HHS Public Access

Author manuscript

J Invest Dermatol. Author manuscript; available in PMC 2014 March 01.

Published in final edited form as:

J Invest Dermatol. 2013 September ; 133(9): 2152–2160. doi:10.1038/jid.2013.21.

Propionibacterium acnes strain populations in the human skin microbiome associated with acne

Sorel Fitz-Gibbon[1,*], **Shuta Tomida**[1,*], **Bor-Han Chiu**[1], **Lin Nguyen**[1], **Christine Du**[1,2], **Minghsun Liu**[3], **David Elashoff**[2], **Marie C. Erfe**[4], **Anya Loncaric**[2], **Jenny Kim**[2,5], **Robert L. Modlin**[2,3], **Jeff F. Miller**[3], **Erica Sodergren**[6], **Noah Craft**[4], **George M. Weinstock**[6], and **Huiying Li**[1,7]

[1]Department of Molecular and Medical Pharmacology, Crump Institute for Molecular Imaging, David Geffen School of Medicine, UCLA

[2]Department of Medicine, David Geffen School of Medicine, UCLA

[3]Department of Microbiology, Immunology and Molecular Genetics, David Geffen School of Medicine, UCLA

[4]Center for Immunotherapeutics Research, Los Angeles Biomedical Research Institute at Harbor-UCLA Medical Center, David Geffen School of Medicine, UCLA

[5]Department of Dermatology, Greater Los Angeles Healthcare System Veterans Affair

[6]The Genome Institute at Washington University in St. Louis

[7]UCLA-DOE Institute for Genomics and Proteomics Los Angeles, California, U.S.A

Abstract

The human skin microbiome plays important roles in skin health and disease. However, bacterial population structure and diversity at the strain level is poorly understood. We compared the skin microbiome at the strain level and genome level of *Propionibacterium acnes*, a dominant skin commensal, between 49 acne patients and 52 healthy individuals by sampling the pilosebaceous units on their noses. Metagenomic analysis demonstrated that while the relative abundances of *P. acnes* were similar, the strain population structures were significantly different in the two cohorts. Certain strains were highly associated with acne and other strains were enriched in healthy skin. By sequencing 66 previously unreported *P. acnes* strains and comparing 71 *P. acnes* genomes, we

Users may view, print, copy, and download text and data-mine the content in such documents, for the purposes of academic research, subject always to the full Conditions of use:http://www.nature.com/authors/editorial_policies/license.html#terms

Corresponding author: Huiying Li, Address: 4339 CNSI, 570 Westwood Plaza, Building 114, UCLA, Los Angeles, CA 90095-1770, Phone: (310) 206-5585, Fax: (310) 206-8975, huiying@mednet.ucla.edu.
*These authors contributed equally to this work.

CONFLICT OF INTEREST

The authors state no conflict of interest.

The data reported in this paper are tabulated in Supplementary Information and archived at GenBank.

The authors declare no competing financial interests.

S.F. and S.T. analyzed the data. B.C., L.N. and C.D. performed experiments. D.E. performed some of the initial statistical analyses. M.C.E., A.L., J.K., R.L.M. and N.C. collected samples. E.S. and G.M.W. directed sequencing, genome assembly and annotation. H.L., M.L., R.L.M. and J.F.M. conceived the demonstration project in the initial phase. H.L. designed and directed the project, analyzed the data and wrote the paper. S.T., S.F. and N.C. co-wrote the paper.

Author Manuscript Author Manuscript Author Manuscript Author Manuscript

Author Manuscript Author Manuscript Author Manuscript Author Manuscript

identified potential genetic determinants of various *P. acnes* strains in association with acne or health. Our analysis suggests that acquired DNA sequences and bacterial immune elements may play roles in determining virulence properties of *P. acnes* strains and some could be future targets for therapeutic interventions. This study demonstrates a previously unreported paradigm of commensal strain populations that could explain the pathogenesis of human diseases. It underscores the importance of strain level analysis of the human microbiome to define the role of commensals in health and disease.

INTRODUCTION

The diversity of the human microbiota at the strain level and its association with human health and disease is largely unknown. However, many studies have shown that microbe-related human diseases are often caused by certain strains of a species, rather than the entire species being pathogenic. Examples include methicillin-resistant *Staphylococcus aureus* (MRSA) (Chambers and Deleo, 2009; Chen *et al.*, 2010; Hansra and Shinkai) and *Escherichia coli* O157 (Chase-Topping *et al.*, 2008; Tarr *et al.*, 2005). Acne vulgaris (commonly called acne) is one of the most common skin diseases with a prevalence of up to 85% of teenagers and 11% of adults (White, 1998). Although the etiology and pathogenesis of acne are still unclear, microbial involvement is considered one of the main mechanisms contributing to the development of acne (Bojar and Holland, 2004; Cunliffe, 2002). In particular, *Propionibacterium acnes* has been hypothesized to be an important pathogenic factor (Webster, 1995). Antibiotic therapy targeting *P. acnes* has been a mainstay treatment for more than 30 years (Leyden, 2001). However, despite decades of study, it is still not clear how *P. acnes* contributes to acne pathogenesis while being a major commensal of the normal skin flora (Bek-Thomsen *et al.*, 2008; Cogen *et al.*, 2008; Costello *et al.*, 2009; Dominguez-Bello *et al.*, 2010; Fierer *et al.*, 2008; Gao *et al.*, 2007; Grice *et al.*, 2009). Whether *P. acnes* protects the human skin as a commensal bacterium or functions as a pathogenic factor in acne, or both, remains to be elucidated.

Here we compared the skin microbiome at the strain level and genome level in 49 acne patients and 52 normal individuals using a combination of metagenomics and genome sequencing. First, for each sample, 16S ribosomal DNA (rDNA) was amplified, approximately 400 clones were sequenced, and an average of 311 nearly full length 16S rDNA sequences were analyzed. The population structure of *P. acnes* strains was determined in each sample. Second, each *P. acnes* strain was assigned an “acne index” by calculating its prevalence in acne patients based on the 16S rDNA metagenomic data. The *P. acnes* strains associated with the acne patient group were identified, as well as the strains enriched in the individuals with normal skin. This metagenomic approach is fundamentally different than prior approaches in determining disease associations; it is more powerful and less biased than traditional methods by bypassing the biases and selection in strain isolation and culturing. To our knowledge this study has the largest number of individual skin microbiomes reported at the strain level to date. Lastly, we sequenced 66 previously unreported *P. acnes* strains and compared 71 *P. acnes* genomes covering the major lineages of *P. acnes* found in the skin microbiota. By combining a metagenomic study of the skin microbiome and genome sequencing of this major skin commensal, this study provides

Author Manuscript Author Manuscript Author Manuscript Author Manuscript

insight into potential bacterial genetic determinants in acne pathogenesis and emphasizes the importance of strain level analysis of the human microbiome to understand the role of commensals in health and disease.

RESULTS

P. acnes dominates the pilosebaceous unit

We characterized the microbiome in pilosebaceous units ("pores") on the nose collected from 49 acne patients and 52 individuals with normal skin. Nearly full length 16S rDNA sequences were obtained using Sanger method, which allowed us to analyze the *P. acnes* at the strain level. After quality filtering, our final dataset contained 31,461 16S rDNA sequences ranging from position 29 to position 1483. 27,358 of the sequences matched to *P. acnes* with greater than 99% identity. Our data demonstrated that *P. acnes* dominates the microbiota of pilosebaceous units, accounting for 87% of the clones (Figure 1). Other commonly found species in pilosebaceous units included *Staphylococcus epidermidis*, *Propionibacterium humerusii*, and *Propionibacterium granulosum*, each representing 1% – 2.3% of the total clones. A total of 536 species level operational taxonomic units (SLOTUs) belonging to 42 genera and six phyla were identified in the samples (Table S1).

To bypass the potential biases due to PCR amplification and due to uneven numbers of 16S rDNA gene copies among different species, we performed a metagenomic shotgun sequencing of the total DNA pooled from the pilosebaceous unit samples of 22 additional normal individuals. Microbial species were identified by mapping metagenomic sequences to reference genomes. The results confirmed that *P. acnes* was the most abundant species (89%) (Figure 1). This is consistent with the results obtained from 16S rDNA sequencing (87%).

Different *P. acnes* strain populations in acne

There was no statistically significant difference in the relative abundance of *P. acnes* when comparing acne patients and normal individuals. We next examined whether there were differences at the strain level of *P. acnes* by extensively analyzing the *P. acnes* 16S rDNA sequences. We define each unique 16S rDNA sequence as a 16S rDNA allele type, called a ribotype (RT). The most abundant *P. acnes* sequence was defined as ribotype 1 (RT1); all other defined ribotypes have 99% or greater sequence identity to RT1. Similar to the distributions seen at higher taxonomical levels (Bik *et al.*), at the strain level a few ribotypes were highly abundant in the samples with a significant number of rare ribotypes (Figure S1). After careful examination of the sequence chromatograms and manual correction of the sequences, a total of 11,009 ribotypes were assigned to the *P. acnes* 16S rDNA sequences. Most of the minor ribotypes were singletons. On average, each individual harbored 3±2 *P. acnes* ribotypes with three or more clones. Based on the genome sequences described below, all the sequenced *P. acnes* strains have three identical copies of 16S rDNA genes (note in Supplementary Information). This allowed us to compare the *P. acnes* strain populations in individuals based on the 16S rDNA sequences. The top ten major ribotypes with more than 60 clones and found in multiple subjects are shown in Table 1.

Author Manuscript Author Manuscript Author Manuscript Author Manuscript

Analysis of the top ten ribotypes showed both disease-specific and health-specific associations. The three most abundant ribotypes (RT1, RT2 and RT3) were fairly evenly distributed among acne and normal individuals. However, the next seven major ribotypes were significantly skewed in their distribution (Table 1). Ribotypes 4, 5, 7, 8, 9 and 10 were found predominantly in acne patients, with four of these six statistically significantly enriched in acne ($p<0.05$, Wilcoxon test). Ribotypes 4, 5 and 10 contain a nucleotide substitution G1058C in the 16S rDNA sequences, which has previously been shown to confer increased resistance to tetracycline (Ross *et al.*, 1998a; Ross *et al.*, 2001). However, only a small percentage of the subjects in our study harboring these ribotypes had been treated with antibiotics (Table S2), therefore enrichment of these three ribotypes in the acne group was not correlated with antibiotic treatment. This is consistent with previous studies, which showed that previous use of antibiotics was not always associated with the presence of antibiotic resistant strains and that some patients who were not previously treated with antibiotics harbored strains already resistant to antibiotics (Coates *et al.*, 2002; Dreno *et al.*, 2001). On the other hand, one ribotype, RT6, although detected in only 11 subjects, was strongly associated with normal skin ($p=0.025$, Wilcoxon test) (Table 1). Its relative abundance in our normal group was similar to that found in the healthy cohort data from the Human Microbiome Project (HMP) (Supplementary Information, Figure S2). The percentage of positive subjects (11/52) was similar as well. Three of the 14 HMP subjects had RT6 found in the anterior nares, and one additional subject had RT6 in the left retroauricular crease.

Based on the distributions of the top ten ribotypes, statistical analysis using several different tests showed significant differences in *P. acnes* population structure between acne and normal skin (Table S3). This is consistent with a principal coordinate analysis, where acne samples and normal skin samples were separated by mostly principal coordinates 1 and 2 (Figure S3), explaining 44% and 20% of the variation, respectively.

To examine whether different individuals share similar *P. acnes* population structures, we clustered the samples based on the relative abundance of the top ten ribotypes. Five main microbiome types were observed at the *P. acnes* strain level (microbiome types I to V). Types IV and V, which are dominated by *P. acnes* RT4 and RT5, respectively, were mainly found in acne patients (Figures 2 and S4). The same five main microbiome types were observed in the HMP data and the data from Grice et al. (Grice *et al.*, 2009) (Supplementary Information, Figure S5).

Genome sequence analysis of 71 *P. acnes* strains

All of the top ten most abundant ribotypes differ from RT1 by only one or two nucleotide changes in the 16S rDNA sequence (Table 1). To determine whether such small changes in the 16S rDNA sequence reflect the lineages and evolutionary history at the genome level, we selected 66 *P. acnes* isolates representing major ribotypes 1, 2, 3, 4, 5, 6, and 8 as well as two minor ribotypes, 16 and 532, for genome sequencing. The genomes of these 66 isolates were fully sequenced and assembled to high quality drafts or complete genomes with 50X coverage or more. Five other *P. acnes* genomes, KPA171202 (Bruggemann *et al.*, 2004), J165, J139, SK137, and SK187, were publicly available and were included in our analysis.

Author Manuscript Author Manuscript Author Manuscript Author Manuscript

We constructed a phylogenetic tree based on 96,887 unique single nucleotide polymorphism (SNP) positions in the core genome obtained from these 71 *P. acnes* genomes. Most of the genomes with the same ribotypes clustered together. The tree suggests that the 16S rDNA ribotypes do represent the relationship of the lineages to a large extent and that16S rDNA sequence is a useful molecular marker to distinguish major *P. acnes* lineages (Figures 3 and S6).

Genetic elements detected in *P. acnes*

We further performed comparative genome analysis among all 71 genomes grouped by ribotypes. Our analysis revealed potential genetic elements by which acne-associated strains could contribute to acne pathogenesis and the elements by which health-associated strains could contribute to maintaining skin health. Specifically, we describe here the unique genome regions of RT4 and RT5, which had a strong association with acne, and RT6, which was found enriched in normal skin. Three distinct regions, loci 1, 2 and 3, were found almost exclusively in strains that belong to clade IA-2 in the phylogenetic tree. Clade IA-2 consists of mainly RT4 and RT5 (Figures 3 and S7). Loci 1 and 2 are located on the chromosome. Locus 1 contains prophage-related genes and appears to be a genomic island. Locus 2 has plasmid integration sites and thus could be derived from a plasmid sequence. Locus 3 appears to be on a large mobile genetic element, likely a plasmid. The plasmid is approximately 55 Kb long and has inverted terminal repeats according to our finished genome HL096PA1 (Supplementary Information). The sequence data suggest that the plasmid is linear and possibly originated from a phage (Hinnebusch and Tilly, 1993). All but one of the fifteen genomes of RT4 and RT5 have at least 60% of the genes of the plasmid represented, and all of them have regions homologous to the inverted terminal repeat in the plasmid, suggesting that they harbor the same or a similar linear plasmid (Figure 3). The copy number of the plasmid in the genomes ranges from 1 to 3 based on genome sequencing coverage, which was confirmed by quantitative PCR (Figures S8 and S9).

The fact that acne-enriched RT4 and RT5 carry a linear plasmid and two unique loci of genomic islands suggests that these plasmid and chromosomal regions may play a role in acne pathogenesis. In fact, the linear plasmid encodes a tight adhesion (Tad) locus, which has been suggested to play a role in virulence in other organisms (Kachlany *et al.*, 2000; Schreiner *et al.*, 2003). The complete Tad locus is found in all but one of the fifteen genomes of RT4 and RT5, and is only occasionally found in other ribotypes. Additionally, in locus 2, a Sag gene cluster is encoded, which has been shown to contribute to hemolytic activity in pathogens (Fuller *et al.*, 2002; Humar *et al.*, 2002; Nizet *et al.*, 2000). Table S4 summarizes the genes that are mostly unique to RT4 and RT5, several of which play essential roles in virulence in other organisms. We speculate that some of these genes encoded in RT4 and RT5 may increase virulence, promote stronger adherence to the human host, or induce a pathogenic host immune response.

In genome comparison analysis, we found that all the genomes of RT2 and RT6 encode Clustered Regularly Interspaced Short Palindromic Repeats (CRISPR). Among the sequenced genomes, RT2 and RT6 are the only ribotypes encoding CRISPR. CRISPR have been shown to confer protective "immunity" against viruses, phage and plasmids (Horvath

Author Manuscript
Author Manuscript
Author Manuscript
Author Manuscript

and Barrangou, 2010; Makarova *et al.*, 2011). The CRISPR locus encoded in *P. acnes* consists of a series of *cas* genes - *cas3, cse1, cse2, cse4, cas5e, cse3, cas1* and *cas2*, which are homologous to the CRISPR locus reported in *E. coli* (Figure S10) and the CRISPR4 locus in *Streptococcus thermophilus* (Horvath and Barrangou, 2010).

CRISPR arrays are composed of a cluster of identical repetitive sequences separated by spacer sequences of similar length but with different nucleotide sequences. Spacer sequences have been found identical or with one or two mismatches to phage or plasmid DNA sequences. A total of 39 spacer sequences were found in eight *P. acnes* strains, 25 of which were unique as shown in Table 2. As expected, most of the identifiable spacers target to known *P. acnes* phage sequences. However, among the unique CRISPR spacer sequences, one matched locus 2 on the chromosome and three matched the plasmid region (locus 3) in *P. acnes* genomes of mainly RT4 and RT5. This suggests that these loci may have been acquired by RT4 and RT5, while the genomes of RT2 and RT6 may be capable of protecting against the invasion of the plasmids or other foreign DNA through the CRISPR mechanism.

DISCUSSION

Our study of the human skin microbiome associated with acne provides a previously unreported portrait of the microbiota of pilosebaceous units at the bacterial strain level. Since *P. acnes* is the major skin commensal bacterium found in both acne and healthy skin, this strain-level analysis is important to help understand the role of *P. acnes* in acne pathogenesis and in skin health. We demonstrate a strong association between strains of RT4 and RT5 with acne and a strong association between strains of RT6 and healthy skin, each with unique genetic elements. Other *P. acnes* strains, including ribotypes 7, 8, 9 and 10, or interactions among different strains, may also contribute to the development of the disease. In addition, host factors, such as hormone level, sebum production and physical changes in the pilosebaceous unit, may also play a role in acne pathogenesis. Further studies aimed at identifying the specific functions of these strains, host factors in the development of acne, as well as the associations of microbiome characteristics with the sub-types of acne (comedonal, pustular, inflammatory, cystic, etc.) with larger cohort sizes may improve our understanding of the molecular mechanisms of the disease. These studies may also help to develop a targeted therapeutic approach to treat this extremely common and sometimes disfiguring skin disease.

Our metagenomic approach in revealing the association of *P. acnes* strains with the disease or health is to our knowledge previously unreported, and is more powerful than previous studies using traditional methods (Lomholt and Kilian, 2010; McDowell *et al.*, 2011). Because the skin microbiota of each individual and each skin site may harbor “good”, “neutral” and “bad” strains at the same time, which may have different growth rates under *in vitro* culturing conditions, culturing a few isolates from a disease lesion or healthy skin site may not provide an accurate and unbiased measurement of the association of the strains with the disease or health. The sampling technique and disease associations in this study do not depend on sampling locations, on the presence of lesions in the sampling field, or on inherently biased culture techniques. While sampling lesional skin intentionally may yield

Author Manuscript Author Manuscript Author Manuscript Author Manuscript

interesting results, these results would not be capable of defining the disease associations that unbiased sampling can. The metagenomic approach employed in this study to identify underlying strain differences in acne might also be applied to the study of other disease/health associations with commensal or pathogenic bacteria. Ultimately, these studies could lead to targeted therapeutics to restore natural commensal population structures, and could help determine if therapeutic modulations of the microbiota can return the host to a state of health.

MATERIALS AND METHODS

Subjects

Subjects with acne and subjects with normal skin were recruited from various clinics in Southern California including private practice, managed care, and public hospital settings, as well as outside of dermatology clinics, to best represent the diversity of populations and history of medical care. The subject data are available at dbGaP (http://www.ncbi.nlm.nih.gov/projects/gap/cgi-bin/study.cgi?study_id=phs000263.v1.p1). The diagnosis of acne was made by board-certified dermatologists. The presence of acne was graded on a scale of 0 to 5 relating closely to the Global Acne Severity Scale (Dreno *et al.*, 2011). Grades were recorded for both the face and the nose separately where zero represents normal skin and 5 represents the most severe inflammatory cystic acne. In acne patients, the grades of the face ranged from 1 to 5 with an average of 2.1, and the grades of the nose ranged from 0 to 2 with an average of 0.3. The presence of scarring was also noted. Subjects with normal skin were determined by board-certified dermatologists and were defined as people who had no acneiform lesions on the face, chest, or back. They were also excluded if they had other skin problems that the investigators felt would affect sampling or the microbial population on the skin. Among the 101 subjects, 59 were female (31 acne patients and 28 normal subjects) and 42 were male (18 acne patients and 24 normal subjects). The average age of the acne cohort was 22.2 and the average age of the normal cohort was 29.6. There was no significant difference in ethnicity between the acne and normal populations. The subjects responded to a written questionnaire, administered by a physician or a well trained study coordinator who went over each question with the subjects. Most of the subjects had not been treated for acne in the past or were not being treated when samples were collected (Table S2). Only nine out of 78 subjects, who provided treatment information, were being treated for acne when samples were taken. Among the nine subjects, two were being treated with antibiotics, five were being treated with topical retinoids, one was being treated with both antibiotics and retinoids, and one did not list the treatment. We also asked subjects for acne treatment history in the past (anytime in their life). Eighteen out of 73 subjects, who provided treatment history, had been treated for acne in the past. Among them, seven had been treated with antibiotics, eight had been treated with retinoids, two had been treated with both antibiotics and retinoids, and one did not list the treatment. All subjects provided written informed consent. All protocols and consent forms were approved by both the UCLA and Los Angeles Biomedical Research Institute IRBs. The study was conducted in adherence to the Helsinki Guidelines.

Author Manuscript Author Manuscript Author Manuscript Author Manuscript

Samples

Skin microcomedone (white head or black head) samples were taken from the nose of the subjects using Bioré Deep Cleansing Pore Strips (Kao Brands Company, Cincinnati, OH) following the instruction of the manufacturer. Clean gloves were used for each sampling. After being removed from the nose, the strip was immediately placed into a 50 mL sterile tube and kept on ice or at 4 °C. The cells were lysed within four hours in most of the cases.

Metagenomic DNA extraction, 16S rDNA amplification, cloning and sequencing

Individual microcomedones were isolated from the adhesive nose strip using sterile forceps. Genomic DNA was extracted using QIAamp DNA Micro Kit (Qiagen). 16S rDNA was amplified and cloned according to the protocol by HMP, which is described in detail in Supplementary Information. Nearly full length sequences were obtained by Sanger method.

16S rDNA sequence analysis

Base calling and quality was determined with Phred (Ewing and Green, 1998; Ewing *et al.*, 1998). Bidirectional reads were assembled and aligned to a core set of NAST-formatted sequences (rRNA16S.gold) using AmosCmp16Spipeline and NAST-ier. Suspected chimeras were identified using ChimeraSlayer and WigeoN (Haas *et al.*, 2011). 16S rDNA sequences were extensively manually examined. Chromatograms were visually inspected at all bases with a Phred quality score < 30. Appropriate corrections were applied. QIIME (Caporaso *et al.*, 2010b) was used to cluster the sequences into OTUs.

P. acnes isolation and genotyping

Colonies with the macroscopic characteristics of *P. acnes* were picked from each sample plate and were passed twice. The ribotype of each isolate was determined by PCR amplification and sequencing of the full length of the 16S rDNA gene by Sanger method.

Whole genome shotgun sequencing, assembly and annotation

Genome HL096PA1 was sequenced using Roche/454 FLX and was assembled using a combination of PHRAP/CONSED (Gordon *et al.*, 1998) and GSMAPPER (Roche) with extensive manual editing in CONSED. The remaining 65 genomes were sequenced using Illumina/Solexa GAIIx. Sequence datasets were processed by quality trimming and were assembled using Velvet (Zerbino and Birney, 2008). Coding sequences were predicted using GeneMark (Borodovsky and McIninch, 1993) and GLIMMER (Salzberg *et al.*, 1998). The final gene set was processed through a suite of protein categorization tools consisting of Interpro, psort-b and KEGG. A more detailed protocol can be found at http://hmpdacc.org/doc/sops/reference_genomes/annotation/WUGC_SOP_DACC.pdf.

Comparative genome analysis

Seventy-one *P. acnes* genome sequences were compared using Nucmer (Kurtz *et al.*, 2004). Phylogenetic analysis was performed using MEGA5 (Tamura *et al.*, 2007). CRISPRFinder (Grissa *et al.*, 2007) was used to identify the CRISPR repeat-spacer sequences.

Author Manuscript Author Manuscript Author Manuscript Author Manuscript

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

We thank G. Kasimatis, B. Shi, E. E. Curd, R. Yan, M. Wong and J. Liu for comments and technical support. We thank C. Lee for performing statistical analyses in the initial phase. We also thank Z. Guo and C. S. Miller for critical reading of the manuscript. This research was funded as one of the Demonstration Projects by the NIH Human Microbiome Project (HMP). It was supported by Grant Number 1UH2AR057503 from NIH/NIAMS and U54HG004968 from NIH.

References

Altschul SF, Gish W, Miller W, Myers EW, Lipman DJ. Basic local alignment search tool. J Mol Biol. 1990; 215:403–10. [PubMed: 2231712]

Bek-Thomsen M, Lomholt HB, Kilian M. Acne is not associated with yet-uncultured bacteria. J Clin Microbiol. 2008; 46:3355–60. [PubMed: 18716234]

Bik EM, Long CD, Armitage GC, Loomer P, Emerson J, Mongodin EF, et al. Bacterial diversity in the oral cavity of 10 healthy individuals. Isme J. 4:962–74. [PubMed: 20336157]

Bojar RA, Holland KT. Acne and Propionibacterium acnes. Clin Dermatol. 2004; 22:375–9. [PubMed: 15556721]

Borodovsky M, McIninch J. Recognition of genes in DNA sequence with ambiguities. Bio Systems. 1993; 30:161–71.

Brosius J, Palmer ML, Kennedy PJ, Noller HF. Complete nucleotide sequence of a 16S ribosomal RNA gene from Escherichia coli. Proc Natl Acad Sci U S A. 1978; 75:4801–5. [PubMed: 368799]

Bruggemann H, Henne A, Hoster F, Liesegang H, Wiezer A, Strittmatter A, et al. The complete genome sequence of Propionibacterium acnes, a commensal of human skin. Science. 2004; 305:671–3. [PubMed: 15286373]

Caporaso JG, Bittinger K, Bushman FD, DeSantis TZ, Andersen GL, Knight R. PyNAST: a flexible tool for aligning sequences to a template alignment. Bioinformatics. 2010a; 26:266–7. [PubMed: 19914921]

Caporaso JG, Kuczynski J, Stombaugh J, Bittinger K, Bushman FD, Costello EK, et al. QIIME allows analysis of high-throughput community sequencing data. Nature methods. 2010b; 7:335–6. [PubMed: 20383131]

Chambers HF, Deleo FR. Waves of resistance: Staphylococcus aureus in the antibiotic era. Nat Rev Microbiol. 2009; 7:629–41. [PubMed: 19680247]

Chase-Topping M, Gally D, Low C, Matthews L, Woolhouse M. Super-shedding and the link between human infection and livestock carriage of Escherichia coli O157. Nat Rev Microbiol. 2008; 6:904–12. [PubMed: 19008890]

Chen CJ, Su LH, Lin TY, Huang YC. Molecular analysis of repeated methicillin-resistant Staphylococcus aureus infections in children. PLoS One. 2010; 5:e14431. [PubMed: 21203439]

Chou HH, Holmes MH. DNA sequence quality trimming and vector removal. Bioinformatics. 2001; 17:1093–104. [PubMed: 11751217]

Coates P, Vyakrnam S, Eady EA, Jones CE, Cove JH, Cunliffe WJ. Prevalence of antibiotic-resistant propionibacteria on the skin of acne patients: 10-year surveillance data and snapshot distribution study. Br J Dermatol. 2002; 146:840–8. [PubMed: 12000382]

Cogen AL, Nizet V, Gallo RL. Skin microbiota: a source of disease or defence? Br J Dermatol. 2008; 158:442–55. [PubMed: 18275522]

Cole JR, Wang Q, Cardenas E, Fish J, Chai B, Farris RJ, et al. The Ribosomal Database Project: improved alignments and new tools for rRNA analysis. Nucleic Acids Res. 2009; 37:D141–5. [PubMed: 19004872]

Costello EK, Lauber CL, Hamady M, Fierer N, Gordon JI, Knight R. Bacterial community variation in human body habitats across space and time. Science. 2009; 326:1694–7. [PubMed: 19892944]

Author Manuscript Author Manuscript Author Manuscript Author Manuscript

Cunliffe WJ. Looking back to the future - acne. Dermatology. 2002; 204:167–72. [PubMed: 12037442]

Dominguez-Bello MG, Costello EK, Contreras M, Magris M, Hidalgo G, Fierer N, et al. Delivery mode shapes the acquisition and structure of the initial microbiota across multiple body habitats in newborns. Proc Natl Acad Sci U S A. 2010; 107:11971–5. [PubMed: 20566857]

Dreno B, Poli F, Pawin H, Beylot C, Faure M, Chivot M, et al. Development and evaluation of a Global Acne Severity Scale (GEA Scale) suitable for France and Europe. Journal of the European Academy of Dermatology and Venereology : JEADV. 2011; 25:43–8. [PubMed: 20456560]

Dreno B, Reynaud A, Moyse D, Habert H, Richet H. Erythromycin-resistance of cutaneous bacterial flora in acne. Eur J Dermatol. 2001; 11:549–53. [PubMed: 11701406]

Edgar RC. Search and clustering orders of magnitude faster than BLAST. Bioinformatics. 2010; 26:2460–1. [PubMed: 20709691]

Eisen MB, Spellman PT, Brown PO, Botstein D. Cluster analysis and display of genome-wide expression patterns. Proc Natl Acad Sci U S A. 1998; 95:14863–8. [PubMed: 9843981]

Ewing B, Green P. Base-calling of automated sequencer traces using phred. II. Error probabilities. Genome Res. 1998; 8:186–94. [PubMed: 9521922]

Ewing B, Hillier L, Wendl MC, Green P. Base-calling of automated sequencer traces using phred. I. Accuracy assessment. Genome Res. 1998; 8:175–85. [PubMed: 9521921]

Excoffier L, Lischer HE. Arlequin suite ver 3.5: a new series of programs to perform population genetics analyses under Linux and Windows. Mol Ecol Resour. 2010; 10:564–7. [PubMed: 21565059]

Fierer N, Hamady M, Lauber CL, Knight R. The influence of sex, handedness, and washing on the diversity of hand surface bacteria. Proc Natl Acad Sci U S A. 2008; 105:17994–9. [PubMed: 19004758]

Fuller JD, Camus AC, Duncan CL, Nizet V, Bast DJ, Thune RL, et al. Identification of a streptolysin S-associated gene cluster and its role in the pathogenesis of Streptococcus iniae disease. Infect Immun. 2002; 70:5730–9. [PubMed: 12228303]

Gao Z, Tseng CH, Pei Z, Blaser MJ. Molecular analysis of human forearm superficial skin bacterial biota. Proc Natl Acad Sci U S A. 2007; 104:2927–32. [PubMed: 17293459]

Gordon D. Viewing and editing assembled sequences using Consed. Curr Protoc Bioinformatics. 2003; Chapter 11(Unit11):2. [PubMed: 18428695]

Gordon D, Abajian C, Green P. Consed: a graphical tool for sequence finishing. Genome research. 1998; 8:195–202. [PubMed: 9521923]

Grice EA, Kong HH, Conlan S, Deming CB, Davis J, Young AC, et al. Topographical and temporal diversity of the human skin microbiome. Science. 2009; 324:1190–2. [PubMed: 19478181]

Grissa I, Vergnaud G, Pourcel C. CRISPRFinder: a web tool to identify clustered regularly interspaced short palindromic repeats. Nucleic Acids Res. 2007; 35:W52–7. [PubMed: 17537822]

Haas BJ, Gevers D, Earl AM, Feldgarden M, Ward DV, Giannoukos G, et al. Chimeric 16S rRNA sequence formation and detection in Sanger and 454-pyrosequenced PCR amplicons. Genome Res. 2011; 21:494–504. [PubMed: 21212162]

Hansra NK, Shinkai K. Cutaneous community-acquired and hospital-acquired methicillin-resistant Staphylococcus aureus. Dermatol Ther. 24:263–72. [PubMed: 21410616]

Hinnebusch J, Tilly K. Linear plasmids and chromosomes in bacteria. Mol Microbiol. 1993; 10:917–22. [PubMed: 7934868]

Horvath P, Barrangou R. CRISPR/Cas, the immune system of bacteria and archaea. Science. 2010; 327:167–70. [PubMed: 20056882]

Humar D, Datta V, Bast DJ, Beall B, De Azavedo JC, Nizet V. Streptolysin S and necrotising infections produced by group G streptococcus. Lancet. 2002; 359:124–9. [PubMed: 11809255]

Kachlany SC, Planet PJ, Bhattacharjee MK, Kollia E, DeSalle R, Fine DH, et al. Nonspecific adherence by Actinobacillus actinomycetemcomitans requires genes widespread in bacteria and archaea. J Bacteriol. 2000; 182:6169–76. [PubMed: 11029439]

Kurtz S, Phillippy A, Delcher AL, Smoot M, Shumway M, Antonescu C, et al. Versatile and open software for comparing large genomes. Genome biology. 2004; 5:R12. [PubMed: 14759262]

Author Manuscript Author Manuscript Author Manuscript Author Manuscript

Leyden JJ. The evolving role of Propionibacterium acnes in acne. Semin Cutan Med Surg. 2001; 20:139–43. [PubMed: 11594668]

Li H, Durbin R. Fast and accurate long-read alignment with Burrows-Wheeler transform. Bioinformatics. 26:589–95. [PubMed: 20080505]

Li H, Durbin R. Fast and accurate short read alignment with Burrows-Wheeler transform. Bioinformatics. 2009; 25:1754–60. [PubMed: 19451168]

Li H, Ruan J, Durbin R. Mapping short DNA sequencing reads and calling variants using mapping quality scores. Genome Res. 2008; 18:1851–8. [PubMed: 18714091]

Lomholt HB, Kilian M. Population genetic analysis of Propionibacterium acnes identifies a subpopulation and epidemic clones associated with acne. PLoS One. 2010; 5:e12277. [PubMed: 20808860]

Makarova KS, Haft DH, Barrangou R, Brouns SJ, Charpentier E, Horvath P, et al. Evolution and classification of the CRISPR-Cas systems. Nat Rev Microbiol. 2011; 9:467–77. [PubMed: 21552286]

McDowell A, Gao A, Barnard E, Fink C, Murray PI, Dowson CG, et al. A novel multilocus sequence typing scheme for the opportunistic pathogen Propionibacterium acnes and characterization of type I cell surface-associated antigens. Microbiology. 2011; 157:1990–2003. [PubMed: 21511767]

McDowell A, Perry AL, Lambert PA, Patrick S. A new phylogenetic group of Propionibacterium acnes. J Med Microbiol. 2008; 57:218–24. [PubMed: 18201989]

McDowell A, Valanne S, Ramage G, Tunney MM, Glenn JV, McLorinan GC, et al. Propionibacterium acnes types I and II represent phylogenetically distinct groups. J Clin Microbiol. 2005; 43:326–34. [PubMed: 15634990]

Nizet V, Beall B, Bast DJ, Datta V, Kilburn L, Low DE, et al. Genetic locus for streptolysin S production by group A streptococcus. Infect Immun. 2000; 68:4245–54. [PubMed: 10858242]

Pop M, Phillippy A, Delcher AL, Salzberg SL. Comparative genome assembly. Brief Bioinform. 2004; 5:237–48. [PubMed: 15383210]

Price MN, Dehal PS, Arkin AP. FastTree: computing large minimum evolution trees with profiles instead of a distance matrix. Mol Biol Evol. 2009; 26:1641–50. [PubMed: 19377059]

Ross JI, Eady EA, Cove JH, Cunliffe WJ. 16S rRNA mutation associated with tetracycline resistance in a gram-positive bacterium. Antimicrob Agents Chemother. 1998a; 42:1702–5. [PubMed: 9661007]

Ross JI, Eady EA, Cove JH, Jones CE, Ratyal AH, Miller YW, et al. Clinical resistance to erythromycin and clindamycin in cutaneous propionibacteria isolated from acne patients is associated with mutations in 23S rRNA. Antimicrob Agents Chemother. 1997; 41:1162–5. [PubMed: 9145890]

Ross JI, Eady EA, Cove JH, Ratyal AH, Cunliffe WJ. Resistance to erythromycin and clindamycin in cutaneous propionibacteria is associated with mutations in 23S rRNA. Dermatology. 1998b; 196:69–70. [PubMed: 9557230]

Ross JI, Snelling AM, Eady EA, Cove JH, Cunliffe WJ, Leyden JJ, et al. Phenotypic and genotypic characterization of antibiotic-resistant Propionibacterium acnes isolated from acne patients attending dermatology clinics in Europe, the U.S.A., Japan and Australia. The British journal of dermatology. 2001; 144:339–46. [PubMed: 11251569]

Saitou N, Nei M. The neighbor-joining method: a new method for reconstructing phylogenetic trees. Molecular biology and evolution. 1987; 4:406–25. [PubMed: 3447015]

Salzberg SL, Delcher AL, Kasif S, White O. Microbial gene identification using interpolated Markov models. Nucleic acids research. 1998; 26:544–8. [PubMed: 9421513]

Schloss PD, Westcott SL, Ryabin T, Hall JR, Hartmann M, Hollister EB, et al. Introducing mothur: open-source, platform-independent, community-supported software for describing and comparing microbial communities. Appl Environ Microbiol. 2009; 75:7537–41. [PubMed: 19801464]

Schreiner HC, Sinatra K, Kaplan JB, Furgang D, Kachlany SC, Planet PJ, et al. Tight-adherence genes of Actinobacillus actinomycetemcomitans are required for virulence in a rat model. Proc Natl Acad Sci U S A. 2003; 100:7295–300. [PubMed: 12756291]

Tamura K, Dudley J, Nei M, Kumar S. MEGA4: Molecular Evolutionary Genetics Analysis (MEGA) software version 4.0. Molecular biology and evolution. 2007; 24:1596–9. [PubMed: 17488738]

Tarr PI, Gordon CA, Chandler WL. Shiga-toxin-producing Escherichia coli and haemolytic uraemic syndrome. Lancet. 2005; 365:1073–86. [PubMed: 15781103]

Webster GF. Inflammation in acne vulgaris. J Am Acad Dermatol. 1995; 33:247–53. [PubMed: 7622652]

White GM. Recent findings in the epidemiologic evidence, classification, and subtypes of acne vulgaris. J Am Acad Dermatol. 1998; 39:S34–7. [PubMed: 9703121]

Zerbino DR, Birney E. Velvet: algorithms for de novo short read assembly using de Bruijn graphs. Genome research. 2008; 18:821–9. [PubMed: 18349386]

Author Manuscript

Author Manuscript

Author Manuscript

Author Manuscript

Author Manuscript

Author Manuscript

Author Manuscript

Author Manuscript

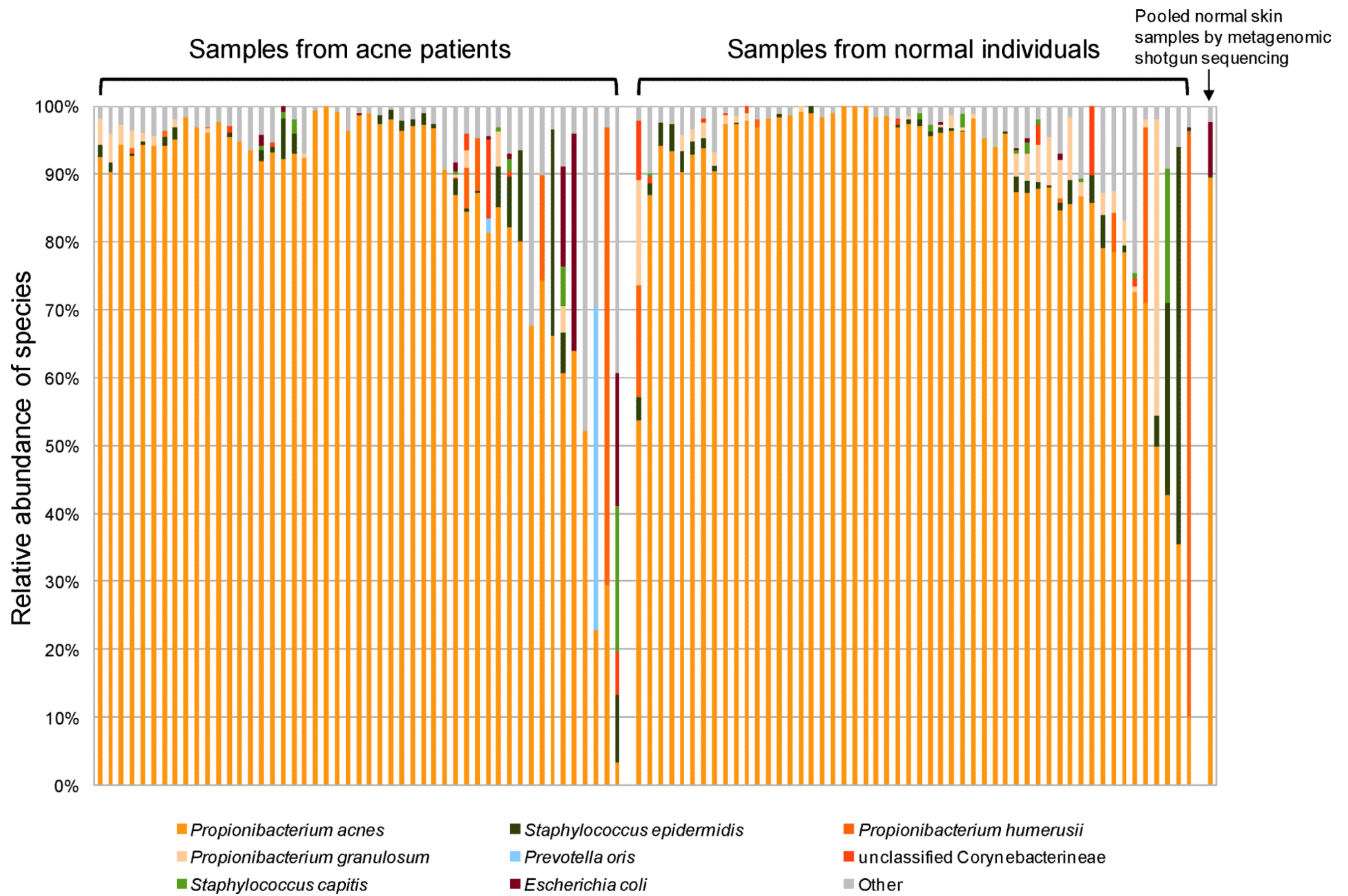

Figure 1.

P. acnes was dominant in pilosebaceous units in both acne patients and individuals with normal skin. By 16S rDNA sequencing, *P. acnes* sequences accounted for 87% of all the clones. Species with a relative abundance greater than 0.35% are listed in order of relative abundance. Species distribution from a metagenomic shotgun sequencing of pooled samples from normal individuals confirmed the high abundance of *P. acnes* in pilosebaceous units, as shown on the far right column.

Author Manuscript
Author Manuscript
Author Manuscript
Author Manuscript

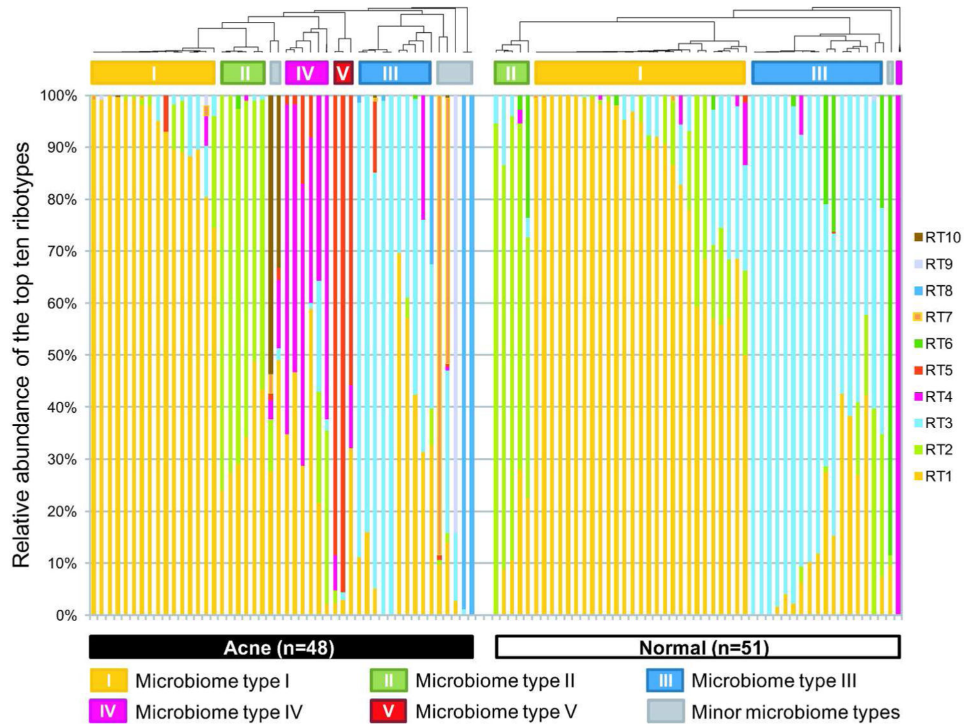

Figure 2.

Distribution of the top ten most abundant *P. acnes* ribotypes in acne patients and individuals with normal skin. Each column represents the percentage of the top ten ribotypes identified in each subject. The average *P. acnes* clone number per subject was 262 and the average clone number of top ten ribotypes was 100. Five major microbiome types at the *P. acnes* strain level were observed in the data. Types IV and V were mostly found in acne patients. Two samples (one from acne, one from normal skin) with fewer than 50 *P. acnes* 16S rDNA sequences are not displayed.

Author Manuscript

Author Manuscript

Author Manuscript

Author Manuscript

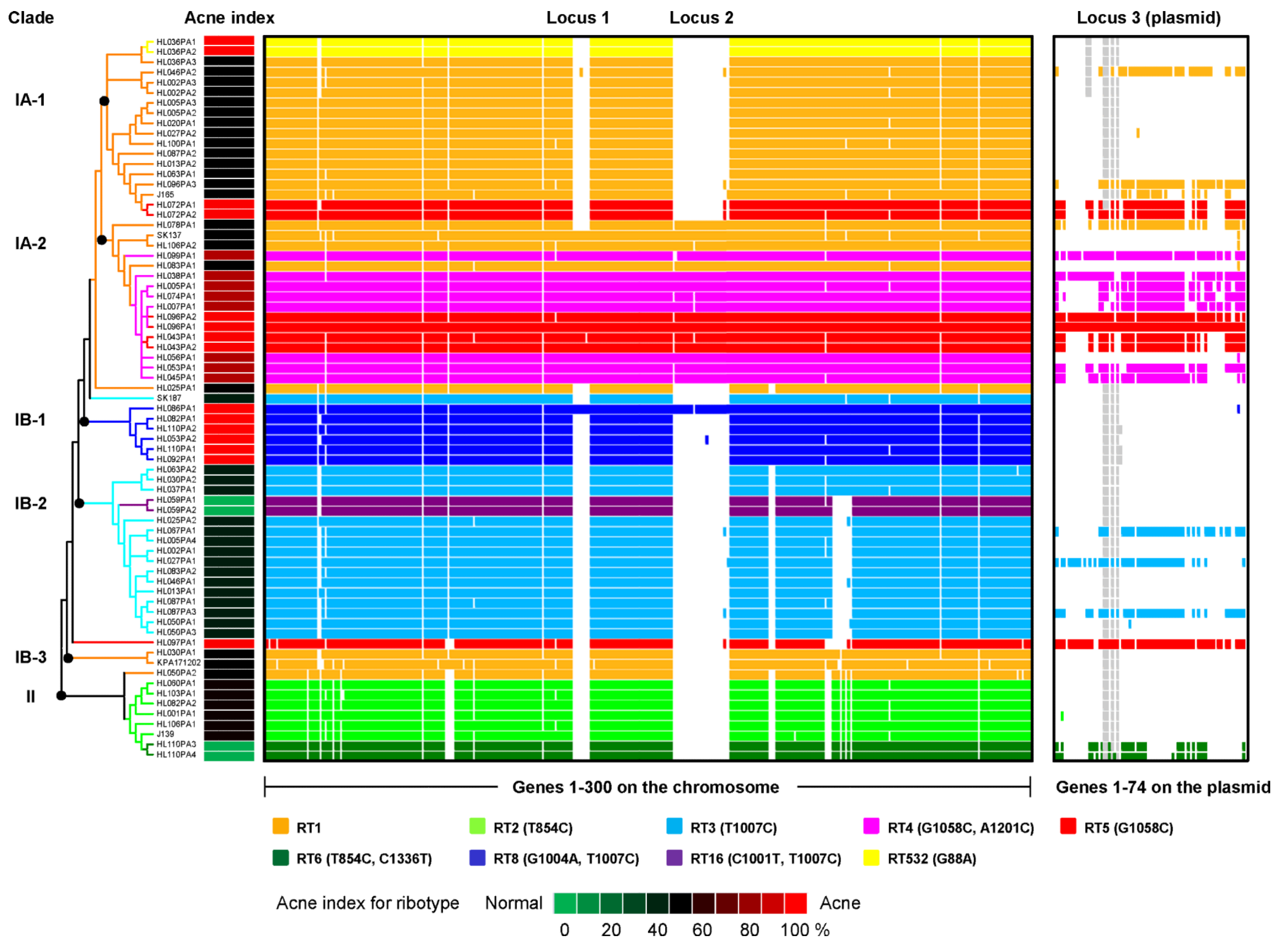

Figure 3.

Genome comparison of 71 *P. acnes* strains showed that the genomes of RT4 and RT5 are distinct from others. Two chromosomal regions, loci 1 and 2, are unique to clade IA-2 and one other genome HL086PA1. Clade IA-2 consists of mainly RT4 and RT5 that were highly enriched in acne. The presence of a plasmid (locus 3) is also characteristic of RT4 and RT5. Each row represents a *P. acnes* genome colored according to the ribotypes. Rows are ordered by the phylogeny calculated based on the SNPs in the *P. acnes* core genome. Only the topology is shown. The clades were named based on their *recA* types (IA, IB and II). Columns represent predicted open reading frames (ORFs) in the genomes and are ordered by ORF positions along the finished genome HL096PA1, which encodes a 55 Kb plasmid. Only the first 300 ORFs on the chromosome (on the left) and all the ORFs on the plasmid (on the right) are shown. The colored plasmid regions represent genes on contigs that match exclusively to the HL096PA1 plasmid region. The genes that fall on contigs that clearly extend beyond the plasmid region are likely to be chromosomally located and are colored in grey. Acne index for the ribotypes was calculated based on the percentage of clones of each ribotype found in acne as shown in column 5 in Table 1.

Author Manuscript Author Manuscript Author Manuscript Author Manuscript

Table 1

Top ten most abundant ribotypes found in pilosebaceous units

Ribotype	Nucleotide changes from RT1	Number of subjects	Number of clones	Percentage of clones from acne patients[a]	Percentage of clones from normal individuals[b]	*p*-value[c]
RT1	-	90	5536	48%	52%	0.84
RT2	T854C	48	1213	51%	49%	0.36
RT3	T1007C	60	2104	40%	60%	0.092
RT4	G1058C, A1201C	23	275	84%	16%	0.049
RT5	G1058C	15	205	99%	1%	0.00050
RT6	T854C, C1336T	11	262	1%	99%	0.025
RT7	G529A	10	188	99%	1%	0.12
RT8	G1004A, T1007C	5	239	100%	0%	0.024
RT9	G1268A	4	68	99%	1%	0.29
RT10	T554C, G1058C	5	61	100%	0%	0.024

[a]The percentage was calculated after the number of clones of each ribotype was normalized by the total number of clones in acne patients (acne index).

[b]The percentage was calculated after the number of clones of each ribotype was normalized by the total number of clones in normal individuals.

[c]Mann-Whitney-Wilcoxon rank sum test.

Author Manuscript Author Manuscript Author Manuscript Author Manuscript

Table 2

CRISPR spacer sequences found in the genomes of RT2 and RT6

Ribotype	Strain	Spacer number	Spacer sequence	BLAST result	Match found
RT2	HL001PA1	1	CATGGCCTGCACACCAGGCGCTTTTAGCACCT	No hits	
		2	CATGGCCTGCACACCAGGCGCTTTTAGCACCT	No hits	
		3	CATGGCCTGCACACCAGGCGCTTTTAGCACCT	No hits	
		4	GGCGTATGACGAGTTGTGGTCGGCGTTTCCTC	*P. acnes* phage PA6 gp15 (minor tail protein)	
		5	CGGTGTTAACGGCTTGCCTGGCTTGGATGGAG	No hits	
RT2	HL060PA1	1	CGCCTACCGTCAGCTGACTCACGCCTCCGCGTT	No hits	
		2	TCACACCAGTCATCAGCGTCATAGTCCTCTCGG	No hits	
RT2	HL082PA2	1	GGCTCAGCCCTGCCCGATGCCTACGCCAAATGG	*C. leptum* DSM 753 CLOLEP_00129 (cell wall-associated hydrolases (invasion-associated proteins))	Locus 3
		2	TCACACCAGTCATCAGCGTCATAGTCCTCTCGG	No hits	
RT2	HL103PA1	1	CACCGGGCCCATCCCGGTCGGCCTCCTGAAAGG	*C. leptum* DSM 753 CLOLEP_00135	Locus 3
RT2	HL106PA1	1	GATCGAGTTGGCTGAGTCGAAGGTGTTGCGGTT	*P. acnes* phage PA6 gp16 (conserved protein) *P. acnes* phage PAD20 gp16	
		2	CTGCTCATCGCTCAGCTCCTGCGCCTCATCACA	No hits	
		3	CTGCGCCAACAGCCGCATCTGATCCGAATACGG	*P. acnes* phage PA6 gp3 (phage portal protein)	
		4	CGCAGCAATCTCAGAAGGCCACAACAAGTTCGT	*P. acnes* phage PA6 gp7 (conserved protein) *P. acnes* phage PAD20 gp7 *P. acnes* phage PAS50 gp7	
		5	CAAATCACCCAAGCCCAACACGCCGCCACCACC	No hits	
		6	TGTCACCGATTCAATGTATCTATGAGTGGTGTA	No hits	
		7	TTGGGTGGGTGAGGTCGGGTCGTCAGTCATGAG	No hits	
		8	GTCGATGTCGAGATTGGCCTGGGGGTCCATGTC	*Clostridium leptum* DSM 753 CLOLEP_00142	Locus 3
		9	ACGTCGTGAACGTACCCCTTGACGGAGACGGCA	No hits	
RT2	J139	1	CGAGGGCTACCACGTGGTCGATTTGGACTGTCG	*C. leptum* DSM 753 CLOLEP_00167 *P. acnes* SK137 HMPREF0675_3193 (domain of unknown function)	Locus 2
		2	CAGGCGCTCCACTCCCTCGCCCTGGCCACCAAC	No hits	
RT6	HL110PA3 HL110PA4	1	CTATGTGGACAGTGTTGGTTACTGTGGGGGGAA	*P. acnes* phage PA6 intergenic region between gp45 and gp46	
		2	GCACTGCACCGATATCGTCGTGGCTGTCACTTG	No hits	
		3	CCCAGACAACCTCGACAACCTGTTCAGGGGATG	*P. acnes* phage PAS50 gp25	

Author Manuscript Author Manuscript Author Manuscript Author Manuscript

Ribotype	Strain	Spacer number	Spacer sequence	BLAST result	Match found
		4	CATGGCTAGCCCGGATTTTTGGCTGCCTGAGCG	*P. acnes* phage PA6 gp34 (mutidrug resistance protein-like transporters) *P. acnes* phage PAD20 gp34 (DNA helicase)	
		5	CGGCCTGCGGCAGATTTTTGTTGCGTTGAATCC	*P. acnes* phage PA6 gp14 (tape measure protein) *P. acnes* phage PAD20 gp14 (tape measure protein) *P. acnes* phage PAS50 gp14 (tape measure protein)	
		6	CGGGCAGAGGATGTGTTGCTCGTTCCTGGATGG	*P. acnes* phage PA6 gp32 (CHC2 zinc finger) *P. acnes* phage PAD20 gp32 (DNA primase) *P. acnes* phage PAS50 gp32 (DNA primase)	
		7	GTTACGCTGGAACCCCCAATGAACACGCGAGAA	*P. acnes* phage PAD42 major head protein gene *P. acnes* phage PAD20 major head protein gene *P. acnes* phage PAD9 major head protein gene *P. acnes* phage PAS40 major head protein gene *P. acnes* phage PAS12 major head protein (gene *P. acnes* phage PAS10 major head protein gene *P. acnes* phage PAD21 major head protein gene *P. acnes* phage PAS2 major head protein gene *P. acnes* phage PA6 gp6 (Phage capsid family) *P. acnes* phage PAS50 gp6 major head protein gene	
		8	CGAGGGCTACCACGTGGTCGATTTGGACTGTCG	*C. leptum* DSM 753 CLOLEP_00167 *P. acnes* SK137 HMPREF0675_3193 (Domain of unknown function)	Locus 2
		9	CAGGCGCTCCACTCCCTCGCCCTGGCCACCAAC	No hits	

Abbreviations: BLAST, Basic Local Alignment Search Tool; CRISPR, Clustered Regularly Interspaced Short Palindromic Repeat; *C. leptum, Clostridium leptum; P. acnes, Propionibacterium acnes*; RT, ribotype.