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Original Article

Antimicrobial Susceptibility and Characterization of *Propionibacterium acnes* by Multilocus Sequence Typing and Repetitive-Sequence-Based PCR

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Propionibacterium acnes, a gram-positive, anaerobic, and aerotolerant bacterium that is found frequently in the skin as part of the human microbiome causes inflammatory acne, shoulder infection, and the contamination of medical devices. The study goals were the antibiotic resistant and molecular epidemiological characterization of the *P. acnes* isolates in Korea. A total of 22 *P. acnes* isolates originated from diverse patients were obtained from three National Culture Collections for Pathogens in South Korea. The hemolytic properties and minimum inhibitory concentrations (MIC) of five antibiotics (tetracycline, doxycycline, clindamycin, erythromycin, and minocycline) were determined. Only one isolate showed high MIC values and resistance to all five antibiotics. Genotypic characterization was achieved by multilocus sequence typing (MLST) for eight loci (*aroE*, *guaA*, *tly*, *camp2*, *atpD*, *gmk*, *lepA*, and *sodA*) and repetitive-sequence-based PCR (rep-PCR) analysis using the DiversiLab kit. MLST revealed four phylogroups that were type IA_1 (27.3%), type IA_2 (18.2%), type IB (13.6%), and type II (40.9%). Rep-PCR results demonstrated three clusters that were cluster I (39.1%), cluster II (45.5%), and cluster III (13.6%). The isolates of cluster I were part of phylogroup type IA (both IA_1 and IA_2), and the isolates of cluster II belonged to phylogroup type II. All isolates of phylogroup type IB were hemolytic and belonged to cluster III. The results of rep-PCR clustering analysis showed a good correlation with those of MLST phylogroups, suggesting that rep-PCR could be an alternative method to track *P. acnes* subtype lineages.

Key Words: *Propionibacterium acnes*, Antimicrobial susceptibility, Multilocus sequence typing, rep-PCR

INTRODUCTION

Propionibacterium acnes is an anaerobic, aerotolerant, and gram-positive bacterium that is frequently isolated from skin samples of acne vulgaris patients and healthy people (1, 2). This species is part of the human skin microbiome and sometimes triggers inflammatory responses as an opportunistic pathogen. Recently, *P. acnes* was determined to be a major pathogen of skin inflammatory disease, acne vulgaris, and shoulder infection. It can also contaminate medical devices, and has been implicated in sarcoidosis and prostate cancer (3~5).

Because *P. acnes* is a human commensal, especially of

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the skin, it has been difficult to determine if *P. acnes* is the causative agent of certain infections (6, 7). However, in recent studies, molecular epidemiologic information using multi-locus sequence typing (MLST), ribotyping, and PCR techniques were used to predict the association of specific subtypes of *P. acnes* with certain diseases and/or infections. Among those, MLST has demonstrated particularly high discriminatory power and convincing subtyping results to determine the associations between acne vulgaris strains and contamination of medical devices, soft tissue infections, and commensal properties (8~10). Recently, Johnson *et al*., reported that phylogroup type IA_2 strains have a greater ability to produce porphyrin than phylogroup type II, and therefore type IA_2 strains could trigger potent inflammatory responses as porphyrin can produce reactive oxygen species (11).

In Korea, studies on *P. acnes* are rare. Only antimicrobial susceptibility data have been reported; several papers in Korea identified *P. acnes* isolates with antibiotic resistance below the break points (12, 13). In this study, we characterized 22 *P. acnes* isolates, obtained from three National Culture Collections for Pathogens (NCCP) in South Korea, for their hemolytic abilities, antimicrobial susceptibilities, and genotypes. Genotype analysis was performed with two different epidemiological tools, MLST and repetitive-sequence-based PCR (rep-PCR). From the results, we characterized the subtypes of Korean *P. acnes* isolates and compared the two molecular subtyping methods.

MATERIALS & METHODS

Bacterial isolates

A total of 23 *P. acnes* isolates including American Type Culture Collection (ATCC) 11828 *P. acnes* strain were examined. Twenty-two clinical isolates were obtained from three (Kyoungpook, Chonbuk, and Gyeongsang National University Hospitals) Korean NCCP. *P. acnes* isolates were collected from skin and perioperative tissue samples from 2010 to 2015. The bacteria were cultured in blood agar plates with 5% sheep blood (BAP) or Brain Heart Infusion (BHI, Becton-Dickinson, Canada) broth anaerobically for 3~4 days using a GasPak EZ Anaerobic Pouch System (Becton-Dickinson, Canada) or an anaerobic chamber (Bactron, USA) connected to anaerobic gas (90% N_2, 5% CO_2, 5% H_2). *P. acnes* isolates were genetically confirmed by performing 16S rRNA sequencing (14). Hemolysis of each *P. acnes* isolate was observed on BAP.

Minimum inhibitory concentration (MIC)

To determine the MICs of five antibiotics (tetracycline, doxycycline, clindamycin, erythromycin, and minocycline), corresponding E-test strips (bioMérieux, France) were used and placed on Brucella agar containing 5% sheep blood, vitamin K, and hemin. The MIC was measured after 48 h anaerobic incubation at 37℃ according to the manufacturer's instructions.

MLST

MLST was performed according to McDowell's expanded MLST scheme (9). In brief, eight gene loci, *aroE*, *atpD*, *gmk*, *guaA*, *lepA*, *sodA*, *tly*, and *camp2*, were amplified using specific primer pairs and sequenced with sequencing primers according to the *P. acnes* expanded MLST scheme. Each sequences information was inputted into the *P. acnes* database (http://pubmlst.org/pacnes/) and the sequence type was determined accordingly.

Rep-PCR

Genomic DNAs from 23 isolates were purified using the HIGene™ Genomic DNA Prep Kit (Biofact, Korea) and diluted to approximately 50 ng/μl for the rep-PCR templates. The genomic DNA was added for amplification using the DiversiLab Propionibacterium Fingerprinting kit (bioMérieux, France) including patented primers following the manufacturer's instructions. According to the manufacturer's standardized protocol, AmpliTaq polymerase (Applied Biosystems, USA) and GeneAmp PCR System 9700 (Applied Biosystems, USA) were used for the DNA amplification. The PCR conditions were 94℃ for 2 min for initial denaturation, and 35 cycles of 94℃ for 30 s, 60℃ for 30 s, and 70℃ for 90 s, with a final polymerization at 70℃ for 3 min. The amplified DNA fragments were analyzed using the auto-

Table 1. Minimum inhibitory concentrations (μg/ml) of five antimicrobial agents using 22 clinical isolates and one standard strain of *P. acnes*

P. acnes isolate	Tetracycline (RB[1]: 2.0)	Doxycycline (RB: 1.0)	Clindamycin (RB: 4.0)	Erythromycin (RB: 0.5)	Minocycline (RB: 1.0)
KB105	0.064	0.125	≤ 0.016	≤ 0.016	0.047
KB106	0.25	0.19	≤ 0.016	≤ 0.016	≤ 0.016
KB109	0.25	0.38	0.023	≤ 0.016	0.094
KB112	8	4	≥ 256	≥ 256	3
KB113	0.125	0.125	≤ 0.016	0.023	0.047
KB114	0.25	0.25	≤ 0.016	0.023	0.125
KB116	≤ 0.016	0.19	≤ 0.016	≤ 0.016	≤ 0.016
KB117	0.064	0.064	0.023	≤ 0.016	0.023
KB118	0.064	0.032	≤ 0.016	≤ 0.016	0.125
KB119	0.5	0.25	≤ 0.016	≤ 0.016	0.047
JB391	0.125	0.047	≤ 0.016	≤ 0.016	0.023
JB893	0.25	0.5	0.023	≤ 0.016	0.19
JB1475	0.125	0.094	≤ 0.016	≤ 0.016	0.047
JB1490	0.125	0.125	≤ 0.016	≤ 0.016	0.023
JB1912	0.25	0.25	0.023	≤ 0.016	0.047
JB3672	0.047	0.032	≤ 0.016	≤ 0.016	≤ 0.016
JB3496	0.064	0.064	≤ 0.016	≤ 0.016	0.023
KS2874	0.25	0.064	≤ 0.016	≤ 0.016	≤ 0.016
KS2875	0.5	0.125	0.032	≤ 0.016	0.094
KS2876	0.19	0.19	0.023	≤ 0.016	0.047
KS2877	0.25	0.19	0.032	≤ 0.016	0.032
KS2878	0.125	0.125	0.023	≤ 0.016	≤ 0.016
ATCC 11828	0.19	0.125	≤ 0.016	≤ 0.016	0.047

[1] Resistance breakpoint according to the recommendation by the European Committee on Antimicrobial Susceptibility Testing (EUCAST) for *P. acnes* (μg/ml).

mated microbial genotyping DiversiLab system (bioMérieux, France). The Unweighted Pair Group Method with Arithmetic mean (UPGMA) was applied to generate a dendrogram.

RESULTS

MIC for antimicrobial agents

All isolates except one were susceptible to five antimicrobial agents according to the EUCAST criteria of resistance breakpoints for tetracycline (TET), doxycycline (DOX), clindamycin (CLI), erythromycin (ERY), and minocycline (MIN) (12) (Table 1). Only one isolate, KB112, showed higher resistances to those antimicrobial agents, and the MICs of TET, DOX, CLI, ERY, and MIN were 8 μg/ml, 4 μg/ml, ≥ 256 μg/ml, ≥ 256 μg/ml, and 3 μg/ml, respectively.

MLST and hemolytic activity of *P. acnes*

According to the expanded MLST scheme for *P. acnes*, nine STs were revealed from 22 clinical isolates through constructing concatenated sequences of the eight MLST genes (Table 2). The most common ST, ST69, was shared by five clinical isolates and the standard ATCC 11828 strain.

Table 2. Hemolysis, multilocus sequence typing (MLST), and repetitive-sequence-based PCR (rep-PCR) for 22 clinical isolates and 1 type strain of *P. acnes*

Isolate	Hemolysis[1]	MLST				Rep-PCR
		Allelic profile (*aroE-guaA-tly-camp2-atpD-gmk-lepA-sodA*)	ST	CC	Phylogroup	
KB105	−	1-5-8-2-1-1-1-4	2	CC2	IA_2	Cluster I
KB106	−	1-5-8-7-1-1-1-5	22	Singleton	IA_2	Cluster I
KB109	+	1-3-1-1-1-1-1-1	1	CC1	IA_1	Cluster I
KB112	+	1-3-22-2-1-1-1-1	115	CC3	IA_1	Cluster I
KB113	+	1-4-8-6-1-9-1-4	53	CC5	IB	Cluster III
KB114	+	1-5-8-7-1-1-1-5	22	Singleton	IA_2	Cluster I
KB116	−	17-4-10-12-4-2-4-6	69	CC72	II	Cluster II
KB117	+	1-4-8-6-1-1-1-4	5	CC5	IB	Cluster III
KB118	+	1-3-1-1-1-1-1-1	1	CC1	IA_1	Cluster I
KB119	+	1-3-1-1-1-1-1-1	1	CC1	IA_1	Cluster I
JB391	+	17-4-10-12-4-2-4-6	69	CC72	II	Cluster II
JB893	+	1-3-1-1-1-1-1-1	1	CC1	IA_1	Cluster I
JB1475	−	17-4-10-12-4-2-4-6	69	CC72	II	Cluster II
JB1490	−	17-4-10-10-4-2-2-3	6	CC6	II	Cluster II
JB1912	−	17-4-10-10-4-2-2-3	6	CC6	II	Cluster II
JB3496	+	1-3-22-2-1-1-1-1	115	CC3	IA_1	Cluster I
JB3672	−	17-4-10-12-4-2-4-6	69	CC72	II	Cluster II
KS2874	−	1-5-8-2-1-1-1-4	2	CC2	IA_2	Cluster I
KS2875	−	17-4-10-10-4-2-2-3	6	CC6	II	Cluster II
KS2876	−	17-4-10-12-4-2-4-6	69	CC72	II	Cluster II
KS2877	−	17-4-10-10-9-2-2-3	25	CC6	II	Cluster II
KS2878	+	1-4-8-6-1-9-1-4	53	CC5	IB	Cluster III
ATCC 11828	−	17-4-10-12-4-2-4-6	69	CC72	II	Cluster I

[1] +: hemolysis positive, −: hemolysis negative.

The second most prevalent ST was ST1 (n = 4), followed by ST6, ST2, ST22, ST53, ST115, (n = 2, each) ST5, and ST25 (n = 1, each). Clonal complexes were identified by inputting the concatenated sequence information of the eight genes into the *P. acnes* MLST website (http://pubmlst.org/pacnes/). The phylogroups of *P. acnes* isolates were determined according to the expanded MLST scheme for *P. acnes* with the phylogenomic analysis of multi-housekeeping gene datasets gathered from the completed *P. acnes* whole genome sequencing data. The phylogroups of the 22 clinical isolates were type IA_1 (n = 6, 27.3%), IA_2 (n = 4, 18.2%), type IB (n = 3, 13.6%), and type II (n = 9, 40.9%) (Table 2). The results of the hemolysis test are showed in Table 2.

Rep-PCR

Repetitive-sequence based PCR was performed using DiversiLab for *P. acnes*. Rep-PCR generated four to five discrete DNA bands with several faint bands that could be

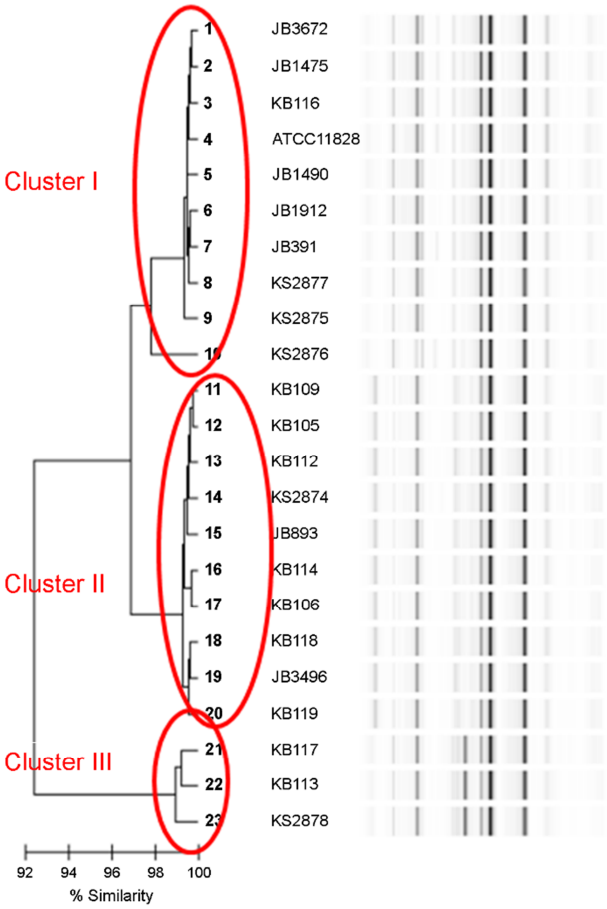

Figure 1. Dendrogram and three similarity clusters generated from repetitive-sequence-based PCR results for 22 clinical isolates of *P. acnes* and one type strain, *P. acnes* ATCC 11828.

detected automatically and clustered into three groups (cluster I, II, and III) (Fig. 1).

All band patterns of the isolates exhibited more than 92% similarity. Cluster I (n = 9, 39.1%) and II (n = 10, 45.5%) were differentiated based on the criteria of approximately 97% similarity and cluster III (n = 3, 13.6%) was approximately 92% different than the others. The band pattern of *P. acnes* ATCC 11828 strain was identified as cluster I.

DISCUSSION

P. acnes isolates have been shown to be susceptible to all antibiotic agents since antibiotic susceptibility testing was initiated in Korea in 1995 (12). In this study, the KB112 isolate was highly resistant to clindamycin and erythromycin. The isolates showing high resistance to these two antibiotics were reported in USA and their phylogroup types were IA_1 like KB112 (9). Clindamycin is the most widely prescribed antibiotic agent in Korean dermatology, so the emergence of this multidrug resistant (MDR) isolate could indicate a warning sign for the overuse of antibiotic agents by acne patients. *P. acnes* was typically resistant to macrolides and clindamycin in other countries; however, tetracycline is still likely to be effective for *P. acnes* treatment worldwide (15).

In this study, we performed a genotypic analysis of *P. acnes* clinical isolates with two different epidemiological tools, specifically, MLST and rep-PCR. From the results, we characterized the subtypes of Korean isolates of *P. acnes* and compared the two molecular subtyping methods. The isolates that were common to cluster I based on Rep-PCR were predominantly of the phylogroup type IA (both IA_1 and IA_2) based on MLST, with the exception of the type strain, ATCC 11828. All isolates belonging to cluster II and III based on rep-PCR were identified as phylogroup type II and IB, respectively, through MLST. Even though rep-PCR could not discriminate IA_1 and IA_2, this result demonstrated a good correlation between MLST and rep-PCR. The genotypic analyses results could not generate any correlation with the antibiotic susceptibility characteristics of the isolates because all isolates except one did not show any resistance to the five antimicrobial reagents.

MLST is considered a gold standard to differentiate and track pathogenic bacterial lineages among health-associated and non-pathogenic *P. acnes*. As a molecular phylogenetic tool, MLST has showed confidence because of the accuracy and reproducibility. MLST type IA_2 isolates have been associated with acne, and have multiple virulence genes contained on extra genomic elements. Type II isolates are rarely related to acne and are known as health-associated isolates (9, 11). Type IB *P. acnes* has been isolated from medical devices and soft tissues (9, 16). Nodzo *et al*. (17) reported that the hemolytic characteristics of *P. acnes* could be a clinical marker for orthopedic infection. In this study, all three type IB isolates and seven of ten type IA isolates were

hemolytic, whereas all isolates of phylogroup type II, except one (JB391), showed no hemolytic characteristics on BAP.

Davidsson *et al*. (18) used DiversiLab to subtype *P. acnes* and first demonstrated the possibility of studying the molecular epidemiology of *P. acnes* using this method instead of MLST. An improved PCR method was developed to differentiate *P. acnes* subtypes, to substitute for MLST methods (19). We could not present further detailed analysis of the clinical importance of these subtyping methods, due to a lack of clinical information. However, rep-PCR could be a predictive method for the efficient subtyping of *P. acnes* isolates, and could be used instead of more expensive and laborious MLST methods.

In conclusion, this is the first report regarding the molecular epidemiological characteristics of *P. acnes* isolates in Korea using MLST and rep-PCR. Rep-PCR methods could be performed for rapid determination of *P. acnes* subtypes. One MDR isolate of *P. acnes* was identified, and this phenomenon requires constant monitoring.

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