NOTE *Bacteriology*

Development of a PCR Test for the Identification of *Staphylococcus intermedius* Based on the 16S rDNA Sequence

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ABSTRACT. The development of a PCR assay based on the 16S ribosomal RNA gene (rDNA) sequence was carried out for the identification of *Staphylococcus intermedius*. Sixty-six strains of *S. intermedius*, 70 of *Staphylococcus aureus* and 2 of *Staphylococcus hyicus* were examined for the assay. The 16S rDNA, of which the PCR target fragment makes up 901 bp corresponding to the sequence data of the gene, was detected in all strains of *S. intermedius*, but it was not detected in any strains of either *S. aureus* or *S. hyicus*. These results suggest that the PCR allows a simple and precise identification of *S. intermedius*.
KEY WORDS: identification, PCR, *Staphylococcus intermedius*.

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A coagulase-positive species *Staphylococcus intermedius* was first described as a new species in 1976 and was differentiated from *Staphylococcus aureus* based on its biochemical and microbiological characteristics [9]. *S. intermedius* causes a variety of canine infections such as otitis externa, pyoderma and wound infections [1, 3]. Recently, methicillin-resistant *S. intermedius* strains have been isolated from dogs [6]. The infections in human beings are usually associated with dog-bite wounds [19]. Furthermore, the organisms have been implicated in a food-poisoning outbreak involving butter-blend products [11]. These circumstances make precise identifications of the organisms more important.

For veterinary medical science, coagulase-positive bacteria of *S. aureus*, *S. intermedius* and *Staphylococcus hyicus* are considerably important. In routine laboratory practice, the production of acid anaerobically from mannitol, acetoin production, hyaluronidase, protein A and β-galactosidase activity are recommended for distinguishing *S. intermedius* from *S. aureus* [5, 7, 12, 16]. The key phenotypic characteristics that are used to differentiate *S. intermedius* from *S. hyicus*, and that could be analyzed in a routine diagnostic laboratory, are aerobic acid production from maltose, hyaluronidase and β-galactosidase [5, 12, 15, 16], but these phenotypic character analyses are not often conducive for the identification of *S. intermedius* strains.

The API-Staph system (Bio Merieux S. A., France), a commercially available kit for biochemical tests, has been used widely as a means of identifying the species of staphylococci, but precise identification of *S. intermedius* cannot be achieved unless additional biochemical tests are performed.

Recently, molecular methods such as ribotyping [2, 4], pulsed-field gel electrophoresis [14, 17], 16S ribosomal RNA gene (rDNA) sequencing [18], and 16S-23S rDNA intergenic spacer PCR [13], have been evaluated for identifying staphylococci. In this study we evaluated a PCR method designed to amplify species-specific sequences in the 16S rDNA to identify the strains of *S. intermedius*.

A total of 66 *S. intermedius* strains were used in this study (Table 1). Sixty-five strains were isolated from 5 different animals. Type strain ATCC 29663 was also used for a reference. Strains of *S. aureus* (n=70) and *S. hyicus* (n=2) were used to confirm the specificity of the primers designed in this study. *S. intermedius* strains were identified by means of the API-Staph system and additional characteristics such as colony pigment, staphylocoagulase, clumping factor, heat-stable DNase, hemolysins, hyaluronidase, β-galactosidase and anaerobic acid production from mannitol.

The bacteria were grown on a heart infusion agar plate (Nissui Pharmaceutical Co., Ltd., Tokyo, Japan) at 37°C overnight. Bacterial DNA was extracted by a standard method as described previously [10].

A pair of primers were designed to amplify the 901 bp region of the 16S rDNA of *S. intermedius* (DDBJ accession number D83369). Primer-1 (5'-CCGTATTAGCTAGTTGGTGG-3') and primer-2 (5'-GAATGATGGCAACTAAGTTC-3') correspond to the nucleotide residues 221 to 240 and 1102 to 1121, respectively.

The PCR was performed in a 20-μl reaction volume. Each reaction mixture contained 1 μl (50 to 150 *n*g) of the sample DNA solution, 2 pmol of each primer, 0.5 units of *Taq* DNA polymerase (TaKaRa *Taq*, TaKaRa Shuzo Co., Ltd., Shiga, Japan), and 800 μM of deoxyribonucleoside triphosphate (TaKaRa) in a PCR buffer [100 mM Tris-HCl (pH 8.3), 50 mM KCl, 1.5 mM $MgCl_2$; TaKaRa]. The amplification reaction was carried out in a GeneAmp® PCR System 9700 (Perkin-Elmer Applied Biosystems, U. S. A.) by using the following program: 5 min at 94°C followed by 25 cycles of 1 min at 94°C, 1 min at 50°C and 1 min 30 sec at 72°C. The program finished with an additional 7-min extension step at 72°C. After the amplification, 2 μl of the

Table 1. Origins of coagulase-positive staphylococci used in this study

Bacterial species	Animal species	Source (no. of strains)
S. intermedius (n=66)	Dog	Otitis externa (4), Pyoderma (3) , Dermatitis (2) Eczema (2), Alveolar blennorrhea (1) Folliculitis (6), Impetigo (1), Skin (5), Mouth (1)
	Pigeon	Naris (10) Type strain ATCC 29663
	Fox	Naris (10)
	Mink	Naris (10)
	Horse	Skin (10)
S. aureus (n=70)	Human	Hand (13), Skin(3) Reference FDA 209P
	Cow	Mastitis (7), Milk (3), Skin (1), Nasal mucus (1)
	Chicken	Dermatitis (4), Septicemia (5), Liver (1), Bone marrow (2)
	Horse	Skin (7)
	Pig	Skin (5)
	Rat	Skin (7)
	Mouse	Skin (10)
S. hyicus (n=2)	Chicken	Naris (1)
	Pig	Type strain NCTC 10350

Table 2. PCR results of *S. intermedius*, *S. aureus*, and *S. hyicus* strains

Bacterial species	No. of strains tested	No. of strains positive on PCR
S. intermedius	66	66
S. aureus	70	0
S. hyicus	2	0

reaction mixture was analyzed by electrophoresis on a 1.5% agarose gel in Tris-acetate-EDTA buffer at 100V for 30 min and stained with ethidium bromide. The correct identity of the PCR products was confirmed by the size.

The 16S rDNA of *S. intermedius*, of which the PCR target fragment makes up 901 bp corresponding to the sequence of the gene, was detected in all of the strains of *S. intermedius* that were tested (Table 2 and Fig. 1). For the strains of *S. aureus* and *S. hyicus*, the specific amplification product was not observed, although larger fragments, approximately 1.1 kbp in size, were detected in 11 of 12 *S. aureus* strains from

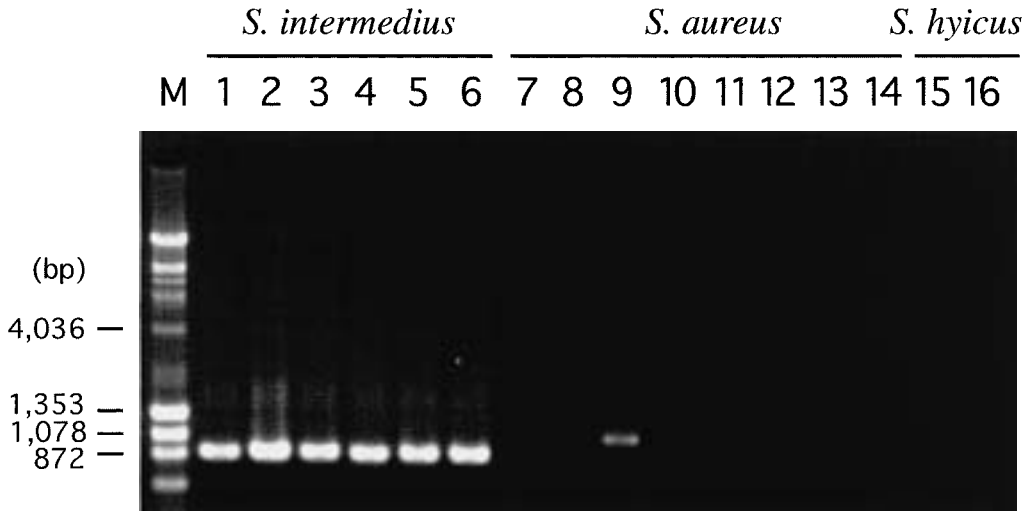

Fig. 1. Agarose gel electrophoresis of the PCR products from representative strains of *S. intermedius*, *S. aureus*, and *S. hyicus*. Lanes: M, molecular size markers; 1, *S. intermedius* dog strain; 2, pigeon strain; 3, fox strain; 4, mink strain; 5, horse strain; 6, type strain ATCC 29663; 7, *S. aureus* human strain; 8, cow strain; 9, chicken strain; 10, horse strain; 11, pig strain; 12, rat strain; 13, mouse strain; 14, reference FDA 209P; 15, *S. hyicus* chicken strain; 16, type strain NCTC 10350.

chickens. Furthermore, the atypical PCR products showed much weaker intensity in the agarose gel as shown in lane 9 of Fig. 1. It was not hard to distinguish the 1.1 kbp fragment from the specific PCR products when the positive reaction was analyzed simultaneously.

The PCR primers designed in this study gave precise identifications of *S. intermedius* strains. A similar method has been evaluated for identifying eight major species of coagulase-negative staphylococci involved in hospital infections [8]. The PCR provides the rapidity and accuracy in diagnosis in the hospital. Our results suggest that the PCR presented in this paper also allows a simple and precise identification of *S. intermedius*, to be used alone or in combination with phenotypic identification.

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