

# The natural history of cutaneous propionibacteria, and reclassification of selected species within the genus *Propionibacterium* to the proposed novel genera *Acidipropionibacterium* gen. nov., *Cutibacterium* gen. nov. and *Pseudopropionibacterium* gen. nov.

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The genus *Propionibacterium* in the family *Propionibacteriaceae* consists of species of various habitats, including mature cheese, cattle rumen and human skin. Traditionally, these species have been grouped as either classical or cutaneous propionibacteria based on characteristic phenotypes and source of isolation. To re-evaluate the taxonomy of the family and to elucidate the interspecies relatedness we compared 162 public whole-genome sequences of strains representing species of the family *Propionibacteriaceae*. We found substantial discrepancies between the phylogenetic signals of 16S rRNA gene sequence analysis and our high-resolution core-genome analysis. To accommodate these discrepancies, and to address the long-standing issue of the taxonomically problematic *Propionibacterium propionicum*, we propose three novel genera, *Acidipropionibacterium* gen. nov., *Cutibacterium* gen. nov. and *Pseudopropionibacterium* gen. nov., and an amended description of the genus *Propionibacterium*. Furthermore, our genome-based analyses support the amounting evidence that the subdivision of *Propionibacterium freudenreichii* into subspecies is not warranted. Our proposals are supported by phylogenetic analyses, DNA G+C content, peptidoglycan composition and patterns of the gene losses and acquisitions in the cutaneous propionibacteria during their adaptation to the human host.

## Introduction

The genus *Propionibacterium* was described by Orla-Jensen in 1909 and constitutes species widely known for producing propionic acid as their end product of fermentation (Goodfellow *et al.*, 2012). Most notable are the type species *Propionibacterium freudenreichii* for its valued contribution to Swiss cheese (Abeijón Mukdsi *et al.*, 2014; Skerman *et al.*, 1980; Thierry *et al.*, 2011), *Propionibacterium acidipropionici* for its beneficial effects on the metabolism in the bovine rumen (Francisco *et al.*, 2002) and *Propionibacterium acnes* for its implications in the common skin disorder acne vulgaris and its notorious habit of contaminating human samples (Lomholt & Kilian, 2010; Mollerup *et al.*, 2016; Williams *et al.*, 2012). Traditionally, species within the genus have been

grouped as either classical or cutaneous propionibacteria (Stackebrandt *et al.*, 2006). The informal term ‘classic propionibacteria’ has been ascribed to the species isolated from dairy products in contrast to the skin-associated, reclassified corynebacteria (Stackebrandt *et al.*, 2006). The classic propionibacteria include the species *Propionibacterium freudenreichii*, *Propionibacterium acidifaciens* (Downes & Wade, 2009), *Propionibacterium cyclohexanicum* (Kusano *et al.*, 1997), *Propionibacterium australiense* (Bernard *et al.*, 2002), *Propionibacterium acidipropionici*, *Propionibacterium jensenii*, *Propionibacterium thoenii*, *Propionibacterium microaerophilum* (Koussémon *et al.*, 2001), *Propionibacterium olivae* (Lucena-Padrós *et al.*, 2014), *Propionibacterium damnosum* (Lucena-Padrós *et al.*, 2014) and *Propionibacterium propionicum* (Charfreitag *et al.*, 1988). The cutaneous group comprises the species *Propionibacterium acnes*, *Propionibacterium avidum* and *Propionibacterium granulosum*. In addition to these, a novel cutaneous species, tentatively named ‘*Propionibacterium humerusii*’, was reported by Butler-Wu *et al.* (2011), but this name was neither effectively nor validly published. Subspecies

Abbreviations: GABA, gamma-aminobutyrate; LL-A<sub>2</sub>PM, LL-2,6-diaminopimelic acid; meso-A<sub>2</sub>PM, meso-2,6-diaminopimelic acid.

Six supplementary figures and one supplementary table are available with the online Supplementary Material.

classification has been used in two species. The *Propionibacterium freudenreichii* subspecies (*Propionibacterium freudenreichii* subsp. *freudenreichii* and *Propionibacterium freudenreichii* subsp. *shermanii*) are separated by two biochemical reactions important for cheese production (Stackebrandt *et al.*, 2006). *P. acnes* has been divided into three phylogenetic types: I, II and III (Kilian *et al.*, 2012; McDowell *et al.*, 2005, 2008). Recently, it was proposed that *P. acnes* should be divided into two subspecies, *Propionibacterium acnes* subsp. *acnes* representing types I and II, and *Propionibacterium acnes* subsp. *elongatum* constituting type III (Dekio *et al.*, 2015). A more comprehensive review of species, including historical changes in taxonomy has been presented by Stackebrandt *et al.* (2006).

The unexplored transmission from food products or live-stock rumen to the human host or vice versa is an exciting scientific case, which may provide new insights into conditions of life on the human skin. As of February 2016, NCBI hosts 162 genome sequences under the taxonomic id corresponding to *Propionibacterium* (txid1743), and representing 10 of the 16 species. It is now possible to re-evaluate the taxonomy of the propionibacteria and to take a first look at their evolutionary adaptation to different habitats. In this study, we explored the genomic foundation of the current taxonomic classification of the genus *Propionibacterium*, along with seeking insights into the adaptive processes affecting the cutaneous group of propionibacteria during their transition to human skin.

## Methods

**Analysis of 16S rRNA gene sequences.** Nucleotide sequences of the gene encoding 16S rRNA were downloaded from NCBI for each type strain within *Propionibacteriaceae* as indicated by LPSN (www.bacterio.net), with the exception of *Propionibacterium propionicum* DSM 43307<sup>T</sup> (see Discussion) where the sequence of accession number NR\_114803 was used. Phylogenetic analysis using minimum-evolution (Fig. 1) was conducted on sequences aligned with MUSCLE (Edgar, 2004) in MEGA v. 7 (Kumar *et al.*, 2016). Additional trees using the algorithms maximum-likelihood, maximum-parsimony and neighbour-joining were reconstructed in MEGA v. 7. Detailed information on the phylogenetic analysis for each algorithm is provided in the supplementary information (available in the online Supplementary Material) for Fig. 2. The same settings were used for reconstructing Fig. 1.

**Reconstruction of core-genome trees.** Genomes of all strains belonging to the family *Propionibacteriaceae* (NCBI taxonomic id 31957), in addition to *Corynebacterium diphtheriae* DSM 43988, were downloaded from NCBI. The root, *Corynebacterium diphtheriae*, was chosen as a compromise between a taxon being distant enough to be a safe root irrespective of possible errors in the current classification and being as close as possible to give the largest core genome to analyse. Shared sequences were identified using tblastx (coverage <50%) (Camacho *et al.*, 2009) on all coding sequences identified by annotation of the *Propionibacterium acnes* KPA171202 genome (Brüggemann *et al.*, 2004). All homologous gene sequences were aligned using MUSCLE (Edgar, 2004) and concatenated using a Python script. This initial dataset was used to identify relevant unclassified strains, to correct wrong species assignments (Fig. S4) and to select genomes for inclusion in our analysis. The trees were built on the included genomes in MEGA 7 (Kumar *et al.*, 2016) using 100 bootstrap replications and complete deletion.

Algorithms used were maximum-likelihood, maximum-parsimony, minimum-evolution and neighbour-joining (see figure legends). Detailed information on the phylogenetic analysis is provided in the supplementary information.

### Comparative analysis of DNA G+C content and genome sizes.

All genome sequences were downloaded from NCBI Genomes. Strain genomes were taken from all genome groups provided on the species page of NCBI Genomes to reduce the number of redundant strains. Wrongly identified genomes were identified and excluded by comparing core-genome sequences to those of related species. Genomic DNA G+C content and genome size were calculated on genome sequences using a Python script.

**Software for drawing figures.** Phylogenetic trees were made in MEGA 7 (Kumar *et al.*, 2016). Illustration of comparative genome sizes and DNA G+C contents was drawn using matplotlib (Michael Droettboom *et al.*, 2015). Circular genome maps were made in BRIG (Ali-khan *et al.*, 2011).

## Results

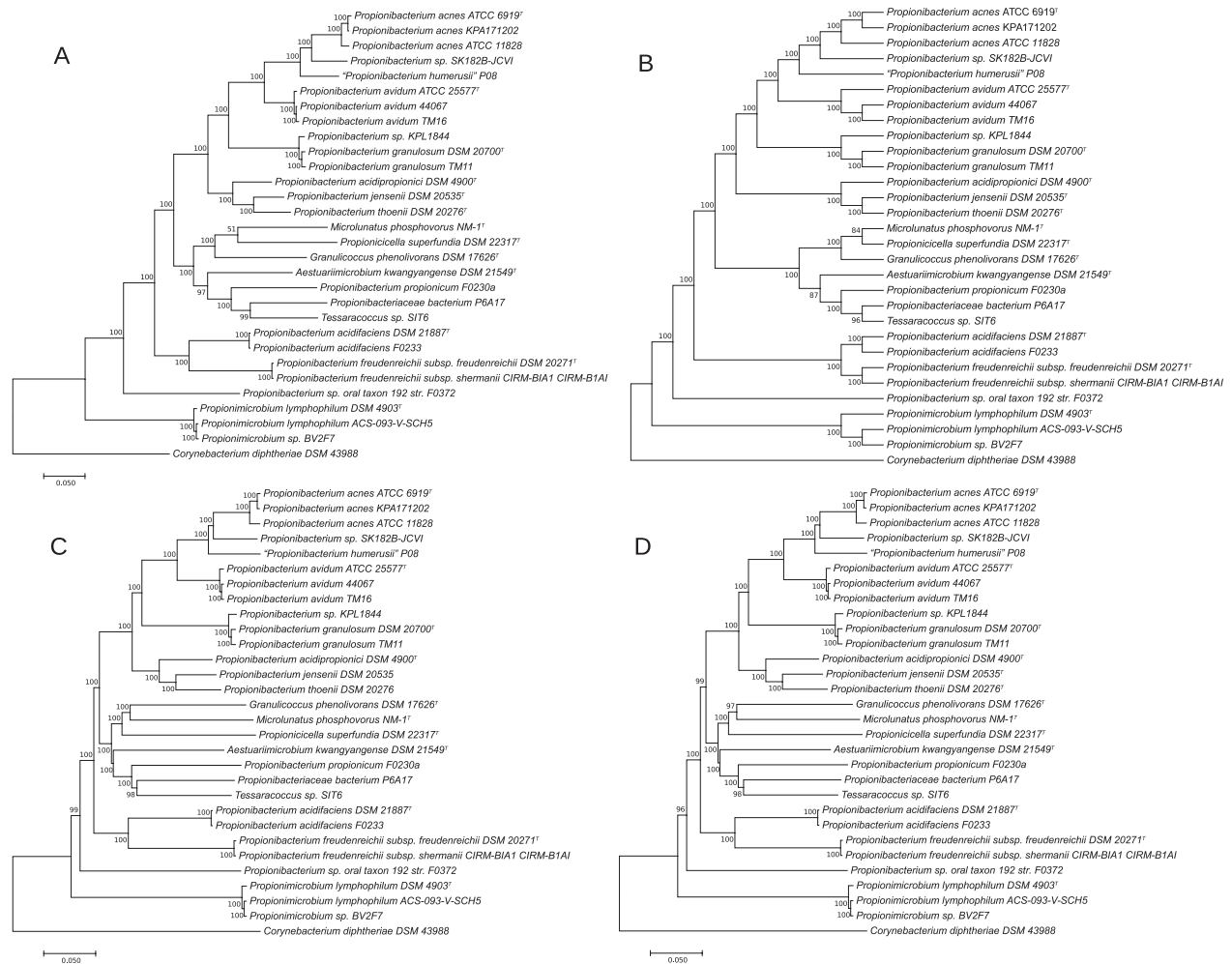
### Topology determined by 16S rRNA gene sequences

In accordance with the current taxonomy, phylogenetic analysis of 16S rRNA gene sequences downloaded from NCBI for all type strains belonging to the family *Propionibacteriaceae* revealed a distinct and coherent *Propionibacterium* clade supported by the bootstrap analysis (bootstrap value 79) with the exception of *Propionibacterium propionicum*, which formed a distinct lineage (Fig. 1). The overall topology of the phylogenetic trees was confirmed in trees reconstructed by the algorithms maximum-likelihood, maximum-parsimony and neighbour-joining in MEGA v. 7 (see Figs S1–S3).

### Topology of the family *Propionibacteriaceae* based on genome analysis

To better resolve the interspecies relatedness we extracted shared nucleotide sequences from all whole-genome-sequenced species, together with one out-group species, *Corynebacterium diphtheriae* (from the family *Corynebacteriaceae*). The concatenated sequences amounted to 50.9 kb and were used to reconstruct phylogenetic trees using four different algorithms (Fig. 2). Based on these phylogenetic analyses, the genus *Propionibacterium* does not form a monophyletic cluster. All four algorithms revealed two major branches of species of the genus *Propionibacterium*, one of which is connected to a branch representing a cluster composed of the genera *Granulococcus*, *Microlunatus*, *Propionicicella*, *Aestuarii-microbium* and *Tessaracoccus*, in addition to one strain of *Propionibacterium propionicum* and an unidentified member of the family *Propionibacteriaceae* (strain P6A17). One of the *Propionibacterium* clades consists of the representatives of ‘classic species of the genus *Propionibacterium*’, the type species *Propionibacterium freudenreichii* and *Propionibacterium acidifaciens*. The other *Propionibacterium* clade consists of two sub-clades, one including the skin-associated species of the genus *Propionibacterium*, *Propionibacterium acnes*,





**Fig. 2.** Phylogenetic trees based on 50 899 bp shared sequences among 30 genomes of strains belonging to the family *Propionibacteriaceae* and one strain of *Corynebacterium diphtheriae*. The trees display the number of supporting bootstrap replications for each branch (of 100). Trees were reconstructed in MEGA v. 7 using complete deletion and the following algorithms: (a) maximum-likelihood, (b) maximum-parsimony, (c) minimum-evolution and (d) neighbour-joining. Bars, number of base substitutions per site. See supplementary information for further details on the evolutionary models used.

*Propionibacterium avidum*, *Propionibacterium granulosum* and '*Propionibacterium humerusii*', and the other including other 'classic species of the genus *Propionibacterium*', *Propionibacterium acidipropionici*, *Propionibacterium jensenii* and *Propionibacterium thoenii*. *Propionimicrobium lymphophilum* (Stackebrandt *et al.*, 2002) and an unclassified strain, *Propionibacterium* sp. oral taxon 192 str. F0372 and the root, *Corynebacterium diphtheriae*, all form distinct lineages. The two clades of species of the genus *Propionibacterium* with *Propionibacterium propionicum* as an outlier are also supported by the 16S rRNA gene sequence tree.

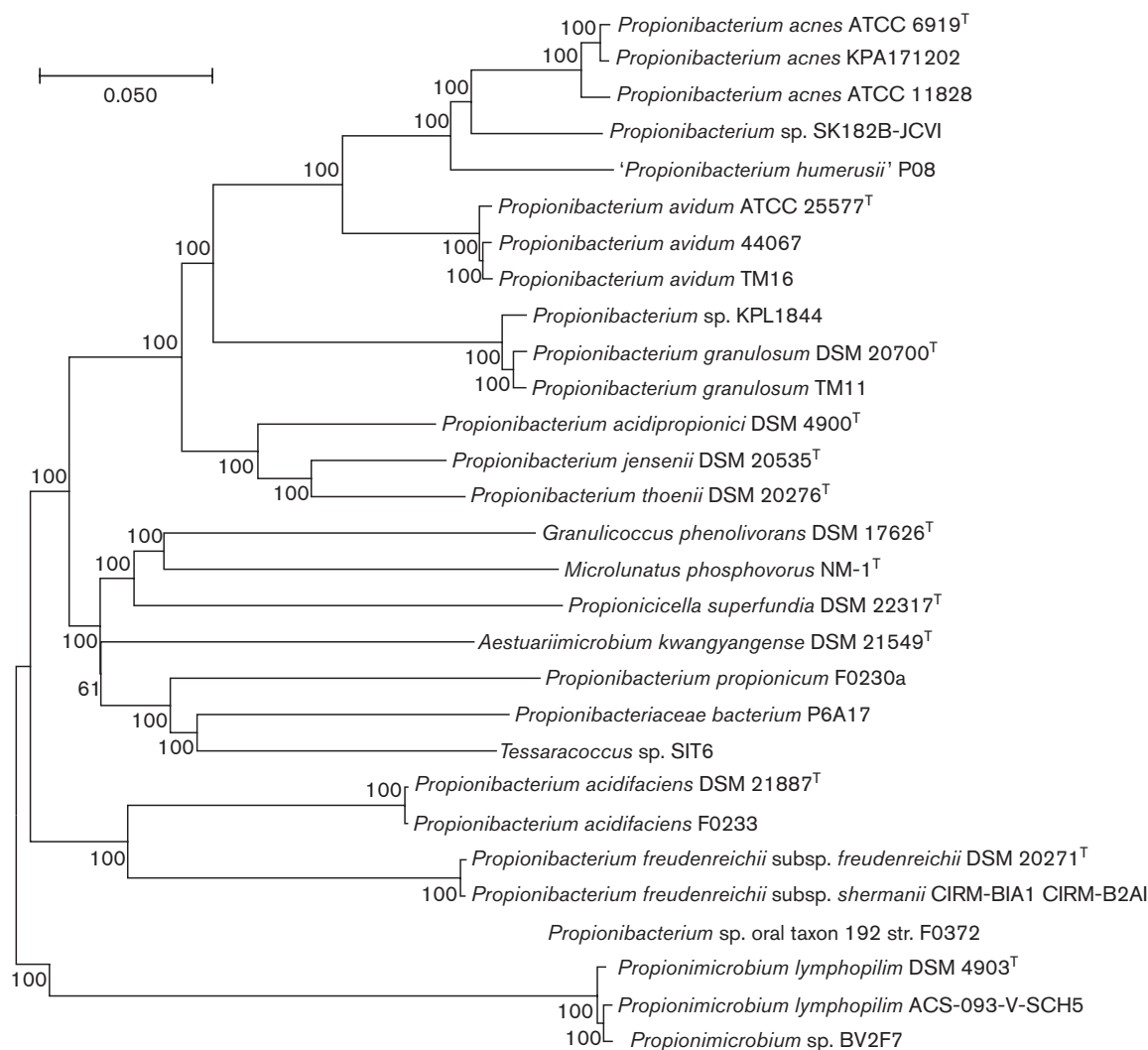
### Phylogenetic analysis with improved resolution

In the next step to improve the resolution, the outgroup *Corynebacterium diphtheriae* was eliminated from the process of extracting core genome sequences. A new tree

reconstructed on an almost three times larger dataset of shared sequences (135 kb) supports the findings represented in Fig. 2 and clearly highlights the non-monophyletic nature of the genus *Propionibacterium* (Fig. 3). It becomes apparent that the cutaneous propionibacteria form a distinct and coherent clade and that the 'classic propionibacteria' constitute two separate clusters. Furthermore, it is evident from Fig. 3 that the current classification does not reflect the relative phylogenetic distances to the type species *Propionibacterium freudenreichii*.

### DNA G+C content and genome size

To further elucidate the intra-genus diversity we mapped the DNA G+C-content and size of genomes of species in the family *Propionibacteriaceae* and *Corynebacterium diphtheriae* (Fig. 4). The lowest DNA G+C content was found in

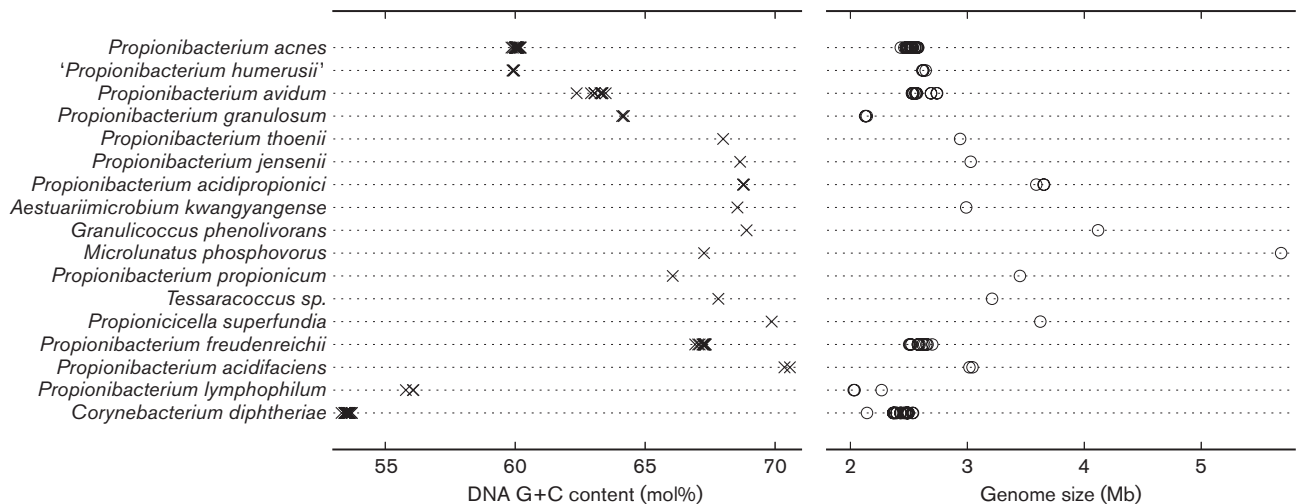


**Fig. 3.** Minimum-evolution tree reconstructed on 135 kb shared sequences displaying results of 100 bootstrap replications. Bar, 0.05 base substitutions per site. See supplementary information for further details on the evolutionary model used.

*Corynebacterium diphtheriae* (53.5 mol%) followed by *Propionibacterium lymphophilum* (56.0 mol%), supporting their separate position in the trees shown in Figs 1, 2 and 3. Interestingly, Fig. 4 displays a movement towards lower DNA G+C contents in the cutaneous species (*Propionibacterium acnes*, '*Propionibacterium humerusii*', *Propionibacterium avidum* and *Propionibacterium granulorum*). These four species have lower DNA G+C contents (59.9–64.2 mol%) than the remaining propionibacteria (66.1–70.6 mol%), with an evident decrease in the species *Propionibacterium acnes* and '*Propionibacterium humerusii*' that, according to the parsimonious analysis, are end-branches (Fig. 2b). Furthermore, the cutaneous species have smaller genomes than the other propionibacteria, with the only exception being *Propionibacterium freudenreichii*.

### Adaptation of cutaneous propionibacteria to their human host

To examine potential adaptive changes in cutaneous propionibacteria we searched the dataset of homologous genes (tblastx hits covering >50% of query) for genes uniquely present in strains of the four species of cutaneous propionibacteria. A total of 108 genes were found (Fig. S5). Most notable, many of these genes clustered in pairs of two or more on the spatial representation in Fig. S5. Many of these clusters encode sugar transporters in forms of ABC transporters, symporters and phosphotransferase systems (PTS). Another interesting theme is iron-acquisition, of which we identified three clusters of genes in addition to a small siderophore-related gene. We identified an island of seven genes encoding several aminomutases related to ornithine metabolism. Another smaller island was found encoding three



**Fig. 4.** Plot of genomic DNA G+C content calculated for each strain in each species. Species are listed in accordance with the topology of Fig. 3.

subunits of a potassium-transporting ATPase, along with a hypothetical protein. In addition, two lipase genes encoding 'triacylglycerol lipase' and 'lysophospholipase' were identified as specific to the four cutaneous species.

To identify genes lost in the process of adaptation to human skin we used the same method for extracting homologous genes with *Propionibacterium jensenii* as reference instead of *Propionibacterium acnes*. The search for homologous sequences present in at least one strain of each of the species *Propionibacterium jensenii*, *Propionibacterium thoenii* and *Propionibacterium acidipropionici* and none of the cutaneous species resulted in 26 annotated genes (Fig. S6). Interestingly, levanase was found among the absent genes. Levanase catalyses the degradation of fructose polymers of the levan type (or phlein) found excessively in grasses (Martin *et al.*, 1987; Suzuki & Chatterton, 1993). Furthermore, cutaneous propionibacteria lack the genes for transporting branched-chain amino acids. Another noteworthy absence is the lack of a glutamate decarboxylase catalysing the conversion of L-glutamate to the neurotransmitter gamma-aminobutyrate (GABA).

The initial enzyme for shunting L-rhamnose into the glycolysis pathway is absent, and the KEGG database confirms that all three enzymes of the rhamnose pathway are missing in *Propionibacterium acnes* and *Propionibacterium avidum* (Kanehisa *et al.*, 2014).

The gene encoding 'glutamate-cysteine ligase' is the first step in de novo synthesis of glutathione and its analogue ophthalmic acid; this rate-limiting enzyme is not present in the cutaneous species.

The end product of the mevalonate pathway is isopentenyl diphosphate, and the final step is catalysed by diphosphomevalonate decarboxylase. This end product is essential for the biosynthesis of polyisoprenoids and peptidoglycan

(Barta *et al.*, 2012). The diphosphomevalonate decarboxylase is absent in the cutaneous species; however, in contrast to *Propionibacterium acidipropionici*, *Propionibacterium acnes* has an intact MEP/DOXP pathway, providing an effective alternative production of isopentenyl diphosphate.

## Discussion

The group of cutaneous propionibacteria constitutes species of a distinct phylogenetic clade in the genus *Propionibacterium* as indicated by Figs 2 and 3, which is also largely supported by the 16S rRNA gene sequence tree displayed in Fig. 1. The maximum-parsimony tree displayed in Fig. 2b illustrates the evolutionary history of these cutaneous species. Evidently, *Propionibacterium granulosum* is the oldest, followed by *Propionibacterium avidum*, then '*Propionibacterium humerusii*' and the intermediate strain SK187B-JCVI and, finally, *Propionibacterium acnes* of which phylotype I is suggested as the most recent lineage. We envisage an evolutionary model where the initial transmission into the new niche, human skin, was followed by an iterative process of better and better adapted clones expanding at the expense of the founders. It was previously reported that clonal complex 18 (MLST8) in type IA<sub>1</sub> includes clones that have been able to spread worldwide (Kilian *et al.*, 2012; Lomholt & Kilian, 2010). Conceivably, this process was driven by the large potential benefit of even the smallest adaptive changes that follow a 'recent' transmission to a new habitat. This hypothesized model fits the staircase-like tree structure of the maximum-parsimony tree and the scarcity of isolated 'older' species compared with the wide-spread lineages of the more recent *Propionibacterium acnes*.

The adaptation of propionibacteria to a human host involves new challenges, but also new benefits (Figs S5 and S6). Iron is essential for many processes in bacteria, while

the human proteins transferrin and ferritin reduce the accessibility of free iron. It is therefore not surprising to find genes related to iron absorption among the acquisitions. Similarly, the change in environment-provided carbon sources is expectedly reflected in the acquisition of several sugar-uptake-related genes and lipases. The change in carbon sources is also reflected in the adaptive deletions. The two lipases acquired by species associated with human skin, triacylglycerol lipase and lysophospholipase, conceivably facilitate their survival in the hostile environment of sebaceous follicles.

Strikingly, levanase (or phleinandase), an enzyme catalysing the degradation of levan-type fructans, unlikely to be encountered on human skin, was lost. To our knowledge, levanase activity has not been described for propionibacteria, but may be an uncounted benefit, in addition to the propionate production, when *Propionibacterium acidipropionici* is added to livestock fodder (Francisco *et al.*, 2002; Peng *et al.*, 2011). In addition, L-rhamnose is widely found among plants and bacteria (Giraud & Naismith, 2000), but is not produced by humans, which may explain the deletion of this metabolic pathway in the cutaneous species. The cutaneous species lack genes used in the production of glutathione/ophthalmate and GABA, both of which are produced by the human host. It is conceivable that at least glutathione/ophthalmate is scavenged to mitigate oxidative stress.

The method employed for identification of gene acquisition and losses is based on BLAST with limitations illustrated especially by the low number of genes lost. The risk of finding false negatives by reference-based sequence searches increases with increasing phylogenetic distance. However, the method is relatively conservative in the sense that all four species of cutaneous propionibacteria were required to have a gene that was absent in all of the out-group strains (*Propionibacterium acidipropionici*, *Propionibacterium jensenii* and *Propionibacterium thoenii*) and vice versa. With few closed genome sequences available and the limitation of using BLAST searches across species borders it is not surprising that the number of genes identified is low. It is expected that future studies based on more closed genomes of a higher quality will reveal additional differences.

Overall, it is evident that the cluster of cutaneous propionibacteria constitutes species occupying a markedly different habitat than the classical propionibacteria and that this is reflected in the gene contents of their genomes.

### Reclassification of *Propionibacterium propionicum*

Stackebrandt *et al.* (2006) discussed the issue of conflicting results regarding the phylogenetic topology of *Propionibacterium propionicum*. They concluded that there are several laboratory stock cultures of the type strain that are non-authentic. At least three studies have found *Propionibacterium propionicum* to be almost identical to *Propionibacterium avidum* (Bernard *et al.*, 2002; Dasen *et al.*, 1998; Koussémon *et al.*, 2001), while others have shown the out-

group topology (Charfreitag & Stackebrandt, 1989; Kusano *et al.*, 1997; Stackebrandt *et al.*, 2006) in agreement with our findings (Figs 1, 2 and 3). We have used the reference genome from the Human Microbiome Project and the 16S rRNA sequence from accession number NR\_114803, both consistent with the results presented by Charfreitag *et al.* (1988) in the context of the reclassification to *Propionibacterium propionicum* in 1988. In 2006, Stackebrandt *et al.* (2006) sequenced a new culture of the type strain DSM 43307<sup>T</sup> and confirmed the results from the 1988 reclassification (Charfreitag *et al.*, 1988). With confirmation of the type strain DSM 43307<sup>T</sup> by Stackebrandt *et al.* (2006), it is futile to await a resolution of contaminations in all laboratory stock cultures, as the ultimate authority of bacterial taxonomy is the designated type strain. The risk of contamination with cutaneous propionibacteria is significant, even more so in the investigation of related species, as contaminated results may be more similar to the expected results. Despite reports of laboratory stock collections being contaminated, the definitive authority is in the repository stock of the type strain. Stackebrandt *et al.* (2006) found that the *Propionibacterium propionicum* 16S rRNA gene sequence was more closely related to that of *Tessaracoccus bendigoensis* than to that of any other species. Our 16S rRNA gene sequence analysis includes more species of the genus *Tessaracoccus* and shows that *Propionibacterium propionicum* forms a unique branch separate from the *Tessaracoccus* clade. As a consequence of the phylogenetic topology of the type strain (Figs 1, 2 and 3) we propose that *Propionibacterium propionicum* should be reclassified to a new genus in the family *Propionibacteriaceae*.

### Reclassification of the genus *Propionibacterium*

During the last century, the genus *Propionibacterium* has been populated with species based on phenotypic traits and 16S rRNA gene sequence similarity. With the advent of whole-genome sequencing, the possibility of high-resolution phylogenetic analysis merits a revision of the taxonomy. From our genome analysis of species in the family *Propionibacteriaceae*, it is evident that 16S rRNA gene sequences largely reflect the true topology of subclades within the genus *Propionibacterium*, but are insufficient to describe the genetic distance from species of other genera. This discrepancy in resolution has resulted in some related species being assigned to the genus *Propionibacterium* and others not, while showing the same level of sequence similarity to *Propionibacterium freudenreichii*. Our results show that the taxonomy of propionibacteria does not reflect the genetic constitution of its species.

Recognizing the uncertainties involved in defining the level of differentiation of taxa in the taxonomic hierarchy, there are two ways the taxonomy of propionibacteria could be changed to reflect the genetic constitution of the species. One option is to include the members of the genera *Granulicoccus*, *Microlunatus*, *Propioniceella*, *Aestuariusmicrobium* and *Tessaracoccus* in the genus *Propionibacterium*. That would render the genus *Propionibacterium* monophyletic,

and even allow *Propionibacterium propionicum* to remain unchanged. The drawback is that the genus *Propionibacterium* would then encompass species of such diverse habitats as activated sludge, flat-water sediment, matured cheeses and human skin/oral cavity. In addition to being inconsistent with the biology, 16S rRNA gene sequence based analysis will no longer display a distinct and coherent *Propionibacterium* clade, rendering classification of novel species by this method error prone. The other option is a more practical approach that retains the functionality of sequencing 16S rRNA genes and provides a more coherent genus with regard to DNA G+C content, genome size, phenotypic traits and habitat. This option consists of reducing the genus *Propionibacterium* to accommodate one branch of the classic propionibacteria and creating a new genus for the other branch. In this way, the cutaneous group will be collected under a separate genus. Finally, the outlier *Propionibacterium propionicum* will be accommodated in a third genus. We propose that the genus *Propionibacterium* contains *Propionibacterium freudenreichii* (type species), *Propionibacterium cyclohexanicum*, *Propionibacterium acidifaciens* and *Propionibacterium australiense*. The novel genus *Acidipropionibacterium* gen. nov. will encompass the former species *Propionibacterium jensenii* (type species), *Propionibacterium thoenii*, *Propionibacterium acidipropionici*, *Propionibacterium microaerophilum*, *Propionibacterium damnosum* and *Propionibacterium olivae*. The novel genus *Cutibacterium* gen. nov. will contain the cutaneous species formerly known as *Propionibacterium acnes* (type species), *Propionibacterium avidum*, *Propionibacterium granulorum* and '*Propionibacterium humerusii*'. Finally, the novel genus *Pseudopropionibacterium* gen. nov. will accommodate the former species *Propionibacterium propionicum* (type species). Table S1 summarizes all novel and former species names.

All species of the proposed reduced genus *Propionibacterium* are characterized by *meso*-2,6-diaminopimelic acid (*meso*-A<sub>2</sub>PM) as the diagnostic amino acid in the peptidoglycan, which separates them from species of the proposed novel genus *Acidipropionibacterium* gen. nov. characterized by *LL*-2,6-diaminopimelic acid (*LL*-A<sub>2</sub>PM) (Goodfellow *et al.*, 2012). The proposed new genus *Cutibacterium* gen. nov. is mainly characterized by *LL*-A<sub>2</sub>PM in the peptidoglycan, with few strains using *meso*-A<sub>2</sub>PM, but the genus is readily separated from the proposed genera *Propionibacterium* and *Acidipropionibacterium* gen. nov. by 5–10 mol% lower genomic DNA G+C content (Fig. 4).

### Abandoning subspecies of *Propionibacterium freudenreichii*

According to the descriptions, *Propionibacterium freudenreichii* subsp. *freudenreichii* is able to reduce nitrate but does not ferment lactose, and vice versa for *Propionibacterium freudenreichii* subsp. *shermanii*. These are the only two distinguishing characteristics of these subspecies (Goodfellow *et al.*, 2012). However, fermentation of lactose is not genetically coupled with the inability to reduce nitrate and, accordingly, four phenotypes have been

observed, two of which fit neither of the two subspecies (de Freitas *et al.*, 2015). Comparing phenotypic traits to multilocus sequence typing (MLST) data, Dalmasso *et al.* (2011) conclude that characteristics of the subspecies are prone to recurrent changes and that these do not reflect the ancestral relationship. Furthermore, de Freitas *et al.* (2015) have shown that the subspecies do not correlate with aroma compounds important for cheese production. By whole-genome sequencing and comparison of genomes, Loux *et al.* (2015) found that the lactose fermentation is encoded by an island flanked by highly repeated transposase sequences. Furthermore, Loux *et al.* (2015) using core-genome analysis demonstrated that the phenotypes do not follow the phylogenetic history (in accordance with Fig. S4), definitively proving that the subdivision is not warranted. It is evident that the current definition of the two subspecies of *Propionibacterium freudenreichii* does not allow the classification of all strains belonging to the species. Therefore, and in accordance with our perceived impression of a consensus within the field, we propose that the subspecies are later synonyms of the species *Propionibacterium freudenreichii*.

## Conclusion

In conclusion, we propose a reclassification of the genus *Propionibacterium*, on the basis of genomic evidence. The proposal is consistent with the 16S rRNA gene sequence tree and allows novel species to be assigned by 16S rRNA gene sequence homology. The proposed changes are consistent with species habitats, genomic topology, DNA G+C content and peptidoglycan composition.

### Emended description of the genus *Propionibacterium* (Approved Lists 1980)

The description is as given for the genus *Propionibacterium* in *Bergey's Manual of Systematic Bacteriology* (Goodfellow *et al.*, 2012) with the following amendments. DNA G+C content ranges from 67 to 71 mol%. Diagnostic amino acid in peptidoglycan is *meso*-A<sub>2</sub>PM.

### Description of *Acidipropionibacterium* gen. nov.

*Acidipropionibacterium* (A.ci.di.pro.pi.o.ni.bac.te'ri.um. N. L. n. *acidum propionicum* propionic acid; N.L. neut. bacterium, a rod; N.L. neut. n. *Acidipropionibacterium* a propionic acid bacterium).

The description is as given for the genus *Propionibacterium* in *Bergey's Manual of Systematic Bacteriology* (Goodfellow *et al.*, 2012) with the following amendments. DNA G+C content is 68 mol%. Diagnostic amino acid in peptidoglycan is *LL*-A<sub>2</sub>PM. Some strains are haemolytic. The type species is *Acidipropionibacterium jensenii*.



**Description of *Cutibacterium* gen. nov.**

*Cutibacterium* (Cu.ti.bac.te'ri.um. L. n. *cutis* skin; N.L. neut. n. *bacterium* a rod; N.L. neut. n. *Cutibacterium* a skin bacterium).

The description is as given for the genus *Propionibacterium* in *Bergey's Manual of Systematic Bacteriology* (Goodfellow *et al.*, 2012) with the following amendments. Members of the genus are predominately isolated from human skin. DNA G+C content is 59–64 mol%. Diagnostic amino acid in peptidoglycan is LL-A<sub>2</sub>PM, but may be *meso*-A<sub>2</sub>P in occasional strains. Strains are non-haemolytic. The type species is *Cutibacterium acnes*.

**Description of *Pseudopropionibacterium* gen. nov.**

*Pseudopropionibacterium* gen. nov. (Pseu.do.pro.pi.o.ni.bac.te'ri.um. Gr. adj. *pseudes* false; N.L. n. *Propionibacterium* a bacterial genus; N.L. neut. n. *Pseudopropionibacterium* a false propionibacterium).

The description is as given for *Propionibacterium propionicum* in *Bergey's Manual of Systematic Bacteriology* (Goodfellow *et al.*, 2012). The type species is *Pseudopropionibacterium propionicum*.

**Description of *Cutibacterium acnes* comb. nov.**

Basonym: *Propionibacterium acnes* (Gilchrist 1900) Douglas and Gunter 1946 (Approved Lists 1980).

The description is as given for *Propionibacterium acnes* in *Bergey's Manual of Systematic Bacteriology* (Goodfellow *et al.*, 2012).

The type strain is deposited in repositories under the following strain identification numbers: ATCC 6919<sup>T</sup>=CCUG 1794<sup>T</sup>=CIP 53.117<sup>T</sup>=DSM 1897<sup>T</sup>=JCM 6425<sup>T</sup>=LMG 16711<sup>T</sup>=NCTC 737<sup>T</sup>=NRRL B-4224<sup>T</sup>=VKM Ac-1450<sup>T</sup>.

**Description of *Cutibacterium avidum* comb. nov.**

Basonym: *Propionibacterium avidum* (Eggerth 1935) Moore and Holdeman 1969 (Approved Lists 1980).

The description is as given for *Propionibacterium avidum* in *Bergey's Manual of Systematic Bacteriology* (Goodfellow *et al.*, 2012).

The type strain is deposited in repositories under the following strain identification numbers: ATCC 25577<sup>T</sup>=CCUG 36754<sup>T</sup>=CIP 103261<sup>T</sup>=DSM 4901<sup>T</sup>=IFO (now NBRC) 15671<sup>T</sup>=NCTC 11864<sup>T</sup>.

**Description of *Cutibacterium granulosum* comb. nov.**

Basonym: *Propionibacterium granulosum* (Prévot 1938) Moore and Holdeman 1970 (Approved Lists 1980).

The description is as given for *Propionibacterium granulosum* in *Bergey's Manual of Systematic Bacteriology* (Goodfellow *et al.*, 2012).

The type strain is deposited in repositories under the following strain identification numbers: ATCC 25564<sup>T</sup>=CCUG 32987<sup>T</sup>=CIP 103262<sup>T</sup>=DSM 20700<sup>T</sup>=JCM 6498<sup>T</sup>=LMG 16726<sup>T</sup>=NCTC 11865<sup>T</sup>.

**Description of *Acidipropionibacterium jensenii* comb. nov.**

Basonym: *Propionibacterium jensenii* van Niel 1928 (Approved Lists 1980).

The description is as given for *Propionibacterium jensenii* in *Bergey's Manual of Systematic Bacteriology* (Goodfellow *et al.*, 2012).

The type strain is deposited in repositories under the following strain identification numbers: ATCC 4868<sup>T</sup>=CCUG 48883<sup>T</sup>=CIP 103028<sup>T</sup>=DSM 20535<sup>T</sup>.

**Description of *Acidipropionibacterium thoenii* comb. nov.**

Basonym: *Propionibacterium thoenii* van Niel 1928 (Approved Lists 1980).

The description is as given for *Propionibacterium thoenii* in *Bergey's Manual of Systematic Bacteriology* (Goodfellow *et al.*, 2012).

The type strain is deposited in repositories under the following strain identification numbers: ATCC 4874<sup>T</sup>=CCUG 28149<sup>T</sup>=CIP 103029<sup>T</sup>=DSM 20276<sup>T</sup>=HAMBI 247<sup>T</sup>=JCM 6437<sup>T</sup>=LMG 16455<sup>T</sup>.

**Description of *Acidipropionibacterium acidipropionici* comb. nov.**

Basonym: *Propionibacterium acidipropionici* Orla-Jensen 1909 (Approved Lists 1980).

The description is as given for *Propionibacterium acidipropionici* in *Bergey's Manual of Systematic Bacteriology* (Goodfellow *et al.*, 2012).

The type strain is deposited in repositories under the following strain identification numbers: ATCC 25562<sup>T</sup>=CIP 103025<sup>T</sup>=DSM 4900<sup>T</sup>.

**Description of *Acidipropionibacterium microaerophilum* comb. nov.**

Basonym: *Propionibacterium microaerophilum* Koussémon *et al.* 2001.

The description is as given for *Propionibacterium microaerophilum* in *Bergey's Manual of Systematic Bacteriology* (Goodfellow *et al.*, 2012).

The type strain is deposited in repositories under the following strain identification numbers: M5<sup>T</sup>=CNCM I-2360<sup>T</sup>=DSM 13435<sup>T</sup>.

### Description of *Acidipropionibacterium olivae* comb. nov.

Basonym: *Propionibacterium olivae* Lucena-Padró *et al.* 2014.

The description is as given for *Propionibacterium olivae* in *Bergey's Manual of Systematic Bacteriology* (Goodfellow *et al.*, 2012).

The type strain is deposited in repositories under the following strain identification numbers: IGBL1<sup>T</sup>=CECT 8061<sup>T</sup>=DSM 25436<sup>T</sup>.

### Description of *Acidipropionibacterium damnosum* comb. nov.

Basonym: *Propionibacterium damnosum* Lucena-Padró *et al.* 2014.

The description is as given for *Propionibacterium damnosum* in *Bergey's Manual of Systematic Bacteriology* (Goodfellow *et al.*, 2012).

The type strain is deposited in repositories under the following strain identification numbers: IGBL13<sup>T</sup>=CECT 8062<sup>T</sup>=DSM 25450<sup>T</sup>.

### Description of *Pseudopropionibacterium propionicum* comb. nov.

Basonym: *Propionibacterium propionicum* (Buchanan and Pine 1962) Charfreitag *et al.* 1988.

The description is as given for *Propionibacterium propionicum* in *Bergey's Manual of Systematic Bacteriology* (Goodfellow *et al.*, 2012) with the following amendment. The accession number for the 16S rRNA gene sequence of type strain DSM 43307<sup>T</sup> is NR\_114803.

The type strain is deposited in repositories under the following strain identification numbers: ATCC 14157<sup>T</sup>=CCUG 4939<sup>T</sup>=CIP 101941<sup>T</sup>=DSM 43307<sup>T</sup>=IFO (now NBRC) 14587<sup>T</sup>=JCM 5830<sup>T</sup>=NCTC 12967<sup>T</sup>=VKM Ac-1449<sup>T</sup>.

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