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Fatty acid metabolism by cutaneous bacteria and its role in axillary malodour

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Summary

It is generally accepted that short (C_2–C_5) and medium (C_6–C_{11}) chain volatile fatty acids (VFAs) are among the primary causal molecules of axillary malodour. It is also widely acknowledged that malodour generation is attributable to the biotransformation of odourless natural secretions, into volatile odorous products, by cutaneous bacteria. However, little information is available on the biochemical origins of VFAs on axillary skin. In these studies, assay systems were developed to investigate the generation of VFAs from lipid substrates readily available to the bacteria resident on axillary skin. A major route to short and medium chain VFAs in the axilla was shown to be the partial catabolism of structurally unusual (e.g. methyl-branched) longer chain fatty acids by a previously uncharacterized sub-group of the *Corynebacterium* genus, corynebacteria (A). In contrast, corynebacteria (B) are incapable of growth on fatty acid. Structurally unusual fatty acids originate from the triacylglycerol component of sebum, and probably also apocrine sweat, by the action of bacterial lipases. Interestingly, VFA formation in the axilla is a dynamic process, with some cutaneous microorganisms, specifically micrococci and brevibacteria, capable of fully catabolizing these odorants. The results of these studies provide new understanding on the biochemical origins of VFA-based axillary malodour.

Introduction

The generation of malodour on various sites of the human body is caused by the microbial biotransformation of odourless natural secretions into volatile odorous molecules. On the skin surface, distinctive odours emanate, in particular, from the underarm (axilla), where a large and permanent population of microorganisms thrives on secretions from the eccrine, apocrine, apoeccrine and sebaceous glands (Leyden *et al.* 1981; Parekh 2002). It is generally accepted that short-medium chain (C_2–C_{11}) volatile fatty acids (VFAs) and 16-androstene steroids are among the causal molecules of axillary malodour (Labows *et al.* 1982; Zeng *et al.* 1991; Gower *et al.* 1994). New information on the biochemical origins of 16-androstenes on axillary skin, and the extent of their contribution to malodour, have recently been reported (Austin & Ellis 2003). The involvement of short chain (C_2–C_5) VFAs in human body odours, including axillary malodour, has long been acknowledged; latterly, medium chain (C_6–C_{11}) acids, in particular the *trans* (*E*) isomer of 3-methyl-2-hexenoic acid (3M2H), have also been implicated (Zeng *et al.* 1991). While little has been reported on the biochemical origins of VFAs on axillary skin, 3M2H is a notable exception. This branched, unsaturated C_7 VFA was shown by Spielman *et al.* (1995) to be carried to the skin surface non-covalently bound to two proteins, apocrine secretion odor-binding proteins 1 and 2 (ASOB1 and ASOB2). The ASOB2 protein was subsequently identified as apolipoprotein D (apoD), a member of the lipocalin family of carrier proteins (Zeng *et al.* 1996). Recently, an apparently contradictory study by Natsch *et al.* (2003) indicated that 3M2H, and the chemically related 3-hydroxy-3-methylhexanoic acid, are instead covalently bound to free glutamine residues in fresh axillary secretions, and released by the action of corynebacterial *N*-acylglutamine aminoacylase. However, it was postulated that, once cleaved from their glutamine conjugates, the acids might subsequently associate non-covalently with apoD. The Natsch *et al.* (2003) study was pioneering by its success in purifying and characterizing corynebacterial aminoacylase, cloning and sequencing its encoding gene, and preparing recombinant enzyme, the first time such a process has been achieved for an enzyme involved in human body odour. Nevertheless, while the contribution of 3M2H and 3-hydroxy-3-methylhexanoic acid to axillary malodour is not disputed, there remains a lack of information on the ability of axillary bacteria to generate VFAs via the biotransformation of readily available cutaneous substrates.

Potential metabolic routes to VFAs in the axilla include the fermentation of glycerol, from triacylglycerol hydrolysis, and lactic acid, naturally abundantly present on skin (Petersen 1999), to acetic and propionic acids by resident microaerophilic *Propionibacterium* and

facultatively anaerobic *Staphylococcus* species (Moat & Foster 1988). Several amino acids also have the potential to be converted to these C_2–C_3 VFAs by propionibacteria and staphylococci, via similar fermentation routes. However, certain amino acids, specifically the branched aliphatic family (valine, leucine and isoleucine), may be metabolized, by alternative pathways, to the highly odorous short chain (C_4–C_5) methyl-branched VFAs, such as isovaleric (3-methylbutyric) acid (Massey *et al*. 1976; Thierry *et al*. 2001), traditionally associated with the acidic note of axillary malodour (Labows *et al*. 1982; Zeng *et al*. 1991). While amino acids are present in eccrine sweat, they may also be provided, on axillary skin, by the breakdown of proteinaceous material from the keratinizing epidermis and apocrine secretion, by proteolytic cutaneous microorganisms, including *Staphylococcus epidermidis* and some propionibacteria (Holland 1993). The metabolism of glycerol, lactic acid and amino acids to VFAs by axillary bacteria is reported in a separate article (A.G. James, D. Hyliands & H. Johnston, *International Journal of Cosmetic Science in press*.

A further potential biotransformation route to VFAs in the axilla is the partial catabolism of longer chain (C_{14}–C_{30}), structurally unusual (odd carbon number and/or methyl-branched) fatty acids present in sebum (Nicolaides 1974; Nicolaides & Apon 1977), and possibly also apocrine sweat (A.G. James, unpublished observation). However, the ability of cutaneous bacteria to degrade fatty acids by β-oxidation, thus using them as a carbon and energy source, has not previously been reported. Sebaceous lipid contains, in particular, large amounts of methyl-branched fatty acids, the majority with the branch in the iso (n−1) or anteiso (n−2) position (Nicolaides 1974; Nicolaides & Apon 1977). Potentially, these present metabolic problems to microorganisms, as the end-products of β-oxidation, isobutyric (methylpropionic), isovaleric and 2-methylbutyric acids, may be non-utilizable. Also present are fatty acids with internal methyl branches on even-numbered carbon atoms. These may also present metabolic problems, as the 2-methylacyl-CoA intermediates of β-oxidation may either be non-utilizable, or hinder chain-shortening such that metabolite leakage occurs (Pirnik 1977). Many of the methyl-branched VFAs implicated in axillary malodour can be accounted for by the partial degradation of longer chain (C_{14}–C_{30}), structurally unusual fatty acids known to be present in sebum (Nicolaides 1974; Nicolaides & Apon 1977; Labows *et al*. 1982; Zeng *et al*. 1991). In the axilla, these fatty acids are presumed to arise principally from the hydrolysis of the triacylglycerol component of sebum, and potentially also apocrine lipid (A.G. James, unpublished observation), by the action of bacterial lipases. While certain cutaneous bacteria, notably propionibacteria and staphylococci, are known to exhibit lipase activity (Holland 1993), previous studies have failed to quantify and compare the specific activity of different genera or species.

In this article, we describe *in vitro* studies on the formation of VFAs from lipid substrates readily available to axillary bacteria. In particular, we report on the identification of a previously uncharacterized subgroup of the *Corynebacterium* genus responsible for an important new pathway to VFA-based axillary malodour.

Materials and methods

General chemicals

Unless indicated otherwise, all general laboratory chemicals were obtained from Sigma-Aldrich Company (UK) Ltd.

Organisms and growth media

A library of Gram-positive bacteria (*Corynebacterium*, *Brevibacterium*, *Propionibacterium*, *Staphylococcus* and *Micrococcus* species), characteristic of the normal flora of axillary skin (Leyden *et al*. 1981; Taylor *et al*. 2003), was established, including both culture collection strains (National Collection of Type Cultures [NCTC]; National Collection of Industrial and Marine Bacteria Ltd. [NCIMB]) and in-house axillary isolates. Bacterial isolates were classified, to genus level, by their ability to grow on selective agar plates (Taylor *et al*. 2003), and, where possible, to species level, by the use of 'api STAPH', 'api CORYNE' and 'rapid ID 32 A' identification systems [bioMerieux]. For long-term maintenance, all cultures were stored on 'Protect' cryogenic beads [Technical Service Consultants Ltd.] at −80 °C; in the shorter term, maintenance was on TSAT plates or TSBT medium (30 g l^{-1} Tryptone Soya Broth [Merck]; 10 g l^{-1} Yeast Extract [Oxoid]; 10 g l^{-1} Tween 80; ±20 g l^{-1} bacteriological agar [Oxoid]) under aerobic or, for the propionibacteria, anaerobic conditions (9–13% CO_2, <1% O_2). For the biotransformation assays, a semi-synthetic medium was employed (g l^{-1}): KH_2PO_4 (1.6), $(NH_4)_2HPO_4$ (5.0), Na_2SO_4 (0.38), Yeast Nitrogen Base [Difco] (3.35), Yeast Extract (0.5), $MgCl_2 \cdot 6H_2O$ (0.5), Tween 80 (0.1), glucose (1.0).

Fatty acid biotransformation assay

The *in vitro* assay system, for investigating VFA formation and utilization by axillary bacteria, consisted of 250 ml baffled shake flasks, to which were added 25–30 ml semi-synthetic medium, supplemented with 0.5–2.0 g l^{-1} fatty acid substrate, either oleic (C18:1, *cis*-9), petroselenic (C18:1, *cis*-6), palmitic (C16:0), pentadecanoic (C15:0), isostearic (Me-C16:0-C18:0), isopalmitic (14-Me-C15:0), 4-methylcaprylic (4-Me-C8:0), 2-methylcaproic (2-Me-C6:0), isocaproic (4-Me-C5:0), 2-methylbutyric (2-Me-C4:0), isovaleric (3-Me-C4:0), isobutyric (Me-C3:0), propionic (C3:0) or acetic (C2) acid. Following inoculation with fresh bacterial biomass, to give starting optical densities (A_{590}) of 1.0–2.0,

equivalent to ~$\log_{10}$ 8–9 viable cells ml^{-1}, assays were incubated aerobically at 35 °C, with agitation (shaking incubator [Infors AG] at 125 rev min^{-1}), and analysed after 24–72 h. Fresh biomass was obtained by pregrowing bacteria aerobically at 35 °C in 25–50 ml TSBT, using 250 ml baffled shake flasks (agitated at 125 rpm in shaking incubator [Infors AG]), then harvesting by centrifugation and resuspending in a minimal volume of semi-synthetic medium. For the propionibacteria, anaerobic conditions (9–13% CO_2, <1% O_2) were used, both for the assay incubations, and to pregrow bacterial biomass. At the beginning and end of each assay incubation, culture purity and viability were determined, either qualitatively, by streaking 1.0 μl culture medium onto TSAT plates, or quantitatively, by total viable count analysis on TSAT plates, following serial dilution in quarter-strength Ringers solution. Plates were incubated aerobically (or anaerobically, for the propionibacteria) at 35 °C for 48–72 h before being assessed. Upon completion of each assay incubation, fatty acid (including VFA) levels were determined by capillary gas chromatography (GC).

GC determination of fatty acids

Fatty acid (including VFA) levels in the biotransformation assays were quantitatively determined by capillary GC analysis. Initially, 5 ml aliquots from each flask were transferred into universal tubes, a known amount of caproic (C6:0) or lauric (C12:0) acid (from a stock solution in ethyl acetate) added as an internal standard, and the culture medium acidified (pH 1–2) by the addition of 3 M HCl. Liquid-liquid extraction was then carried out using 10 ml ethyl acetate, with the organic and aqueous phases being resolved by centrifugation. An aliquot of each organic (upper) phase was then transferred to a sampling tube, prior to analysis on a Perkin Elmer 8000 (Series 2) GC fitted with a 15 m × 0.32 mm (internal diameter) nitroterephthalic acid-modified PEG/siloxane copolymer (FFAP) fused silica capillary column (film thickness, 0.25 μm) [Quadrex Scientific]. The column was attached to the split–splitless injector and flame ionization detector (FID) of the GC; injector and detector temperatures were each 300 °C. Carrier gas for the column was helium (0.4 kg cm^{-2}), while hydrogen (1.2 kg cm^{-2}) and air (1.6 kg cm^{-2}) supplied the FID. The temperature programme for fatty acid analysis was 80 °C (2 min); 80–250 °C (20 °C min^{-1}); 250 °C (5 min). On some occasions, VFAs were resolved using an alternative programme: 80 °C (2 min); 80–155 °C (7.5 °C min^{-1}); 155 °C (1 min). Sample size for injection was 1 μl. Fatty acid levels were quantified by comparison of peak areas with known concentrations of both internal (caproic or lauric acid) and externally run standards (tailored mixtures of fatty acids, of known concentration, in ethyl acetate).

Lipase assay

High biomass bacterial suspensions were generated by growth on complex medium, specifically a modified version of TSBT (20 g l^{-1} Tryptone Soya Broth; 10 g l^{-1} Yeast Extract; 2.5 g l^{-1} Tween 80), for 48 h with agitation (shaking incubator [Infors AG] at 120 rev m^{-1}), at which point triacylglycerol (1.0 g l^{-1} high oleate sunflower oil) was added to stimulate lipase activity, and the cells incubated for a further 24 h. Incubations were carried out aerobically or, for the propionibacteria, under anaerobic conditions (9–13% CO_2, <1% O_2). Bacteria were harvested by centrifugation, washed in 10 mM potassium phosphate buffer, pH 7.0, and resuspended in a minimal volume of this buffer. Cell dry weights were determined for each preparation by drying a known volume (1–5 ml) of sample at 98 °C, and adjusting to allow for the weight of buffer salts. The lipase activity of each suspension was determined by use of a Radiometer autotitrator, with glyceryl tributyrate substrate, titrating with NaOH. Specific activities were calculated as lipase units (μmol free fatty acid released min^{-1}) g^{-1} cell dry weight (LU g^{-1}).

Results and discussion

Fatty acid biotransformations

Initially, in these studies, representative axillary bacteria (*Staphylococcus*, *Corynebacterium*, *Micrococcus*, *Brevibacterium* and *Propionibacterium* species) were screened for their ability to metabolize fatty acids, using oleic acid (2.0 g l^{-1}) as a model substrate. The results demonstrated, for the first time, that some cutaneous bacteria, specifically members of the *Corynebacterium*, *Micrococcus* and *Brevibacterium* genera, are capable of utilizing fatty acids as a source of carbon and energy (Table 1). In contrast, staphylococci and propionibacteria were unable to grow on oleic acid. Of most interest were the results obtained with members of the *Corynebacterium* genus, which clearly fell into two distinct groups, namely those capable of catabolizing fatty acids, which were defined as sub-group (A), and those lacking this biochemical capability, defined as sub-group (B). This

Table 1. Summary of oleic acid (2.0 g l^{-1}) utilization by axillary bacteria, as determined by GC.

Genus	Strains tested	Oleic acid catabolizing strains	Non-oleic acid catabolizing strains
Corynebacterium	26	16	10
Staphylococcus	17	0	17
Propionibacterium	3	0	3
Micrococcus	6	6	0
Brevibacterium	3	3	0

new phenotyping of axillary corynebacteria is distinct from the traditional reference to lipophilic and non-lipophilic coryneforms or diphtheroids (Leyden *et al.* 1981), which relates to a requirement, or otherwise, for minor lipid components in the medium (usually satisfied by Tween 80) to support growth, probably to overcome a biosynthetic deficiency. As indicated in Table 2, some non-lipophilic corynebacteria can catabolize fatty acids, while certain lipophilic species lack this biochemical capability.

In a subsequent study, representative cutaneous corynebacteria (A), micrococci and brevibacteria were tested for their ability to catabolize a range of straight-chain fatty acid substrates of varying chain lengths and positions of desaturation, typical of those present in axillary skin lipid. Assay systems were inoculated with *Corynebacterium* (A) isolates (G40, G41 or NCIMB 40928), *Micrococcus* spp. (NCIMB 40927 or isolate DH3) or *B. epidermidis* (NCTC 11083 or isolate W11), and supplemented with oleic, petroselenic, palmitic, or pentadecanoic acid, each at 2.0 g l^{-1}. All of these substrates were fully degraded by each of the tested *Micrococcus* and *Brevibacterium* strains, and this was also true for the *Corynebacterium* (A) strains, but only on even carbon number substrates, i.e. oleic, petroselenic and palmitic acid. The ability to catabolize LCFAs with double bonds at even-numbered carbon atoms (e.g. petroselenic acid), indicating 2,4-dienoyl-CoA reductase activity (Dommes *et al.* 1983), is important, as most unsaturated sebaceous fatty acids are members of the *cis*-6, rather than the usual *cis*-9 family (Nicolaides 1974). The data (not shown) for corynebacteria (A) on the odd carbon number substrate, pentadecanoic acid, provided the first evidence that fatty acid biotransformations may contribute to VFA production on axillary skin. In each case, an essentially stoichiometric amount of the end-product of β-oxidation, propionic acid, was formed.

A further study was undertaken to investigate the catabolism of a range of methyl-branched fatty acid substrates by corynebacteria (A), micrococci and brevibacteria. Assay systems were inoculated with appropriate bacteria, and supplemented with 2.0 g l^{-1} isostearic acid, 1.0 gl^{-1} isopalmitic acid, or 1.0 gl^{-1} 4-methyl caprylicacid Isostearic acid is a heterogeneous substrate, consisting of a poorly-defined cocktail of branched and unbranched C_{16}–C_{18} fatty acids; it does, however, represent a good model for the structurally unusual fatty acid component of skin lipid (Nicolaides 1974; Nicolaides & Apon 1977). Isopalmitic (14-methylpentadecanoic) and 4-methylcaprylic acids are defined homogeneous substrates representing, respectively, the iso and internally branched fatty acid components of sebum. The results of this study (Table 3) clearly showed the inability of corynebacteria (A) to fully degrade the isostearic acid cocktail, resulting in the generation of significant levels of chain-shortened metabolites, mainly medium chain (C_6–C_{14}) VFAs. In the *Micrococcus* and *Brevibacterium* systems, substrate removal was more efficient, and only small amounts of catabolic intermediates and/or end-products were apparent. In the isopalmitic acid-supplemented systems, similar results were obtained. Each of the tested *Corynebacterium* (A) strains only partially catabolized this substrate, resulting in the formation of essentially stoichiometric amounts of the intermediate and non-utilizable end-product of β-oxidation, isocaproic (4-methylvaleric) and isobutyric (methylpropionic) acid, respectively (4-Me-C5:0 & Me-C3:0 VFAs). In contrast, the tested *Micrococcus* and *Brevibacterium* species fully degraded isopalmitic acid. Incomplete catabolism of 4-methylcaprylic acid was also apparent with corynebacteria (A), resulting in the production of essentially stoichiometric amounts of 2-methylcaproic (2-Me-C6:0) acid, the non-utilizable product of one round of β-oxidation. Again, *M. luteus* NCIMB 40927 and both *B. epidermidis* strains fully catabolized this methyl-branched acid. However, with *Micrococcus* sp. DH3, degradation of this substrate was incomplete, resulting in the generation of 2-methylcaproic acid and two other VFAs, possibly the *cis*-3 and *trans*-2 isomers of the β-oxidation intermediate, 4-methyloctenoic (4-Me-C8:1) acid.

Table 2. Comparison of lipophilicity and fatty acid catabolism by axillary corynebacteria. Lipophilicity was determined by growth (non-lipohilic, −), or otherwise (lipophilic, +), on TSAT plates, minus the Tween 80 component. Fatty acid catabolism was determined by the ability to utilise (+) oleic acid (2.0 g l^{-1}), or not (−), as determined by GC.

Corynebacterium species (code)	Lipophilicity (+/−)	Fatty acid catabolism (+/−)
C. xerosis (NCTC 7243)	−	+
Unspeciated (G96)	−	+
Unspeciated (G97)	−	+
Unspeciated (G40)	+	+
Unspeciated (G41)	+	+
Unspeciated (NCIMB 40928)	+	+
Unspeciated (G36)	+	−
Unspeciated (G37)	+	−
Unspeciated (G38)	+	−

The results of these studies provide evidence that the partial catabolism of structurally unusual fatty acids, by a previously undefined sub-group (A) of the *Corynebacterium* genus, contributes to the levels of short and medium chain VFAs on axillary skin. Furthermore, *in vitro* kinetic data (A.G. James, D. Hyliands & H. Johnston, *International Journal of Cosmetic Science*), in press as well as *in vivo* studies showing an association between corynebacterial numbers and malodour intensity (Taylor *et al.* 2003), suggest that this may represent the major biotransformation route to VFA-based axillary malodour.

VFA utilization

As net VFA levels on axillary skin are probably a balance between genesis and trophy, a further study was

Table 3. Biotransformation of methyl-branched fatty acids by axillary bacteria, as determined by GC. Percentage conversions were calculated on a mol:mol basis, except for the isostearic acid biotransformations, which were estimated on weight:weight basis.

Genus (group)	Species (code)	Substrate (g l^{-1})	VFA product(s)	Conversion (% mol:mol)
Corynebacterium (A)	Unspeciated (G40)	Isostearic acid (2.0)	C_6–C_{14}	~20%
		Isopalmitic acid (1.0)	Me-C3:0 & 4-Me-C5:0	95%
		4-Methylcaprylic Acid (1.0)	2-Me-C6:0	94%
	Unspeciated (G41)	Isostearic acid (2.0)	C_6–C_{14}	~25%
		Isopalmitic acid (1.0)	Me-C3:0 & 4-Me-C5:0	90%
		4-Methylcaprylic acid (1.0)	2-Me-C6:0	105%
	Unspeciated (NCIMB 40928)	Isostearic acid (2.0)	C_6–C_{14}	~20%
		Isopalmitic Acid (1.0)	Me-C3:0 & 4-Me-C5:0	94%
		4-Methylcaprylic acid (1.0)	2-Me-C6:0	90%
Micrococcus	*M. luteus* (NCIMB 40927)	Isostearic acid (2.0)	C_8–C_{14}	~3%
		Isopalmitic acid (1.0)	None detected	–
		4-Methylcaprylic acid (1.0)	None detected	–
	unspeciated (DH3)	Isostearic acid (2.0)	C_8–C_{14}	~4%
		Isopalmitic acid (1.0)	None detected	–
		4-Methylcaprylic acid (1.0)	2-Me-C6:0 & others	~100%
Brevibacterium	*B. epidermidis* (NCTC 11083)	Isostearic acid (2.0)	C_8–C_{14}	~5%
		Isopalmitic acid (1.0)	None detected	–
		4-Methylcaprylic acid (1.0)	None detected	–
	B. epidermidis (W11)	Isostearic acid (2.0)	C_8–C_{14}	~4%
		Isopalmitic acid (1.0)	None detected	–
		4-Methylcaprylic acid (1.0)	None detected	–

undertaken to determine the ability of representative cutaneous bacteria to catabolize a range of VFAs (C_2–C_7), representing the intermediates and end-products of longer chain fatty acid biotransformations. Laboratory model systems were inoculated with appropriate bacteria and supplemented with acetic (C_2), propionic (C3:0), isobutyric (Me-C3:0), isovaleric (3-Me-C4:0), 2-methylbutyric (2-MeC4:0), or 2-methylcaproic (2-MeC6:0) acid, each at 0.5 g l^{-1}. The results of this study (Table 4) demonstrated that all tested bacteria could utilize acetic acid as a source of carbon and energy. However, in the systems supplemented with odd carbon number and methyl-branched VFAs, the metabolic deficiencies of certain axillary bacteria became apparent. With the exception of isovaleric acid utilization by isolate G41, corynebacteria (A) were incapable of degrading structurally unusual VFAs, thus clarifying previous results obtained in incubations with longer chain odd carbon number (pentadecanoic acid) and methyl-branched (isostearic, isopalmitic and 4-methylcaprylic acid) substrates. In contrast, micrococci and brevibacteria readily catabolized propionic acid and most methyl-branched VFAs, although the inability of *Micrococcus* sp. DH3 to metabolize internally branched fatty acids was confirmed. These results confirmed that VFA formation in the axilla is a dynamic process, with some cutaneous microorganisms, specifically micrococci and brevibacteria, capable of fully catabolizing these odorants and their precursors. However, both the prevalence and density of these bacteria in the axilla are probably too low for them to have a significant effect on net VFA levels (Taylor *et al.* 2003).

Lipase activity

The first key step in the generation of VFAs on axillary skin, via fatty acid biotransformations, is the hydrolysis of the triacylglycerol component of sebaceous and apocrine lipid by bacterial lipases. In this study, concentrated cell suspensions of a range of representative axillary bacteria were screened for lipase activity, using

Table 4. Utilization (+) and non-utilization (–) of VFAs by axillary bacteria, as determined by GC.

Genus (group)	Species (code)	VFA (0.5 g l^{-1})					
		C_2	C3:0	Me-C3:0	3-Me-C4:0	2-Me-C4:0	2-Me-C6:0
Corynebacterium (A)	Unspeciated (G40)	+	–	–	–	–	–
	Unspeciated (G41)	+	–	–	+	–	–
	Unspeciated (NCIMB 40928)	+	–	–	–	–	–
Micrococcus	*M. luteus* (NCIMB 40928)	+	+	+	+	+	+
	unspeciated (DH3)	+	+	+	+	+	–
Brevibacterium	*B. epidermidis* (NCTC 11083)	+	+	+	+	+	+
	B. epidermidis (W11)	+	+	+	+	+	+

Table 5. Lipase activity of axillary bacteria, as determined by autitration.

Genus (group)	Species (code)	Lipase activity (LU g^{-1})
Corynebacterium (A)	Unspeciated (G40)	151
	Unspeciated (NCIMB 40928)	251
Corynebacterium (B)	Unspeciated (G36)	17
	Unspeciated (G80)	0
Micrococcus	*M. luteus* (NCIMB 40927)	5
	Unspeciated (DH3)	0
Brevibacterium	*B. epidermidis* (NCTC 11083)	13
	B. epidermidis (W11)	2
Propionibacterium	*P. avidum* (NCTC 11864)	173
	P. avidum (G104)	306
	P. acnes (G61)	1070
	P. acnes (G62)	966
Staphylococcus	*S. epidermidis* (W7)	20
	S. epidermidis (DH1)	69

an autotitrator assay. The results (Table 5) demonstrate that the most lipase-active axillary microrganisms are propionibacteria, in particularly *P. acnes*, followed by corynebacteria (A) and staphylococci. Corynebacteria (B), micrococci and brevibacteria were found to be lipase-negative, or exhibited only very low levels of activity. In the case of *M. luteus* NCIMB 40927 and *B. epidermidis* (NCTC 11083 and isolate W11), separate studies indicated that these low levels of activity are probably due to bacterial esterases, rather than lipases, hydrolysing the glyceryl tributyrate substrate (C. Austin & D. Hyliands, personal communication). Although these results are in broad agreement with those reported previously (Holland 1993), they also represent the first successful attempt to quantify and compare the specific lipase activity of different genera, groups or species of axillary bacteria.

Concluding comments

The highly active lipase of corynebacteria (A) gives further indication of the pivotal role these previously unclassified organims have, in the formation of malodorous short-medium chain (C_2–C_{11}) VFAs in the axilla. While the metabolism of glycerol, lactic acid and amino acids to short chain (C_2–C_5) VFAs by staphylococci and propionibacteria has also been shown, *in vitro* kinetic data indicates that these pathways contribute less to axillary VFA levels, than fatty acid biotransformations by corynebacteria (A) (A.G. James, D. Hyliands & H. Johnston, *International Journal of Cosmetic Science*, in press). This view is further supported by *in vivo* studies, showing an association between corynebacterial numbers and malodour intensity in the axilla (Taylor *et al.* 2003); significantly, no such relationship exists for staphylococci or propionibacteria. Meanwhile, the combined results of these studies provide new understanding on the biochemical origins of VFA-based axillary malodour (Figure 1) which, in turn, should lead to the development of novel deodorant systems. For example, the identification of a previously undefined sub-group of bacteria, and a new metabolic pathway, involved in malodour generation, opens up the possibility of using inhibitors which either target this pathway (Casey *et al.* 2001), or the organisms themselves (Casey *et al.* 2000). Furthermore, consideration of this article alongside those published by Austin & Ellis (2003), on 16-androstene steroids, and Natsch *et al.* (2003), on 3M2H and 3-hydroxy-3-methylhexanoic acid, implies a recent step-change in our understanding of axillary malodour.

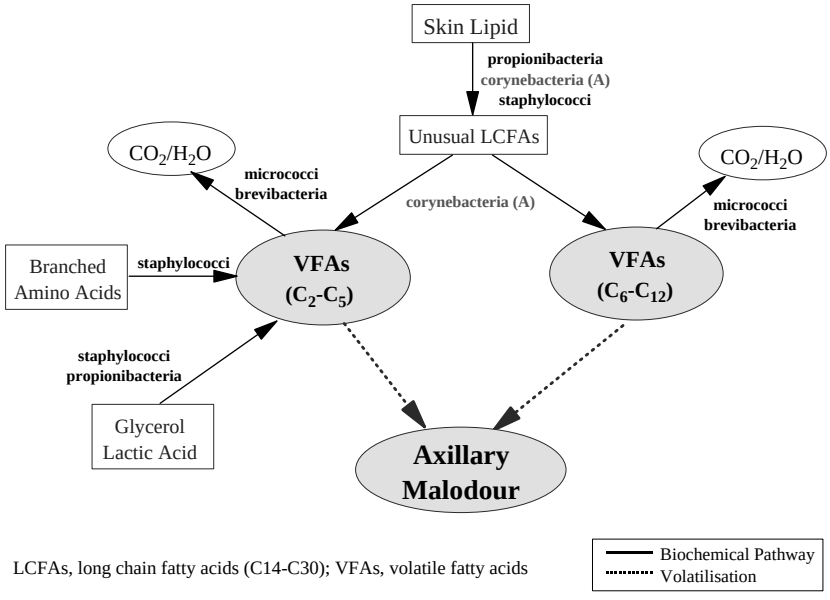

Figure 1. Formation and utilization of VFAs by axillary bacteria.

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