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(54) Title: MICROBIAL ENZYMES TO REDUCE MALODOUR OF SKIN AND TEXTILES

(57) Abstract: The present invention relates to the field of reducing malodour which is due to bacterial conversion of molecules which are present in sweat. The present invention discloses purified enzymes, lyophilized or non-living bacteria, bacterial lysates, and/or bacterial fragments which contain the functional potential to fully catabolize the human skin lipids and fully catabolize squalene, so no malodour arises. The present invention also discloses enzymes that produce a natural fragrance from squalene breakdown products. The compounds of the present invention can thus be used in deodorants, washing powders, clothing finishing agents or in any method to reduce malodour.

Microbial enzymes to reduce malodour of skin and textiles

Field of the invention

The present invention relates to the field of reducing malodour which is due to bacterial conversion of molecules which are present in sweat. The present invention discloses purified enzymes, lyophilized or non-living bacteria, bacterial lysates, and/or bacterial fragments which contain the functional potential to fully catabolize the human skin lipids and squalene, so no malodour arises. The present invention also discloses enzymes that produce a natural fragrance from squalene breakdown products. The compounds of the present invention can thus be used in deodorants, washing powders, clothing finishing agents or in any method to reduce malodour.

Background of the invention

The skin microbiome is rich and complex and every skin niche has its own specific microbiome (1). Most of the microbiota living on the skin are harmless. Some bacteria are associated with the production of malodours, while others are not associated to malodours or are even associated to good odours (2). In the case of osmidrosis, body odour, bromhidrosis, the skin microbiome is different and considered responsible for the malodour formation (3).

Treatments of axillary body odour include the use of deodorants and antiperspirants (4), botulinum toxin injections (5), axillary liposuction (6), laser- or microwave-based ablation of sweat glands (7,8), antidepressants and antipsychotics (9), and topical antibiotics (10). Most methods focus on the sweat precipitation and not on the bacterial impact. To date, there is no lasting solution focusing against the microbiome of underarm body odour.

Deodorants and antiperspirants are widely used to mask body odour. Both use perfumes, to mask the malodour production, and generally use antimicrobial compounds, to limit the bacterial growth. Compounds with an antimicrobial and/or antifungal function that are commonly used are triclosan, triclocarsan, quaternary ammonium compounds, metal salts, aliphatic alcohols and glycols and other fragrances (11)(12). Antiperspirants generally contain aluminium salts, which blocks the sweat pores by mechanical obstruction. The ingredients with such action are aluminium chloride hexahydrate (ACH), aluminium chlorohydrate, aluminium sesquichlorohydrate, aluminium chlorohydrex and aluminium zirconium tetrachlorohydrate. The deodorants and antiperspirants of today do not adjust the microbiome. Contrary, most deodorants and antiperspirants will infer a shock to the microbial community and will lead to

an increase in microbial diversity (13). This is unwanted as a higher microbial diversity is associated with a stronger underarm malodour. The abundance of corynebacteria may increase, which can lead to more malodour formation (13)(14).

The current deodorant and antiperspirants aims to reduce the microbial load, and thus also the enzymatic load.

Deodorant formulations upon today indeed target and suppress biochemical reactions that transform odour precursors into malodorous volatiles. For example, US5213791 discloses amino acid β-lyase inhibitors in deodorants. The latter document discloses this amino acid β-lyase as a malodour-forming enzyme and identifies ways to inhibit it. In other words, this document describes the inhibition of enzymes to block biochemical conversions. Also US 5676937 and US 5643559 describe inhibitors of bacterial sulfatases and glucuronidases, which are thought to reduce the steroidal malodour. Also here, agents to block and inhibit the enzymes responsible for biochemical conversion are disclosed. WO 00/01355 (17) further describes agents to inhibit steroid reductase and thus malodour. EP 1258531A1) (18) describes the enzyme Nα-acyl-glutamine-aminoacylase which is responsible for the release of thioalcohol-based malodour and a general formula containing ingredients to inhibit said enzyme. This enzyme can also be present in *Staphylococcus* spp., namely *S. hominis* (19), and is therefore responsible for the release of the typical oniony sulphurous thioalcohol based malodour in the axillae. This document thus describes agents to inhibit the afore mentioned enzyme, in order to avoid the biochemical conversion with the release of the malodorous thioalcohol as result. Similarly, US 6951729B1 describes a high throughput screening method for agents affecting the enzymes involved in the fatty acid biosynthesis.

However, the use of inhibitors in deodorants and applied on skin fall short. The biochemical reaction already largely occurred in the sweat glands and the hair roots, where the deodorant ingredients cannot or can barely penetrate. The inhibitors of microbial enzymes will therefore only be partially effective.

Hence, there is still a need to find alternative products and methods which are useful to combat malodour.

Description of the Figures

Figure 1. Metagenome prediction collapsed to level 2 correlation plot with most important axillary taxa of the armpit observation study. A black colour means a low enzymatic load; a white colour refers to a higher enzymatic load. The genetic content is divided into classes such as lipid metabolism, energy metabolism, etc. Enzyme families collapsed to level 2 of KEGG pathways.

Figure 2. Correlations between KEGG bacterial predicted metabolic fat metabolism pathways (from PICRUSt) and bacterial abundances. The most odour-contributing and high abundant underarm genera are presented. KEGG metabolic pathway are only presented for fat metabolising enzymes. The colour of bubble, along with size, is indicative of the Kendall rank correlation coefficient between matrices. White designates a positive correlation while black designates a negative correlation. *Staphylococcus* has a positive correlation for fatty acid biosynthesis, and thus has a higher abundance of enzymes to utilize fatty acids breakdown products.

Figure 3. Lipid degradation and biosynthesis pathways: axillary microbiomes associated with good odours show enrichment in functional composition (genes) as compared to microbiomes associated with malodour. (Unsaturated) fatty acids are easily metabolized by *Corynebacterium* in the first step, but further lack gene abundances to fully metabolize or *de novo* synthesis, as compared to *Staphylococcus*. Pathway of β-oxidation and fatty acid synthesis of *Staphylococcus* and *Corynebacterium* spp. are coloured in full and dashed arrows, respectively.

Figure 4. Squalene degradation pathway: axillary microbiomes associated with good odours show enrichment in functional composition (genes) as compared to microbiomes associated with malodour. *Staphylococcus* spp. enzymes have more enzymes to fully degrade squalene. Squalene degradation of *Staphylococcus* and *Corynebacterium* spp. are in full and dashed arrows, respectively. Thickness of arrow indicates relative amount of enzymes present.

Figure 5. Subjects with bromhidrosis treated with lyophilized *Staphylococcus* bacteria. Odour panel verified the odour intensity, on five consecutive weeks. The first week (week 0 –

W0) was status without treatment (grey boxplot), while the last four weeks (week 1 W1, week 2 W2, week 3 W3, week 4 W4) was during treatment (white boxplots). The lyophilized viable bacteria were tested on three different subjects and showed a significant decrease in underarm odour intensity upon use of the spray containing the bacterial lysates. Difference in odour intensity were assessed using the Mann-Whitney U-test; values shown on top of the plots.

Figure 6. Subjects with bromhidrosis treated with *Staphylococcus* lysates containing the active enzymes, as verified by the enzymatic assay. Odour panel verified the odour intensity, on five consecutive weeks. The first week (week 0 – W0) was status without treatment (grey boxplot), while the last four weeks (week 1 W1, week 2 W2, week 3 W3, week 4 W4) was during treatment (white boxplots). The bacterial lysates were tested on three different subjects and showed a significant decrease in underarm odour intensity upon use of the spray containing the bacterial lysates. Difference in odour intensity were assessed using the Mann-Whitney U-test; values shown on top of the plots.

Summary of the invention

Contrary to the inhibition of enzymes, the use of enzymes to foster or encourage the biochemical conversion of sweat precursors has not been disclosed before.

The present invention discloses the addition of extra enzymes to fully convert the biochemical compounds in –as an example– the underarm. This invention does thus not describe the inhibition of microbial enzymes. In contrast, the present invention aims to add extra active enzymes to selectively steer the biochemical reaction towards the release of non-odorous volatiles. By promoting one biochemical reaction, we selectively create breakdown of sweat molecules that are not odorous, and avoid the biochemical breakdown of sweat molecules by the armpit microbiome into malodorous molecules.

In one aspect, the present invention discloses the use of fatty acid degrading enzymes to enable fatty acid breakdown and sweat molecules on skin and clothes to encourage the complete breakdown of fatty acids release from sebaceous and apocrine sweat glands.

The present invention also further describes the use of enzymes to fully catabolize squalene and biochemically break it down towards non-malodorous molecules or useful building blocks.

The present invention further discloses a series of enzymes that use squalene and converts it into farnesyl, mevalonate and acetyl. In other words, the present invention discloses the usage of enzymes to convert naturally secreted molecules on skin to produce other useful compounds on skin, rather than degrading and producing malodorous volatiles.

As indicated above, the current deodorants and antiperspirants aims to reduce the microbial load, and thus also to reduce the enzymatic load. In contrast, the present invention discloses to add extra enzymes to deodorants and antiperspirants in order to help the biochemical breakdown of sweat secretions. Indeed with the addition of extra enzymes, the present invention discloses to dissolve the sweat secretions and lipids into small compounds which don't have a bad odour.

In fact, the present invention relates to the finding that enzymes from *Staphylococcus epidermidis* have the ability to fully convert lipids secreted on the skin whereas Corynebacteria and other malodour associated bacteria lack the ability the fully catabolize the lipids. This is in contrast with the prior art wherein it is indicated that Corynebacteria contain a rich set of enzymes and that *Corynebacterium* spp. predominantly feed on skin secretions containing a variety of different lipid compounds (21). It is further indicated that to compensate for its incapability in synthesizing fatty acids, *Corynebacterium* spp. harbour a comprehensive set of enzymes involved in lipid metabolism encoded by multiple paralogous genes (22,23). This feature enables the organism to metabolize a broader range of lipid compounds (24). In contrast, the present invention shows that *Staphylococcus epidermidis* has a broader set of lipolytic enzymes than *Corynebacterium* spp. In addition, the present invention discloses that *Staphylococcus epidermidis* also has a broader set of squalene degrading enzymes. Squalene can convert into steroids, which can biochemically be broken down and can cause malodour (25).

The present invention thus discloses the usage of purified enzymes, bacterial fragments, dead or viable lyophilized bacteria, and/or bacterial lysates to combat skin malodour. The enzymes will fully catabolize skin lipids and squalene so that no malodour is generated. The enzymes may originate from *Staphylococcus* spp., and more in particular from *Staphylococcus epidermidis*.

Lyophilization refers to the process in which bacteria are cultured and preserved by freezing very quickly and subsequently subjecting to a vacuum to remove the ice. Lyophilization is one of the most effective methods for the long-term preservation of cells. Freeze drying, known

as lyophilization, is used to prepare bacterial culture that can be revived upon contact with moisture (26).

Hence, the present invention relates to the usage of lipolytic enzymes obtained from a bacterial *Staphylococcus* species to reduce the amount of malodorous short-chain fatty acids released from unusual, methyl-branched, odd-numbered long-chain fatty acids present in sweat. The human fatty acids present on the stratum corneum are often called "unusual", as no other animal contains such mixture of methyl-branched and odd-numbered fatty acids on the skin.

Fatty acids of the human skin consists of saturated fatty acids, among which and not limited to palmitic acid (C16), myristic acid (C14), stearic acid (C18), pentadecanoic acid (C15), heptadecanoic acid (C17); unsaturated fatty acids, among which and not limited to cis-hexadec-6-enoic acid (16:1 Ω6), cis-octadec-8-enoic acid (18:1 Ω8), oleic acid (18:1 Ω9), petroselenic acid (18:1 Ω6), cis-heptadec-6-enoic acid (17:1 Ω6), cis-tetradec-6-enoic acid (14:1 Ω6), sebaleic acid (18:2 Ω5,8), cis-heptadec-8-enoic acid (17:1 Ω8), linoleic acid (18:2 Ω9,12), cis-eicos-10-enoic acid (20:1 Ω10), cis-eicos-7,10-dienoic acid (20:2 Ω7,10); iso-branched fatty acids, among which and not limited to 4-methyltetradecanoic acid, cis-14-methylpentadec-6-enoic acid, cis-16-methylheptadec-8-enoic acid; anti-iso-branched fatty acids, among which and not limited to 12-methyltetradecanoic acid, cis-14-methylhexadec-6-enoic acid (21,27,28).

Malodorous short-chain fatty acids or 'malodorous fatty acids' are defined as, but not limited to, 3-methyl-2-hexenoic acid (3M2H), 3-hydroxy-3-methylhexanoic acid (HMHA) (29–31), 3-methyl-3-octenoic acid, 4-methyl-3-nonenoic acid, 3-hydroxy-4-methylhexanoic acid, 3-hydroxy-3-methylheptanoic acid, 3-hydroxy-4-heptanoic acid, 3-hydroxyoctanoic acid, 3-hydroxy-3-methyloctanoic acid, 3-hydroxy-4-methyloctanoic acid, 3-hydroxy-4-methylnonacoic acid, 3-hydroxydecanoic acid (31), isovaleric acid (32), 4-ethyloctanoic acid, 7-octenoic acid (29), 1-octen-3-ol, 1,5-octadien-3-one (33). Thorough reviews on the topic of short-chain fatty acids and other malodorants derived from human sweat can be found in Takeuchi *et al.* (33) and Martin *et al.* (34).

The present invention thus relates to the usage of lipolytic or squalene degrading enzymes obtained from a bacterial *Staphylococcus* species to reduce the amount of malodorous fatty acids, preferably short-chain fatty acids, in sweat.

The present invention further relates to the usage of lipolytic or squalene degrading enzymes as described above wherein said *Staphylococcus* species is *Staphylococcus epidermidis*.

The present invention also relates to the usage of lipolytic or squalene degrading enzymes as described above wherein said enzymes are administered as purified enzymes or as part of a bacterial fragment, dead bacterium or bacterial lysate or a viable lyophilized bacterium.

The present invention relates to the usage of lipolytic enzymes as described above wherein said enzymes are part of the beta-oxidation pathway or the fatty acid biosynthesis pathway or the synthesis and degradation of ketone bodies pathway or terpenoid backbone biosynthesis pathway or steroid biosynthesis pathway of a bacterial *Staphylococcus* species.

The present invention further relates to the usage of lipolytic enzymes as described above wherein said lipolytic enzymes which are part of the beta-oxidation pathway (or fatty acid degradation pathway) are chosen from the list consisting of: FadE (acyl CoA dehydrogenase), FadB (enoyl CoA hydratase), FadJ (3-hydroxyacyl-CoA dehydrogenase), FadA (β-ketothiolase).

The present invention also relates to the usage of lipolytic enzymes as described above wherein said lipolytic enzymes which are part of the fatty acid biosynthesis pathway are chosen from the list consisting of: AccA (acetyl-CoA carboxylase), AccB (acetyl-CoA carboxylase), AccC (acetyl-CoA carboxylase), AccD (acetyl-CoA carboxylase), FabD (malonyl-CoA:ACP transacylase), FabH (β-ketoacyl-ACP synthases), FabG (NADPH-dependent β-ketoacyl-ACP reductase), FabZ (3-hydroxyacyl-ACP dehydratase), FabA (β-hydroxydecanoyl-ACP dehydrase), FabB (3-ketoacyl-ACP synthases I), FabF (β-ketoacyl-ACP synthase (chain elongation)), FabI (enoyl-ACP reductase), FabL (enoyl-ACP reductase) and FabK (enoyl-ACP reductase).

The present invention further relates to the usage of lipolytic enzymes as described above wherein said enzymes which are part of the synthesis and degradation of ketone bodies are chosen from the list consisting of: acetyl-CoA C-acetyltransferase, hydroxymethylglutaryl-CoA synthase, hydroxymethylglutaryl-CoA lyase, 3-oxoacid CoA-transferase, acetoacetate decarboxylase, 3-hydroxybutyrate dehydrogenase.

The present invention also relates to the usage of squalene degrading enzymes as describe above, wherein said squalene degrading enzymes are chosen from the list consisting of: farnesyl-diphosphate farnesyltransferase, farnesyl diphosphate synthase, diphosphomevalonate decarboxylase, phosphomevalonate kinase, mevalonate kinase, hydroxymethylglutaryl-CoA reductase, hydroxymethylglutaryl-CoA synthase, acetyl-CoA C-acetyltransferase. Through usage of the enzymes indicated above, the breakdown products of the lipid and squalene metabolism can be further used into the metabolism of bacteria or

bacterial cell mass. Indeed, the present invention relates to the usage of squalene catabolizing enzymes to degrade to building blocks such as farnesyl, mevalonate and acetyl. Through the use of said enzymes, squalene is completely broken down, and no longer converted to steroids and can no longer lead to malodorous compounds.

The present invention further relates to the usage of lipolytic enzymes or squalene degrading enzymes as described above wherein lipases, amylases, proteases and/or cellulases which are obtained from any microbial species are further added to said lipolytic or squalene degrading enzymes.

The present invention also relates to the usage of lipolytic or squalene degrading enzymes as described above wherein said sweat is present on skin and/or textiles.

In addition, the present invention relates to the usage of farnesyl diphosphatase to produce a natural fragrance on skin.

Moreover, the present invention relates to the usage of farnesyl diphosphatase as described above, wherein said farnesyl diphosphatase is obtained from a *Saccharomyces* species.

The present invention relates to the usage of farnesyl diphosphatase as described above, wherein said Saccharomyces species is *Saccharomyces cerevisiae*.

Indeed, the present invention relates to the usage of farnesyl diphosphatase to convert farnesyl-PP to farnesol and farnesal, which has natural perfuming properties. Farnesyl-PP is a breakdown product from squalene. Through this enzyme, naturally present in *Saccharomyces*, the intermediate compound is converted to a natural perfume. Converting these intermediates into perfuming agents also prevents the breakdown into malodorous compounds.

Detailed description of the invention

The present invention relates to the following enzymes that fully catabolize sweat secretions into non malodorous side-products. The enzymes are part of the fatty acid degradation and fatty acid biosynthesis pathways: FadE (acyl CoA dehydrogenase), FadB (enoyl CoA hydratase), FadJ (3-hydroxyacyl-CoA dehydrogenase), FadA (β-ketothiolase), AccA (acetyl-CoA carboxylase), AccB (acetyl-CoA carboxylase), AccC (acetyl-CoA carboxylase), AccD (acetyl-CoA carboxylase), FabD (malonyl-CoA:ACP transacylase), FabH (β-ketoacyl-ACP synthases - initial condensation with acetyl-CoA and branched acyl-CoAs), FabG (NADPH-dependent β-ketoacyl-ACP reductase), FabZ (3-hydroxyacyl-ACP dehydratase), FabA (β-

hydroxydecanoyl-ACP dehydrase), FabB (3-ketoacyl-ACP synthases I), FabF (β-ketoacyl-ACP synthase (chain elongation)), FabI (enoyl-ACP reductase), FabL (enoyl-ACP reductase) and FabK (enoyl-ACP reductase).

More specifically, the present invention relates to the following enzymes which are part of the KEGG synthesis and degradation of ketone bodies (and use acetyl-CoA for further uptake into bacterial metabolism): acetyl-CoA C-acetyltransferase, hydroxymethylglutaryl-CoA synthase, hydroxymethylglutaryl-CoA lyase, 3-oxoacid CoA-transferase, acetoacetate decarboxylase, 3-hydroxybutyrate dehydrogenase. Through usage of these enzymes, the breakdown products of the lipid and squalene metabolism can be further used into the metabolism of bacteria or bacterial cell mass.

The present invention further relates to the usage of lipolytic enzymes as described above wherein lipases, amylases, proteases and/or cellulases which are obtained from any microbial species are further added to said lipolytic enzymes.

More specifically, the present invention relates to the following enzymes which degrade squalene into smaller non-odorous compounds: farnesyl-diphosphate farnesyltransferase, farnesyl diphosphate synthase, diphosphomevalonate decarboxylase, phosphomevalonate kinase, mevalonate kinase, hydroxymethylglutaryl-CoA reductase, hydroxymethylglutaryl-CoA synthase, acetyl-CoA C-acetyltransferase. The usage of squalene catabolizing enzymes promotes the conversion into useful building blocks for microbial biomass, non-malodorous molecules or even good odorous molecules, such as farnesyl, mevalonate and acetyl. Through the use of said enzymes, squalene is completely broken down, and no longer converted to steroids and can no longer lead to malodorous compounds. These enzymes are naturally present in *Staphylococcus* spp. and can be obtained and purified from their cells.

The present invention further relates to the usage of lipolytic and squalene degrading enzymes as described above wherein farnesyl-diphosphate which is obtained from a *Saccharomyces* species is further added to said enzymes. Farnesyl diphosphatase converts farnesyl-PP to farnesol and farnesal, which has natural perfuming properties. Farnesyl-PP is a breakdown product from squalene. Through this enzyme, highly present in *Saccharomyces*, the intermediate compound is converted to a natural perfume. Converting these intermediates into perfuming agents also prevents the breakdown into malodorous compounds.

Detailed information about the above described enzymes can –for example– be found in Fujita *et al.* (2007) (35).

The present invention thus relates in first instance to composition which degrade lipids and/or fatty acids secreted by the skin into smaller compounds that do not have a bad smell. The composition can be enzymes and/or bacterial lysates and/or inactive bacteria and/or lyophilized bacteria that have the enzymatic potential to catabolize the precursors that normally lead to underarm malodour into molecules which no longer smell bad. The enzymes and/or bacterial lysates and/or inactive bacteria (i.e. bacterial fragment and or dead bacteria) and/or living or lyophilized bacteria of the present invention can be obtained by any manner known in the art and/or as specified further in the examples.

Underarm malodour is characterized by a sour, musty, sharp, oniony and/or faecal-like smell. The enzymes described in this invention can convert the precursor and/or intermediate breakdown products into molecules which no longer have the above described odour.

Skin and textile malodour is generated due to bacterial biotransformation of sweat secretions. There are four major routes that lead to underarm/skin malodour.

(1) Typical human, methyl-branched, odd-numbered long-chain fatty acids (LCFA) are degraded via β-oxidation into short-chain, volatile fatty acids (36,37).

(2) Additionally, the release of short-chain fatty acids, such as E-3-methyl-2-hexenoic acid (3M2H), 3-hydroxy-3-methyl-hexanoic acid (HMHA), and a wide range of other structurally unusual VFAs, secreted as L-glutamine conjugates in apocrine glands, are considered as major components of the axillary malodour (29–31). After secretion by apocrine sweat glands, bacteria remove the L-glutamine residue with N^{α}-acyl-glutamine aminoacylase and consequently releasing the VFAs.

(3) Several thioalcohols, such as 3-methyl-3-sulfanyl-hexan-1-ol (3M3SH) and 2-methyl-3-sulphanylbutan-1-ol (2M3SB), as well as their isomers were also reported as important contributors to axillary malodour (38–40).

(4) The biotransformation of steroids. Steroids secreted in the underarm through the apocrine glands secrete a series of steroids, through the ABCC11 gene (41). The bacterial breakdown products, although not fully characterized, are known to cause a specific malodour (25).

The present invention relates to methods that mainly impacts the first and second route to malodour. The present invention does not relate to the inhibition of specific enzymes that cleave off L-glutamine residues or cysteine residues from malodour precursors.

The present invention reduces the formation of malodorous (short-chain) fatty acids (released from human methyl-branched, odd-numbered long-chain fatty acids). The long-chain fatty acids present in human sweat, released from sebaceous and apocrine sweat, is with this invention completely broken down into ATP and building blocks for bacterial *de novo* fatty

acid synthesis. The pathways leading to malodour are converted towards pathways that no longer cause malodorous volatiles. The fatty acids are fully catabolized by the administered enzymes, when applied in an effective amount of a composition as defined above to a mammal in need thereof.

Examples

1 MATERIALS AND METHODS

1.1 ARMPIT OBSERVATION STUDY: UNDERARM MICROBIOME ANALYSIS OF 189 PEOPLE

1.1.1 Subjects

Triplicate bacterial samples were taken from the axillary skin of 189 healthy subjects. Sample demographic characteristics can be found in **Table 1**. Subjects younger than 18 years were accompanied by a parent. All participants were Belgian, except two participants from The Netherlands and one from France. Samples were collected on three different days spread over a period of six weeks. The participants were invited to Ghent and were asked not to use deodorants or antiperspirants three days prior to sampling and not to wash their axillae on the day of sampling. No attempts were made to control the subjects' diet. All participants were asked to fill in their personal metadata and a questionnaire regarding the effect of the malodour on their daily lives. All participants attached a sterile cotton pad under their left axilla for 3h for odour assessment. All participants gave their written and informed consent to this research.

Table 1. Sample demographic characteristics of the armpit screening study.

	Total	M	F
N	189	62	127
Age			
14-24	48	9	39
25-34	51	14	37
35-44	29	12	17
45-54	32	11	21
55-64	19	10	9
65+	8	6	2

BMI			
<20	14	1	13
20-25	101	31	70
25-30	53	21	32
>30	19	8	11
Nationality			
Belgian	186	61	125
The Netherlands	2	0	2
France	1	1	0
Ethnicity			
Caucasian	184	62	122
Asian	2	0	2
Black	3	0	3

1.1.2 Sampling

A sterile cotton swab (Biolab, Belgium) was moistened in physiological water and swabbed for 15s in the axillary region to detach and absorb the microorganisms. The tip was suspended and broken in a sterilized reaction tube filled with 1 ml of sterile physiological water (42). The bacterial samples were immediately stored at 4°C not longer than 48h prior to further analysis. The participants attached a sterile cotton pad with a dressing under their left axilla for 3h to absorb the odour. Afterwards, the cotton pad was brought into a closed numbered goblet until odour assessment by an odour panel.

1.1.3 Odour assessment

The odour panel was trained and selected and samples were rated as previously described (43). Odour measurement was performed by means of a cotton pad worn in the axilla for 3h. The cotton pads were presented in a numbered odourless sealed glass goblet in a random manner to a panel of six selected and screened human assessors (four men, four women). Assessors were selected by means of sensitivity to dilutions of n-butanol and wastewater, and by means of the triangle test (44). In the triangle test, each member of the panel was presented three flasks, two of which were the same but the third contained a different odour. The flask was shaken, the stopper was removed, after which the vapours were evaluated. The panellists had to correctly

identify the flask with the different odour. In the dilution test, each member of the panel was presented six flasks with increasing concentration of n-butanol and wastewater, starting with a flask without addition of n-butanol or wastewater. The panellists had to correctly place the flasks according concentration. The triangle and dilution test was repeated three times, with a minimum of two days in between each measurement. Assessors with minimum 85% correct answers were selected for the panel. A representative panel was recruited from a pool of the 20 people. Training of the assessors was conducted through odour references (ammonia, cheese, axillary sample) and experience. The references were assessed in group to compromise hedonic value and intensity. The room in which the tests were conducted, was free from extraneous odour stimuli e.g. caused by smoking, eating, soaps, perfume, etc. The odour assessors were familiar with the olfactometric procedures and met the following conditions: (i) older than 16 years and willing to follow the instructions; (ii) no smoking, eating, drinking (except water) or using chewing gum or sweets 30 min before olfactometric measurement; (iii) free from colds, allergies or other infections; (iv) no interference by perfumes, deodorants, body lotions, cosmetics or personal body odour; (v) no communication during odour assessment. About 60 odour measurements were performed per day, spread over a timeframe of 4h, to prevent fatigue. The samples of the observational study were assessed by the following odour characteristic: intensity (scale 0 to 10). The intensity indicates the quantity of the odour and varies between 0 (no odour) to 10 (very strong / intolerable). Each time, a control odour measurement, a clean cotton pad with random number, was served to the odour panel together with the other samples. Odour assessment was in the observational study additionally performed via direct odour assessment (first 60 participants), and a self-evaluation (all participants).

1.1.4 DNA extraction, PCR

Total DNA extraction was performed as previously described (45). Briefly, the bacterial sample, dissolved in 1 ml of saline water, was centrifuged (12 min, 13,000 rpm) to obtain a pellet, while the supernatant was discarded. The pellet was washed and resuspended in 100 µL 6% Chelex 100 resin (BioRad, Munich, Germany) and incubated at 56 °C for 20 min. The removal of PCR inhibitors and metal ions was accomplished by means of Chelex-100. The sample was then firmly vortexed and boiled at 100 °C for 8 min. Subsequently, the sample was mixed and cooled for 5 min on ice. Next, a centrifugation step (10 min, 13,000 rpm) was performed. The supernatant containing the DNA was removed and stored at -20°C until further analysis.

The polymerase chain reaction (PCR) is a technique to amplify a piece of DNA, generating millions of copies of a particular DNA sequence. This method consists of repeated cycles of heating and cooling, which promotes the melting and separation of the double-stranded DNA. A target region of interest in the 16S rRNA bacterial gene is selected as a marker for thermal cycling, consisting of the reaction for DNA melting and enzymatic replication of the DNA. At present, the bacterial ribosomal RNA operon, encompassing the 16S rRNA gene, is the most frequently used molecular marker. The bacterial ribosomal RNA operon, encompassing the 16S rRNA gene, is the most frequently used molecular marker. The hypervariable regions of the gene (V regions) has a high discriminatory potential (46) and contains the signatures of phylogenetic groups and even species. It enables an accurate description of the microbial populations in a community (47). Therefore, molecular techniques based on 16S rDNA can be useful tools to gain insight on phylogenetic and functional relationships among the microbiota of any given environment. Two primers were used to amplify a target region of the 16S rRNA gene. As PCR progresses, the sequences generated are used as a template for replication and exponentially amplified with each cycle.

1.1.5 Illumina MiSeq sequencing

Genomic DNA axillary samples from 189 participants were amplified with conserved 16S rRNA gene primers generating 140-bp amplicons. The 16S rRNA gene regions were amplified by PCR using the 27F and 338R primers, targeting the V1 region and libraries for Illumina sequencing were constructed as previously described (48). Briefly, the forward primer contains a 6 nucleotide (nt) barcode45 and a 2 nt CA linker (49). Both primers comprised sequences complementary to the Illumina specific adaptors to the 5'-ends. Amplification was performed in a total volume of 50 μL with 5x PrimeSTAR buffer, containing 2.5 mM deoxynucleoside triphosphate, 0.2 μM of each primer, 1 μL template DNA and 0.5 μL PrimeSTAR HS DNA polymerase (2.5U). PCR was performed with the following conditions: initial denaturation (95°C - 3 min), followed by 15 cycles of denaturation, annealing and elongation (98°C - 10 sec; 55°C - 10 sec and 72°C - 45 sec). One μL of this reaction mixture served as template in a second PCR performed under the same conditions, but for 20 cycles using PCR primers designed to integrate the sequence of the specific Illumina multiplexing sequencing primers and index primers. PCR amplicons were verified by agarose gel electrophoresis, purified using Macherey-Nagel 96-well plate purification kits (Macherey-Nagel, Germany) following the manufacturer's instructions and quantified with the Quant-iT PicoGreen dsDNA reagent and kit (Invitrogen, UK). Libraries were prepared by pooling equimolar ratios of amplicons (200

ng of each sample), all tagged with a unique barcode. Each library was precipitated on ice for 30 min after addition of 20 µl of 3M NaCl and 3 volumes of ice-cold 100% ethanol to remove contaminants or PCR artefacts. The precipitated DNA was centrifuged at 13,000×g for 30 min at 4°C. The supernatant was removed, the pellet air dried, resuspended in 30 µL of double-distilled water and separated on a 2% agarose gel. PCR products of the correct size were extracted and recovered using the QIAquick gel extraction kit (Qiagen, Belgium). Negative controls (water as template) were performed and were free of any amplification products after PCR. Libraries were sent for paired-end sequencing on a MiSeq Genome Analyzer (Illumina Inc., California, USA). Image analysis and base calling were accomplished using the Illumina Pipeline (version 1.7).

1.1.6 Downstream analysis, taxonomy, alpha- and beta-analysis

Demultiplexed and quality-controlled sequences were clustered against the Greengenes (50) database using the closed reference OTU picking protocol (51) as implemented in QIIME1.9.0 (52). These processing steps were performed using default parameters. The OTU table used for primary analysis was filtered. Finally, the table was rarefied to normalize for sample effort at 1000 sequences per sample (53). Taxonomy was determined and alpha- and beta-diversity calculations were performed using QIIME version 1.9.1.

1.1.7 Metagenome analysis

Indications of functionality from phylogenetic information was obtained with PICRUSt (54). The open-source software allows reconstruction of the bacterial metagenomes of the obtained results based on the bacterial 16S rRNA gene sequence. The generated OTU table from the 16S sequencing data was used as an input. The copy number per OTU was normalized before the metagenome was predicted using Kyoto Encyclopedia of Genes and Genomes (KEGG) database (55). The metagenome prediction provided an annotated table of predicted gene family counts for each sample, where gene families were grouped by KEGG Orthology (KO) identifiers (**Figure 1**).

1.1.8 Production of bacteria

The bacterial cells are grown to late log or early stationary phase for about 18h in LB medium till an OD_{600} of about 0.8. The cells are harvested by centrifugation (6000 rpm/5min), after which the supernatant is discarded. The cells are washed in sterile M9 medium. To create 1L of M9 medium, one needs at first a salt solution (1L) that consists of: 42g $Na_2HPO_4.7H_2O$, 15g KH_2PO_4, 2.5g NaCl, 5g NH_4Cl. All compounds are added to a Schott bottle of 1L which is

diluted with distilled water until one reaches 1L. This solution is autoclaved prior to use. Again, the cells are harvested by centrifugation; after which the supernatant is discarded. The bacterial biomass is suspended in an equal volume of lyophilization medium. The lyophilization medium contains suitable lyoprotectants and excipients, preferably sucrose and mannitol. The mixture is mixed thoroughly by vortexing. The cell suspension is distributed over a flat surface prior to freezing. The cell suspension is kick-frozen in -80°C in liquid nitrogen prior to the eventual freeze-drying. The frozen suspension is freeze dried and lyophilized (under vacuum pressure). A cell count is performed after the freeze-drying protocol (dilution to extinction protocol). The final concentration should be around 10^{12} CFU/g.

1.1.9 Production of bacterial enzymes or lysates

The bacterial culture is grown for 18h in LB medium till an OD_{600} of about 0.8; till the bacteria are in plateau phase. The cells are harvested by centrifugation (6000 rpm/5min), after which the supernatant is discarded. The dense culture (pellet) is frozen at -80°C. About 1.5ml of the pellet is resuspended in 0.75ml lysis buffer. The lysis buffer contains 50mM Tris pH 8.0; 10% glycerol; 0.1% Triton X-100; 100ug/ml lysozyme; 1mM PMSF and /or more anti-proteases; DNAse 3U and 2mM MgCl on the final concentration. The pellet and lysis buffer are incubated on 30°C for 15 minutes or 30min on ice. The resulting solution is sonicated 3x20'' till sample is no longer viscous. Ultrasonication at frequencies around 20kHz was used to kill bacteria and to release their enzymatic contents. It was for many years has been standard technique in microbiology for the disruption of living cells (56). The solution is subsequently centrifuged at 12,000rpm for 20min at 4°C. The solution is transferred to new tubes and re-suspended in 0.75ml lysis buffer. Then 60ul of the solution is centrifuged, after which 20µl 4X sample buffer with 100mM DTT. The rest of the lysates is frozen in -20°C. A cell count is performed after the lysis protocol (dilution to extinction protocol). The final concentration should be 0 CFU/g.

1.1.10 Production of purified enzymes

Staphylococcal enzymes were produced as follows: 1/ sonication to disrupt the bacterial cells (57) as described before, 2/ centrifugation to separate the large particles from the enzymes, 3/ bringing it in solution, 4/ purification and precipitation of proteins (enzymes) using ammonium sulphate and crystallization by changing the pH (58).

1.1.11 Enzymatic tests

The activity of the enzymes of the bacterial lysates and the purified enzymes was verified using a turbidity assay. We tested for the ability to degrade lipids using a turbidity assessment assay as adopted from Lawrence *et al.* (59), and further modifications (60). Human lipids, as obtained from liposuction and as described earlier (61), were emulsified in agar and poured into Petri dishes. The agar consistency was very turbid as a result of the lipids in the agar. The bacterial lysates and purified enzymes were brought onto the solid agar in droplets. Hydrolysis of the lipid emulsion was observed by formation of a zone of clearance around the droplet. Lipophilic enzyme activity was measured by the clearing zone on and around the droplet. Degradation of dense human lipids and conversion into different compounds led to a change in turbidity. A positive control with living bacteria and a negative control using no bacteria or enzymes was also assessed.

1.1.12 Study design sprays with lyophilized bacteria or bacterial enzymes

A spray was assembled using living lyophilized bacteria, inactive/dead bacterial lysates containing viable enzymes and the purified enzymes. Subjects were selected with above-average malodorous axillae (**Table 1**), as determined by the odour panel and six subjects were recruited (**section 1.1.3**). A one-month follow-up was scheduled, where the underarm odour was measured every week. A spray was prepared containing the live bacteria, the bacterial lysates/purified enzymes. This spray was used on a daily basis (once or twice application per day).

1.2 *IN VIVO* EXPERIMENTS

1.2.1 Spray experiment with lyophilized bacteria

Subjects with malodorous axillae were recruited to conduct an experiment, where they applied a spray solution containing lyophilized *S. epidermidis* bacteria. The content of the spray was as follows:

- 2.00g *S. epidermidis* lyophilized 50-200um
- 8.00g cyclopentasiloxane
- 0.20g isopropyl myristate
- 0.20g stearalkonium hectorite
- 0.10g propylene carbonate
- 40.00g butane, propane, isobutane pre-mix

- 100ml spray bottle, under pressure

It was tested and verified on agar plate that the final concentration of bacteria with one normal spray is about 10^8 CFU/spray. The subject's underarm odour was assessed, as described above (1.1.3), before, during and after application of the spray. The subject applied the spray on a daily basis during four consecutive weeks, where the armpit was sampled every week during that period.

1.2.2 Spray experiment with bacterial enzymes

Ultrasonication at frequencies around 20kHz was used to kill bacteria and to release their enzymatic contents. It was for many years has been standard technique in microbiology for the disruption of living cells (56). A spray was created containing the non-viable *S. epidermidis* bacterial lysates, containing active enzymes. Other enzymes, such as lipases, amylases, proteases, cellulases, farnesyl-diphosphate, were purchased through normal commercial canals: Christian Hansen, Denmark; Novozymes, Denmark; DuPont, USA; Lallemand, France; or DSM, The Netherlands. The enzymes were either obtained as purified enzymes or as microbial lysate. The content of the spray was similar as before:

- 2.00g ultrasonicated *S. epidermidis* lysates containing active enzymes
- 0.20g lipases, amylases, proteases, cellulases, farnesyl-diphosphate
- 8.00g cyclopentasiloxane
- 0.20g isopropyl myristate
- 0.20g stearalkonium hectorite
- 0.10g propylene carbonate
- 40.00g butane, propane, isobutane pre-mix
- 100ml spray bottle, under pressure

The subject's underarm odour was assessed, as described above (1.1.3), before, during and after application of the spray. The subject applied the spray on a daily basis during four consecutive weeks, where the armpit was sampled every week during that period.

2 RESULTS

2.1 SCREENING OF UNDERARMS

Figure 1 shows the enzymatic load of each bacterial group, which originates from the armpit observation study. It can be noticed that in the class of lipid metabolism enzymes

Staphylococcus epidermis has a white colour (higher enzymatic content), while Corynebacterium has a black colour (lower enzymatic content). **Figure 2** shows a more detailed where the KEGG pathways were collapsed to level 3, for the fat metabolising enzymes only. Again, clear differences are seen between staphylococcal and corynebacterial enzymes for fat metabolism. In **Figure 3** finally, the lipid degradation and synthesis have been highlighted onto enzyme level. It can clearly be seen that the white lines (enzymes) are more abundant for *Staphylococcus* than for *Corynebacterium*. *Staphylococcus* is correlated with better underarm odours, while *Corynebacterium* is correlated with unpleasant body odours (fecal-like, sour, dirty, pungent, etc). In conclusion: a higher load of lipid degrading/catabolizing/synthetizing enzymes are needed to improve body odour.

Hence, the present invention discloses the following enzymes which are used in the battle against body odour:

- FadE (acyl CoA dehydrogenase)
- FadB (enoyl CoA hydratase)
- FadJ (3-hydroxyacyl-CoA dehydrogenase
- FadA (β-ketothiolase)
- AccA (acetyl-CoA carboxylase)
- AccB (acetyl-CoA carboxylase)
- AccC (acetyl-CoA carboxylase)
- AccD (acetyl-CoA carboxylase)
- FabD (malonyl-CoA:ACP transacylase)
- FabH (β-ketoacyl-ACP synthases (initial condensation with acetyl-CoA and branched acyl-CoAs))
- FabG (NADPH-dependent β-ketoacyl-ACP reductase)
- FabZ (3-hydroxyacyl-ACP dehydratase)
- FabA (β-hydroxydecanoyl-ACP dehydrase)
- FabB (3-ketoacyl-ACP synthases I)
- FabF (β-ketoacyl-ACP synthase (chain elongation))
- FabI (enoyl-ACP reductase)
- FabL (enoyl-ACP reductase)
- FabK (enoyl-ACP reductase)
- farnesyl-diphosphate farnesyltransferase

- farnesyl diphosphate synthase
- diphosphomevalonate decarboxylase
- phosphomevalonate kinase
- mevalonate kinase
- hydroxymethylglutaryl-CoA reductase
- hydroxymethylglutaryl-CoA synthase
- acetyl-CoA C-acetyltransferase
- hydroxymethylglutaryl-CoA lyase
- 3-oxoacid CoA-transferase
- acetoacetate decarboxylase
- 3-hydroxybutyrate dehydrogenase
- farnesyl-diphosphate
- Lipases
- Amylase
- Protease
- Cellulase

The function of each of the enzymes is given hereunder:

-Enzymes involved in fatty acid synthesis (see **Figure 3**):

Acetyl CoA:ACP transacylase: Activates acetyl CoA for reaction with malonyl-ACP

Malonyl CoA:ACP transacylase: Activates malonyl CoA for reaction with acetyl-ACP

3-ketoacyl-ACP synthase: Reacts priming acetyl-ACP with chain-extending malonyl-ACP

3-ketoacyl-ACP reductase: Reduces the carbon 3 ketone to a hydroxyl group

3-Hydroxyacyl ACP dehydrase: Removes water

Enoyl-ACP reductase: Reduces the C2-C3 double bond.

-Enzymes involved in fatty acid metabolism (beta-oxidation, see **Figure 3**):

Acetyl CoA:ACP transacylase: Activates acetyl CoA for reaction with malonyl-ACP

Malonyl CoA:ACP transacylase: Activates malonyl CoA for reaction with acetyl-ACP

3-ketoacyl-ACP synthase: Reacts priming acetyl-ACP with chain-extending malonyl-ACP

3-ketoacyl-ACP reductase: Reduces the carbon 3 ketone to a hydroxyl group

3-Hydroxyacyl ACP dehydrase: Removes water

Enoyl-ACP reductase: Reduces the C2-C3 double bond.

- Enzymes involved in synthesis and degradation of ketone bodies:
  - hydroxymethylglutaryl-CoA lyase: converts 3-hydroxy-3-methylglutaryl-coenzyme A into acetoacetate.

- 3-oxoacid CoA-transferase: converts acetoacetyl coenzyme A into acetoacetate.
- acetoacetate decarboxylase: converts acetoacetate into acetone.
- 3-hydroxybutyrate dehydrogenase: converts acetoacetate into 3-hydroxybutanoate.
- hydroxymethylglutaryl-CoA synthase: converts acetyl coenzyme A into 3-hydroxy-3-methylglutaryl-coenzyme A.

- Enzymes involved in squalene degradation (full pathway: see **Figure 4**):
  - farnesyl-diphosphate farnesyltransferase: converts squalene into presqualene-diphosphate (presqualene-PP) and converts presqualene-diphosphate (presqualene-PP) into farnesyl diphosphate (farnesyl-PP)
  - farnesyl diphosphate synthase: converts farnesyl diphosphate (farnesyl-PP) into isopentenyl-diphosphate (isopentenyl-PP)
  - diphosphomevalonate decarboxylase: converts isopentenyl-diphosphate (isopentenyl-PP) into 5-diphosphomevalonate (mevalonate-5PP)
  - phosphomevalonate kinase: converts 5-diphosphomevalonate (mevalonate-5PP) into 5-phosphomevalonate (mevalonate-5P)
  - mevalonate kinase: converts 5-phosphomevalonate (mevalonate-5P) into mevalonate
  - hydroxymethylglutaryl-CoA reductase: converts mevalonate into 3-hydroxy-3-methyl-glutaryl coenzyme A.
  - hydroxymethylglutaryl-CoA synthase: converts 3-hydroxy-3-methyl-glutaryl coenzyme A into acetoacetyl coenzyme A.
  - acetyl-CoA C-acetyltransferase: converts acetoacetyl coenzyme A into acetyl coenzyme A.
- Enzyme involved in production of farnesol or farnesal, a natural fragrance:
  - farnesyl diphosphatase: converts farnesyl-diphosphate (breakdown product of squalene) to farnesol
  - production of a natural fragrance from squalene breakdown compounds.
  - This enzyme is present in *Saccharomyces cerevisiae*

-Function of lipase, amylase, protease, cellulase:
Breakdown of carbohydrate, proteolytic or fatty matrix compounds the sweat precipitations.

A more thorough review on the bacterial enzymes as described above can be found with Fujita et al. (2007) (35).

2.2 SPRAY WITH LYOPHILIZED BACTERIA

Three subjects with above-average malodorous axillae were selected, as determined by the odour panel. The subjects used the lyophilized bacterial spray on a daily basis during four consecutive weeks. In the week preceding the treatment, no bacteria were applied in the underarms. The subjects wore a cotton pad in the underarm to capture the odour, after which the sample was frozen till odour assessment by the trained odour panel. The subjects self-assessed the underarm odour and marked significant improvements during the use of the spray. The improved odour was also reported on clothing and elsewhere on the skin when the bacterial spray were applied. The trained and selected odour panel reported a significant decrease (as determined by the Mann-Whitney U-test; $p<0.05$) in underarm intensity when the bacteria were applied, as compared to when no bacteria were applied (**Figure 5**).

2.3 SPRAY WITH BACTERIAL ENZYMES

Three subjects with above-average malodorous axillae, as determined by the odour panel, were selected for the bacterial enzyme spray. It was confirmed that the bacterial lysates had active enzymatic activity and no viable bacterial cells. The subjects used the enzyme spray on a daily basis during four consecutive weeks. In the week preceding the treatment, no enzymes were applied in the underarms. The subjects wore a cotton pad in the underarm to capture the odour during five consecutive weeks, after which the sample was frozen till odour assessment by the trained odour panel. The subjects self-assessed the underarm odour and marked significant improvements during the use of the enzymes. Participants also reported a decreased body on clothing and elsewhere on the skin when the enzymes were applied. The trained and selected odour panel reported a significant decrease (as determined by the Mann-Whitney U-test; $p<0.05$) in underarm intensity when the enzymes were applied, as compared to when no enzymes were applied (**Figure 6**).

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Claims

1. Use of lipolytic enzymes or squalene degrading enzymes obtained from a bacterial *Staphylococcus* species to reduce the amount of malodorous fatty acids in sweat.

2. Use of lipolytic enzymes or squalene degrading enzymes according to claim 1 wherein said *Staphylococcus* species is *Staphylococcus epidermidis*.

3. Use of lipolytic enzymes or squalene degrading enzymes according to claim 1-2 wherein said enzymes are administered as purified enzymes or as part of a bacterial fragment, dead bacterium or bacterial lysate or a viable lyophilized bacterium.

4. Use of lipolytic enzymes or squalene degrading enzymes according to claims 1-3 wherein said lipolytic enzymes are part of the beta-oxidation pathway or the fatty acid biosynthesis pathway or the synthesis and degradation of ketone bodies pathway or terpenoid backbone biosynthesis pathway or steroid biosynthesis pathway of a bacterial *Staphylococcus* species.

5. Use of lipolytic enzymes or squalene degrading enzymes according to any of claims 1-4 wherein said lipolytic enzymes, which are part of the beta-oxidation pathway, are chosen from the list consisting of: FadE (acyl CoA dehydrogenase), FadB (enoyl CoA hydratase), FadJ (3-hydroxyacyl-CoA dehydrogenase), FadA (β-ketothiolase).

6. Use of lipolytic enzymes or squalene degrading enzymes according to any of claims 1-5 wherein said lipolytic enzymes, which are part of the fatty acid biosynthesis pathway, are chosen from the list consisting of: AccA (acetyl-CoA carboxylase), AccB (acetyl-CoA carboxylase), AccC (acetyl-CoA carboxylase), AccD (acetyl-CoA carboxylase), FabD (malonyl-CoA:ACP transacylase), FabH (β-ketoacyl-ACP synthases), FabG (NADPH-dependent β-ketoacyl-ACP reductase), FabZ (3-hydroxyacyl-ACP dehydratase), FabA (β-hydroxydecanoyl-ACP dehydrase), FabB (3-ketoacyl-ACP synthases I), FabF (β-ketoacyl-ACP synthase (chain elongation)), FabI (enoyl-ACP reductase), FabL (enoyl-ACP reductase) and FabK (enoyl-ACP reductase).

7. Use of lipolytic enzymes or squalene degrading enzymes according to claims 1-6 wherein said enzymes, which are part of the synthesis and degradation of ketone bodies,

are chosen from the list consisting of: acetyl-CoA C-acetyltransferase, hydroxymethylglutaryl-CoA synthase, hydroxymethylglutaryl-CoA lyase, 3-oxoacid CoA-transferase, acetoacetate decarboxylase, 3-hydroxybutyrate dehydrogenase.

8. Use of lipolytic enzymes or squalene degrading enzymes according to claims 1-7 wherein said squalene degrading enzymes are chosen from the list consisting of: farnesyl-diphosphate farnesyltransferase, farnesyl diphosphate synthase, diphosphomevalonate decarboxylase, phosphomevalonate kinase, mevalonate kinase, hydroxymethylglutaryl-CoA reductase, hydroxymethylglutaryl-CoA synthase, acetyl-CoA C-acetyltransferase.

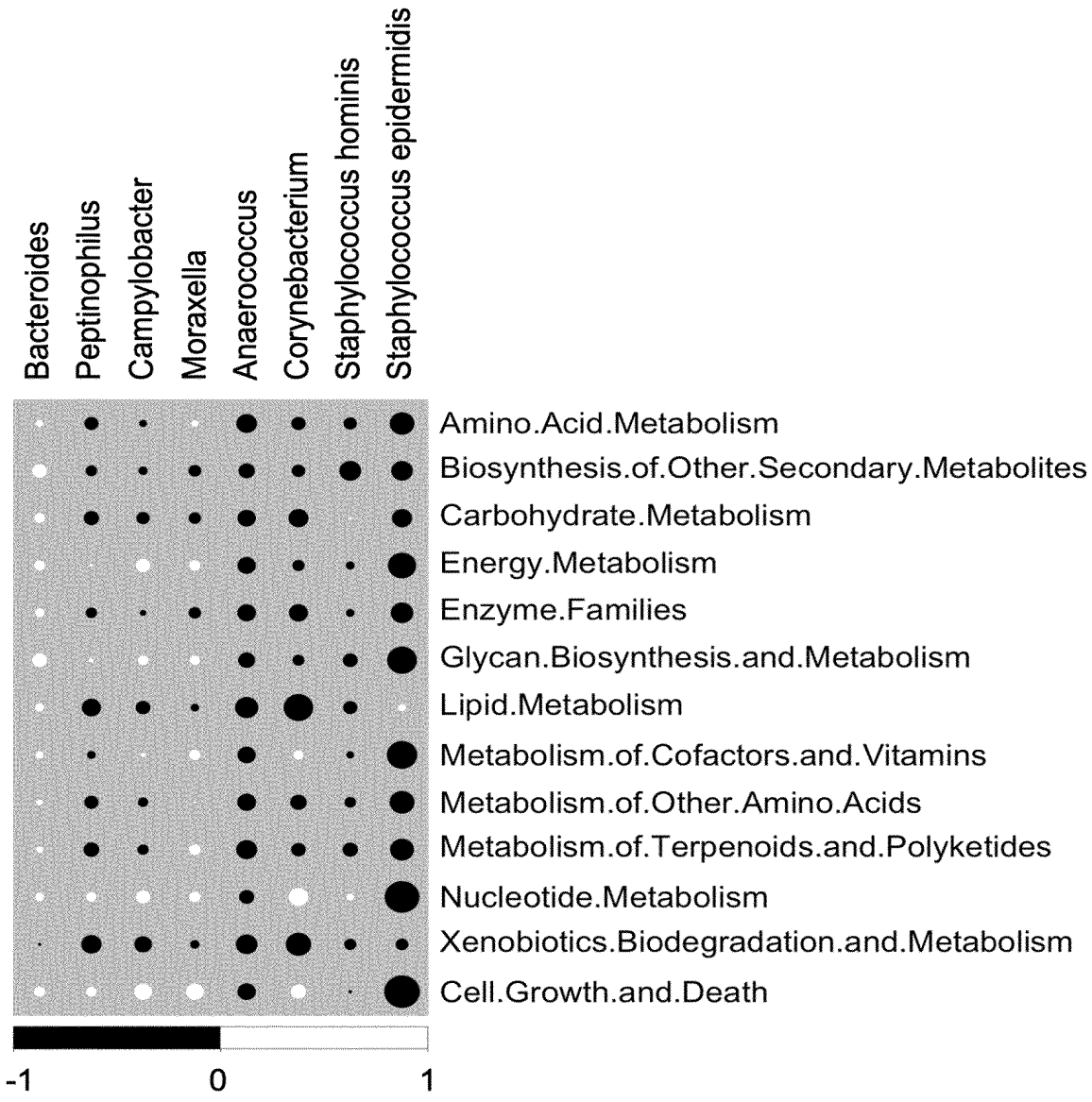
9. Use of lipolytic enzymes or squalene degrading enzymes according to any of claims 1-8 wherein lipases, amylases, proteases and/or cellulases which are obtained from any microbial species are further added to said lipolytic or squalene degrading enzymes.

10. Use of lipolytic or squalene degrading enzymes according to any of claims 1-9 wherein said sweat is present on skin and/or textiles.

11. Use of farnesyl diphosphatase to produce a natural fragrance on skin.

12. Use of farnesyl diphosphatase according to claim 11, wherein said farnesyl diphosphatase is obtained from a *Saccharomyces* species.

13. Use of farnesyl diphosphatase according to claim 12, wherein said Saccharomyces species is *Saccharomyces cerevisiae*.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Campylobacter
Moraxella
Anaerococcus
Corynebacterium
Staphylococcus hominis
Staphylococcus epidermidis
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Carbohydrate.Metabolism
Energy.Metabolism
Enzyme.Families
Glycan.Biosynthesis.and.Metabolism
Lipid.Metabolism
Metabolism.of.Cofactors.and.Vitamins
Metabolism.of.Other.Amino.Acids
Metabolism.of.Terpenoids.and.Polyketides
Nucleotide.Metabolism
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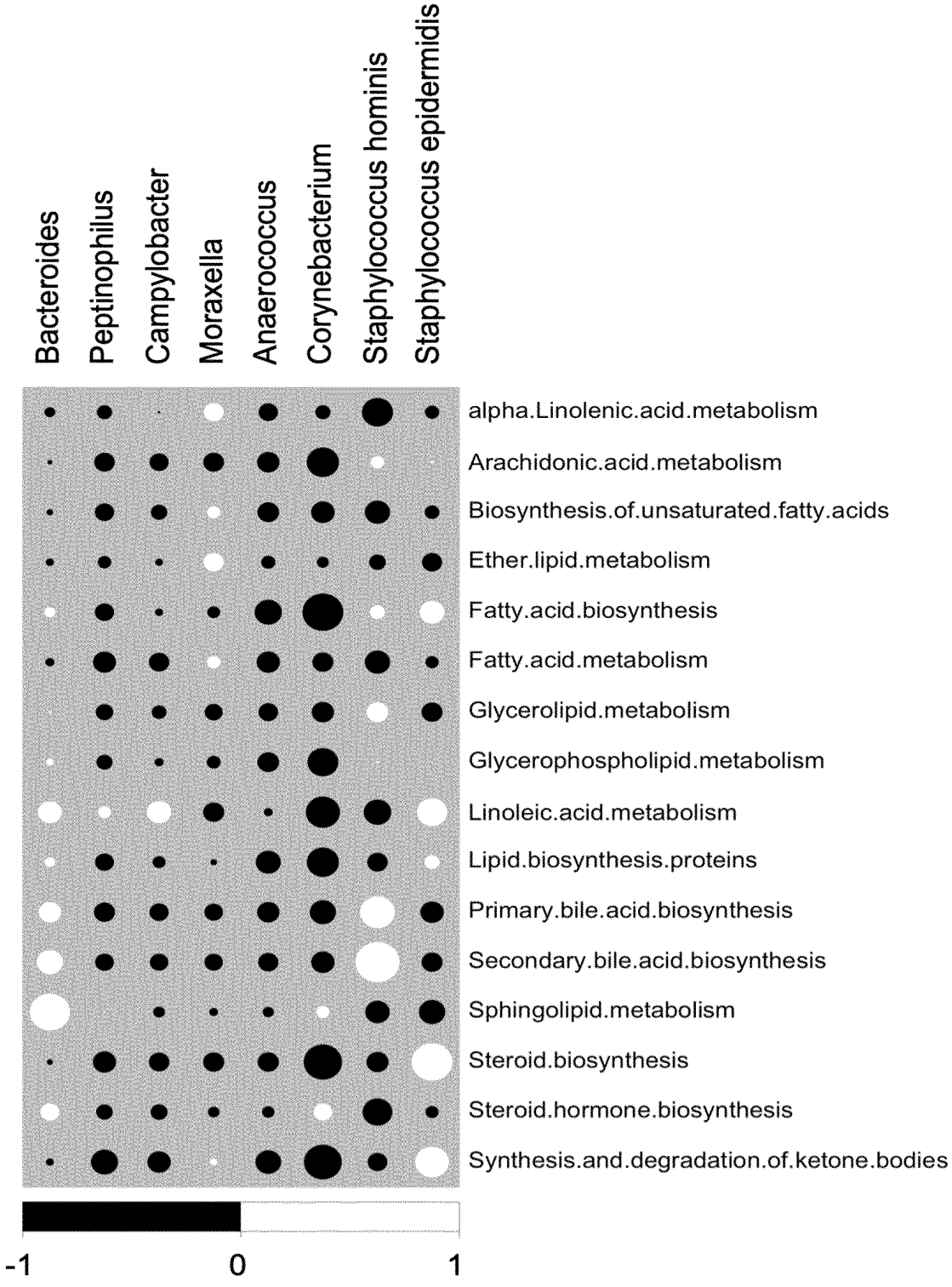
Cell.Growth.and.Death
-1
0
1

Figure 1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Campylobacter
Moraxella
Anaerococcus
Corynebacterium
Staphylococcus hominis
Staphylococcus epidermidis
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Arachidonic.acid.metabolism
Biosynthesis.of.unsaturated.fatty.acids
Ether.lipid.metabolism
Fatty.acid.biosynthesis
Fatty.acid.metabolism
Glycerolipid.metabolism
Glycerophospholipid.metabolism
Linoleic.acid.metabolism
Lipid.biosynthesis.proteins
Primary.bile.acid.biosynthesis
Secondary.bile.acid.biosynthesis
Sphingolipid.metabolism
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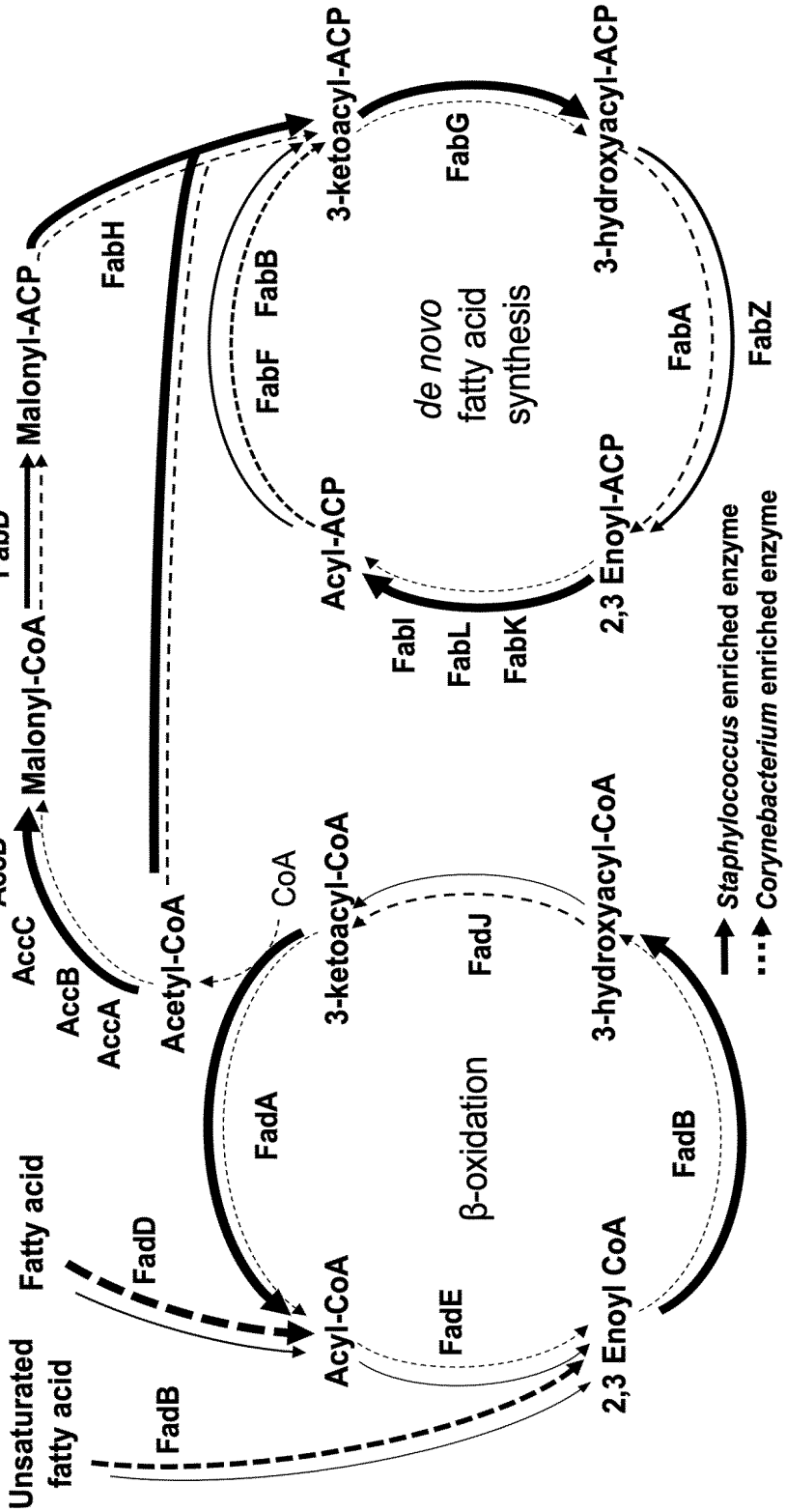
1

Figure 2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FabH
FabD
Malonyl-CoA
AccD
AccC
AccB
AccA
Acetyl-CoA
CoA
3-ketoacyl-CoA
FadJ
3-hydroxyacyl-CoA
FadA
β-oxidation
FadB
Fatty acid
FadD
Acyl-CoA
FadE
2,3 Enoyl CoA
Unsaturated fatty acid
FadB
3-ketoacyl-ACP
FabG
3-hydroxyacyl-ACP
FabF
FabB
de novo fatty acid synthesis
FabA
FabZ
Acyl-ACP
2,3 Enoyl-ACP
FabI
FabL
FabK
Staphylococcus enriched enzyme
Corynebacterium enriched enzyme

Figure 3

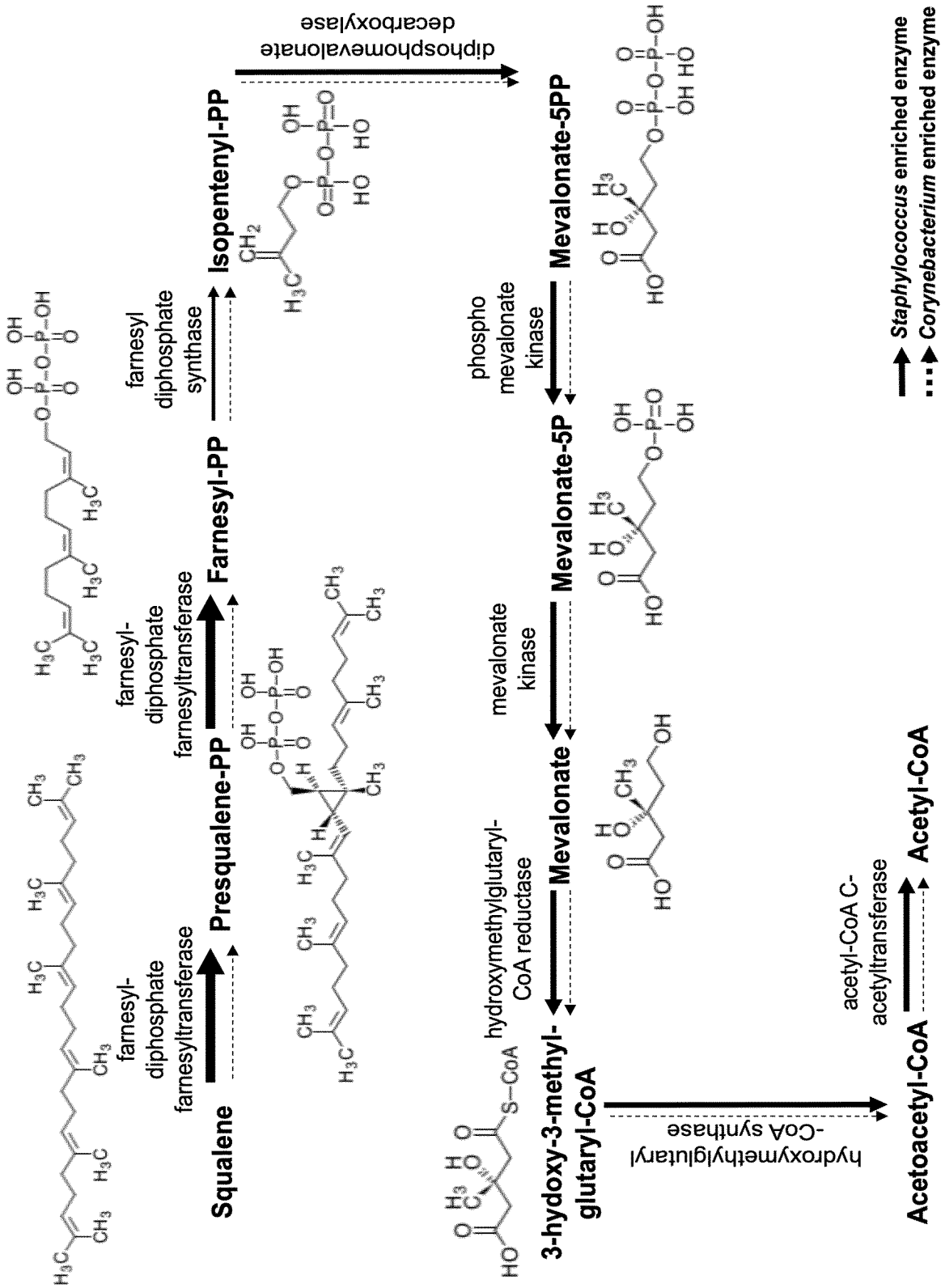
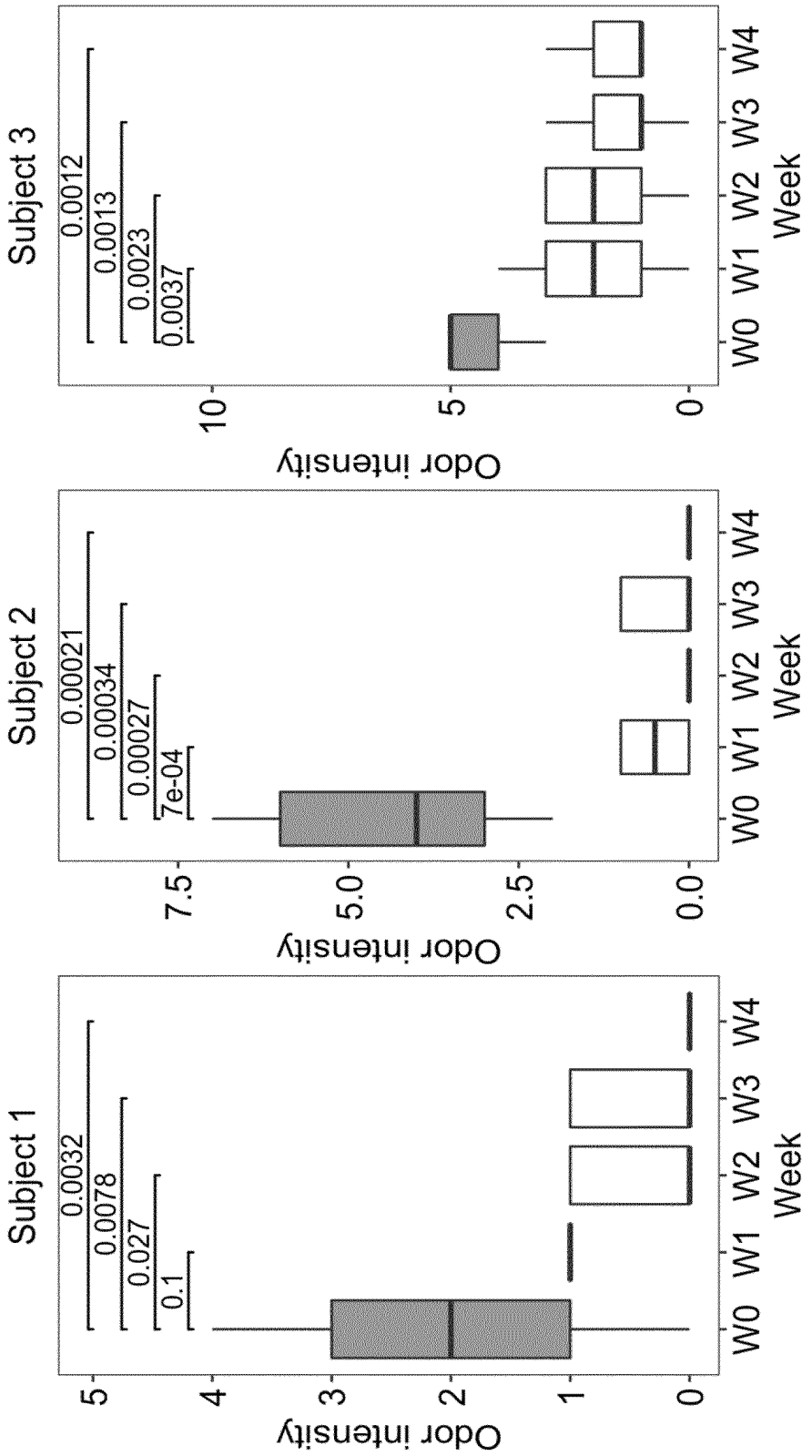

Squalene
farnesyl-diphosphate farnesyltransferase
Presqualene-PP
farnesyl-diphosphate farnesyltransferase
Farnesyl-PP
farnesyl diphosphate synthase
Isopentenyl-PP
diphosphomevalonate decarboxylase
3-hydoxy-3-methyl-glutaryl-CoA
hydroxymethylglutaryl-CoA reductase
Mevalonate
mevalonate kinase
Mevalonate-5P
phospho mevalonate kinase
Mevalonate-5PP
hydroxymethylglutaryl -CoA synthase
Acetoacetyl-CoA
acetyl-CoA C-acetyltransferase
Acetyl-CoA
Staphylococcus enriched enzyme
Corynebacterium enriched enzyme

Figure 4


Subject 1
Subject 2
Subject 3
Odor intensity
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W1
W2
W3
W4
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0.0078
0.027
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0.00021
0.00034
0.00027
7e-04
0.0012
0.0013
0.0023
0.0037

Figure 5

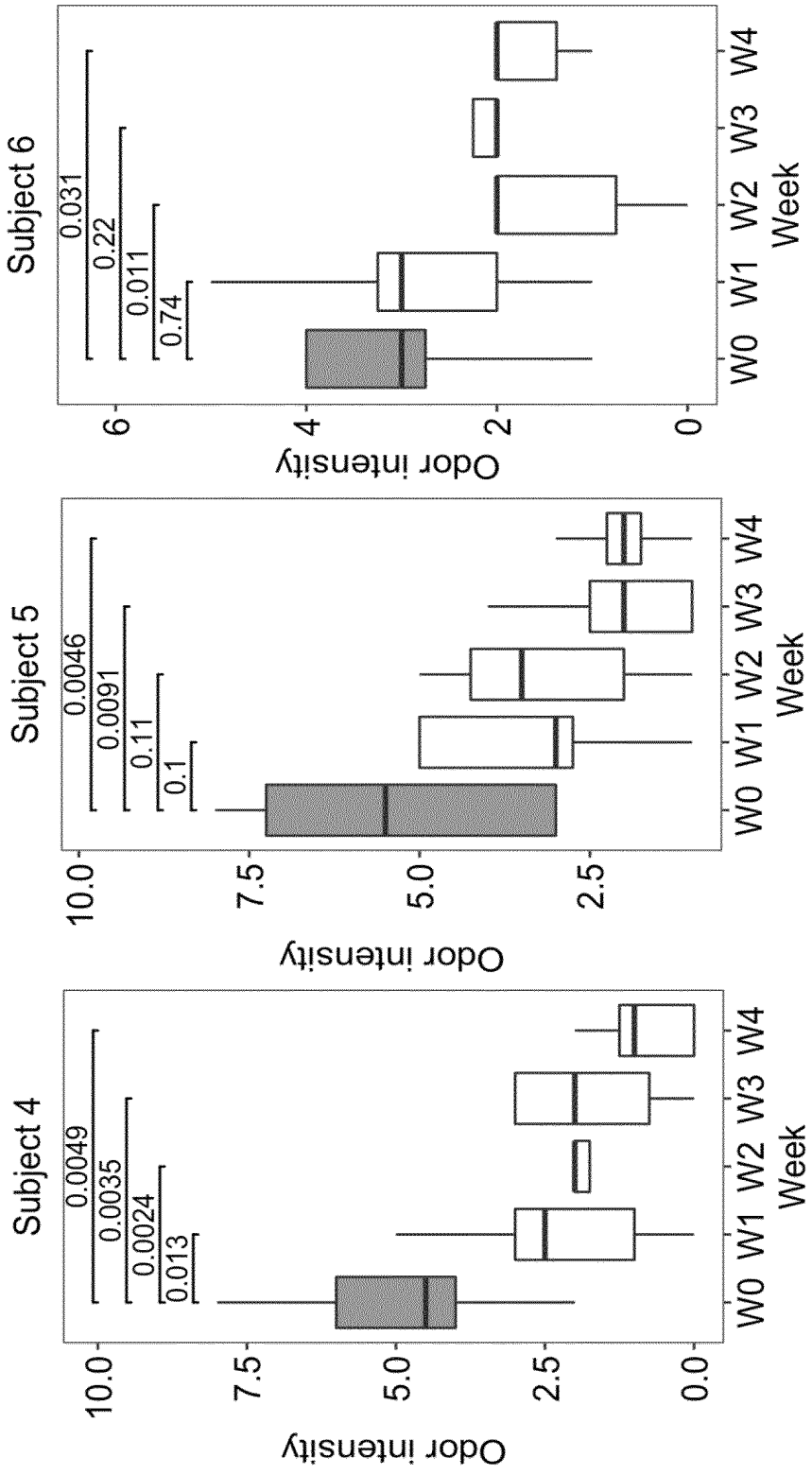
Subject 4
Subject 5
Subject 6
Odor intensity
Week
W0
W1
W2
W3
W4
0.0049
0.0035
0.0024
0.013
0.0046
0.0091
0.11
0.1
0.031
0.22
0.011
0.74

Figure 6

INTERNATIONAL SEARCH REPORT

International application No
PCT/EP2020/058897

A. CLASSIFICATION OF SUBJECT MATTER
INV. A61K8/33 A61Q15/00 A61K8/99
ADD.

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
A61K A61Q

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)
EPO-Internal, WPI Data, BIOSIS, EMBASE

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	US 5 213 791 A (LYON SUE B [US] ET AL) 25 May 1993 (1993-05-25)	1,3,4,10
Y	column 1, line 27 - line 30; claims	1-13
X	EP 1 258 531 A1 (GIVAUDAN SA [CH]) 20 November 2002 (2002-11-20)	1,3,4,10
Y	paragraph [0001]; claims; examples	1-13
Y	WO 00/01355 A1 (UNILEVER PLC [GB]; UNILEVER NV [NL]; LEVER HINDUSTAN LTD [IN]) 13 January 2000 (2000-01-13) page 1, line 6 - line 11; claims	1-13

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[X] Further documents are listed in the continuation of Box C.

[X] See patent family annex.

* Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier application or patent but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
29 May 2020	09/06/2020

Name and mailing address of the ISA/	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Fax: (+31-70) 340-3016	Heller, Dorothée

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Form PCT/ISA/210 (second sheet) (April 2005)

INTERNATIONAL SEARCH REPORT

International application No
PCT/EP2020/058897

C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	TROCCAZ M ET AL: "3-METHYL-3-SULFANYLHEXAN-1-OL AS A MAJOR DESCRIPTOR FOR THE HUMAN AXILLA-SWEAT ODOUR PROFILE", CHEMISTRY & BIODIVERSITY, HELVETICA CHIMICA ACTA, ZUERICH, CH, vol. 1, no. 7, 1 July 2004 (2004-07-01), pages 1022-1035, XP002392824, ISSN: 1612-1872, DOI: 10.1002/CBDV.200490077 cited in the application abstract discussion -----	1-13
Y	HASEGAWA Y ET AL: "IDENTIFICATION OF NEW ODORIFEROUS COMPOUNDS IN HUMAN AXILLARY SWEAT", CHEMISTRY & BIODIVERSITY, HELVETICA CHIMICA ACTA, ZUERICH, CH, vol. 1, no. 12, 1 December 2004 (2004-12-01), pages 2042-2050, XP002392811, ISSN: 1612-1872, DOI: 10.1002/CBDV.200490157 cited in the application conclusion -----	1-13
A	US 6 951 729 B1 (DEWOLF JR WALTER E [US] ET AL) 4 October 2005 (2005-10-04) column 1, line 12 - line 15; claims -----	1-13

1

INTERNATIONAL SEARCH REPORT

Information on patent family members

International application No
PCT/EP2020/058897

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
US 5213791 A	25-05-1993	US 5213791 A	25-05-1993
		US 5487886 A	30-01-1996
		US 5595728 A	21-01-1997
EP 1258531 A1	20-11-2002	AT 356876 T	15-04-2007
		AU 2002308337 A1	25-11-2002
		BR 0209628 A	22-06-2004
		DE 60218837 T2	15-11-2007
		EP 1258531 A1	20-11-2002
		EP 1387891 A2	11-02-2004
		EP 1811034 A2	25-07-2007
		MX PA03010323 A	17-02-2004
		US 2004241795 A1	02-12-2004
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US 6951729 B1	04-10-2005	NONE	