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Original Article

Isolation and Cloning of an ABC Transporter-Like Gene of *Haemophilus parasuis* and Its Use in a New Diagnostic PCR

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The aim of this study was to identify a new gene of *Haemophilus parasuis* that could be used to develop a polymerase chain reaction (PCR) test for this porcine pathogen. *H. parasuis* genomic DNA was cloned into a set of expression vectors, and transformants expressing His-tagged polypeptides were identified by colony blotting. An ABC transporter-like gene was isolated. The cloned DNA fragment is 1,105 base pair and shows 78% similarity at the nucleotide level with an ABC transporter gene of *H. ducreyi*. Based on this sequence, two PCR primers were designed to amplify the entire 1,105-bp fragment in the proposed diagnostic PCR test. PCR amplification was able to detect a minimum of 1×10^4 CFU/ml of *H. parasuis* organisms. Fifteen different *H. parasuis* serovars were positive using the PCR test. No amplification was observed when the test was done using DNA from 16 other bacterial species commonly isolated from swine.

Key Words: ABC transporter-like gene, *Haemophilus parasuis*, Diagnostic PCR

INTRODUCTION

Haemophilus parasuis is a small, non-motile, pleomorphic gram-negative rod of the family *Pasteurellaceae* (1, 2). It is the causative agent of Glässer's disease, which is characterized by a fibrinous polyserositis, polyarthritis and meningitis. This illness is considered a sporadic, stress-associated disease of young pigs (2, 3).

Diagnosis of *H. parasuis* infection is generally made by isolation of the organism from a clinical specimen and subsequent biochemical analysis (2, 4, 5). However, recovery of *H. parasuis* from specimens is low, due in part to the fragility and fastidious growth requirements of *H. parasuis* relative to other bacteria that may also be present in the specimen (2, 6). In fact, a retrospective analysis conducted in diagnostic laboratories in Ontario, Canada indicated that the inability to confirm the presence of *H. parasuis* from submitted specimens (7) may have resulted in the true incidence of the disease being underestimated by as much as 10-fold. The polymerase chain reaction (PCR) provides fast and accurate identification of *H. parasuis* not only in pure culture but also in clinical samples, even when nonviable organisms are present (8). Recently, several PCR

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methods for *H. parasuis* have been developed (8, 9). Oliveira *et al*. (8) introduced a PCR method to detect 16S ribosomal DNA sequences. However, the 16S rDNA sequence is highly conserved and PCR primers designed to amplify the *H. parasuis* cognate may hybridize with 16S rDNA sequence of *Pasteurellaceae*. In the PCR, replicon with same size of *H. parasuis* was found for *Actinobacillus indolicus* (8). This makes the interpretation of a positive reaction difficult, especially when testing samples were obtained from the upper respiratory tract. However, recent report of Angen *et al*. (9) improved the weakness of 16S rDNA PCR of Oliveira *et al*. (8) by modifying primers for 16S rDNA region. In general, diverse PCR amplifying different regions of amplicon were developed in order to detect accurately even one pathogen such as *Salmonella*, *Brucella* and so on. Therefore, another PCR test based on specific gene sequences of *H. parasuis* is necessary to identify this porcine pathogen.

In this study, we cloned and sequenced a new gene of *H. parasuis* using a colony blot technique to determine an immunogenic antigen. Based on gene sequence comparison using GenBank data, the newly identified gene has 83% amino acid sequence similarity with an ABC transporter of *H. ducreyi*, which is an ATP-binding protein and permease. Since this species-specific gene was selected by comparative analysis among the gene from the bacteria within the *Pasteurellaceae* family, the PCR technique presented in this study could be a valuable method for diagnosis of *H. parasuis* infection from clinical materials.

MATERIALS AND METHODS

Bacterial strains and culture conditions

Fourteen reference strains for *H. parasuis* serovars 1 to 15, excluding serovar 8, were obtained from Dr. Chimmel in Germany (Bundesinstitut für gesundheitlichen Verbrauchershcutz und Veterinarmedizin, Fachberich Bakterielle Tierseuchen und Bekampfung von Zoonosen, J-Jena) and one *H. parasuis* standard strain (ATCC No. 19417) was purchased from American Tissue Culture Collection (ATCC). For propagation, all strains were inoculated on chocolate agar and incubated at 37℃ for 48 h. In this study, *H. parasuis* standard strain was used for genomic DNA extraction. All v-factor dependent strains were grown on chocolate agar, whereas all other strains were grown on sheep blood agar and nutrient agar. These cultures were incubated at 37℃ overnight.

Clinical samples

Seventy nine clinical samples from pigs suspected for being infected with *H. parasuis*, showing polyserositis or arthritis at necropsy were collected from 2007 to 2009 in Korea. Swabs were vortexed in 300 μl of PBS. DNA was extracted from the suspension using genomic DNA extraction kit (Intron Co., Sungnam, Korea).

Isolation of genomic DNA from *H. parasuis*

Genomic DNAs were obtained using DNAzol reagent (Invitrogen, Carlsbad, CA, USA). *H. parasuis* in culture medium [4 ml Tryptic soy broth with 5 μg/ml nicotinamide dinucleotide (NAD)] was centrifuged at 12,000 x g for 5 min. After centrifugation, the pellet was resuspended in 200 μl distilled water, and 1 ml of DNAzol reagent was added and mixed by gentle vortexing. The lysate was centrifuged at 10,000 x g for 10 min and the supernatant (1 ml) was transferred to a new tube. Genomic DNA from the supernatant was precipitated by adding 100% ethanol (500 μl) and washed with 75% ethanol. The dried DNA pellet was dissolved in 200 μl 0.1 N NaOH.

Library construction and screening

Standard molecular cloning techniques were used for library construction (10). *Sau*3A1 (Promega, Madison, WI, USA) fragments (0.5~3 kb) of partially digested *H. parasuis* genomic DNA were recovered from an agarose gel by using an Accuprep Agarose Gel Extraction Kit (Bioneer, Daejon, Korea) and cloned into the *Bam*H1 site of pET-28a, pET-28b and pET-28c expression vectors containing the T7 promoter/lac operator and 6X His tag (Novagen, Madison, USA). For analysis of *Escherichia coli* transformants, the transformation mixture was plated on LB plates containing 50 μg/ml kanamycin and incubated overnight (16 h) at

37℃ until the colonies were 1~2 mm in diameter. The colonies were transferred to nitrocellulose membrane filters (BIO-RAD, Hercules, CA, USA). The identification of clones on colony blots was performed according to the manufacturer's protocol (The QIA expressionist, Qiagen, Valencia, CA, USA). After induction with 1 mM isopropyl-β-thiogalactopyranoside (IPTG; Promega) at 37℃ for 4 h, the colonies on the membranes were treated with denaturing solution (0.5 M NaOH, 1.5 M NaCl) and neutralization solution (1.5 M NaCl, 0.5 M Tris-HCl, pH 7.4). After washing with Tris-buffered saline (TBS: 10 mM Tris-HCl, pH 7.5, 150 mM NaCl) the membranes were blocked with blocking buffer [3% bovine serum albumin (BSA) in TBS] for 1 h. After washing twice in TTBS [100 mM Tris-HCl, 0.9% (w/v) NaCl, 0.1% (v/v) Tween 20, pH 7.5], for 10 min, the membrane was washed again in TBS for 10 min. The prepared membranes were reacted with an anti-6X His-tag mouse IgG monoclonal antibody (0.1 μg/ml; Novagen) and horseradish peroxidase (HRP)-conjugated anti-mouse IgG goat monoclonal antibody (Sigma, St. Louis, MO, USA). The HRP conjugate-bound membranes were developed with HRP staining solution (0.018% H_2O_2, 0.06% 4-chloro-1-naphthol, 20% methanol in 25 ml TBS), and the reaction was terminated by washing with tap water. The developed membranes were aligned with the original transformation plates and the anti-His tag positive clones were picked with sterile toothpicks and transferred into cluster tubes (1.2 ml, 96 wells, Costar 4411) containing LB broth with 50 μg/ml kanamycin. The cluster tube cultures were grown overnight at 37℃ and the sequences of the cloned genes were analyzed.

Sequence analysis of cloned genes

An ABI3730xl automatic sequencer (Applied Biosystems, Foster City, CA, USA) was used for analysis of the cloned inserts. SP6 promoter and T7 terminator primers were used as sequencing primers. DNA sequences were analyzed using the nucleotide BLAST algorithm [National Center for Biotechnology Information server (http://www.ncbi.nlm,nih.gov)] to identify homologous genes in the GenBank database.

PCR primers and PCR conditions

Based on the sequence data, a pair of primers specific for *H. parasuis* was designed: HP forward (5'- GAT CGA GTT GGA TGC CTG CAC GGT-3') and HP reverse (5'-GAT CCA TTG CAA ACC CTT CGC CCG-3'). The tubes containing bacteria were boiled for 10 min. After boiling, the tubes were centrifuged at 12,000 rpm for 5 min. The supernatant was used as the DNA template. A PCR was carried out in a 50 μl mixture containing 3 U *Taq* DNA polymerase (Promega), 3 mM $MgCl_2$, 1X reaction buffer [5 mM Tris-HCl (pH 8.0), 10 mM NaCl, 0.01 mM EDTA, 0.1 mM DTT, 5% glycerol, 0.1% Triton X-100], 0.3 mM each dNTP (Promega) and 5 μl of extracted DNA. The PCR mixture was subjected to a 35-cycle PCR profile of 94℃ for 30 sec, 53℃ for 90 sec, 72℃ for 1 min, followed by a final extension step of 72℃ for 5 min. The PCR products were run in a 1% agarose gel for 50 min at 100 V. Gels were stained with 0.5 μg/ml ethidium bromide solution and photographed.

PCR sensitivity and specificity

Serial 10-fold dilutions of 24-h broth cultures containing 1.0×10^7 CFU/ml of *H. parasuis* (strain: ATCC 19417) were tested by PCR to determine the sensitivity of the proposed test. One milliliter of each dilution was used for DNA extraction. The tubes containing bacteria were boiled for 10 min. After boiling, the tubes were centrifuged at 12,000 rpm for 5 min. The supernatant was used as the DNA template and was also tested to determine the minimum amount of bacteria that could be detected in the samples. Specificity of the PCR test was determined by testing serovars of *H. parasuis* and 16 bacterial species commonly isolated from pigs (Table 1).

RESULTS

Isolation of *H. parasuis* polypeptide-expressing plasmid constructs

A total of 9 colonies out of about 2,500 showed a positive reaction against anti-His tag monoclonal antibody.

Table 1. PCR amplification using DNA extracted from *H. parasuis* strains of 15 serovars and 16 other bacterial species commonly isolated from pigs.

Species	Strain ID	Origin	Serotype or serogroup	PCR results
Haemophilus parasuis	H409	Dr. Chimmel	1	+
	H410	Dr. Chimmel	2	+
	H411	Dr. Chimmel	3	+
	H412	Dr. Chimmel	4	+
	H413	Dr. Chimmel	5	+
	H780	Dr. Chimmel	6	+
	H781	Dr. Chimmel	7	+
	IVK	Field isolate (pericardium, pigs)	8	+
	H553	Dr. Chimmel	9	+
	H555	Dr. Chimmel	10	+
	H465	Dr. Chimmel	11	+
	H425	Dr. Chimmel	12	+
	H793	Dr. Chimmel	13	+
	H792	Dr. Chimmel	14	+
	H790	Dr. Chimmel	15	+
	ATCC19417	ATCC[1]		+
Actinobacillus pleuropneumoniae	Serotype 2	NVRQS[2]	2	−
	Serotype 5	NVRQS	5	−
Actinobacillus suis	ATCC 15557	ATCC		−
Pasteurella multocida	Serotype A	NVRQS	A	−
	Serotype D	NVRQS	D	−
Proteus mirabilis		Field isolate[8]		−
Escherichiae coli		NVQRS		−
Escherichia fergusonii		Field isolate		−
Enterobacter cloacae		ATCC 13047		−
Erysipelothrix rhusiopathiae		Field isolate		−
Klebsiella pneumoniae		Field isolate		−
Moraxella spp		Field isolate		−
Salmonella enteritidis		ATCC 13712		−
Staphylococcus hyicus		Field isolate		−
Staphylococcus aureus		ATCC 65389		−
Streptococcus suis	Type [4]2 [5]EF+	Field isolate	2	−
	[6]Type 7	Field isolate	7	−
	[7]Type 9	Field isolate	9	−
Bordetella bronchiseptica		Field isolate		−
Clostridium perfringens	Type A	Field isolate	A	−
Clostridium perfringens	Type B	Field isolate	B	−
Clostridium perfringens	Type C	Field isolate	C	−

[1]ATCC, American Type Culture Collection; [2]NVRQS, National Veterinary Research Quarantine Service; [3]KCCM, Korean Culture Center of Microorganisms; [4]type 2, Capsule polysaccharide of sero types 2; [5]EF, Extracellular facter; [6]Type 7, Capsule polysaccharide of serovar 7; [7]Type 9, Capsule polysaccharide of serovar 9; [8]all field isolates were isolated in Korea.

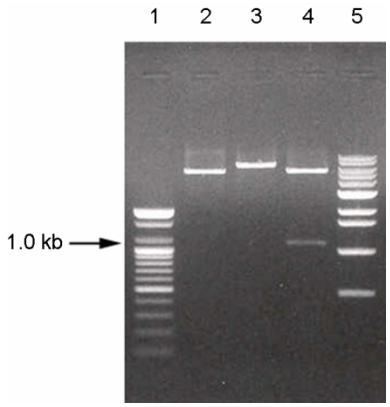

Figure 1. Agarose gel electrophoresis (1%, v/w) of the plasmid DNA extracted from the putative transformants showing positive reaction against anti-His tag antibody. Lanes 1, molecular weight marker type 100 bp DNA ladder. Lane 2, pET-28b after being linearized with *Bam*H1 restriction enzyme. Lane 3, linearized pET-28b-2A3 inserted plasmid with *Bam*H1 restriction enzyme. Lane 4, pET-28b-2A3 after being digested with restriction enzyme (*Not* 1-double cut). Lane 5, molecular weight marker type 1 kb DNA ladder.

Four colonies showing the strongest signals were picked. Plasmid DNA was extracted from the 4 colonies and the sequences of the inserts were analyzed. One of these clones, named 2A3, showed significant similarity to sequences in GenBank (see below) and was therefore chosen for further characterization. To verify the presence of an insert in the pET vector, clone 2A3 was digested with restriction enzymes (Fig. 1). Clone 2A3 digested with *Not* I (which cuts once in the vector) was linearized to a fragment larger than linearized pET vector (Fig. 1, lane 3), thus indicating the presence of an insert in 2A3. Because the *H. parasuis* genomic DNA was originally subjected to partial digestion with *Sau*3A1 and ligated into *Bam*HI-digested vector, the 2A3 clone should have at least 2 *Sau*3A1 restriction enzyme sites (GATC), one or both (or neither) of which could also be part of a *Bam*HI site (GGATCC). Digestion with *Bam*HI released a fragment of about 1 kb from the vector (Fig. 1, lane 4), thus establishing the size of the insert.

Sequence of the 2A3 DNA insert

The 2A3 DNA insert-containing plasmid was subjected to further sequence analysis. Analysis of the determined DNA sequence (1,105 bp) revealed an open reading frame encompassing the entire DNA length but no putative gene in other frames. The sequence of the cloned DNA was submitted to GenBank (accession number DQ153243). BLAST analysis of the nucleotide sequence showed it to have 78% similarity with the ABC (ATP binding cassette) transporter/ATPase binding protein/permease gene of the *H. ducreyi* 35000HP strain. The size of *H. ducreyi* ABC transporter gene is 1,956 bp, and the cloned 2A3 sequence matched the *H. ducreyi* ABC transporter gene from nucleotide 142 to 1,247. Moreover, BLAST analysis showed 83% amino acid sequence similarity with that of *H. ducreyi*. Latest BLAST analysis showed this sequence showed following similarity with ABC transporter genes from some bacterial species; 95% similarity with *H. parasuis*, 76% similarity with *Actinobacillus pleuropneumonia*, and 74% similarity with *H. somni*.

PCR specificity and sensitivity

Two *H. parasuis*-specific primers were designed based on a species-specific gene and, as predicted, an *H. parasuis*-specific 1,105-bp PCR product was amplified from *H. parasuis* genomic DNA (Fig. 2). The PCR test detected 15 different *H. parasuis* serovars whereas the PCR result was negative for 16 other bacterial species that were tested (Table 1). The 2A3 PCR primer pair detected a minimum concentration of 1×10^4 CFU/ml of *H. parasuis* organisms (Fig. 3). The 2A3 DNA insert-specific bands were observed when the PCR was performed using DNA extracted from bacterial suspensions in the range of $10^7 \sim 10^4$ CFU/ml. The bands at the bottom of the gel were observed in all samples, indicating unincorporated primers.

We also tested clinical samples from pigs suspected for being infected with *H. parasuis*. Of 79 samples tested, 30 samples (38.0%) were positive by PCR with 2ABC, while *H. parasuis* was recovered from only 8 (10.1%) samples, which all were positive by PCR test (Table 2).

DISCUSSION

Because of the fastidious growth requirements of *H. parasuis*, isolation and identification of this pathogen from

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1    GATCCATTGCAAACCCTTCGCCCAGTTCAAGCACCAAATGCAGATTACTGGTTTGGGACA
      |||| || ||||| ||  | || |||||||||||| | | |||| ||||  ||||| |||
       ATCCTTTACAAACACTGAGACCGGTTCAAGCACCGAGTACAGACTACTTATTTGGTACA

61   GACCGTCTTGGACGTGATATTTTATCTCGCTTAATTTACGGTGCTCAAACATCACTCTTT
     ||||| || ||  | ||||||||| || ||  || | |  || ||   |||||| ||||||
     GACCGCCTAGGTAGAGATATTTTCTCGCGGATACTATGGGGCGCAAGAACATCGCTCTTT

121  ATCGGACTTGGTGCAGTAGCACTTGCCATTGTACTCGGCAGTATCTTAGGGGCAACTGCT
     || ||  | ||||| || ||  |||| ||| || | ||| || | ||||| |||||||||
     ATTGGTTTAGGTGCGGTTGCTTTTGCTATTCTATTGGGCGGTGTATTAGGTGCAACTGCT

181  GCGACCGCAGACAAGCTCGGTAACGAAGTCATTATGCGTTTTATGGACATCTTAATGGCA
     || ||||| || |  || || || ||||| |||||||| |  ||||| ||||||||||||
     GCAACCGCTGATAGACTTGGCAATGAAGTGATTATGCGATCAATGGATATCTTAATGGCA

241  TTCCCTGGTATTGCACTTGCAGCGGTATTACTTGCAACCTTAGGTAACGCAGTACCGATT
     || || ||||| ||| | || || |||||||| ||||| || |||||  | |||||| ||
     TTTCCAGGTATCGCATTAGCTGCCGTATTACTCGCAACTTTTGGTAATTCTGTACCGGTT

301  ATCATCTTAGCCATTGCGATTGTTTATACCCCACAACTTGCTCGTGTGGTGCGTGCAAAC
     || ||  |  |||| ||| | |||||||| |||||||||| ||||| || ||||| ||
     ATTATTATTACCATAGCGGTCGTTTATACGCCACAACTTGCACGTGTTGTACGTGCTAAT

361  GTAGTTTCTCAATGGGAAGAAGATTATGTTCGTGCAGAACGTGTTATCGGTGGTAGCCGT
     || ||  | |||||||| |||||||||||||||||| ||||| ||  | || |||||||||
     GTCGTAGCACAATGGGAGGAAGATTATGTTCGTGCTGAACGAGTATTAGGAGGTAGCCGT

421  ACCTACATCTTAGTCAAACACGTTGTGCGCAACTCTGCCGCACCAGTGTTAGTCTTTGCA
     || || ||  | || |||||||| || || ||  | || || || || || || |||||
     ACTTATATTCTCGTAAAACACGTAGTACGTAATACCGCTGCGCCTGTATTCGTTTTTGCC

481  ACCGTAATGGTGGCTGATGCGATTGTACTTGAAGCCTCTCTCTCTTTCTTAGGTGCAGGT
     ||||| ||||| || ||||| |||||  ||||||| || |||||||||||||| || ||
     ACCGTTATGGTTGCCGATGCAATTGTTTTTGAAGCTTCGCTCTCTTTCTTAGGGGCGGGA

541  GTACAACTTCCACAACCATCTTGGGGAAATATCCTGTCAGAAGGACGTAACATCGTATTA
     ||||| |  ||    ||||| ||||||||||  |  | ||||| |||||  | || |||
     GTACAGCCACCTTTCCCATCGTGGGGAAATATTTTAGCGGAAGGCCGTAATTTGGTGTTA

601  AGTGGCTTCTGGTGGGCAACAACCTTTGCAGGTTTAGCGATACTCTTAACGGTACTCTCG
     ||||| || |||||||||| || ||||||||  |   ||| ||  | ||||||||| |
     AGTGGTTTTTGGTGGGCAACGACTTTTGCAGGCCTGATGATTCTACTCACGGTACTCGCA

661  CTCAACATTCTCTCTGAAGGCTTAACAGATGCGTTAGTCAATCCAAAACTCAAACGTAGC
      | || ||  |  | |||||||||| ||||| |||||||||||||||| ||||   ||
     TTAAATATCTTAGCAGAAGGCTTAACTGATGCCTTAGTCAATCCAAAACTAAAAC---GC
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Figure 2. Sequences of 2A3 cloned gene in *H. parasuis* (Genebank DQ153243) and ABC transporter gene in *H. ducreyi* 35000HP. Sequence of the gene in *H. parasuis* is shown in upper lane and those in *H. ducreyi* is shown in lower lane. When this sequence was compared with database, it showed 78% similarity with ABC transporter, ATPase binding protein, permease gene of *H. ducreyi* 3500HP strain analyzed by Conserved Domain Database (CDD). The length of cloned gene was 1,105 bp and underlined sequences were the primer sets used in this study.

Table 2. Sensitivity of 2A3 gene PCR and isolation of *H. parasuis* from field clinical samples

Sample No.	Detection method	
	2A3 gene PCR (%)	Bacterial isolation (%)
79	30 (38.0%)	8 (10.1%)

clinical specimens have been challenging. To overcome these difficulties, several PCR methods were developed for rapid and accurate diagnosis of the bacterium (8, 11, 12). In addition, gene amplification by PCR can provide other important information, including data on pathogenicity or serovar, when the amplified PCR product is characterized by RFLP or sequence analysis (13). To diagnose *H. parasuis* infection by PCR, primers were designed based on the 16S small ribosomal DNA subunit of *H. parasuis* (8). However, the authors reported that their primers showed a weak false positive reaction against another member of the *Pasteurellaceae* family, *Actinobacillus indolicus* (8). Recently, the PCR using modified 16S rDNA primer reported by Angen *et al*. (9) improved the weakness of Oliveira's PCR (8) and the formal PCR was found to be complete species specific for *H. parasuis*. Oliveira reported a detection rate of 48.6% based on 16S ribosomal DNA PCR, and a bacterial isolation rate of 12.3% (14). The PCR detection rate and the

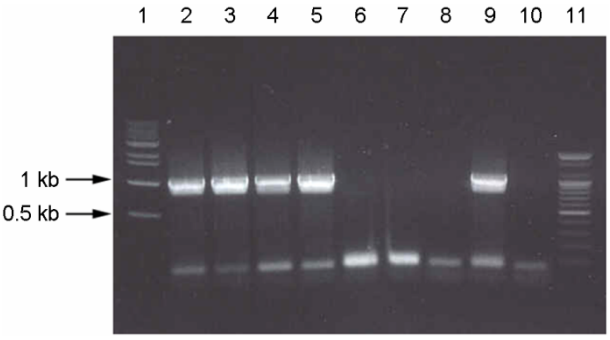

Figure 3. The PCR sensitivity test using DNA extracted from serial dilution of a 1 × 10^7 CFU/ml *H. parasuis* culture. Lanes 1, DNA molecular weight marker type 1 kbp DNA ladder; 2, PCR product from 1 × 10^7 CFU/ml *H. parasuis* culture; 3, 1 × 10^6 CFU/ml *H. parasuis* culture; 4, 1 × 10^5 CFU/ml *H. parasuis* culture; 5, 1 × 10^4 CFU/ml *H. parasuis* culture; 6~8, showing negative PCR amplification; 9, a positive control for *H. parasuis*; 10, distilled water as a negative control; 11, DNA molecular weight marker type 100 bp DNA ladder.

bacterial isolation rate in our study were 38.0% and 10.1%, respectively. Although the samples tested were different in both studies, Oliveira's detection rate and isolation rate are 10.6% and 2.2% higher, respectively, than those reported in the present study. All samples testing positive in cultivation were also positive by PCR. However, 7 of 49 samples testing negative by PCR with 2A3 primers were positive by PCR with 16S rDNA primers (14). We cannot explain this difference in PCR results obtained with the two different primer pairs. The 16S rDNA sequences of *A. pleuropneumoniae* and *H. parasuis* have 89.8~90.9% similarity. Thus, some bacteria of this family may show false positive PCR results when *H. parasuis* 16S rDNA-based primers are used (9).

Genes encoding ABC transporter proteins are highly specific to the particular species (15). The ABC superfamily of active transporters is composed of about 50 functionally diverse prokaryotic and eukaryotic transmembrane proteins (16). These proteins transport a variety of substrates, including amino acids, lipids, inorganic ions, peptides, sugars, metals, drugs, and proteins (16). These proteins utilize energy derived from the hydrolysis of ATP to transport the substrate across the membrane against a concentration gradient (17). Because the amino acid sequence of the 2A3 polypeptide shows 83% similarity with that of an *H. ducreyi* ABC transporter, it is expected that the 2A3 protein also acts as a transporter. The similarity of nucleotide sequence between the 2A3 and the ABC transporter gene of *H. ducreyi* is 78%, and 99% identity with ABC type oligopeptide transport system in *H. parasuis* in recent BLAST analysis. However, the 2A3 sequence has 76% identity with *A. pleuropnumoniae* strains, which shows less similarity for 16S rDNA sequence. Therefore, PCR primers based on the putative ABC transporter gene 2A3 is thought to detect *H. parasuis* specifically. In a diagnostic real-time PCR for *Mycoplasma hyopneumoniae*, the agent of swine mycoplasma pneumonia, a pair of primers amplifying a putative ABC transporter gene was used (15). It showed high sensitivity and specificity. Likewise, the ABC transporter gene in this study could be a valuable PCR target for *H. parasuis* detection.

In conclusion, we analyzed a previously unknown gene encoding an ABC transporter-like protein of *H. parasuis* and developed a new PCR diagnostic tool using primers designed from the aforementioned sequence. The PCR product seems to be highly specific for *H. parasuis*, allowing the pathogen to be differentiated from other bacteria with appreciable sensitivity. Since this species-specific gene was selected by comparative analysis among the gene from the bacteria within the *Pasteurellaceae* family, the PCR technique could be a valuable method for diagnosis of *H. parasuis* infection from clinical materials.

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