Acne is Not Associated with Yet-Uncultured Bacteria

M. Bek-Thomsen, H. B. Lomholt, and M. Kilian*

Institute of Medical Microbiology and Immunology, The Bartholin Building, Wilhelm Meyer’s Allé 1240, Aarhus University, Aarhus C DK-8000, Denmark

Received 28 April 2008/Returned for modification 15 July 2008/Accepted 7 August 2008

Current clinical and microbiological information on acne fails to demonstrate a clear association between particular species, including Propionibacterium acnes, and disease, and the disease continues to be a considerable problem. To test if acne is associated with hitherto uncultured bacteria residing in diseased skin follicles, sequencing and phylogenetic analysis of approximately 5,700 amplified and cloned 16S rRNA genes were used to determine the microbial diversity in follicles from acne patients and healthy individuals and from the superficial skin of acne patients. Follicles from healthy skin were exclusively colonized by P. acnes, whereas the follicular microbiota of acne patients included, in addition, Staphylococcus epidermidis and minor proportions of other species. In comparison, samples from superficial skin showed a complex microbiota represented by 12 to 16 bacterial species. The findings of the study exclude the possibility that acne is associated with yet-uncultured bacteria and shows that healthy skin follicles constitute a remarkably exclusive habitat allowing colonization only by P. acnes.

Acne is a significant clinical problem with severe social, psychological, and emotional implications. The disease affects up to 80% of teenagers and frequently continues into adulthood. Clinically, acne is characterized by distended sebaceous follicles seen as open or closed comedones and inflamed lesions presenting as papules, pustules, and cysts (2, 19). The pathogenesis of acne appears to be multifactorial, although it is yet only partly understood. A mainly genetically determined host response pattern (3) combined with bacterial “triggering” is generally accepted as being important for the apparently unbalanced inflammatory activity.

Propionibacterium acnes, a regular inhabitant of human skin and sebaceous follicles, has been suspected to be an important triggering agent (4–6, 16). A significant increase in P. acnes colonization density is observed at puberty, concurrent with the onset of the disease (25). A preference for anaerobic conditions and the production of lipid-degrading lipases are believed to make P. acnes well suited to colonize the sebaceous follicles and to sustain this harsh environment (26). In addition, several studies have shown that P. acnes has potent immunostimulating properties, reflected in its ability to induce several proinflammatory cytokines and defensins through Toll-like receptors on keratinocytes, sebocytes, and monocytes (21, 29, 30, 37).

Although some indirect evidence supports the role of P. acnes as the causative agent of acne, other findings are in apparent conflict with this hypothesis. Some researchers have suggested that the involvement of P. acnes in the inflammation is relatively minor and that the predominance of P. acnes in the sebaceous ducts may be a side effect of the inflammation rather than the cause. P. acnes colonizes the skin and follicles of both acne-affected and healthy individuals, and no correlation has been observed between the absolute number of bacteria and the severity of disease (8, 9, 23, 25). It has even been reported that not all comedones or early inflamed lesions harbor microorganisms (23, 24). In addition, beta-lactam antibiotics are ineffective against acne, although P. acnes is fully sensitive to these drugs (36). Finally, prolonged periods of treatment with tetracyclines and macrolides are often necessary for the treatment of acne, in contrast to the shorter courses used for standard infections (9, 34). Studies that have attempted to explain these divisive findings have shown variations in host reactions to P. acnes colonization (37), differences among P. acnes strains (20, 27, 30, 31), and the ability of these bacteria to form local biofilms with reduced susceptibility to antimicrobials (7). In any case, the current treatment options are far from ideal. The use of broad-spectrum antibiotics has led to widespread resistance, and the alternative treatment, isotretinoin, is highly teratogenic and induces pronounced subjective side effects when it is used at therapeutic doses (11). A better understanding of the etiology of acne is essential to develop more efficient treatment options with fewer side effects.

Recent application of molecular techniques has demonstrated that the microbial diversity of most habitats is significantly underestimated by conventional culture-based methods. By amplification and sequencing of cloned libraries of the phylogenetically informative 16S rRNA gene, numerous hitherto unknown species were demonstrated in complex habitats such as the human oral cavity (1, 32) and intestines (12). Therefore, the possibility exists that previously uncultured bacteria resident in the sebaceous follicles play an etiologic role in the development of acne. This hypothesis was evaluated in the present study by mapping the microbial diversity of sebaceous follicles of acne-affected and healthy individuals. The study was based on sequencing of approximately 5,700 cloned PCR amplicons of the 16S rRNA gene generated from samples collected from diseased and healthy individuals.

**MATERIALS AND METHODS**

Patients. Five patients and three healthy controls were recruited in Aarhus, Denmark, in 2006 and 2007 by advertising in local newspapers and by contact
with local dermatologists. All patients were diagnosed and scored by the same specialist in dermatology (H.B.L.). The severity of disease was assessed by using the Leeds scoring system and grading of closed comedones, open comedones, papules, pustules, nodules and scar tissue into four grades: none (score 0), slight (score 1), moderate (score 2), or severe (score 3). Records of disease and medication were obtained, while the use of cosmetic products was not recorded. The clinical data are summarized in Table 1. None of the subjects included in the study were prescribed any form of acne therapy. All subjects were included on the basis of informed consent. The study protocol was approved by the Ethics Committee of the County of Aarhus, and the study was conducted according to the principles of the Declaration of Helsinki.

Sample processing. Follicular bacteria were sampled from acne-affected skin areas of the face by cyanoacrylate biopsy (17, 28). In control subjects, follicles on other areas of facial skin of healthy subjects were too few and small to generate samples with a sufficient number of bacteria. A thin layer of cyanoacrylate adhesive (Sikabond-585; Sika Danmark A/S Lynge, Denmark) was cyanoacrylate adhesive (Sikabond-585; Sika Danmark A/S Lynge, Denmark) was used to cover the full spectrum of acne follicles with regard to the age and the types of the lesions in the individual patient. As a control for the ability of the method to map a complex microbiota, an acne-affected skin area of superficial skin on the cheeks of two patients was sampled by the detergent scrub technique of Williamson and Kligman (38) over an area of approximately 6 cm². Two milliliters of wash fluid (0.075 M sodium phosphate [NaH₂PO₄], pH 7.9, 0.1% Triton X-100) was used for each sample. After the skin was scrubbed, the wash fluid was transferred into a microcentrifuge tube. The bacteria were harvested by centrifugation and were resuspended in lysis buffer. DNA from all samples was extracted with a DNeasy tissue kit (Qiagen, Valencia, Calif.).

DNA from all samples was extracted with a DNeasy tissue kit (Qiagen, Valencia, Calif.). For each sample, molecular-grade water was added to compute the rarefaction curve. The bias-corrected Chao1 estimator was used to compute the rarefaction curve. The bias-corrected Chao1 estimator was used to compute the rarefaction curve. All sequences were checked for the presence of an insert of the correct size by vector-target PCR with universal primers M13F and M13R. A total of 576 clones from each library underwent sequence analysis with primers M13F and M13R, resulting in sequence reads of approximately 700 to 800 bp. Plasmid purification and sequencing were performed at the Beijing Genomics Institute (Beijing, China).

Sequence analysis. Vector trimming and primer removal were performed manually. Alignment of the sequences amplified from each of the individuals was performed by use of the ClustalX program (35). The sequences were manually edited, and for identification purposes, the sequences encoding the first 500 bp of the 16S rRNA gene were obtained. All sequences were checked by use of the Chimera Check program of the RDP II database (http://rdp.cme.msu.edu/index.jsp). A total of 87 chimeric sequences were excluded from the phylogenetic analysis. Corrected and trimmed sequences were grouped into clusters of sequences at least 98% similar to members of their cluster, and representative sequences were determined with the FastgroupII program, accessible at http://biome.sdsu.edu/fastgroup/. The phylogenetic associations of representative sequences were determined with the help of the Sequence Match program, available at the RDP II database (http://rdp.cme.msu.edu/index.jsp). The phylogenetic associations of representative sequences were determined with the help of the Sequence Match program, available at the RDP II database (http://rdp.cme.msu.edu/index.jsp). The phylogenetic associations of representative sequences were determined with the help of the Sequence Match program, available at the RDP II database (http://rdp.cme.msu.edu/index.jsp). The phylogenetic associations of representative sequences were determined with the help of the Sequence Match program, available at the RDP II database (http://rdp.cme.msu.edu/index.jsp). The phylogenetic associations of representative sequences were determined with the help of the Sequence Match program, available at the RDP II database (http://rdp.cme.msu.edu/index.jsp). The phylogenetic associations of representative sequences were determined with the help of the Sequence Match program, available at the RDP II database (http://rdp.cme.msu.edu/index.jsp). The phylogenetic associations of representative sequences were determined with the help of the Sequence Match program, available at the RDP II database (http://rdp.cme.msu.edu/index.jsp). The phylogenetic associations of representative sequences were determined with the help of the Sequence Match program, available at the RDP II database (http://rdp.cme.msu.edu/index.jsp). The phylogenetic associations of representative sequences were determined with the help of the Sequence Match program, available at the RDP II database (http://rdp.cme.msu.edu/index.jsp). The phylogenetic associations of representative sequences were determined with the help of the Sequence Match program, available at the RDP II database (http://rdp.cme.msu.edu/index.jsp). The phylogenetic associations of representative sequences were determined with the help of the Sequence Match program, available at the RDP II database (http://rdp.cme.msu.edu/index.jsp). The phylogenetic associations of representative sequences were determined with the help of the Sequence Match program, available at the RDP II database (http://rdp.cme.msu.edu/index.jsp). The phylogenetic associations of representative sequences were determined with the help of the Sequence Match program, available at the RDP II database (http://rdp.cme.msu.edu/index.jsp). The phylogenetic associations of representative sequences were determined with the help of the Sequence Match program, available at the RDP II database (http://rdp.cme.msu.edu/index.jsp). The phylogenetic associations of representative sequences were determined with the help of the Sequence Match program, available at the RDP II database (http://rdp.cme.msu.edu/index.jsp). The phylogenetic associations of representative sequences were determined with the help of the Sequence Match program, available at the RDP II database (http://rdp.cme.msu.edu/index.jsp). The phylogenetic associations of representative sequences were determined with the help of the Sequence Match program, available at the RDP II database (http://rdp.cme.msu.edu/index.jsp). The phylogenetic associations of representative sequences were determined with the help of the Sequence Match program, available at the RDP II database (http://rdp.cme.msu.edu/index.jsp). The phylogenetic associations of representative sequences were determined with the help of the Sequence Match program, available at the RDP II database (http://rdp.cme.msu.edu/index.jsp). The phylogenetic associations of representative sequences were determined with the help of the Sequence Match program, available at the RDP II database (http://rdp.cme.msu.edu/index.jsp). The phylogenetic associations of representative sequences were determined with the help of the Sequence Match program, available at the RDP II database (http://rdp.cme.msu.edu/index.jsp). The phylogenetic associations of representative sequences were determined with the help of the Sequence Match program, available at the RDP II database (http://rdp.cme.msu.edu/index.jsp). The phylogenetic associations of representative sequences were determined with the help of the Sequence Match program, available at the RDP II database (http://rdp.cme.msu.edu/index.jsp). The phylogenetic associations of representative sequences were determined with the help of the Sequence Match program, available at the RDP II database (http://rdp.cme.msu.edu/index.jsp). The phylogenetic associations of representative sequences were determined with the help of the Sequence Match program, available at the RDP II database (http://rdp.cme.msu.edu/index.jsp). The phylogenetic associations of representative sequences were determined with the help of the Sequence Match program, available at the RDP II database (http://rdp.cme.msu.edu/index.jsp). The phylogenetic associations of representative sequences were determined with the help of the Sequence Match program, available at the RDP II database (http://rdp.cme.msu.edu/index.jsp). The phylogenetic associations of representative sequences were determined with the help of the Sequence Match program, available at the RDP II database (http://rdp.cme.msu.edu/index.jsp). The phylogenetic associations of representative sequences were determined with the help of the Sequence Match program, available at the RDP II database (http://rdp.cme.msu.edu/index.jsp). The phylogenetic associations of representative sequences were determined with the help of the Sequence Match program, available at the RDP II database (http://rdp.cme.msu.edu/index.jsp).

Estimation of microbial diversity. The rarefaction curve and richness estimations were calculated with the EstimateS program (version 8.0), available at http://viceroy.eeb.uconn.edu/EstimateS. The sampled-based Mao Tau method was used to compute the rarefaction curve. The bias-corrected Chao1 estimator of species richness was calculated after 100 randomizations of sampling without replacement.

RESULTS

Clone analysis. We analyzed a total of ca. 5,700 cloned bacterial 16S rRNA gene sequences from gene libraries generated from samples collected from healthy individuals and acne-affected patients. Approximately 4,600 of these clones were from samples of follicles from five acne patients with Leeds scores ranging from 2 to 5 and from three healthy subjects (Table 1). The remaining approximately 1,100 cloned sequences were from acne-affected areas of superficial skin.

<table>
<thead>
<tr>
<th>Subject</th>
<th>Age (yr)</th>
<th>Gender</th>
<th>Leeds score</th>
<th>Closed comedones</th>
<th>Open comedones</th>
<th>Papules</th>
<th>Pustules</th>
<th>Scarring</th>
</tr>
</thead>
<tbody>
<tr>
<td>1P</td>
<td>15</td>
<td>M</td>
<td>3</td>
<td>2</td>
<td>1</td>
<td>2</td>
<td>2</td>
<td>0</td>
</tr>
<tr>
<td>2P</td>
<td>33</td>
<td>F</td>
<td>3</td>
<td>3</td>
<td>1</td>
<td>3</td>
<td>1</td>
<td>3</td>
</tr>
<tr>
<td>3P</td>
<td>23</td>
<td>M</td>
<td>5</td>
<td>1</td>
<td>1</td>
<td>2</td>
<td>3</td>
<td>2</td>
</tr>
<tr>
<td>4P</td>
<td>36</td>
<td>F</td>
<td>5</td>
<td>1</td>
<td>1</td>
<td>3</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>5P</td>
<td>17</td>
<td>M</td>
<td>2</td>
<td>1</td>
<td>2</td>
<td>2</td>
<td>2</td>
<td>0</td>
</tr>
<tr>
<td>1C</td>
<td>25</td>
<td>F</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>2C</td>
<td>30</td>
<td>F</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>3C</td>
<td>24</td>
<td>M</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>

Abbreviations: F, female; M, male; P, acne patient; C, healthy control.
from two acne patients. A sequence stretch corresponding to nucleotide positions 28 to 528 in the 16S rRNA gene of *E. coli*, which phylogenetically is the most informative part of the molecule, was analyzed and was used to determine identity or an approximate phylogenetic affiliation. The exclusion of truncated sequences and chimeras resulted in a total of 5,040 valid sequences, which were grouped into operational taxonomic units (OTUs). OTUs were defined as clusters of sequences that were at least 98% similar to members of their respective cluster. The taxonomic affiliation of OTUs was determined with the programs Sequence Match at the RDP II database and BLAST at NCBI, based on a comparison of the generated sequences to the 16S rRNA sequences of recognized species. Sequences of designated type strains were preferred as references, unless they were of insufficient quality.

**Follicle samples.** After exclusion of unsuccessful and chimeric sequences, a total of 4,082 sequences generated from the follicles of acne patients and controls remained. Thus, a mean of 510 sequences (range, 475 to 537 sequences) from each of the eight subjects were analyzed. Compared to the bacterial community present on superficial skin, the bacterial community present in follicles was strikingly homogeneous. Thus, all sequences generated from follicles from the three healthy controls represented sequences of recognized species. OTUs were defined as clusters of sequences that were at least 98% similar to members of their respective cluster. The taxonomic affiliation of OTUs was determined with the programs Sequence Match at the RDP II database and BLAST at NCBI, based on a comparison of the generated sequences to the 16S rRNA sequences of recognized species. Sequences of designated type strains were preferred as references, unless they were of insufficient quality.

**Superficial skin samples.** To validate the nonculture technique, a total of 958 sequences obtained from acne-affected areas of the faces of two patients, patients 2P and 4P, were analyzed. The sequences clustered into 25 OTUs, 18 of which showed ≧98% similarity to the designated type strains of recognized species. The remaining OTUs showed ≧98% similarity to non-type strains of recognized species or to the PCR-generated 16S rRNA gene sequences of yet-uncultured bacteria. The taxonomic affiliations of the OTUs are listed in Table 2. The 25 OTUs identified belonged to four bacterial phyla, *Actinobacteria*, *Firmicutes*, *Proteobacteria*, and *Bacteroidetes*, and were distributed in 18 recognized genera and one yet-uncultured taxon belonging to the Betaproteobacteria. A total of 12 and 16 species were identified in samples from superficial acne-affected skin of the two subjects, respectively. As shown in Table 2, significant differences were observed between the two individuals. Only 3 of a total of 25 identified species were detected in both individuals. However, the three species carried by both of the acne patients, *S. epidermidis*, *P. acnes*, and *Propionibacterium granulosum*, accounted for 95% of all the sequences analyzed. In both subjects, the majority of sequences represented those of *S. epidermidis*, while *P. acnes* constituted the second most dominant species. Both species are known to be universal members of the bacterial community resident on human skin. Several of the species detected in minor proportions, e.g., *Veillonella parvula*, *Streptococcus peregrinatus*, and *Capnocytophaga gingivalis*, are known to be regular residents of the oral cavity.

The total number of OTUs present on the superficial skin from the two acne-affected patients was estimated by the non-parametric estimator of total richness, Chao1 (Fig. 1). As expected, on the basis of the numerous species represented by a single clone (Table 2), the number of species present in the samples from superficial skin was not exhaustively revealed by the number of sequences analyzed. On the basis of these calculations, the combined bacterial communities in the samples from the two patients contained 43 OTUs (95% confidence interval ([CI]) range, 30 to 90). Thus, the 25 OTUs detected (95% CI range, 18 to 32) represent 58.1% (95% CI range, 35.5 to 60%) of the estimated number of species present in the superficial samples from acne-affected areas of the skin. Extrapolation of the Mao Tau graph in Fig. 1 indicates that sequencing of an additional ~1,600 clones would be required to reveal the remaining OTUs, which were all present at proportions below 1%.

**DISCUSSION**

Our study evaluated the hypothesis that yet-uncultured bacteria play a role in the pathogenesis of acne. This was made possible by applying a PCR-based strategy with a sensitivity and a discriminatory power surpassing those of previously used culture-based methods. The 16S rRNA-based method was recently developed and has been applied in several studies of complex bacterial communities associated with humans, including the microbiota of healthy skin (1, 10, 12–14, 32) The 16S rRNA gene is present in all known bacteria and contains an array of regions perfect for species assignment. The sensitivity of the method depends on primer specificity and the number of sequences determined. The coverage of the primers used in our study was found to be significant, with the primers matching more than 55,000 bacteria representing 1,061 genera in the RDP II database. Similar primers were used in previous studies and revealed that the microbiota of the superficial skin of healthy individuals has considerable diversity (10, 13, 14).

As a validation of the method, the overall composition of the microbiota of superficial acne-affected skin that was demonstrated was very similar to that previously shown both for healthy human skin by a standard culture technique and for skin from the forearm and the forehead by the sequence-based technique used in this study (6, 10, 13). The results of these studies combined show that the skin microbiota of both healthy and acne-affected individuals contains a ubiquitous and dominant core of bacterial species, i.e., *S. epidermidis* and *P. acnes*. As an exception, Grice et al. (14) found *Pseudomonas* and *Janthinobacterium* species to be dominant in the more humid skin of the healthy human antecubital fossa, suggesting that significant regional differences may exist in the core bacterial species present on various skin surfaces. In addition, numerous species are present in minor proportions, and these proportions vary among individuals. The latter include bacteria that

**Table 1.** OTUs generated by sequence analysis of 16S rRNA gene regions from follicles and superficial skin from acne patients. *P. acnes* was also dominant in the follicles from acne-affected patients, comprising 52.7 to 91.6% of all clones. Additional species were detected. *Staphylococcus epidermidis* constituted 6.8 to 47.3% of the clones generated from the five patients. Minor proportions of additional species were detected in three of the patients (patients 2P, 3P, and 4P). Among these, *Corynebacterium tuberculostearicum* was detected in all three subjects and constituted 0.2 to 1.3% of the clones. In addition, patient 3P was colonized by a previously uncultured *Actinobacterium* sp. and by *Staphylococcus hominis* subsp. *hominis*. Patient 4P housed a yet uncultured *Anaerococcus* sp. and *Propionibacterium granulosum*.

<table>
<thead>
<tr>
<th>OTUs</th>
<th>Superficial skin samples</th>
<th>Follicle samples</th>
</tr>
</thead>
<tbody>
<tr>
<td>25</td>
<td>958</td>
<td>1600</td>
</tr>
<tr>
<td>18</td>
<td>958</td>
<td>1600</td>
</tr>
<tr>
<td>12</td>
<td>958</td>
<td>1600</td>
</tr>
<tr>
<td>16</td>
<td>958</td>
<td>1600</td>
</tr>
</tbody>
</table>
are recognized commensals of the oral cavity, which are presumably transiently present on the facial skin. In addition, our results and those reported by previous investigators (10, 13) clearly show that yet-uncultured phylotypes may be present but do not belong to the dominant core microbiota of the skin either in healthy individuals (10, 13) or in patients with acne (this study).

Sebaceous follicles, which are generally recognized to be the sites relevant to acne, must represent a uniquely harsh environment for bacteria, as evidenced by the surprising finding that only \( P. \) acnes colonized the follicles of healthy individuals.

Our coverage analysis demonstrates that sequencing of more clones would not have revealed additional diversity. No other exposed microenvironment of the human body is known to selectively allow colonization by a single bacterial species. It is conceivable that the more heterogeneous microbiota found in
previous culture-based studies of healthy follicles (15, 23, 33) was the result of differences in sampling methods. The cyanoacrylate method employed in the present study has the advantage, in addition to being noninvasive, that the upper part of the stratum corneum is captured in the applied cyanoacrylate, which minimizes the possibility of contamination with skin surface microorganisms when follicles are isolated by dissection. Microdissection of single follicles from biopsy punch specimens inevitably results in contamination with surface skin colonizers. Interestingly, Grice et al. (14) detected the same composition of skin microbiota on the antecubital fossa by use of swap, scrape, and punch biopsy techniques, all of which are likely to include surface bacteria.

The bacterial microbiota of follicles from acne-affected subjects showed more, although still very limited, diversity (Table 2). The microbiota was dominated by *P. acnes* and *S. epidermidis*, which were the only species consistently found. These results clearly exclude the possibility that yet-uncultured bacteria are associated with acne-affected skin follicles. Although the number of patients was limited, there was a complete absence of previously uncultured bacteria common to acne-affected follicles. It is unlikely that potential members of the microbiota present in numbers below the detection threshold of the method play a role in generating the intense inflammatory reaction typical of acne. We therefore conclude that yet-uncultured bacteria do not play an etiologic role in acne.

In contrast to previous culture-based studies (6, 15, 23), our molecular analysis revealed differences in the microbiota of follicles from healthy and acne-affected subjects. The main difference was the presence of *S. epidermidis* in acne-affected follicles (Table 2). There is evidence from histological studies with fluorescently labeled species-specific antibodies that the majority of staphylococci, when they are present, colonize the dilated openings of follicles, an area that is unlikely to be included by our method as a result of the previously mentioned effect of cyanoacrylate (18). Our detection of *S. epidermidis* and occasional other bacterial species exclusively in acne-affected follicles may be explained by the facilitated access of the bacteria to dilated, inflamed follicles and by the previous histological demonstration that staphylococci infiltrate tissues surrounding inflamed follicles (18). A possible alternative or additional explanation may be that the inflammatory reaction in the skin follicles of acne patients interferes with their apparently uniquely potent antimicrobial defense mechanisms.

The presence of *S. epidermidis* exclusively in acne-affected follicles raises the question of the potential role of this species. Previous studies excluded staphylococci as agents that play a role in the pathogenesis of acne on the basis of their rapid development of resistance to therapeutic antibiotics (15). It is possible that staphylococci are secondary colonizers, as inflamed and dilated follicles seem to be more conducive to colonization. However, on the basis of the findings of the present study, a reevaluation of the role of *S. epidermidis* may be advisable. It is widely accepted that microbes interact and that such interactions may influence their production of biologically reactive substances. Further studies are required to elucidate the potential symbiotic effects of the two species.

In conclusion, by using a genetically based strategy with a sensitivity and a discriminatory power surpassing those of culture-based methods, we demonstrated that the follicles of healthy skin constitute a uniquely selective habitat for *P. acnes*, which was the only species detected. A microbiota that was more heterogeneous but that had limited complexity was demonstrated in acne-affected follicles. The results exclude the possibility that yet-uncultured bacteria are associated with acne.

**ACKNOWLEDGMENT**

The study was supported by an unconditional grant from LEO Pharma, Ballerup, Denmark.

**REFERENCES**


