Comparative genomics reveals distinct host-interacting traits of three major human-associated propionibacteria

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Abstract

Background: Propionibacteria are part of the human microbiota. Many studies have addressed the predominant colonizer of sebaceous follicles of the skin, Propionibacterium acnes, and investigated its association with the skin disorder acne vulgaris, and lately with prostate cancer. Much less is known about two other propionibacterial species frequently found on human tissue sites, Propionibacterium granulosum and Propionibacterium avidum. Here we analyzed two and three genomes of P. granulosum and P. avidum, respectively, and compared them to two genomes of P. acnes; we further highlight differences among the three cutaneous species with proteomic and microscopy approaches.

Results: Electron and atomic force microscopy revealed an exopolysaccharide (EPS)-like structure surrounding P. avidum cells, that is absent in P. acnes and P. granulosum. In contrast, P. granulosum possesses pili-like appendices, which was confirmed by surface proteome analysis. The corresponding genes were identified; they are clustered with genes encoding sortases. Both, P. granulosum and P. avidum lack surface or secreted proteins for predicted host-interacting factors of P. acnes, including several CAMP factors, sialidases, dermatan-sulphate adhesins, hyaluronidase and a SH3 domain-containing lipoprotein; accordingly, only P. acnes exhibits neuraminidase and hyaluronidase activities. These functions are encoded on previously unrecognized island-like regions in the genome of P. acnes.

Conclusions: Despite their omnipresence on human skin little is known about the role of cutaneous propionibacteria. All three species are associated with a variety of diseases, including postoperative and device-related abscesses and infections. We showed that the three organisms have evolved distinct features to interact with their human host. Whereas P. avidum and P. granulosum produce an EPS-like surface structure and pili-like appendices, respectively, P. acnes possesses a number of unique surface-exposed proteins with host-interacting properties. The different surface properties of the three cutaneous propionibacteria are likely to determine their colonizing ability and pathogenic potential on the skin and at non-skin sites.

Keywords: Cutaneous propionibacteria, Propionibacterium acnes, Propionibacterium granulosum, Propionibacterium avidum, Exopolysaccharide, Pilus/pili, Surfome
parasites or opportunistic pathogens. The pathogenic side of cutaneous propionibacteria, in particular *P. acnes* is slowly gaining attention. Apart from its possible role in acne vulgaris due to its immunostimulatory property, *P. acnes* has been associated with a number of other diseases [8,9]. Recently, *P. acnes* were found in diseased prostatic tissue [5,6,10], and its contribution to prostate pathologies is currently under investigation. In our previous study, we isolated *P. acnes*, *P. avidum* and *P. granulosum* from radical prostatectomy specimens [10]. Little is known about the association of *P. avidum* and *P. granulosum* with human diseases. *P. avidum* has been found to cause abscess formation, in particular after surgical intervention; it has been described as the cause of abdominal wall and intra-peritoneal, perianal, psoas, splenic, and breast abscesses [11-13]. The disease association of *P. granulosum* is less clear, though it has been found in a few cases of endocarditis and endophthalmitis, and has been associated, like *P. acnes*, with sarcoidosis [14,15].

It is not understood if all cutaneous propionibacteria have similar disease-causing potentials. The genome sequence of *P. acnes* and subsequent studies have highlighted host-interacting factors such as CAMP factors, hemolysins, sialidases and dermatan-sulphate adhesins [16-20]. To date it is not clear if these factors are shared in all cutaneous propionibacteria. Here, we provide genomic insight into *P. avidum* and *P. granulosum*, and compare these genomes to *P. acnes*. We also performed electron microscopy and atomic force microscopy analysis on these species to further highlight differences among cutaneous propionibacteria. Together with proteomic data, our study highlights the individuality of each of the three human-associated propionibacterial species. In particular, the distinct surface structures suggest that each species interacts differently with the human host, which likely results in distinct pathogenic potentials.

**Results**

**Comparative genome analysis of cutaneous propionibacteria**

Seven genomes were analyzed and compared, two of each species, *P. acnes* (strains 266 and KPA) and *P. granulosum* (strains DSM20700 and TM11), and three genomes of *P. avidum* (44067, ATCC25577 and TM16). Four genomes were available from public databases (*P. acnes* KPA171202 (KPA) (GenBank: AE017283) [16], *P. acnes* 266 (GenBank: CP002409) [21], *P. avidum* 44067 (CP005287) [22] and *P. avidum* ATCC25577 (GenBank: NZ_AGBA00000000)) and we draft sequenced three additional ones (*P. granulosum* DSM20700 and TM11) and *P. avidum* TM16. *P. granulosum* TM11 and *P. avidum* TM16 were both isolated from radical prostatectomy specimens [10], and *P. avidum* 44067 was isolated from a human skin abscess [22]. *P. avidum* ATCC25577 and *P. granulosum* DSM20700 are both type strains.

First analysis revealed the much smaller genomes of the two *P. granulosum* strains, which is in average 400 kb smaller than *P. acnes* and *P. avidum* (Additional file 1). A bidirectional Blast revealed the core genome and species-specific genes of cutaneous propionibacteria (Figure 1; Additional file 2). 1380 proteins are common to all three species (Figure 1b). KEGG analysis showed that this core genome encodes main metabolic pathways, including the propionate formation pathway, the respiratory chain and the fatty acid metabolism (data not shown). *P. acnes* proteins encoded by the core genome show in average 89% and 73% identity to homologs of *P. avidum* and *P. granulosum*, respectively. This is in agreement with phylogenetic analyses based on 16S rRNA gene sequences, showing that *P. acnes* and *P. avidum* are closely related, whereas *P. granulosum* is more distant (data not shown).

Larger and smaller species-specific genomic islands were identified (Figure 1a; Additional files 2 and 3). Most of these are associated with a significant divergence from the main G + C content, which could indicate horizontal gene transfer (HGT) events. For example, the two genomes of *P. acnes* contain larger regions (>10 kb) not present in *P. avidum* and *P. granulosum*; these encode among others non-ribosomal peptide synthetases (PPA1277-PPA1307), and harbor genes for nitrate reductase and anaerobic dimethyl sulf-oxide reductase (PPA0497-PPA0520) (Additional file 2a). Other interesting results of comparative genome analyses are reported in the next sections.

**Absence of host-interacting, putative virulence factors in the genomes of *P. avidum* and *P. granulosum***

Genome sequencing of *P. acnes* revealed the existence of five Christie-Atkins-Munch-Petersen (CAMP) factors [16,17]. At least three of these, CAMP factors 1, 2 and 4, are produced as either secreted or surface-exposed proteins [17,23]. It has been shown that at least CAMP factor 2 has properties of a co-hemolysin and exotoxin [24,25]. Genome analysis revealed now that the genes camp1, camp2 and camp4 are absent from the genomes of *P. avidum* and *P. granulosum*. The latter genome carries only one CAMP factor gene, designated here CAMP factor 6 (H640_02108 in strain TM11; H641_03053 in strain DSM20700), since it has no strong similarity to one of the five CAMP factors of *P. acnes*. *P. avidum* contains two CAMP factor genes with high similarities to CAMP factors 3 and 5, respectively (HMPREF9153_0708 and HMPREF9153_1759 in strain ATCC25577). Interestingly, when comparing the genomic regions containing the camp1, camp2 and camp4 genes in *P. acnes* with the genome of *P. avidum*, we noticed that the CAMP factor genes are encoded on genomic island-like regions (Figure 2). For example, the camp2 gene is inserted as part of a six-genel...
cluster into the backbone genome. This cluster also contains two genes encoding sialidases (PPA0684, PPA0685) and a sialic acid transporter (PPA0686). The camp1 containing region is replaced in P. avidum with a region of eight genes encoding a putative arsenate reductase and transposases, underlining the mobile nature of this genomic region. The camp4 region is replaced in P. avidum with an island-like region of 13 genes, encoding among others a type I restriction-modification system.

Besides the camp genes also other predicted host-interacting factor-encoding genes of P. acnes are absent from the genomes of P. avidum and P. granulosum,
including a hyaluronate lyase (PPA0380), the two dermatan-sulphate adhesins DsA1 and DsA2 [18,19], and other proline-threonine repeat-motif proteins (PPA1880, PPA1715) (Additional file 4). Furthermore, the characterized sialidase (PPA1560) that has been shown to have a role in \textit{P. acnes} adhesion and cytotoxicity [20], is truncated in \textit{P. avidum} ATCC25577 and absent from \textit{P. avidum} 44067 and \textit{P. granulosum} DSM20700 and TM11. We tested the sequenced strains for hyaluronidase and neuraminidase activities: only the \textit{P. acnes} strains were positive (data not shown), thus confirming findings from genome analyses.

\textbf{P. avidum produces an exopolysaccharide-like structure}

The search for species-specific genomic regions identified a large genomic island, present in all \textit{P. avidum} genomes (HMPREF9153_1223 to HMPREF9153_1257 in strain ATCC25577; PALO_09550 to PALO_09690 in strain 44067), that is absent from \textit{P. acnes} and \textit{P. granulosum} (Additional file 2b). This region harbors 35 genes (in strain ATCC25577), 19 of them encode glycosyl transferases and enzymes involved in mono- or polysaccharide modification. We noticed a similarity of this cluster to a 20-gene cluster found in the genome of \textit{Rothia mucilaginosa}, a Gram-positive, coagulase-negative coccus that is part of the commensal flora of the oral cavity and the upper respiratory tract in humans (Additional file 5a). \textit{R. mucilaginosa} produces a thick exopolysaccharide (EPS)-like structure [26]; thus, we applied electron microscopy to examine the cell morphology of \textit{P. avidum}. We observed a meshwork structure surrounding cells of \textit{P. avidum} that was absent from \textit{P. acnes} and \textit{P. granulosum} (Figure 3), and that is very similar to the meshwork structures surrounding \textit{R. mucilaginosa}.

Atomic force microscopy further confirmed the presence of an extracellular structure of \textit{P. avidum} extending several μm from the cell surface (Figure 4). The AFM imaging excludes the possibility of artifacts caused by the vacuum environment of the electron microscope, but the structure did collapse on to the cell surface and underlying glass substrate upon gentle air drying of the sample. It is probably a highly hydrated and amorphous material when in aqueous solution, as it appears more amorphous rather than filamentous in structure at the cell surface where
some level of hydration remains. To investigate the composition of this structure, we used specific fluorescent stains for DNA and for carbohydrates (Additional file 5b), which revealed that the structure is made of polysaccharides containing β-1,4 or β-1,3 bonds.

P. granulosum possesses pili-like appendices
Electron microscopy further revealed pili- or fimbriae-like appendices on the cell surface of P. granulosum (Figure 3C). Such structures were not detected on the surface of P. acnes and P. avidum. Pili or fimbriae are found in several Gram-positive bacteria, including some species of the related genus Corynebacterium [27]. In Corynebacterium sp., pilin subunits are encoded in several gene clusters that also contain genes for pilin-specific sortases. In a search for such gene clusters in the genome of P. granulosum we found two gene clusters that encode multiple sortases (designated SrtA-D; H641_03530, H641_03545, H641_05823, H641_05838 in strain DSM20700) and homologs of several pilin subunits of Corynebacterium ulcerans and Corynebacterium resistens, respectively (Table 1). The SpaD homolog could represent the major subunit of the pilus, and the SpaB homolog could be a minor pilin, in analogy to the SpaD-type pilus of Corynebacterium diphtheria [27].

Secreted and surface-associated proteins of P. avidum, P. granulosum and P. acnes
We determined main secreted and surface-associated proteins of the three cutaneous propionibacteria, since such proteins could mediate the contact with human tissue sites, and could reveal host-interacting strategies. For determining secreted proteins, we collected culture supernatants, precipitated the secreted proteins and identified prominent bands on a 1D-SDS-PAGE gel by mass spectrometry (Additional file 6). For P. avidum ATCC25577 the main secreted protein, under the applied growth conditions, is a triacylglycerol lipase that is 48% similar to GehA (PPA2105), a characterized lipase of P. acnes. Furthermore, a homolog of CAMP factor 3 and several proteins of unknown function could be detected in the supernatant of P. avidum ATCC25577 (Additional file 7).
All identified eight secreted proteins of *P. avidum* have a homolog in *P. acnes*; five of eight homologs have also been detected in *P. acnes* supernatants [23]. *P. granulosum* DSM20700 abundantly secretes two proteins, with peaks in the beginning of the stationary phase: two predicted lysophospholipases that are 53% and 56% similar to a putative lysophospholipase (PPA2142) of *P. acnes* (Additional files 6 and 7). Homologs of all secreted proteins in *P. granulosum* have been detected in culture supernatants of *P. acnes* [23]. Homologs of one protein (PPA0532) are secreted by all cutaneous propionibacteria; the function of PPA0532 is unknown. Our previous study has shown that *P. acnes*, regardless of the specific subtype, abundantly secretes CAMP factor 2, a putative lysozyme (PPA1662), an endoglycoceramidase (PPA0644), and a protein of unknown function (PPA1939) [23]. Homologs of none of these proteins were detected in culture supernatants of *P. avidum* ATCC25577 or *P. granulosum* DSM20700; the corresponding genes are absent from their genomes (Figure 2; Additional file 2a). This indicates that *P. acnes* secretes a unique set of factors.

The surface-exposed proteins were determined by a surfome-approach that is based on trypsin cleavage of surface-exposed protein moieties [28]. In all three *Propionibacterium* species the most abundantly detected surface-attached proteins were RlpA-domain containing lipoproteins (Table 1; Additional file 7). Two such proteins (PPA2175 and PPA2271) were detected on the surface of *P. acnes*. PPA2175 is exclusively produced by *P. acnes*; it contains a bacterial SH3 and a peptidoglycan-binding domain (Figure 6). The corresponding gene is absent from the genomes of *P. avidum* and *P. granulosum*. Homologs of the other lipoprotein, PPA2271, were also abundantly detected on *P. granulosum* DSM20700 (H641_02938) and *P. avidum* ATCC25577 (HMPREF9153_0971). Additional species-specific surface-attached factors were identified, e.g. CAMP factor 6 of *P. granulosum*. In *P. acnes*, CAMP factors 1 and 2 were detected as well as two endoglycoceramidases and two GroEL chaperonins (Additional file 7). The duplication of surface-exposed factors points to some redundancy, and could indicate that the duplicated factors are of high importance for *P. acnes*. 

Figure 4 Atomic force microscopy of *P. avidum* ATCC25577. A) Amplitude image of the overall morphology of the cells. Extracellular structures are clearly visible, extending out from the cell surface. B) and C) Height images showing extracellular structures collapsed onto the glass coverslip. The fiber-like structures measure 1-3 nm in diameter. X-Y scalebar = 1 μm, z scale is indicated by color.
Discussion

Here, we analyzed and compared the genomes of the three propionibacterial species known to colonize the human skin. Species-specific gene clusters were identified in each genome that encode traits for colonization and host-interaction. Applying high-resolution microscopy and proteomic approaches we could verify the production of these surface-associated functions.

*P. avidum* was found to be surrounded by an EPS-like meshwork. A gene cluster that encodes proteins involved in the biosynthesis and modification of EPS was identified. The cluster encodes several homologs for enzymes involved in LPS and EPS biosynthesis (RfbA, RfbB, RfbD, RfaG, ExoU, NeuA, NeuB) as well as a number of glycosyl transferases with unknown specificities. RfbA, B, D are found in *Lactococcus lactis*, and required for dTDP-rhamnose biosynthesis, which is an important precursor of rhamnose-containing exopolysaccharides [29]. The genes neuA and neuB are found in the LPS biosynthesis gene clusters of several Gram-negative species. NeuA and NeuB have been shown to be important in polysialic acid capsule biosynthesis [30]. The *P. avidum* EPS gene cluster lacks a gene for a flippase, indicating that the EPS structure is formed on the outside of the cell. Interestingly, the cluster also contains genes involved in trehalose biosynthesis (TreY-TreZ pathway). The disaccharide trehalose can protect cells from environmental stresses such as low water availability [31]. 20 of the 35 genes have a homolog in *R. mucilaginosa* that produces a mucilaginous capsular material [26]. Like *P. avidum*, *R. mucilaginosa* is occasionally isolated from disease sites, thus regarded as an opportunistic pathogen, for instance involved in prosthetic device infections. Well-studied EPS in other bacteria, such as in *Pseudomonas aeruginosa*, have several roles in pathogenicity; EPS contributes to biofilm formation, adherence to surfaces and host cells, evasion of phagocytosis, and elicitation of immune response [32-34]. We hypothesize that the EPS structure of *P. avidum* could have a role in biofilm formation, and thereby contribute to its pathogenicity by leading to persistent infections that cannot be cleared by the immune system. This might explain why *P. avidum* is in particular recognized in abscess formation after surgical intervention [11-13,22]. It should be noted that several studies reported the presence of a cell wall-associated polysaccharide of *P. acnes* that can partially be extracted by phenol extraction [35-37]. Such a cell wall polysaccharide was further described as a lipidated macroamphiphile; this lipoglycan cell envelope component of *P. acnes* was found to have a lipid anchor and a polysaccharide moiety containing mannose, glucose and galactose, and probably diaminohexuronic acid [38]. We strongly suspect that the lipoglycan of *P. acnes* is distinct from the EPS of *P. avidum*. We tested different strains of *P. acnes* grown under different conditions and could not

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**Figure 5** Fimbriae/Pili-encoding loci in the genome of *P. granulosum*. **A and B)** Two loci in the *P. granulosum* genome encode sortases (designated here SrtA-D). These genes are clustered with genes encoding proteins similar to fimbriae/pilin subunits of *Corynebacterium* sp. (designated here SpaB, SpaC, SpaD, SpaF and SpaI, based on their sequence similarity to pilin subunits of *Corynebacterium diphtheriae*). These proteins carry a C-terminal LPXTG motif (boxed genes). The two genetic loci are absent from the genomes of *P. acnes* KPA and 266 and *P. avidum* ATCC25577 and 44067. *P. granulosum* TM11 and *P. acnes* KPA were used for this illustration; the genomes of *P. granulosum* DSM20700 and *P. acnes* 266 are highly similar in the depicted genomic regions. Same colors and numbers depict homologies between CDS of different species.
detect any EPS-like structure by EM analyses (data not shown). To our knowledge no study so far could visualize an EPS-like meshwork on \textit{P. acnes} cells. Moreover, the identified putative EPS biosynthesis genes of \textit{P. avidum} are absent from the genomes of \textit{P. acnes} (and \textit{P. granulosum}).

We found that \textit{P. granulosum} possesses pili/fimbriae-like appendices and pilin subunits were identified among cell surface-exposed proteins of \textit{P. granulosum}. We determined two gene clusters encoding pilin subunits in direct vicinity to genes encoding sortases. Such clustering of genes for sortase and pilin subunits has been reported for a number of Gram-positive bacteria, including related actinobacteria such as \textit{Corynebacterium diphtheriae} that produces three distinct pilus structures, SpaA-, SpaD- and SpaH-type pili [27]. In corynebacteria pilins are covalently polymerized and the formed pilus is anchored to the bacterial cell wall; these steps are catalyzed by pilin-specific and housekeeping sortases, respectively. It has been shown that minor pilins (SpaB/SpaC) represent the major adhesins of corynebacteria [27,39]. Thus, we hypothesize that pili of \textit{P. granulosum} could have a role in adhesion to human skin tissue and colonization. They might also have a role in forming a multispecies biofilm, since \textit{P. granulosum} and \textit{P. acnes} are often detected together within sebaceous follicles. The anchorage of the base of the pilus to the cell wall is usually mediated by a housekeeping sortase [27]. A likely candidate for this housekeeping sortase was identified among the surface-associated proteins: H641_09423.

Table 1 Selected surface-associated factors of \textit{P. avidum}, \textit{P. granulosum} and \textit{P. acnes} identified from surface proteome analysis

| Locus tag          | Size (kDa) | Function                                      | Score | Matches | \textit{P. acnes} homolog |
|--------------------|-----------|-----------------------------------------------|-------|---------|--------------------------|
| \textit{P. avidum} ATCC25577 |           |                                               |       |         |                          |
| HMPREF9153_0971    | 53.0      | Rare lipoprotein A (RlpA family)              | 747   | 18      | PPA2271 (76%)            |
| HMPREF9153_0397    | 33.6      | Glutamine/glutamate ABC superfamily ATP binding cassette Transporter | 601   | 15      | no                       |
| HMPREF9153_1303    | 60.9      | Peptidase (SpE)                               | 349   | 10      | PPA0247 (78%)            |
| HMPREF9153_0820    | 87.7      | Penicillin-binding protein PonA                | 321   | 4       | PPA2149 (89%)            |
| HMPREF9153_0967    | 26.2      | Hypothetical protein                          | 292   | 6       | no                       |
| \textit{P. granulosum} DSM20700 |         |                                               |       |         |                          |
| H641_02938         | 63.1      | Rare lipoprotein A (RlpA family)              | 1290  | 24      | PPA2271 (39%)            |
| H641_04819         | 36.9      | Hypothetical protein                          | 1159  | 19      | no                       |
| H641_08540         | 56.6      | Conserved hypothetical protein                 | 1077  | 13      | no                       |
| H641_04393         | 61.1      | Hypothetical protein                          | 921   | 15      | PPA0444 (72%)            |
| H641_03802         | 41.2      | Rare lipoprotein A (RlpA family)              | 803   | 11      | PPA2239 (64%)            |
| H641_06353         | 84.5      | Phosphoesterase                               | 540   | 9       | PPA1745 (66%)            |
| H641_03053         | 29.4      | CAMP factor 6                                 | 533   | 8       | PPA0687 (41%)            |
| H641_07095         | 41.2      | Putative lysophospholipase                     | 509   | 7       | PPA2142 (56%)            |
| H641_05818         | 60.2      | SpaD homolog                                  | 375   | 6       | no                       |
| H641_03535         | 42.2      | SpaB homolog                                  | 222   | 3       | no                       |
| \textit{P. acnes} KPA171202 |        |                                               |       |         |                          |
| PPA2175            | 36.5      | Rare lipoprotein A (RlpA family)              | 2923  | 47      |                          |
| PPA2271            | 52.2      | Rare lipoprotein A (RlpA family)              | 2666  | 42      |                          |
| PPA0644            | 56.6      | Endoglycoceramidase                           | 1113  | 13      |                          |
| PPA2106            | 54.0      | Endoglycoceramidase                           | 1019  | 16      |                          |
| PPA0721            | 40.7      | NPL/P60 family secreted protein               | 674   | 12      |                          |
| PPA1939            | 16.8      | Hypothetical protein                          | 639   | 12      |                          |
| PPA2097            | 73.4      | 5'-nucleotidase/2',3'-cyclic phosphodiesterase or related esterase | 547   | 7       |                          |
| PPA0687            | 28.6      | CAMP factor 2                                 | 394   | 4       |                          |
| PPA2105            | 35.9      | Triacylglycerol lipase precursor              | 353   | 4       |                          |
| PPA2239            | 41.0      | Lipoprotein A-like protein                    | 342   | 5       |                          |
| PPA1340            | 30.3      | CAMP factor 1                                 | 230   | 3       |                          |

Listed are all surface-exposed proteins that possess a typical N-terminal signal peptide. See Additional file 7 for a complete list.
(strain DSM20700), a protein with a sortase E domain. A homolog exists in *P. acnes* KPA (PPA0777) and in *P. avidum* ATCC25577 (HMPREF9153,2132). These sortases likely catalyze the anchoring of other LPXTG-motif proteins of the three propionibacterial species to their cell walls. A genome search revealed that *P. avidum* contains 12, *P. acnes* 15, and *P. granulosum* 18 proteins (including 7 putative pilin subunits) with a C-terminal LPXTG motif. Most of these LPXTG-motif proteins have no or little similarity to known proteins, exceptions are proteins with nucleotidase or phosphoesterase domains. Only few of these LPXTG-motif proteins have been identified in the surfome. That could either indicate that they were not or weakly expressed under the applied growth conditions (liquid culture, complex broth), or were not accessible for trypsin cleavage.

Analysis of the surfome data of *P. granulosum* further revealed the presence of several cytosolic proteins, including ribosomal proteins and those involved in core metabolic functions (methylmalonyl-CoA:pyruvate transcarboxylase 12S subunit; two methylmalonyl-CoA mutases; fumarate hydratase class II; succinyl-CoA ligase). That indicates that *P. granulosum* seems to be more sensitive to trypsin treatment or lyse earlier than *P. avidum* and *P. acnes*.

*P. acnes* has, unlike *P. granulosum* and *P. avidum*, no obvious surface appendages. However, *P. acnes* is by far the most prevalent bacterium in sebaceous follicles of the face and back [2,7]. Thus, this species must have evolved a different strategy to adhere to and colonize human tissues. Surface proteins could act as powerful adhesins. Indeed, the dermanan-sulphate adhesins DsA1 and DsA2 have been identified and partially characterized in *P. acnes* [18,19]. These were not found in the surfome of the strain KPA, most likely because the respective genes are phase variable, but DsA1 and DsA2 are present on the surface of the type la strain 266 (data not shown). In addition, the surfome data revealed an abundance of lipoproteins with RlpA (rare lipoprotein A) domains on the surface of *P. acnes*. The bacterium specifically produces PPA2175 on the surface; it contains a SH3 and a peptidoglycan-binding domain (Figure 6). Bacterial lipoproteins have diverse functions; they play roles in a wide range of physiological processes. They can also function as ligands of the innate immunity host cell receptor Toll-like receptor 2 (TLR2), thus triggering an innate immune reaction [40]. It has been reported that TLR2 was sufficient for NF-kappaB activation in response to *P. acnes* and activation of TLR2 resulted in an inflammatory cytokine response, which is thought to be of crucial importance in acne vulgaris [41,42]. The TLR2 ligand of *P. acnes* is so far unknown. We speculate that one or all of the surface-exposed RlpA-domain lipoproteins of *P. acnes* are TLR2 ligands. These lipoproteins are abundantly produced on the surface of *P. acnes* and they are not covered or protected from host cell contact by other surface structures, such as EPS or pili in *P. avidum* and *P. granulosum*, respectively. It will be interesting to investigate if *P. avidum* and *P. granulosum* are also able to trigger TLR2 responses, or if this is specific to *P. acnes*.

The colonization of human skin by *P. acnes* can be achieved by other strategies, such as factors that allow successful competition with other bacteria, including *P. avidum* and *P. granulosum*. Successful competition might include the efficient acquisition of nutrients from host components. In this respect, only *P. acnes* expressed surface-attached endoglycoceramidases, which might hydrolyze gangliosides on host cell membranes [43]. Another specific feature of *P. acnes* is the presence and
the production of surface-exposed CAMP factors 1 and 2. It has been shown that CAMP factor 2 has properties of a co-hemolysin [17,24,25,44]. Moreover, inhibition of CAMP2 by neutralizing antibodies efficiently attenuated P. acnes-induced inflammation in the mouse ear model [45], suggesting that CAMP2, and probably the other ones as well, are virulence factors of P. acnes. The corresponding camp1 and camp2 genes are absent in the genomes of P. granulosum and P. avidum. CAMP factors have been partially characterized in streptococcal species as co-hemolysins and pore-forming toxins [46]. They are involved in the CAMP reaction, the lysis of sheep erythrocytes by the synergistic action of the sphingomyelinase C from S. aureus and CAMP factor from Group B Streptococcus strains [47]. The sphingomyelinase initially hydrolyzes sphingomyelin to ceramide (and phosphocholine) on the erythrocyte membrane, which renders the erythrocytes susceptible to the lytic activity of CAMP factor. It was recently shown that CAMP factor 2 of P. acnes can act as an exotoxin, exhibiting cytotoxic activity on host cells [25].

The study of Nakatsuji et al. further suggests that CAMP factor 2 acts together with host acid sphingomyelinase to amplify bacterial virulence, thus supporting the degradation and invasion of host cells. The gene for CAMP factor 2 is located within a small gene cluster that also contains genes encoding sialidases and a sialic acid transporter. This cluster seems to be inserted into the P. acnes genome (Figure 2). It is tempting to suggest a functional connection of these factors as host-interacting and/or virulence traits. One possible scenario is that sialidases act directly on host cell membrane exposed gangliosides, thus releasing terminal sialic acid residues that are taken up by the sialic acid transporter and used as energy source. The remaining ceramide moiety could be a binding site for CAMP factor 2, in analogy to the CAMP reaction. Another component in this scenario could be surface-associated endoglycoceramidases of P. acnes that is predicted to hydrolyze gangliosides on host cell membranes into ceramides and oligosaccharides.

Important questions remain to be answered, in particular regarding the host tissue and host cell interactions of these three species. Although all three species are colonizing human skin, it is not known if these species actually compete at those sites or have adapted to occupy unique niches through species-specific host interactions. The different surface properties of the three species suggest that they have different colonization strategies that could be host cell or tissue-specific at skin and non-skin sites.

Conclusions

Taken together, comparative genome analysis showed that CAMP factor 1 and 2 and other host-interacting traits (e.g. DsA1, DsA2, hyaluronate lyase, endoglycoceramidase, sialidase, linoleic acid isomerase) are encoded on smaller genomic regions that are either inserted into the genome of P. acnes or deleted from the genomes of P. avidum and P. granulosum. That might explain the greater versatility of P. acnes to interact with the human host, P. avidum and to a minor extent P. granulosum have their own species-specific genomic regions, that are absent from P. acnes. Among these are the EPS cluster of P. avidum and the pili/fimbriae gene clusters of P. granulosum. Thus, human-associated propionibacteria have evolved different host-interacting strategies which are likely linked to different disease-causing potentials of these three species.

Methods

Bacteria strains and culture

P. avidum strain ATCC25577 and P. granulosum strain DSM20700 were obtained from DSMZ (German Collection of Microorganisms and Cell Cultures). P. acnes KPA171202 (KPA, type 1-2) and P. acnes 266 (type IA) were previously isolated [16,21]. P. avidum TM16 and P. granulosum TM11 were isolated from radical prostatectomy specimens in our previous study [10]. All strains were cultured on Reinforced Clostridial Agar (Oxoid) plates for 3 days at 37°C under anaerobic conditions. For liquid cultures, plate-grown bacteria were resuspended and washed in brain heart infusion (BHI) broth (Sigma-Aldrich); BHI broth was inoculated with P. avidum ATCC25577 and P. granulosum DSM20700 (OD600 0.01) and cultures were grown to exponential (OD600 0.3-0.4) and stationary phases (OD600 0.9-1.2) at 37°C under anaerobic conditions using the Gas-Pak™ system (Oxoid). To ensure identical growth conditions due to the usage of the Gas-Pak system, all strains were cultured in the same GasPak container and the same BHI batch was used.

DNA extraction and genome sequencing

Genomic DNA from all strains of P. avidum TM16 and P. granulosum DSM20700 and TM11 were extracted using the MasterPure™ Gram Positive DNA Purification Kit (Epicentre). The genomes were draft sequenced using Illumina/Solexa GAIIx machines at the Beijing Genomics Institute (BGI) (Shenzhen, China). The whole genome shotgun projects have been deposited at DDBJ/EMBL/GenBank under the accession numbers AOUA00000000 for P. avidum TM16, AOST00000000 for P. granulosum TM11 and AOS00000000 for P. granulosum DSM20700. The versions described in this paper are the first versions, AOUA01000000, AOST00000000 and AOS01000000, respectively.

Sequence analysis

Automatic annotations were performed by PGAAP, NCBI Prokaryotic Genome Automatic Annotation Pipeline [48]. To identify homologs in different species,
comparisons were done with a protein sequence-based bidirectional BLAST approach (blastP version 2.2.18). Sequence homologies were only mentioned in this study for proteins with an amino acid identity of >25% and an overlap of the query and subject sequence of >75%. Genome representations were created by DNA plotter (Sanger Institute). For nucleotide sequence comparisons the Artemis Comparison Tool (ACT) was used [49]. To identify genomic islands the Island Viewer was used, a computational tool that integrates three different genomic island prediction methods, i.e. IslandPick, IslandPath-DIMOB, and SIGI-HMM [50]. For the analysis and mapping of pathways of P. acnes KEGG and KEGG MAPPER were used (http://www.genome.jp/kegg/).

Scanning electron microscopy
Bacterial cells were fixed with 2.5% glutaraldehyde, post-fixed using repeated incubations with 1% osmium tetroxide/1% tannic acid, dehydrated with a graded ethanol series, critical-point dried and coated with 2 nm platinum. After dehydration and critical-point drying, the specimens were coated with 5 nm platinum/carbon and analyzed in a Leo 1550 scanning electron microscope.

Atomic force microscopy, fluorescence staining and confocal microscopy
A NanoWizard II atomic force microscope (JPK Instruments, Germany) combined with an inverted optical microscope (Zeiss Axiovert 200 M, Zeiss, Germany) was used to record AFM images at 512 pixels per line, with 1 Hz scanning speed. Tapping mode in air was performed for imaging, using OMCL-AC160TS cantilevers (Olympus) with spring constant of 26 N/m. A glass coverslip was immersed in bacterial cells suspended in water, gently rinsed with water, briefly dried in air, and mounted for AFM imaging of the cells.

Staining of DNA and carbohydrates in the EPS was done by suspending bacterial cells in PBS and staining simultaneously with Calcofluor white (100 μg/ml, Sigma-Aldrich) and propidium iodide (0.05 mM, Invitrogen) for 30 min. After one wash in PBS, the samples were resuspended with PBS and visualized using a confocal laser scanning microscope (LSM 700, Carl Zeiss), using 405 nm excitation for Calcofluor white, and 555 nm excitation for propidium iodide.

Surfome analysis
The protocol for bacterial surface digestion was adapted from Doro et al. [28]. P. avidum strain ATCC25577, P. acnes KPA and P. granulosum strain DSM20700 strains were grown to OD600 0.3 - 0.4. Bacterial cells were harvested by centrifugation at 3,500 × g for 10 min at 4°C and washed twice with PBS. Cells were resuspended in 800 μl of PBS containing 40% sucrose. Digestions were carried out with 10 μg of trypsin (Promega) for 30 min at 37°C. Bacterial cells were centrifuged at 3,500 × g for 10 min at 4°C and the supernatants were filtered through 0.22-μm pore size filters (Millipore). Protease reactions were stopped with formic acid at 0.1% final concentration. Before protein identification, PBS and sucrose were removed using ZipTip C18, 0.6 μl bed volume (Millipore). Peptides were eluted with 5 μl 60% ACN, 0.1% TFA followed by 5 μl 80% ACN, 0.1% TFA. The combined eluates were concentrated using a Microconcentrator 5301 (Eppendorf) and kept at −20°C until further analysis.

Protein identification by UPLC/MS/MS
The samples were solubilized in 12 μl 2.98 (v/v) acetonitrile/water containing 0.1% TFA (v/v). After concentration on a
Acclaim PepMap 100 trap column at a flow rate of 5 μl/min (75 μm x 2 cm, C18, 3 μm, 100 Å, Thermo Scientific) separation was performed by UHPLC (UltiMate 3000, Dionex) using an Acclaim PepMap RSLC column at a flow rate of 300 nl/min (75 μm x 150 mm, C18, 2 μm, 100 Å, Thermo Scientific). Mobile phase A was 0.1% (v/v) TFA and B was 80:20 (v/v) acetonitrile/water containing 0.08% (v/v) TFA. The elution gradients were 3-15% B for 2 min, 15-60% B for 60 min, 60-98% B for 4 min, 98% B for 2 min and 98-3% B for 3 min. 312 fractions per sample were spotted onto a MALDI template using a Probot microfraction collector (Dionex). Spotting frequency was 10 seconds and α-cyano-4-hydroxycinnamic acid (0.5%, in 70:30 (v/v) acetonitrile/water containing 0.1% (v/v) TFA) was added at a flow rate of 1 μl/min. Mass spectra were acquired with a 4700 Proteomics Analyzer (Applied Biosystems) MALDI-TOF/TOF instrument. The MS mass range was 800–4000 Da. MS/MS precursor selection was performed automatically; using the 4000 Series Explorer Software 3.6 and a maximum of 7 MS/MS measurements per spot were possible. MS/MS data were searched against a manually created propionibacteria database using MASCOT 2.3 (Matrix Science) allowing a peptide mass tolerance of 100-150 ppm and 0.3 Da for the fragment mass tolerance. Enzyme specificity was set to none. N-acetyl (Protein), oxidation (M), pyro-glu (N-term Q) were considered in these searches and standard scoring and ions score cut-off 30 were used for data evaluation.

The criterion for the identification of a protein was a minimum number of 3 peptides fulfilling the Mascot homology criteria.

Availability of supporting data
The Whole Genome Shotgun projects have been deposited at DDBJ/EMBL/GenBank under the accession AOST00000000 (http://www.ncbi.nlm.nih.gov/bioproject/PRJNA189037), AOS50000000 (http://www.ncbi.nlm.nih.gov/bioproject/PRJNA189038) and AOUA00000000 (http://www.ncbi.nlm.nih.gov/bioproject/PRJNA189036). Other supporting data are included as Additional files 1, 2, 3, 4, 5, 6 and 7.

Additional files

Additional file 1: Genome features of the propionibacterial species P. avidum, P. granulosum and P. acnes.

Additional file 2: A) Bidirectional Blast of all CDS of P. acnes KPA against other genomes of P. acnes, P. granulosum and P. avidum. B) Bidirectional Blast of all CDS of P. avidum 44067 against other genomes of P. avidum, P. acnes and P. granulosum. C) Bidirectional Blast of all CDS of P. granulosum TM11 against other genomes of P. granulosum, P. acnes and P. avidum. The color code represents the Blast e-values: White: e<20, Light yellow: e<20 and >e50, Gold: e<50 and >e90, Light orange: e<90 and >e100, Orange: e<100 and >e120, Red: e>120.

Additional file 3: Comparative genome analysis of three cutaneous propionibacteria. A) Genome comparison with P. granulosum TM11 as the reference genome. B) Genome comparison with P. acnes KPA as the reference genome. Color code: CDS of P. granulosum TM11, marine; P. granulosum DSM20700, green; P. avidum ATCC25577, red; P. avidum TM16, orange; P. acnes KPA, blue; P. acnes 266, light blue. The inner ring (in purple and olive) represents the G + C content distribution of the reference genome (window size 10000 bp, step size 200 bp). The most inner circle depicts predicted islands (in red) acquired by horizontal gene transfer (predictions from IslandViewer; results from two different algorithms are included: orange, Sigi-HMM; blue, IslandPath-DIMOB). P. granulosum and P. acnes harbors 10 and 5 genomic regions, respectively, that are predicted to be horizontally acquired.

Additional file 4: Genes encoding host-interacting proteins of P. acnes are absent from the genome of P. avidum. Shown are four examples of genomic regions encoding putative host-interacting proteins of P. acnes that differ or are deleted in the genome of P. avidum ATCC25577. A) This P. acnes-specific region encodes a hyaluronidase (PPA3638) and contains 9 genes (PPA3637-PPA3638); several of them encode oxidoreductases and one encodes a glycosyl transferase. B) DsA1 (PPA2127) is a dermatan-sulphate adenisin with proline-threonine repeats [18]. The corresponding P. acnes-specific gene is replaced in P. avidum ATCC25577 by a larger island, encoding mostly proteins with unknown functions. C) DsA2 (PPA2210) is another dermatan-sulphate adenisin that is encoded in a region of 12 P. acnes-specific genes that includes five genes putatively involved in carnitine catalbolism. D) PPA1560 encodes the characterized sialidase of P. acnes [19]. P. avidum strain ATCC25577 encodes a different sialidase (HMPREF9153_0188; 63% protein identity to PPA1560). In the genome of P. avidum 44067, the sialidase-encoding region is deleted (data not shown). Red bars/lines identify regions with high sequence similarity (>70%).

Additional file 5: P. avidum produces an EPS structure. A) The genome of P. avidum harbors a gene cluster for exopolysaccharide biosynthesis (HMPREF9153_1223 to HMPREF9153_1257 in strain ATCC25577 and PALO_09550 to PALO_09690 in strain 44067). Most of the genes encode glycosyltransferases. See Additional file 2b for the functional assignment of all CDS. A similar gene cluster exists in the genome of Rothia mucilaginosa, a Gram-positive bacterium producing a muclinsuglar capsular material. Same colors and numbers depict homologies between CDS of P. avidum and R. mucilaginosa. B) Staining experiments using calcofluor white and propidium iodide were performed, recorded by confocal microscopy (2-series, layers taken from the surface of the cells (top left) to the center of the cells (bottom right)), to confirm the existence of a polysaccharide structure surrounding P. avidum ATCC25577 cells. Blue, polysaccharide; red, DNA.

Additional file 6: Secreted proteins of P. avidum and P. granulosum. A) P. avidum ATCC25577 and B) P. granulosum DSM20700 were grown in BHI medium to exponential (E) and early stationary (S) phase. Secreted proteins were precipitated from culture supernatants and separated on a SDS-PAGE gel (12%). Abundant bands (numbered) were subjected to MS identification (see Additional file 7 for all identified proteins). Under the applied growth conditions, the most abundantly secreted proteins of P. avidum and P. granulosum are a triacylglycerol lipase (band 5 in section A) and two lysophospholipases (bands 1 and 2 in section B), respectively.

Additional file 7: Identification of secreted and surface-exposed proteins of P. avidum, P. granulosum and P. acnes.

Abbreviations
CAMP: Christie-Akins-Munch-Petersen; EPS: Exopolysaccharide.

Competing interests
The authors declare that they have no competing interests.

Authors’ contributions
TNM and HB conceptualized and designed the study and analyzed data. MS carried out mass spectrometry experiments and analyzed data. EB and HB analyzed the genomes. GZ and RM performed atomic force microscopy. KSS and TFM provided material and advice. VB performed electron microscopy. TNM and HB wrote the manuscript and all authors reviewed and edited the manuscript. All authors read and approved the final manuscript.
References
1. Poonnam, Pophaly SD, Tornar S, De S, Singh R: Multifaceted attributes of dairy propionibacteria: a review. World J Microbiol Biotechnol 2012, 28:3011–3015.
2. Grice EA, Segre JA: The skin microbiome. Nat Rev Microbiol 2011, 9:244–253.
3. Sharon I, Morowitz MJ, Thomas BC, Costello EK, Relman DA, Banfield JF: propionibacteria. Nat Rev Microbiol 2010, 8:565–577.
4. Fassi Fehri L, Mak TN, Laube B, Brinkmann V, Ogilvie LA, Mollenkopf H, Lein S, Dürre P, Gottschalk G: Staphylococcus aureus hijacks a skin commensal to intensify its virulence: immunomodulation targeting hemolysin and CAMP factor. J Invest Dermatol 2011, 131:401–409.
5. Nakatsuji T, Kang DC, Zhang L, Gallo RL, Huang CM: Propionibacterium acnes CAMP factor and host sphingomyelinase contribute to bacterial virulence: potential targets for inflammatory acne treatment. PLoS One 2011, 6:e17497.
6. Yamané K, Nambu T, Yamanaka T, Mashimo C, Sugimoto C, Leung RP, Fukushima M: Complete genome sequence of Rothia mucilaginosa DY-18: a clinical isolate with dense meshwork-like structures from a persistent apical periodontitis lesion. Sequencing 2010, 2010:45723.
7. Rogers EA, Das A, Ton-Thatch: Adhesion by pathogenic corynebacteria. Adv Exp Med Biol 2011, 715:91–103.
8. Doro F, Liberatiori S, Rodriguez-Ortega MJ, Rinoado CD, Rosini R, Mora M, Scarfelli M, Altilus E, D’Aulizio R, Stella M, Margaret I, Marvin D, Telford J, et al: Pathotyping, identification and functional characterization of the trehalose biosynthetic loci of Rothia mucilaginosa. J Med Microbiol 2011, 60:1156–1166.
9. Brzószkiewicz E, Weiner J, Wohlbach A, Hüpeden J, Lomholt HK, Kilian M, Gottschalk G, Daniel R, Mollenkopf HJ, Meyer TF, Brüggemann H: Comparative genomics and transcriptomics of Propionibacterium acnes. PLoS One 2011, 6:e21581.
10. Orduogh L, Hunaadkurti J, Viotti A, Onahsuch B, Szuca A, Uriban E, Kerebreit A, Kondoroshi E, Ngyi J: Complete genome sequence of Propionibacterium acnes strain 40467, isolated from a human skin abscess. Genome Announc 2013, in press.
11. Holland C, Mak TN, Zimny-Arndt U, Schmid M, Meyer TF, Jungblut PR, Brüggemann H: Proteomic identification of secreted proteins of Propionibacterium acnes. BMC Microbiol 2010, 10:230.
12. Lo CW, Lai YK, Liu YS, Gallo RL, Huang CM: Complete genome sequence of Propionibacterium acnes strain DSM 14407 isolated from a human skin abscess. Genome Announc 2013, in press.
38. Whale GA, Sutcliffe IC, Morrisson AR, Pretswell EL, Emmison N: Purification and characterisation of lipoglycan macroamphiphiles from Propionibacterium acnes. Antonie Van Leeuwenhoek 2004, 86:77–85.

39. Mandlik A, Swierczynski A, Das A, Ton-That H: Corynebacterium diphtheriae employs specific minor pilins to target human pharyngeal epithelial cells. Mol Microbiol 2007, 64:111–124.

40. Schmaler M, Jann NJ, Ferracin F, Landolt LZ, Biswas L, Götz F, Landmann R: Lipoproteins in Staphylococcus aureus mediate inflammation by TLR2 and iron-dependent growth in vivo. J Immunol 2009, 182:7110–7118.

41. Kim J: Review of the innate immune response in acne vulgaris: activation of Toll-like receptor 2 in acne triggers inflammatory cytokine responses. Dermatology 2005, 211:193–198.

42. Kim J, Ochoa MT, Krutzik SR, Takeuchi O, Tagaya T, Tsujimoto M, Takeuchi O, Uematsu S, Legaspi AJ, Brightbill HD, Holland D, Cunliffe WJ, Akira S, Sieling PA, Godowski PJ, Medin RL: Activation of Toll-like receptor 2 in acne triggers inflammatory cytokine responses. J Immunol 2002, 169:1535–1541.

43. Sakaguchi K, Okino N, Sueyoshi N, Izu H, Ito M: Cloning and expression of gene encoding a novel endoglycoceramidase of Rhodococcus sp. strain 9. J Biochem 2000, 128:145–152.

44. Sörensen M, Mak TN, Hurwitz R, Ogilvie LA, Mollenkopf HJ, Meyer TF, Brüggemann H: Mutagenesis of Propionibacterium acnes and analysis of two CAMP factor knock-out mutants. J Microbiol Methods 2010, 83:211–216.

45. Liu PF, Nakatsugi T, Zhu W, Gallo RL, Huang CM: Passive immunoprotection targeting a secreted CAMP factor of Propionibacterium acnes as a novel immunotherapeutic for acne vulgaris. Vaccine 2001, 29:3230–3238.

46. Liu GY, Nizet V: Extracellular virulence factors of group B Streptococci. Front Microbiol 2004, 9:1794–1802.

47. Christie R, Atkins NE, Munch-Petersen E: A note on a lytic phenomenon shown by group B streptococci. Aust J Exp Biol 1944, 22:197–200.

48. Angiuoli SV, Gussman A, Klimke W, Cochrane G, Field D, Ganty G, Kodira CD, Kyrpides N, Madupu R, Markowitz V, Tatusova T, Thomson N, White O: Toward an online repository of Standard Operating Procedures (SOPs) for (meta) genomic annotation. OMICS 2008, 12:137–141.

49. Carver TJ, Rutherford KM, Berniman M, Rajandream MA, Barrett BG, Parkhill J: ACT: the Artemis Comparison Tool. Bioinformatics 2005, 21:3422–3423.

50. Langille MG, Brinkman FS: Island viewer: an integrated interface for computational identification and visualization of genomic islands. Bioinformatics 2009, 25:664–665.

51. Komoriya K, Shibano N, Higano T, Azuma N, Yamaguchi S, Aizawa S: Flagellar proteins and type III-exported virulence factors are the predominant proteins secreted into the culture media of Salmonella typhimurium. Mol Microbiol 1999, 34:767–779.