inoculum is put on the plate and spread with a sterile Drigalski spatula. The plates were sealed with airtight caps and parafilm. After aerobic incubation at 37 °C, the simulation was swabbed with a cotton swab (Biolab, Belgium) to collect the bacteria. Several incubation periods were verified, going from 7 to 21 days. The SCIN sweat composition was compared with four other formulations of artificial sweat, prepared according to the International Standard Organization (ISO105-E04-2008E), American Association of Textile Chemists and Colorists (AATCC Test Method 15-2002) and the British Standard (BS EN1811-1999) (Table 1). These formulas are used

* Corresponding author at: Ghent University, Faculty of Bioscience Engineering, Laboratory of Microbial Ecology and Technology (LabMET), Coupure Links 653, B-9000 Ghent, Belgium. Tel.: +32 9 264 59 76; fax: +32 9 264 62 48.
E-mail address: nico.boon@ugent.be (N. Boon).
URL's: http://www.labmet.ugent.be, http://www.drarmpit.com (N. Boon).

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Table 1
The chemical composition of the four types of artificial sweat used to test the reliability of the SCIN sweat formula. The pH was adjusted with NaOH or HCl (Kulthong et al., 2010).

Composition	Concentration (% (w/v))				
	SCIN	AATCC	ISO	ISO	EN
	pH 6.5	pH 4.3	pH 5.5	pH 8.0	pH 6.5
Sodium chloride	2.923	1.000	0.500	0.500	1.080
Lactic acid (88%)	1.441	0.097	–	–	0.120
Urea	1.201	–	–	–	0.130
Human fatty acids	16.40	–	–	–	–
Squalene	2.000	–	–	–	–
Cholesterol	0.800	–	–	–	–
Glycine	0.125	–	–	–	–
L-Leucine	0.026	–	–	–	–
L-Cysteine	0.003	–	–	–	–
L-Serine	0.303	–	–	–	–
L-Alanine	0.081	–	–	–	–
L-Arginine	0.001	–	–	–	–
L-Histidine	0.043	0.025	0.050	0.050	–
L-Threonine	0.066	–	–	–	–
L-Valine	0.032	–	–	–	–
L-Isoleucine	0.020	–	–	–	–
L-Lysine	0.021	–	–	–	–
L-Phenylalanine	0.021	–	–	–	–
L-Tyrosine	0.034	–	–	–	–
L-Asparagine	0.022	–	–	–	–
L-Glutamic acid	0.023	–	–	–	–
L-Methionine	0.007	–	–	–	–
L-Glutamine	0.003	–	–	–	–
L-Aspartic acid	0.055	–	–	–	–
L-Ornithine	0.086	–	–	–	–
L-Citrulline	0.073	–	–	–	–
L-Ascorbic acid	0.001	–	–	–	–
D-Glucose	0.180	–	–	–	–
Creatinine	0.004	–	–	–	–
Sodium pyruvate	0.055	–	–	–	–
Potassium hydrogen carbonate	0.120	–	–	–	–
NaH_2PO_4	0.002	–	0.220	–	–
Na_2HPO_4	–	0.100	–	0.500	–
Calcium sulfate	0.057	–	–	–	–

in the textile industry to test color steadiness (Kulthong et al., 2010). The bacteria were dissolved in 1 ml sterile physiological solution. A DNA extraction was performed on the initial and the simulated communities. The DNA profile of the communities was analyzed using DGGE, as described previously (Callewaert et al., 2013) and compared to the

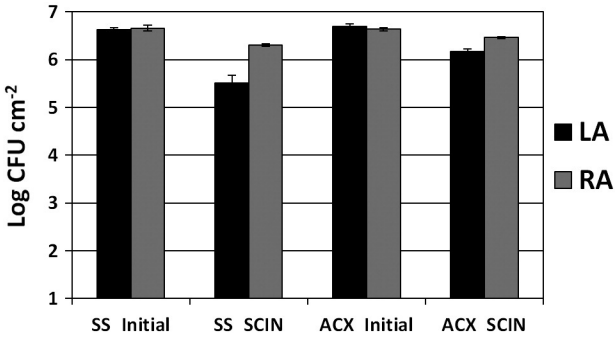

Fig. 2. Growth of staphylococci (SS) and axillary bacteria (ACX) of the autochthonous and SCIN simulated axillary culture, for the left (LA) and right (RA) axillae of subject 2. Data are represented as log CFU per cm^2 axillary skin.

profile of the inoculum. The SCIN sweat composition used in a simulation of 7 days allowed the bacterial community to develop which closely resembled the autochthonous axillary community, with a similarity of 86%. The second closest relationship was found with the EN artificial sweat composition, with a similarity of 67%. The AATCC and ISO pH 5.5 artificial sweat compositions had a similarity of 46% and the ISO pH 8.0 artificial sweat composition had a similarity of 20% (Fig. 1a). The EN, ISO and AATCC compositions contained lower concentrations and a lower diversity of nutrition, as compared with the SCIN composition (Table 1), which can be responsible for the lower similarities. Axillary inocula of another subject grown in the SCIN artificial sweat composition also resulted in a bacterial community closely related with the autochthonous community, for incubations from 4 up to 21 days (Fig. 1b, c and d). Diversity analysis was carried out to verify the differences between simulated and autochthonous axillary community. Richness analysis, as the number of bands present on DGGE gel, indicated an average of 10.4 ± 3.5 and 8.7 ± 2.1 bands for the autochthonous and SCIN simulated community, respectively. Evenness analysis, calculated as the Gini coefficient, indicated an average of 0.48 ± 0.073 and 0.62 ± 0.091 for the autochthonous and SCIN simulated community, respectively. The diversity of the axillary bacteria grown on plate was slightly lower in diversity (less even, less rich), which was expected, as not all bacteria are able to grow on plate. Nevertheless, good results were obtained as compared to other artificial sweat compositions. Plate counts were carried out which indicated comparable log-counts after 6 days of growth on the

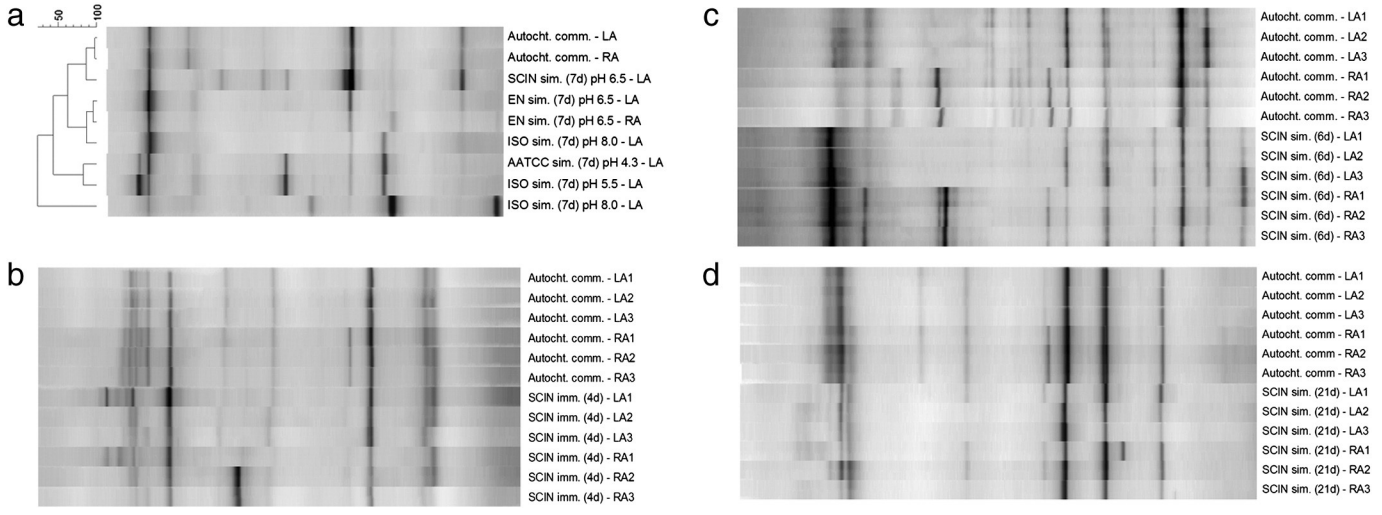

Fig. 1. (a) DGGE comparison of the autochthonous axillary communities with the simulated axillary communities for different artificial sweat compositions (SCIN, ISO pH 8.0, ISO pH 5.5, AATCC and EN pH 6.5) simulated for 7 days for subject 1. (b) Comparison for the left (LA) and right axillary (RA) community for subject 2 between the autochthonous and immersed (liquid) SCIN simulated community (4 day simulation). (c) Comparison for LA and RA for subject 2 between the autochthonous and SCIN simulated community (6 days). (d) Comparison for LA and RA for subject 2 between the autochthonous and SCIN simulated community (21 days).

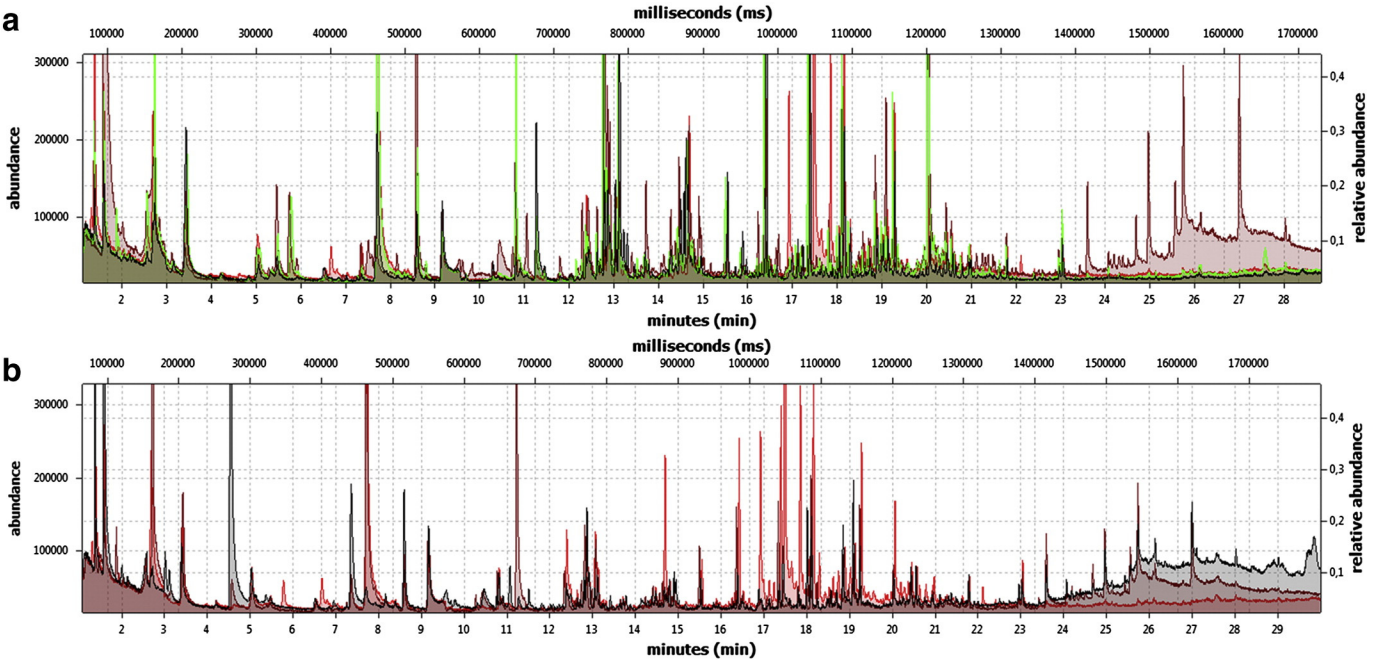

Fig. 3. Overlay chromatogram of different simulations of (a) subject 1 and (b) subject 2. Numerous compounds (volatile fatty acids, aldehydes, ketones, esters) were identified by MS similar to *in vivo* axillary (mal)odor components. Large differences were observed when different axillary inoculations were used.

SCIN simulation as in the autochthonous axillary community (Fig. 2). Bacterial samples were taken from the SCIN simulation plates using sterile cotton swabs (Biolab, Belgium), transferred and diluted in saline and subsequently plated in triplicate. Plates were incubated at 37 °C for 24 h on staphylococcal selective (SS agar) and non-selective axillary culture (ACX agar) agar plates (Taylor et al., 2003). Bacterial growth was confirmed and additional optical density (OD) measurements indicated apparent growth curves of the axillary bacteria. The results indicate that the SCIN artificial sweat is a realistic sweat composition to cultivate a mixed axillary microbial community in the laboratory.

The SCIN artificial sweat composition was analyzed on its ability to develop axillary (mal)odor. Axillary bacterial communities of different males were subjected to immersion in SCIN artificial sweat at 37 °C for 24 h. Head-space samples were extracted and injected into the GC/MS *via* manual injection of a 65-mm polydimethylsiloxane-divinylbenzene SPME fiber (Supelco, Pennsylvania, USA). Extracted volatiles were analyzed using a gas chromatograph (Agilent model 6890 N) coupled to a mass-selective detector (Agilent model 5973, Agilent Technologies, Diegem, Belgium). Chromatography was performed on a HP-5 column (30 m × 250 μm × 1 μm, 5% phenyl methyl siloxane, Agilent Technologies, Diegem, Belgium). The method and GC temperature program were described previously (Curran et al., 2005). A simulation with metallic caps (18 mm magn screw cap sil/ptfe precut) and agarose substrate produced the lowest background noise. The GC/MS results indicated many volatile fatty acids, aldehydes, esters and ketones (Fig. 3) which correspond to the *in vivo* situation (James et al., 2004), among which acetic acid, propanoic acid, isovaleric acid, butanoic acid, myristic acid, pentadecanoic acid, palmitic acid, n-hexadecanoic ester, 2-butanone, 2-pentanone, 2-pentanal, 1-hexanol, octanal, nonanal, 2-nonanone and 2-undecanone. These compounds were not released in the controls.

There is a necessity to analyze the effect of underarm and skin cosmetics on the existing microbial community and the odor development. A uniform simulation of the axillary environment is needed which is not host-dependent. We assembled a new formula for artificial sweat which was found to be useful to cultivate mixed axillary microbiota. As tested for different axillary inocula, the bacterial community and structure were maintained for a period of up to 21 days. An improvement was made when comparing with other available artificial sweat solutions. Initial results revealed that the SCIN artificial sweat can be used for odor analysis. Further optimization is, nevertheless, needed for this purpose.

References

Callewaert, C., Kerckhof, F.M., Granitsiotis, M.S., van Gele, M., van de Wiele, T., Boon, N., 2013. Characterization of Staphylococcus and Corynebacterium clusters in the human axillary region. PLoS ONE 8, e50538.
Curran, A.M., Rabin, S.I., Prada, P.A., Furton, K.G., 2005. Comparison of the volatile organic compounds present in human odor using SPME-GC/MS. J. Chem. Ecol. 31, 1607–1619.
Evans, C.A., Stevens, R.J., 1976. Differential quantitation of surface and subsurface bacteria of normal skin by combined use of cotton swab and scrub methods. J. Clin. Microbiol. 3, 576–581.
Gitlitz, P.H., Fw, Sunderma, Hohnadel, D.C., 1974. Ion-exchange chromatography of amino-acids in sweat collected from healthy subjects during sauna bathing. Clin. Chem. 20, 1305–1312.
Holland, D.B., Bojar, R.A., Jeremy, A.H.T., Ingham, E., Holland, K.T., 2008. Microbial colonization of an in vitro model of a tissue engineered human skin equivalent—a novel approach. FEMS Microbiol. Lett. 279, 110–115.
Hostens, M., Fievez, V., Leroy, J.L.M.R., Van Ranst, J., Vlaeminck, B., Opsomer, G., 2012. The fatty acid profile of subcutaneous and abdominal fat in dairy cows with left displacement of the abomasum. J. Dairy Sci. 95, 3756–3765.
James, A.G., Casey, J., Hyliands, D., Mycock, G., 2004. Fatty acid metabolism by cutaneous bacteria and its role in axillary malodour. World J. Microbiol. Biotechnol. 20, 787–793.
Kulthong, K., Srisung, S., Boonpavanitchakul, K., Kangwansupamonkon, W., Maniratanachote, R., 2010. Determination of silver nanoparticle release from antibacterial fabrics into artificial sweat. Part. Fibre Toxicol. 7.
Leist, M., Hasiwa, N., Daneshian, M., Hartung, T., 2012. Validation and quality control of replacement alternatives—current status and future challenges. Toxicol. Res. 1, 8–22.
Nicolaides, N., 1974. Skin lipids—their biochemical uniqueness. Science 186, 19–26.
Robinson, S., Robinson, A.H., 1954. Chemical composition of sweat. Physiol. Rev. 34, 202–220.
Semlin, L., Schaefer-Korting, M., Borelli, C., Korting, H.C., 2011. In vitro models for human skin disease. Drug Discov. Today 16, 132–139.
Taylor, D., Daulby, A., Grimshaw, S., James, G., Mercer, J., Vaziri, S., 2003. Characterization of the microflora of the human axilla. Int. J. Cosmet. Sci. 25, 137–145.