Detection of *Propionibacterium acnes* 16S rRNA and Lipase Genes from Sebum Samples Collected on Lipid-specific Adhesive Skin Strips and Processed by Pressure Cycling Technology (PCT)

Introduction

Sebum is an oily secretion produced by the sebaceous glands in the skin. In association with certain bacteria, such as *Propionibacterium acnes* (*P. acnes*), the accumulation of sebum can lead to *Acne vulgaris* (commonly known as acne). This bacterium secretes lipases that allow it to break down the fatty acids in sebum. Here we describe a rapid noninvasive method for the extraction of bacterial RNA from human sebum samples. Sebum was collected on lipid-specific pore-cleansing adhesive skin strips, and processed by pressure cycling technology (PCT). Our data show that the16S rRNA gene and the lipase gene of *P. acnes* can readily be detected in samples from both healthy and acne-prone skin using this method.

PCT Sample Preparation System (PCT SPS)

PCT uses alternating cycles of high and ambient pressures to induce cell lysis. Biological samples, such as sebum, are placed in specially designed, single-use processing containers (PULSE Tubes) and are subsequently subjected to alternating cycles of high (up to 35,000 PSI) and ambient pressures in a pressure-generating instrument (Barocycler©) – together, they comprise the PCT Sample Preparation System (PCT SPS). Maximum and minimum pressures, the time at each pressure level, and the number of cycles are set by the user using a programmable logic controller. The reaction chamber of the Barocycler instrument can be temperature controlled using a peripheral circulating water bath [1].

Materials and Methods

Sebum Collection

Sebum samples were collected from human skin using commercially available lipid-specific pore-cleansing adhesive skin strips. Prior to collection, the skin (forehead or neck) was wiped thoroughly using a piece of all-purpose dressing wetted with DEPC-treated water. An adhesive skin strip was then applied to the skin with the smooth side down, and gently pressed to ensure good contact. The strip was allowed to stay on the skin until it was stiff to the touch (~5 to 10 min). The strip was then carefully peeled from the skin and either processed as described below, or stored at –70^0 C for later processing. One sebum sample was collected from a volunteer who was clinically diagnosed with acne. Other sebum samples were collected from four volunteers who appeared to have normal healthy skin.

Pressure Cycling Technology (PCT)

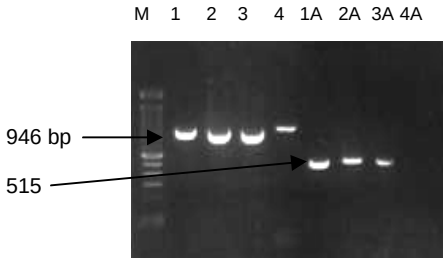
Adhesive skin strips containing sebum were processed using the PCT SPS. PULSE Tube was prepared for each sample by first putting the Ram in place according to the Product Insert (PBI). Saturated Guanidine hydrochloride (1 mL containing 5% Triton X-100, 0.2 M dithioerythritol (DTE) 0.05 M Urea, pH 4.5) was added to each tube. The strips were first trimmed along the edges (~ 0.5 cm), then cut into eight pieces and placed into a PULSE Tube. Each sample was subjected to PCT (10 sec at 35,000 psi followed by 5 sec at ambient pressure at 4^{0}C) for 10 cycles.

Purification of RNA

Following PCT treatment, each PULSE Tube was vortexed for 10 sec and 700 µL of acid phenol (equilibrated with 50 mM sodium acetate pH 4.0) was added (Ambion acid phenol, Cat # 9720 may also be used for this step). The sample was then thoroughly mixed by aspirating ten times with a 1 mL pipette. The solution was transferred to a microcentrifuge tube (2 mL) and 250 mL of 1-Bromo-3-Chloropropane (BCP, MRC Cat# Bp151) was added. Chloroform:isoamyl alcohol (24:1) may be used in place of 1-Bromo-3-Chloropropane. The sample was thoroughly mixed and incubated on ice for 5 min. Debris was removed by centrifugation (13,000 rpm for 10 min at 4^{0}C). The aqueous phase (upper phase) was transferred to a new microcentrifuge tube (2.0 mL), and an equal volume of chloroform:isoamyl alcohol (24:1) was added to remove any residual phenol. The aqueous phase was collected again by centrifugation (10,000 rpm for 10 min at 4^0 C). To precipitate the RNA, an equal volume of pre-cooled isopropanol solution (0.3 mL 3 M sodium acetate pH 4.0, 49.7 ml isopropanol) was added, and the mixture was incubated at – 70^{0}C for 30 min. RNA was pelleted by centrifugation (15 min at 4^{0}C at 13,000 rpm) (note: the RNA pellet may be difficult to see) and the supernatant was carefully removed and discarded. The pellet was briefly dried and dissolved in 50 µL DEPC-treated water.

RT-PCR

DNA was amplified from the RNA by RT-PCR using a GeneAmp 9700 (PE Applied Biosystems) to detect the 16S rRNA gene and the lipase gene of *P. acnes*. Primers were synthesized by Integrated DNA Technologies, Inc. Human B-actin primers were purchased from Clontech. Primers for the 16S rRNA gene of *P. acnes* were PAS9 5'-CCC TGC TTT TGT GGG GTG and PAS11 5'-CGA CCC CAA AAG AGG GAC. Primers for the lipase gene of *P. acnes* were Lip1 5'-TCA CTG ATG AAG ATC AAC GCA C and TGC GAA TGT CCG ACG AAG TCG A [2]. RT-PCR reactions (50 µL) were done using QIAGEN OneStep RT-PCR Kit (QIAGEN Cat# 210212 or



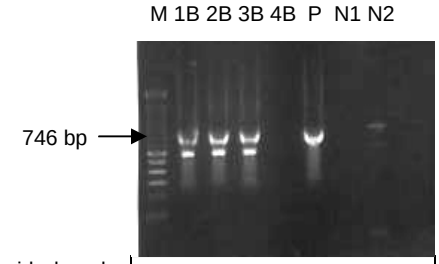


Figure 1. Agarose Gels of RT-PCR Products of *P. acnes* 16S rRNA Gene, Lipase Gene (Upper Gel) and Human B-actin (Lower Gel) from a Subject Diagnosed with Acne. Lane: M (100 bp) DNA marker. Lanes 1, 2, 3 and 4: *P. acnes* 16S rRNA gene amplification. Lanes 1A, 2A, 3A and 4A: *P. acnes* lipase gene amplification. Lanes 1B, 2B, 3B and 4B: Human B-actin gene amplification. Lane P: RT-PCR Positive Control. Lanes N1 and N2: Negative Controls.

210210). The reverse transcription step was conducted at 50^{0}C for 30 min using 5-10 µL of the RNA preparation. PCR amplification was conducted with an initial activation step at 95^0 C for 15 min; followed by 36 cycles of 94^0 C for 45 sec; at 60^0 C for 45 sec and at 72^0 C for 45 sec; followed by a final extension at 72^0 C for 10 min. Negative controls were prepared using DNase and RNase-free water instead of RNA. RT-PCR products were visualized on agarose gels (1.2% agarose, 0.5XTBE) stained with ethidium bromide.

Results

P. acnes is a bacterium found on the skin of most humans. Sebum triglycerides, when released by the action of the bacterial lipase, play an important role in the pathogenesis of acne. Figure 1 shows the RT-PCR products amplified from RNA samples extracted from sebum, collected by adhesive skin strips from a subject diagnosed with acne. The expected RT-PCR amplified products from the *P. acnes* 16S rRNA gene (946 bp) and the lipase gene (515 bp) where observed (Nakamura et al, 2003). The product of the human B-actin gene (746 bp) was also observed, confirming the human source of the sample. No specific bands were detected in the negative control samples.

Since *P. acnes* is present on most humans, including normal skin, samples were also collected from subjects with no observable acne. As expected, RT-PCR products of the predicted sizes were also amplified from healthy individuals (Figure 2). It is interesting to note that a relatively weaker band was observed form a 47 years old female with healthy skin (lane 3). We postulate that the reason for this weak signal may be due to a relatively lower concentration of bacteria on this subject's skin. Alternatively the skin of healthy subjects may be producing less sebum or the sebum itself may harbor fewer bacteria or less RNA. However, other possible explanations for this result have not been rigorously excluded.

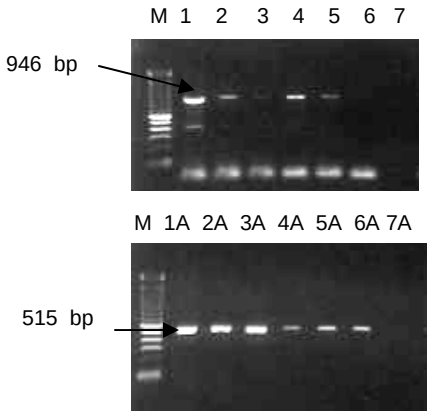


Figure 2. Agarose Gels of RT-PCR Products of *P. acnes* 16S rRNA Gene (Upper Gel) and Lipase Gene (Lower Gel) from Subjects with No Visible Acne. Lane M: (100 bp) DNA marker. Lanes 1 to 7: RT-PCR products of *P. acnes* gene amplification. Lanes 1A to 7A: RT-PCR products of the *P. acnes* lipase gene amplification. Lanes 1, 1A, 5 and 5A: Sebum from 53 year old male. Lanes 2, 2A, 6 and 6A: Sebum from 30 year old female. Lanes 3 and 3A: Sebum from 47 year old female. Lanes 4 and 4A: Sebum from 35 year old male. Lanes 7 and 7A: Negative Control.

Discussion

Using adhesive tape to collect samples from skin for the extraction of RNA and proteins by PCT in order to potentially diagnose diseases with a noninvasive method has previously been reported [3 and 4]. Data presented here show that pressure cycling technology (PCT) can effectively extract RNA from sebum samples, which may be easily collected on lipid-specific pore-cleansing adhesive skin strips. RNA from bacteria in the sebum is readily extracted by PCT, which can be used to identify a specific bacterium. Since the strips can be directly processed by PCT, sebum samples need not be removed from the strips prior to sample processing. As a noninvasive collection and processing method, adhesive tape and PCT may be useful in evaluating acne or assessing the effectiveness of acne treatments.

References

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