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Proteobacteria from the human skin microbiota: Species-level diversity and hypotheses

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Abstract

The human skin microbiota is quantitatively dominated by Gram-positive bacteria, detected by both culture and metagenomics. However, metagenomics revealed a huge variety of Gram-negative taxa generally considered from environmental origin. For species affiliation of bacteria in skin microbiota, clones of 16S rRNA gene and colonies growing on diverse culture media were analyzed. Species-level identification was achieved for 81% of both clones and colonies. Fifty species distributed in 26 genera were identified by culture, mostly belonging to Actinobacteria and Firmicutes, while 45 species-level operational taxonomic units distributed in 30 genera were detected by sequencing, with a high diversity of Proteobacteria. This mixed approach allowed the detection of 100% of the genera forming the known core skin Gram-negative microbiota and 43% of the known diversity of Gram-negative genera in human skin. The orphan genera represented 50% of the current skin pan-microbiota. Improved culture conditions allowed the isolation of Roseomonas mucosa, Aurantronas altamirensis and Agrobacterium tumefaciens strains from healthy skin. For proteobacterial species previously described in the environment, we proposed the existence of skin-specific ecotypes, which might play a role in the fine-tuning of skin homeostasis and opportunistic infections but also act as a shuttle between environmental and human microbial communities. Therefore, skin-associated proteobacteria deserve to be considered in the One-Health concept connecting human health to the health of animals and the environment.

1. Introduction

Various human microbiota are now deciphered in depth thanks to metagenomics and new generation DNA sequencing (NGS) [1,2]. These methods allowed comparative microbial ecology by examining the influence of environmental factors, body sites and pathology on the diversity of microbiome [3–12]. Compared to culture-based approaches, NGS has generally extended the range of microbial diversity and may be more sensitive for the detection of minority bacteria able to grow on artificial media in monoculture [13,14]. Polyphasic studies associating molecular and culture-based analyses remained scarce [15,16] despite the development of high-throughput methods named culturomics [13].

Human skin is colonized by a complex microbial community, considered for a long time as dominated by Gram-positive bacteria such as staphylococci, micrococci, corynebacteria, Propionibacterium spp., Brevibacterium spp., and members of the genus Acinetobacter being the most frequently encountered Gram-negative bacteria in human skin microbiota. These bacteria belong to the long-term resident microbiota, based on the frequency with which they have been detected [2,4,15,17–21]. Beside these well-described bacteria, culture-independent approaches demonstrate that Gram-negative bacteria, particularly Proteobacteria, represent an important component of the skin microbiota [2,12,19–22]. Despite their detection in numerous metagenomic studies and diverse physio-pathological conditions, cutaneous Proteobacteria remained poorly described, mainly because isolates were not available and sequences generated by NGS were generally too short to obtain an accurate species affiliation.
This study aimed to precisely the phylogenetic relationships and taxonomy of Proteobacteria from healthy human skin microbiota by analysis of 16S rRNA gene sequences of more than 800 bp and by strain cultivation. Phylotypes and isolates will be described to the species and/or genotype level in order to compare skin-associated Proteobacteria with related environmental ecotypes.

2. Materials and Methods

2.1. Cutaneous samples, isolates and clones

The present study was an ancillary study proposed beside clinical study on atopy (Institut de Recherche Pierre Fabre, unpublished data). Briefly, in the main study, donors are tertiary workers (no healthcare workers) in urban areas without particular exposure to animals and soil. They took a shower using mild soap between 4 and 6 h before sampling. For each donor, one sample was taken from the inner forearm protected by clean personal garments until sampling. Sampling was performed according to the method described by Fleurette using a transfer fluid able to maintain the viability and to avoid proliferation of the microbiota [23]. Briefly, the open end of a sterile glass cylinder, with an area of 3.14 cm² was manually placed on the skin. Two milliliters of RTF medium [23] sterilized by 0.22 μm filtration was used to collect skin microbiota. Four successive spots were realized each during 1 min with the same liquid or a total forearm skin area of 12.56 cm². One tenth of the liquid was used for microbial culture, the remaining was stored at −20 °C for molecular analysis.

A total of 311 isolates and 278 16S rRNA gene clones obtained from two healthy-donors included in the clinical study were analyzed herein. Isolates were obtained by culture on Columbia agar supplemented with 5% sheep blood (Biomérieux) incubated under aerobic and anaerobic (Anoxomat) conditions at 37 °C for 5 days. One colony of each morphotype observed was harvested, sub-cultured and stored at −20 °C in cryopreservative medium (Eugon broth + 10% glycerol). A selective isolation of Gram-negative bacilli was performed at 30 °C for 5 days using culture media implemented by vancomycin (7.5 mg/L): R2A agar (Pronadisa), Schaedler agar (Difco) and Chocolat agar (Difco) for 8 samples from 2 other healthy donors.

The 16S rRNA clones library had been obtained after total DNA extraction directly from samples (MagNA.Lyser Green beads, Roche Molecular Biochemicals), and purification (QIAamp DNA micro purification kit, Qiagen, Germany). The amplification of a 863-bp-sequence 16SrRNA gene was performed using the universal primers, Universal1E (5’- AGACGCGCCGTRATWC 3’) and Universal2E (5’-ACGGGCGGTGTG TAC 3’) [24,25]. The purified amplicons (QIAquick PCR Purification kit, Qiagen) were ligated into the plasmid vector pGEM®-T Easy, then transformed into JM109 High Efficiency competent cells using the pGEM®-T Easy Vector Systems kit (Promega). JM109 transformed cells were streaked onto Luria–Bertani agar plates containing 100 μg/mL ampicillin, 40 μg/mL X-gal, 0.5 mM IPTG for blue/white screening as previously described [15]. The insert of each selected white clone was amplified and sequenced (ABI PRISM 3130 Genetic Analyzer, Applied Biosystems, USA) using T7 and SP6 primers as previously described [26].

2.2. Bacterial identification

Each colonial morphotype was submitted to identification by molecular methods based on 16S rRNA gene sequencing [15]. For members of the genus Staphylococcus, ITS 16S–23S and tuf gene sequencing were used for species affiliation [27,28].

2.3. Phylogenetic analysis and taxon affiliation

Sequences used for further sequence analysis corresponded to high-quality sequences, i.e. presenting less than 0.5% undetermined positions. Nucleotide sequences were analyzed using the Blast program in NCBI and Greengenes database. Chimera was detected using Bellerophon software in the Greengenes website (greengenes.lbl.gov). For database comparison, we retained the stringent threshold value of 98.7% of similarity with a fully defined cultured strain (type or non-type) as recommended for bacterial species delineation [29], for the affiliation of a clone to a species– or a genus-level operational taxonomic unit (OTU). When a similarity level of more than 98.7% was obtained for an uncultured bacterial clone only, the sequence was classified as 16S rRNA gene clone and affiliated to a genus according Greengenes database. Beside sequences identified herein, the sequences used to re-construct phylogenies were chosen by Blast analysis as follows. For each clone sequence, we included the most related deposited sequence and the most related sequences corresponding to (1) validated species and (2) human skin clone. The dataset of sequences was aligned using ClustalW software [30]. The most appropriate substitution model determined according to Akaike information criterion calculated with Modeltest (v.3.7) was GTR plus gamma distribution, plus invariant sites [31]. ML phylogenetic analysis was performed using PHYML v2.4.6, gamma shape parameter being estimated from the dataset [32]. ML bootstrap support was computed using PhyML after 100 reiterations.

The Shannon-Wiener (H’) and Simpson (D) diversity indexes were calculated for each phylum and according to the type of method (culture or uncultured approaches) [33,34].

3. Results

3.1. Taxonomic diversity in the skin microbiota

Table 1 shows species-level identification of the bacterial isolates and clones. Fig. 1 summarizes the qualitative and quantitative diversity repartition of the skin microbiota according to phylum and type of cell wall structure. Our approach allowed species-level identification for 81% of both clones and colonies. The culture detected 50 species-level OTUs including 39 taxonomic species distributed in 26 genera among 311 colonies tested. The sequencing of 278 clones allowed the identification of 45 species-level OTUs in 30 genera, including 26 taxonomic species, 6 pairs of undifferentiated taxonomic species, 7 groups of related species and 5 unaffiliated OTUs (Table 1).

Gram-positive bacteria belonging to Firmicutes and Actinobacteria represented 90.3% of the colonial morphotypes studied. Genera belonging to Firmicutes (175 isolates) were Bacillus, Streptococcus, Enterococcus, Gemella, Eubacterium and Staphylococcus. The latter was the most diverse genus of the cultivable skin microbiota, with 15 different species identified. Actinobacteria appeared more diverse to the genus level since the 106 isolates affiliated to this phylum belonged to 10 different genera (Table 1; Fig. 1). Uncultured clones affiliated to Firmicutes and Actinobacteria were minority (Table 1; Fig. 1) compared to clones belonged to Gram-negative bacterial phyla (Table 1): Proteobacteria of the alpha (27.0%), beta (23.9%) and gamma (47.2%) subdivisions and Bacteroidetes (1.9%). Most of the Gram-negative genera identified by sequencing were not detected in culture, except for Acinetobacter, Pseudomonas and Sphingomonas. Indeed, only 30 of the total of 311 bacterial colonies tested (9.7%) corresponded to Gram-negative bacteria. Fourteen isolates belonged to Proteobacteria (Enterobacteriaceae and non-fermentative bacilli) (Table 1), and 16 isolates of Gram-negative anaerobes were represented by one unique species, Prevotella buccae in the phylum Bacteroidetes.

Fig. 1 revealed that the distribution of clones and colonies by phylum and type of cell wall structure differed markedly. Cultivating Gram-positive bacteria were quantitatively the most represented (175 positive bacteria were quantitatively the most represented (175

### Table 1: Taxonomic diversity in the skin microbiota

| Phylum                  | Species | OTUs |
|-------------------------|---------|------|
| Firmicutes              | 92      | 15   |
| Actinobacteria          | 72      | 26   |
| Proteobacteria          | 73      | 30   |
| Bacteroidetes           | 45      | 10   |
| Gamma Proteobacteria    | 35      | 7    |
| Beta Proteobacteria     | 25      | 6    |
| Alpha Proteobacteria    | 15      | 5    |
| Uncultured firmicutes   | 10      | 3    |
| Uncultured actinobacteria | 5   | 2    |
| Uncultured proteobacteria | 30   | 10   |
| Uncultured bacteroidetes | 14   | 4    |

Fig. 1 revealed that the distribution of clones and colonies by phylum and type of cell wall structure differed markedly. Cultivating Gram-positive bacteria were quantitatively the most represented (175 positive bacteria were quantitatively the most represented (175
approach (ratio of 0.89) and vice versa for Gram-negative bacteria (Fig. 1). Moreover, most clones had no cultivable counterparts. These results showed major discrepancy between Gram-positive and Gram-negative diversities assessed by each approach. According to these results, the diversity was higher for Gram-negative bacteria retrieved by molecular approach than by culture, in particular for Proteobacteria.

Table 1

| Species or genus | Identification after culture | Phyla | Identification after culture | Bacteria | Identification after culture |
|-----------------|------------------------------|-------|-----------------------------|----------|-------------------------------|
| Bacillus sp.    | Actinomyces sp.              | Firmicutes | Actinobacteria | Actinobacter radioreisistens | Chryseobacterium indologenes |
| Entercoccus faecalis | Cellulomonas sp.             | Enterobacteriaceae | Enterobacter aerogenes | Prevotella buccae |
| Eubacterium lentum | Cellulosimicrobium cellulans | Klebsiella oxytoca |
| Gemella morbillorum | Corynebacterium amycolatum | Moraxella osloensis |
| Staphylococcus sp. | Corynebacterium riegiei | Pasteurella sp. |
| Staphylococcus aureus | Dermabacter hominis | Pseudomonas aeruginosa |
| Staphylococcus auricularis | Kocuria sp. | Serratia ficaria |
| Staphylococcus capitis | Kocuria kristinae | Sphingomonas mucosissima |
| Staphylococcus caprae | Micrococcus sp. | |
| Staphylococcus chondri | Micrococcus luteus | |
| Staphylococcus epidermidis | Micrococcus mucilaginosus | |
| Staphylococcus haemolyticus | Propionibacterium sp. | |
| Staphylococcus hominis | Propionibacterium acnes | |
| Staphylococcus lugdunensis | Propionibacterium avidum | |
| Staphylococcus schleiferi | Propionibacterium granulosum | |
| Staphylococcus sciuri | Rothia dentocariosa | |
| Staphylococcus warneri | | |
| Staphylococcus xylosus | | |
| Streptococcus sp. | | |
| Streptococcus oralis | | |

| No. of isolates | No. of species | No. of genera |
|-----------------|---------------|--------------|
| 175             | 21            | 6            |
| 106             | 10            | 8            |
| 14              | 9             | 8            |
| 16              | 2             | 2            |
| No. of isolates | No. of species | No. of genera |
| 81              | 6             | 4            |
| 33              | 6             | 5            |
| 32              | 1             | 20           |
| 1               | 1             | 1            |

a A group or a pair of described species that could not be discriminated by the markers used.
b Taxonomic name not validly published.
c Phylotypes corresponding to undescribed species.
(H = 0.3384 vs 0.1396 and D = 0.0088 vs 0.0769 for uncultured and culture approaches, respectively) (Table 2).

To address the lack of growing of cutaneous Proteobacteria on blood agar medium, we tested a posteriori their growth on Gram-negative selective media containing vancomycin. We tested the R2A medium, a medium developed to study bacteria that will not readily grow on rich and complex organic media [35] such as bacteria from water or other poor environments, as well as enriched media, chocolate agar and Scheadler broth. In this purpose, 8 additional skin samples were obtained from 2 additional healthy donors volunteers from the research team. Five samples were positive for Proteobacteria: Roseomonas mucosa (n = 2), Agrobacterium tumefaciens (n = 2), Acinetobacter johnsonii (n = 1), Acinetobacter lwofii (n = 1), Aurantimonas altamirensis (n = 2) and Pseudomonas psychrotolerans (n = 1). All strains were found on chocolate plus vancomycin medium except Roseomonas mucosa that only grew in Scheadler medium. No growth was observed onto R2A agar plates.

3.2. Phylogenetic taxonomy of Proteobacteria in skin microbiota

Proteobacterial sequences were classified according to alpha, beta and gamma subdivisions that contained 11, 4 and 17 OTUs representing 43, 38 and 75 different clones, respectively. The phylogenetic trees showed the repartition of the clone sequences in the known proteobacterial diversity (Fig. 2A–C).

The Alphaproteobacteria subdivision was represented by the genus Paracoccus (Rhodobacterales) (34.9%), and the orders Sphingomonadales (44.2%) and Rhizobiales (20.9%). The phylogenetic analysis (Fig. 2A) allowed affiliation to the species Aurantimonas coralicida, A. tumefaciens/Cossea et al. / One Health 2 (2016) 33–41

Table 2

| Culture approach          | Uncultured approach |
|---------------------------|---------------------|
| H'                        | D                   |
| Fimicutes                 | 0.323556959         | 0.005747126 |
| Actinobacteria            | 0.366860144         | 0.00952318  |
| Gram positive             | 0.091633203         | 0.003571429 |
| Proteobacteria            | 0.139582892         | 0.07923077  |
| Bacteroidetes             | 0.152833392         | 0.066666667 |
| Gram negative             | 0.225587993         | 0.034482759 |

Rhizobium pusense, Bradyrhizobium elkanii, Paracoccus yeei, Paracoccus haemadensis, Paracoccus seriniphilus and Paracoccus marinus. Clones affiliated to the genus Sphingomonas were related but at less than 98% identity with several species not discriminated by 16S rRNA gene sequencing including Sphingomonas aquatilis (Fig. 2A). In the Paracoccus clade, two clones (PA + C.C06 and PA + C.C07) corresponded to a yet undescribed species. In Rhizobiales, one clone was related to “Rasbo bacterium”, an undescribed species detected in plasma samples during acute sepsis [36]. In all cases, a clone detected in metagenomic studies on skin microbiota was related to the clone detected herein (Fig. 2A).

The Betaproteobacteria subdivision was dominated by Pseudomonas, which represented 70% of the clones of this sub-division with mainly the species Pseudomonas paruagae. Other clones grouped with the non valid species Imtechium assamisensi. Published cutaneous clones grouped together with this non validate species but differed from Aquabacterium fontiphilum and other related species (Fig. 2B). Other clones grouped in Acidovorax spp. and Neisseria spp. but identification to the species level was not accurate for these genera. The most related but undistinguished species were indicated in the tree (Fig. 2B).

The gamma subdivision was mainly represented by the genera Acinetobacter (26.7%), Idiomarina (16%), Alcanivorax (12%) and Pseudomonas (9.4%) and the species Escherichia coli (20%). In the genus Acinetobacter, the clones were distributed among the species A. lwofii, Acinetobacter baumannii, Acinetobacter junii and A. johnsonii. Seven clones were distributed among pseudomonads (Fig. 2C) mainly in Pseudomonas putida, Pseudomonas stutzeri and Pseudomonas fluorescens groups.

Finally, the phylogeny confirmed that four phylotypes (noted 5 in Table 1) corresponded to undescribed species of Alphaproteobacteria.

3.3. Core and pan Gram-negative skin microbiota

The recent studies of molecular ecology have enriched our knowledge about bacteria associated with the human skin. The Gram-negative diversity described in these studies and herein is compared in Table 3. The genera Acidovorax, Acinetobacter, Pseudomonas and Stenotrophomonas were isolated from independent donors and from different sampling sites (forearm, forehead, inner elbow and back) in all of the 6 studies or in all studies but one. Therefore, one can hypothesize that these bacteria may belong to the permanent core skin microbiota. If we consider the bacteria detected in at least 50% of the studies...
namely core\textsuperscript{50} skin microbiota, ten genera are highlighted (in bold in Table 3). Our approach coupling culture and molecular approach detected 100\% of the genera forming the current core\textsuperscript{50} skin microbiota (Table 3). Moreover, we detected from only 2 subjects 43\% of the known diversity of Gram-negative genera in human skin (Table 3).

4. Discussion

Current metagenomics based on NGS explores in depth the bacterial diversity and avoids the bias of cultivability. It has changed our vision of human microbiota but several limitations worth to be underlined. Particularly, NGS presents a lack of sensitivity for the detection of minority OTUs present at less than 10\textsuperscript{6} bacteria per sample\textsuperscript{37}. The size of sequenced DNA fragments varied greatly among techniques and studies rendering comparison hard to perform and explaining some discrepancies\textsuperscript{38}. In the case of the gut microbiota, the relative abundance of the phyla Bacteroidetes and Firmicutes depends on the 16S rRNA gene hypervariable region analyzed\textsuperscript{39}. These discrepancies observed to the phylum level are probably magnified when genera or species are considered. Generally, in NGS studies, the quality of taxonomic affiliation is low and limited to the genus due to the size of generated sequences. Herein, we used classical methods such as Sanger sequencing and culture with the aim to affiliate to taxonomic species a collection of clones and bacterial colonies isolated from healthy skin. This combined approach was targeted on Proteobacteria that present high diversity in skin microbiota but remain scarcely described to the species-level in previous studies on skin metagenome.

Dekio et al. in 2005 described for the first time the presence of Pseudomonas, Stenotrophomonas, Acidovorax, Bradyrhizobium and Neisseria as proteobacteria inhabiting the skin microbial ecosystem\textsuperscript{15}. Then, Gao et al. (2007) and Grice et al. (2008) reported two major molecular analyses confirming that Gram-negative bacteria are common residents and not contaminants from environment or other microbiota\textsuperscript{19,22}. They reported also the presence of bacteria usually found in the environment such as Methylobacterium, Sphingobium, Diaphorobacter, Enhydrobacter, Serratia, Pedromicrobium, Paracoccus, Halomonas and Delftia. Proteobacteria appears qualitatively the more diverse phylum\textsuperscript{22} even if the quantitative predominance of the Gram-positive genera classically detected in culture is confirmed\textsuperscript{20}. Recently, Probst et al. characterized another underestimated biodiversity, the Archaea of the human skin microbiota using the cloning of 16S rRNA gene PCR products\textsuperscript{40}.

Our study, limited to the forearm of two subjects, confirmed that the skin bacterial community is dominated in diversity by Gram-negative
Similarly, they represented 58% of the pan-microbiota in the study of species, which participate to the skin pan-microbiota expanse. In this study, orphan genera represented 50% of the current pan-microbiota. Similarly, they represented 58% of the pan-microbiota in the study of Grice [22]. Moreover, about 70% (with 84.4% of Proteobacteria) of the genera detected by Gao et al. on the forearm were specifically associated to skin microbiota and that beside the 10 genera found to belong to the core50 species, which participate to the skin pan-microbiota expanse.

By the approaches proposed herein, we detected all the members of the core50 Gram-negative skin microbiota and 43% of the Gram-negative skin pan-microbiota. These data validate our strategy, in spite of the lack of NGS data. It is particularly noteworthy that only a magnitude of 2 was observed between the 87 skin-associated bacterial genera detected in Human Metagenomic Project (4 cutaneous site and 242 subjects) [2] versus 48 genera detected by cloning and culture from one cutaneous site from 2 subjects in this study.

Since long ago, Gram-positive bacteria (Firmicutes and Actinobacteria) are recognized as the major component of the culturable skin microbiota [17]. When blood agar culture at 37 °C was used, we detected 92.0% of Gram-positive bacteria. Conversely, the same culture conditions yielded only few Gram-negative bacteria whereas most of those that had been detected by sequencing are considered to be culturable. The richness of the medium and the incubation temperature could be the causes of growth defect. The incubation at 30 °C of rich and poor media with vancomycin that inhibited Gram-positive bacteria led to the growth of R. mucosa, A. tumefaciens, A. altamirensis, A. johnsonii, A. lwofii and P. psychrotolerans. Growth is observed only for rich media but not onto R2A whereas these strains belong to taxa that generally grow onto poor media such as R2A medium (Bergey’s manual). This result suggests that the species or strains associated to skin microbiota may present particular requirements. Therefore, the existence of particular ectotypes of Proteobacteria specialized in human skin mutualism might be hypothesized.

Sequencing and phylogeny reconstruction detected many Proteobacteria considered to be environmental belonging to the orders Rhizobiales, Sphingomonadales, Burkholderiales, Oceanospirillales, Alteromonadales and Pseudomonadales. However, lifestyle of donors, that are tertiary workers in urban area, excluded regular or recent exposure to animals, plants, fresh or marine waters and soil. Most clones assumed to belong to environmental species have closest relatives sequences detected previously from the human skin (clones in green in Fig. 2) rather than from other ecosystems [4,19,22]. These clones being detected in independent analysis, one can hypothesize again that some of these sequences represent specific ectotypes in the human cutaneous microbiota. The metagenomic study of Mathieu et al. demonstrated functions that clearly illustrate the unique life style of the skin microbial communities and reinforce the hypothesis skin-specific ectotypes [42]. Microbiota description at the species-level completed by strain isolation is a way to study these ectotypes.

Some phenotypic and metabolic traits appear common to several species detected in skin microbiota and could help to define the proteobacterial skin ecotypes. Mainly, several clones correspond to halophilic genera and species of Gammaproteobacteria isolated in marine environments: Alcanivorax diesei, Alcanivorax venustus, Halomonas aquamarina, Halomonas neptunia or Halomonas alkantartica, Marinobacter hydrocarbonoclasticus and Idiomarina loihensis. Halophilic is consistent with the salt rich environment of the skin. Some of halophilic species in Gammaproteobacteria are also hydrocarbonoclastic: Alcanivorax diesei, Halomonas alkantartica, and Marinobacter hydrocarbonoclasticus. Such properties are also described for the genera Acinetobacter and Pseudomonas. Other clones grouped with the non validated species Intemchiassamaniensis were described as forming biofilms on polychlorinated biphenyls surfaces [43]. Out of gammaproteobacteria Pseudonocallaria chlorethenivorans and Sphingomonas spp. are other alkane-degrading bacteria detected herein [44–46].

The cutaneous microbiota is a major bacterial reservoir involved in opportunistic infections, particularly in health-care associated infections (HAI). Consequently, hand washing and skin antisepsis are now considered the most important interventions to prevent the spread of HAI agents [47]. Among Proteobacteria detected in this study, some species such as Stenotrophomonas, Aeromonas, Pseudomonas and Acinetobacter have been associated with opportunistic infections.
To our knowledge, the opportunistic pathogen *Aeromonas* has never been detected in the normal skin microbiota so far. Besides well-known opportunistic pathogens, bacteria assumed to be environmental are more and more described in opportunistic infections. For instance, *A. altamirensis* was described as part of the microbial community that produces deleterious colonization of Paleolithic paintings in Altamira Cave [51] and since then, it has been mainly described in human infections [52]. Finally, the genus *Roseomonas* gathers species mainly isolated from environment but *R. mucosa* and *Roseomonas gilardii* are frequently described in human infections [54]. By analogy with the main lifestyle observed for most members of *Roseomonas* spp., the source of human infections caused by *R. mucosa* is searched into environment and not among endogenous microbial community [54, 55]. These cases are particularly emblematic of the need of species-level identification in human microbiota in order to assess infectious risk and prevent opportunistic infections.

Table 3
Data comparison with the 5 main published studies that characterized healthy skin microbiota by molecular approaches.

| Gram-negative bacteria | D | G | Gr | H | Z | C |
|------------------------|---|---|----|---|---|---|
| *Acidovorax*            |   |   |    |   |   |   |
| *Acinetobacter*         |   |   |    |   |   |   |
| *Actinobacillus*        |   |   |    |   |   |   |
| *Aeromonas*             |   |   |    |   |   |   |
| *Afipia*                |   |   |    |   |   |   |
| *Agrobacterium/Rhizobium* |   |   |    |   |   |   |
| *Alcanivorax*           |   |   |    |   |   |   |
| *Alistipes*             |   |   |    |   |   |   |
| *Aquabacterium*         |   |   |    |   |   |   |
| *Aurantimonas*          |   |   |    |   |   |   |
| *Bacteroides*           |   |   |    |   |   |   |
| *Barnesiella*           |   |   |    |   |   |   |
| *Brachybacterium*       |   |   |    |   |   |   |
| *Bradyrhizobium*        |   |   |    |   |   |   |
| *Brevibacterium*        |   |   |    |   |   |   |
| *Brevundimonas*         |   |   |    |   |   |   |
| *Burkholderia*          |   |   |    |   |   |   |
| *Butyrivibrio*          |   |   |    |   |   |   |
| *Campylobacter*         |   |   |    |   |   |   |
| *Capnocytophaga*        |   |   |    |   |   |   |
| *Chryseobacterium*      |   |   |    |   |   |   |
| *Comamonas*             |   |   |    |   |   |   |
| *Cronobacter*           |   |   |    |   |   |   |
| *Delfia*                |   |   |    |   |   |   |
| *Enhydrobacter*         |   |   |    |   |   |   |
| *Enterobacter*          |   |   |    |   |   |   |
| *Escherichia*           |   |   |    |   |   |   |
| *Flavobacterium*        |   |   |    |   |   |   |
| *Haemophilus*           |   |   |    |   |   |   |
| *Halomonas*             |   |   |    |   |   |   |
| *Idiomarina*            |   |   |    |   |   |   |
| *Inimichthium*          |   |   |    |   |   |   |
| *Jannaschobacterium*    |   |   |    |   |   |   |
| *Klegbeiella*           |   |   |    |   |   |   |
| *Labrya*                |   |   |    |   |   |   |
| *Marinobacter*          |   |   |    |   |   |   |
| *Methylbacterium*       |   |   |    |   |   |   |
| *Mesorella*             |   |   |    |   |   |   |
| *Neteisera*             |   |   |    |   |   |   |
| *Odoribacter*           |   |   |    |   |   |   |
| *Parabacteroides*       |   |   |    |   |   |   |
| *Paracoccus*            |   |   |    |   |   |   |
| *Paraprevotella*         |   |   |    |   |   |   |
| *Parasutterella*        |   |   |    |   |   |   |
| *Pasteurella*           |   |   |    |   |   |   |
| *Pedobacter*            |   |   |    |   |   |   |
| *Pelomonas*             |   |   |    |   |   |   |
| *Phenylobacterium*      |   |   |    |   |   |   |

5. Conclusion

Inter-ecosystem comparisons suggest that the human skin communities possess strong capacities for interacting with their environment.
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