A comparison of culture and PCR methods for identifying *Propionibacterium acnes* in lesions isolated from patients with acne

Negin NAGHDI*, Masood GHANE
Department of Microbiology, Faculty of Biological Sciences, Islamic Azad University, Tonekabon Branch, Tonekabon, Iran

**1. Introduction**
Healthy skin consists of a great diversity of commensal microorganisms (1,2). Different parts of the skin have a different number and diversity of microorganisms. This difference depends on factors such as temperature, UV light (3,4), dryness, skin moisture content, skin fat content (2,3), acidity (2), skin structure (2,4), and the reciprocal reaction of microorganisms (4). Due to dryness and acidity, the epidermis is not suitable for bacterial survival (2,5); however, gram-positive bacteria can better adapt to living in these conditions compared to gram-negative bacteria. *Streptococcus* (2), *Staphylococcus*, *Micrococcus*, *Corynebacterium*, *Propionibacterium* (4,5), *Acinetobacter* (4), and *Brevibacterium* (5) are examples of epidermal bacteria. As a lipophilic yeast found on the surface of the skin, *Malassezia* plays a significant role in the body (4,5).

Acne vulgaris or youth acne is a prevalent, chronic, inflammatory disease of the sebaceous follicles (6–8) observed in 80% of teenagers (9–11). *Propionibacterium acnes* has been proposed as a bacterium involved in the pathogenesis of acne for a century (12,13). Since the majority of patients with acne have oily skin, a relationship appears to exist between the intensity of acne and the rate of sebum production (8,10,12). Clogged sebaceous pores, increased sebum production, *P. acnes*, and inflammation are potential factors involved in the formation of acne (11,14).

The skin is the largest habitat of *P. acnes* (13,15,16), but the bacterium can be isolated from the conjunctiva (16–18), heart, stomach (19), intestines (16,17), respiratory system, urinary system (18), oral cavity, and outer ear (15,16).

*P. acnes* is a polymorphic (9,17,20), gram-positive, facultative anaerobic, sporeless (18,20), nonmotile (13,19), and slow-growing (16,19) bacillus that grows best under limited oxygen concentrations (0%–20%) and has a reduced growth rate with high oxygen concentrations (19,21). These bacilli produce propionic acid (12,17) and acetic acid (19) from fermented carbohydrates. *P. acnes* is an organism with low pathogenesis; however, it may contribute to opportunistic infections (15,17,18) and damage the host by the secretion of extracellular enzymes such as lipase, protease, hyaluronidase (3,21), and phosphatase acid (21).

* Correspondence: negin.naghdi91@yahoo.com

**Background/aim:** One of the factors that affect the occurrence of acne is the presence of *Propionibacterium acnes*. The present study was conducted to compare the culture and polymerase chain reaction (PCR) methods for identifying *P. acnes* in lesions isolated from patients with acne.

**Materials and methods:** To examine the presence of *P. acnes*, 70 samples of acne lesions were collected. Microbial culture and the PCR molecular technique were used to identify *P. acnes*.

**Results:** Of the total of 70 samples, 14 cases (20%) were identified as *P. acnes* positive using microbial culture and 58 cases (82.85%) using PCR. The results obtained showed the lack of a relationship between the frequency of *P. acnes* and factors such as sex, family history of acne, and history of treatment with either of the techniques examined (i.e. the microbial culture and PCR). In contrast, a significant relationship was observed between the frequency of *P. acnes* and age with the culture method.

**Conclusion:** Given the limitations in the identification of *P. acnes* using microbial culture, PCR is proposed as a better method with a higher efficiency.

**Key words:** *Propionibacterium acnes*, culture, polymerase chain reaction, acne
The development of molecular techniques has yielded a new perspective on epidermal microflora (3,4). Given the diversity of epidermal microorganisms, molecular methods provide a great tool for their identification (3). Polymerase chain reaction (PCR) is a powerful, quick, sensitive, and reliable molecular biology technique that relies on the analysis of a particular sequence of DNA molecules (22,23). The present study was conducted to compare the culture and PCR methods in the identification of *P. acnes* isolated from patients with acne.

2. Materials and methods

2.1. Sample Collection

The present cross-sectional analysis was conducted on 70 patients with acne referred to a skin clinic in Tonekabon, Iran, in 2013 and 2014. Of the total of 70 samples collected, 49 were from females and 21 were from males; patients ranged in age from 14 to 32 years old. The samples were collected from the patients' face (forehead, cheek, and chin). The site of sampling was disinfected by 70% ethanol. To collect the samples, sterile cotton swabs (14) were placed inside a test tube containing 2 mL of physiological serum. To remove and extract the closed comedones and the papules, a lancet was used to make a scratch on the surface of the lesion and the content was then extracted with slight hand pressure.

2.2. Microbial culture

The collected samples were transferred into test tubes containing brain heart infusion broth (Merck, Germany) (24,25); after sterile paraffin (Merck, Germany) was added for creating anaerobic conditions (14), the samples were incubated at 37 °C for 48 to 72 h (15). A loop of bacterial suspension was then taken from the liquid medium under sterile conditions next to a fire and was then cultured on plates containing brain heart infusion agar (Merck, Germany) (24,25). The plates were placed in anaerobic conditions and were then incubated at 37 °C for 4 to 5 days (19,25).

2.3. Phenotypic identification

The cultured plates were examined carefully for emerging colonies. After conducting the Gram staining (24), a pure culture was obtained from the samples that showed the intended bacterial morphology. For identifying the bacterium, a variety of biochemical tests were used, including the catalase test, the oxidase test, the indole test, the motility test, SH2, and glucose and maltose fermentation (20,24).

2.4. DNA extraction

A total of 300 µL of the collected samples were dissolved in 200 µL of TE buffer (Tris-EDTA buffer); after adding 2 µL of lysozyme, the solution was kept at room temperature for 30 min. DNA extraction was then carried out according to the instructions in the extraction kit (QIAGEN, Germany). To examine the accuracy of the DNA extraction, the samples' photoabsorption was evaluated at 260 and 280 nm using a biophotometer (Eppendorf, Germany).

2.5. PCR with specific primers

A pair of specific primers synthesized by TAG (Copenhagen, Denmark) were used to perform the PCR. This primer duplicates 1202 base pairs from the genome of *P. acnes*. Table 1 presents the primer sequence used.

A PCR kit made by QIAGEN was used to perform the PCR. According to the instructions provided in the kit, the PCR mix was prepared with a final volume of 20 µL, consisting of 10 µL of Master Mix and 0.5 µL of forward primer, 0.5 µL of reverse primer (10 pmol), 5 µL of the extracted DNA, and 4 µL of sterile distilled water in a 0.2-mL microtube. The mix was then well vortexed and spun and placed in a thermocycler (Eppendorf, Germany). PCR was carried out in 35 cycles, including steps of denaturation at 95 °C for 60 s, primer annealing at 60 °C for 30 s, and elongation at 72 °C for 90 s. The initialization step was carried out at 95 °C for 5 min and the final elongation was at 72 °C for 10 min (26).

2.6. Electrophoresis

To carry out electrophoresis, 5 µL of each sample was transferred to wells of agarose gel (1.5%) along with a 1-kb DNA ladder (QIAGEN) and was then electrophoresed at 75 V for 40 min. The gel was then transferred to a transilluminator (UVIdoc, UK) and the duplicated 1202 base pairs were examined under UV light.

2.7. Sequence determination

To confirm the results obtained from the PCR, four samples were selected for sequencing. A total of 50 µL of the PCR product synthesized by *P. acnes* specific primers (PAR-1 and PAR-2) was transferred to Macrogen Co. in Korea for determining the sequence and identifying the isolated strains. The sequences obtained were ultimately blasted (NCBI) for the final confirmation.

### Table 1. Primer sequence (26).

<table>
<thead>
<tr>
<th>Primer</th>
<th>Primer sequence 5'→ 3'</th>
<th>PCR product</th>
</tr>
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<tbody>
<tr>
<td>PAR-1</td>
<td>5'-AGC TCG GTG GGG TTC TCT CAT C-3'</td>
<td>1202 bp</td>
</tr>
<tr>
<td>PAR-2</td>
<td>5'-GCT TCC TCA TAC CAC TGG TCA TC-3'</td>
<td></td>
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</table>
3. Results

3.1. Isolation and identification of P. acnes using the culture technique and PCR

The present study used the culture technique and PCR to identify P. acnes in lesions isolated from acne samples. Of the total of 70 samples examined, 14 cases (20%) were identified as P. acnes-positive through the culture method, while 58 cases (82.85%) were identified as such using PCR. The Figure presents the PCR product electrophoresis on agarose gel (1.5%). As observed, a significant difference exists between the results obtained through the culture method and those obtained through PCR. Comparison of the results obtained through these two methods for the identification of P. acnes was carried out with SPSS 18 using the chi-square test. Given that the level of statistical significance was set at P < 0.001, the difference in the ability of each method for identifying the bacterium was confirmed. The PCR method is therefore shown to identify a higher percentage of bacteria compared to the culture method (Figure).

3.2. Demographic analysis of the results

The findings obtained from the two methods were analyzed based on participants' demographic information, including sex, age, family history of acne, and history of treatment. The data were interpreted in SPSS 18 using the independent chi-square test. The results showed the lack of a relationship between the frequency of P. acnes and factors such as sex, family history of acne, and history of treatment with either of the techniques examined (i.e. culture and PCR). As shown in Table 2, the P-value obtained for the age groups is 0.013 and smaller than 0.05 using the culture method, which suggests a significant relationship between the frequency of P. acnes and age with the culture method (Table 2).

3.3. Sequencing results

The sequencing results obtained confirmed the PCR results. Table 3 presents the results obtained from the sample sequencing blasted on the NCBI database (Table 3).

4. Discussion

In recent years, our knowledge about epidermal microorganisms has relied on the culture method; however, this method allows for only a minority (less than 1%) of bacteria to be identified (1,4). In contrast with the culture method, molecular methods enable the identification of a greater diversity of microorganisms (3). Molecular methods have great applications in the identification of microorganisms that are unculturable (23,27), slow-growing (23), and obligate anaerobic (27). Moreover, the molecular method allows for a small quantity of the microorganisms' DNA to be identified and so the microorganism does not need to be alive (5,27). Despite all the advancements in molecular methods, the culture technique is still regarded as a standard method (5,22).

P. acnes is a fastidious bacterium that requires a rich medium, special nutritional factors, and anaerobic conditions to grow. Due to the slow pace of growth, identifying P. acnes is not easily possible through the culture technique. The present study examined 70 samples; 14 cases (20%) were found to be P. acnes-positive using the culture method and 58 (82.85%) using PCR. Moreover, a relationship was observed between the frequency of P. acnes and age using the culture method. Research suggests that acne outbreak occurs most frequently in the age group of 13 to 20 (10,14). Given the physiological and anatomical differences between men and women, factors such as age, sex (3,4), sampling site (3), and drug administration (4) affect the microbial flora diversity among individuals.

Hykin et al. conducted a study in 1994 and collected 23 samples. The culture method revealed 4 (17.39%) P. acnes-positive samples, while the PCR method revealed 8 (34.78%) (28). A study conducted by Schabereiter-Gurtner...
et al. on individuals with conjunctiva infection in 2001 collected 60 samples; however, the culture method yielded no positive \( P. acnes \) samples, while PCR yielded 7 (12%) positive samples (29). In 2003, Le Page et al. conducted a study to diagnose vascular prosthesis infections using the amplification of 16S rDNA sequences. Standard culture yielded negative results in all the samples, while the PCR method yielded 5 (25%) \( P. acnes \)-positive samples out of the total of 20 examined (30). In 2006, Bagyalakshmi et al. conducted a study on patients with endophthalmitis and collected a total of 30 samples; however, the culture method yielded no \( P. acnes \)-positive samples while mPCR yielded 4 (13.3%) positive samples (31).

As suggested by the review of literature, Schabereiter-Gurtner et al., Le Page et al., and Bagyalakshmi et al. were unable to identify any positive \( P. acnes \) samples in their studies; their failure may have been due to poor culture conditions, including poor nutritional factors, lack of anaerobic conditions, poor thermal conditions, and insufficient incubation time. Moreover, the presence of certain microorganisms in the culture medium and the previous administration of antibiotics to the samples may have affected the culture results, while no such effect is observed in the results obtained through PCR.

The disparity of results obtained in different studies might be due to the sampling site, the type of samples examined (acne or other \( P. acnes \) infections), culture conditions, and PCR conditions. The results obtained in this study and in other studies conducted on the subject suggest that, in comparison with the culture method, PCR allows for a higher percentage of bacteria to be identified. The use of techniques relying on nucleic acid has eliminated the limitations present in the identification of microorganisms through culture (4,22). Molecular methods have a high sensitivity; however, they also have some limitations, such as showing false negative or positive responses due to contamination (22,23) or the lifelessness of the microorganisms under study (22).

| Table 2. Demographic data pertaining to the patients and the relative and absolute frequency of \( P. acnes \) in terms of the examined factors and by the culture and PCR methods. |
|---|---|---|---|---|---|
| **Index** | **Item** | **Frequency, N (%)** | **Number of positive cultures (%)** | **Number of positive PCRs (%)** | **P-value for culture** | **P-value for PCR** |
| **Sex** | Female | 49 (70%) | 10 (20.4%) | 42 (85.7%) | 0.587 | 0.261 |
| | Male | 21 (30%) | 4 (19%) | 16 (76.2%) | 0.013 | 0.330 |
| **Age (in years)** | | | | | | |
| | 10–15 | 6 (8.57%) | 0 (0%) | 6 (100%) | 0.151 | 0.828 |
| | 15–20 | 32 (45.71%) | 9 (28.1%) | 27 (84.4%) | | |
| | 20–25 | 18 (25.71%) | 3 (16.7%) | 13 (72.2%) | | |
| | 25–30 | 12 (17.14%) | 0 (0%) | 10 (83.3%) | | |
| | 30–35 | 2 (2.86%) | 2 (100%) | 2 (100%) | | |
| **Family history of acne** | Yes | 37 (52.86%) | 5 (35.7%) | 31 (83.8%) | | |
| | No | 33 (47.14%) | 9 (57.1%) | 27 (81.8%) | | |
| **History of treatment** | Yes | 29 (41.43%) | 6 (42.9%) | 22 (37.9%) | 0.904 | 0.215 |
| | No | 41 (58.57%) | 8 (41.1%) | 36 (58.3%) | | |

| Table 3. Results obtained from the sample sequencing blasted on NCBI. |
|---|---|---|
| **Sample** | **\( P. acnes \) strain** | **Sequence ID** |
| 1 | hdn-1 | CP006032.1 |
| 2 | PA44 | KJ572677.1 |
| 3 | hdn-1 | CP006032.1 |
| 4 | PA62 | KJ572678.1 |
Given the limitations discussed for the identification of microorganisms through the culture technique and
the quick and accurate identification of pathogenic factors enabled by the PCR technique, PCR is proposed
as a standard method for identifying the bacterium under study. Nevertheless, it should be noted that PCR allows
only for the identification of bacterial DNA and does not facilitate the study of microorganisms in their alive
form. Despite the advancements made in the molecular techniques used for identifying various infections, the
culture method still holds its place as a standard method.

References


