

Heterogeneous IgE reactivities to *Staphylococcus pseudintermedius* strains in dogs with atopic dermatitis, and the identification of DM13-domain-containing protein as a bacterial IgE-reactive molecule

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One sentence summary: IgE reactivities to *Staphylococcus pseudintermedius*.

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Abstract

Staphylococcus pseudintermedius is one of the major pathogens causing canine skin infection. In canine atopic dermatitis (AD), heterogeneous strains of *S. pseudintermedius* reside on the affected skin site. Because an increase in specific IgE to this bacterium has been reported, *S. pseudintermedius* is likely to exacerbate the severity of canine AD. In this study, the IgE reactivities to various *S. pseudintermedius* strains and the IgE-reactive molecules of *S. pseudintermedius* were investigated. First, examining the IgE reactivities to eight strains of *S. pseudintermedius* using 141 sera of AD dogs, strain variation of *S. pseudintermedius* showed 10–63% of the IgE reactivities. This is different from the expected result based on the concept of *Staphylococcus aureus* clonality in AD patients. Moreover, according to the western blot analysis, there were more than four proteins reactive to IgE. Subsequently, the analysis of the common IgE-reactive protein at ~15 kDa confirmed that the DM13-domain-containing protein was reactive in AD dogs, which is not coincident with any *S. aureus* IgE-reactive molecules. Considering these, *S. pseudintermedius* is likely to exacerbate AD severity in dogs, slightly different from the case of *S. aureus* in human AD.

Keywords: *Staphylococcus pseudintermedius*, atopic dermatitis, IgE, dogs, DM13-domain-containing protein, exacerbation factor

Introduction

Staphylococcus pseudintermedius, which was previously identified as *Staphylococcus intermedius*, is a commensal bacterium on dog skin (Bannoehr et al. 2007, Sasaki et al. 2007, Bannoehr and Guardabassi 2012). The heterogeneous strains of *S. pseudintermedius* reside on different parts of the body (Fazakerley et al. 2010). It is also one of the major pathogens of canine diseases, including skin, ear and urinary tract infections (Lynch and Helbig 2021). The use of antimicrobial drugs is the current first choice for these various *S. pseudintermedius* infections in dogs. Some infections caused by methicillin-resistant *S. pseudintermedius* can be difficult to treat using systemic administration of antibiotics (Lynch and Helbig 2021).

Atopic dermatitis (AD) is a pruritic skin disease in humans (Weidinger et al. 2018), and AD in humans has been reported to af-

fect 15–20% of children and 1–3% of adults (Nuttan 2015). AD can be caused by a combination of genetic predisposition and environmental factors. Exposure to environmental stimuli, such as humidity loss, pH change and exposure to chemicals and molecules, links to the onset and progress of AD (David Boothe et al. 2017). When the skin barrier is broken by the scraping action in AD, the molecules enter the epidermis and bind to IgE (David Boothe et al. 2017). Certain molecules bind to IgEs bound to mast cells and the dendritic cells (such as Langerhans cells). As a consequence, inflammation and itchiness are induced (David Boothe et al. 2017, Weidinger et al. 2018). Thus, IgE-reactive molecules play an important role in the pathophysiology of AD.

Dogs also suffer from AD, and canine AD and human AD share many common features (Marsella and Girolomoni 2009). It has been reported to affect 10–15% of dogs (Gedon and Mueller 2018).

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On the affected skin site of AD dogs, *S. pseudintermedius* becomes dominant, along with a decrease in the microbial diversity of normal microflora (Bradley et al. 2016). Several studies have reported that the level of specific IgE to *S. pseudintermedius* increases in AD dogs (Bexley et al. 2013, Khantavee et al. 2020). Because the scratching behavior due to itching leads to barrier dysfunction, and house dust mite and bacteria on the skin are sensitized in canine AD, *S. pseudintermedius* possibly aggravates the AD severity, as with *Staphylococcus aureus* in human AD (David Boothe et al. 2017). However, few studies are available for the relatedness of *S. pseudintermedius* strains with the prevalence of specific IgE to *S. pseudintermedius* in AD dogs and for the bacterial molecules reactive to specific IgE, to our knowledge. In this study, we investigated the IgE reactivity to *S. pseudintermedius* strains among AD dogs and the IgE-reactive molecule of *S. pseudintermedius*.

Methods and materials

Bacterial strain, culture condition and reagents

All the bacteria used in this study are described in Table 1. Tryptic soy broth (Becton, Dickinson and Co., Franklin Lakes, NJ) and Luria–Bertani medium (Kanto Chemical Co., Tokyo, Japan) were used to culture *S. pseudintermedius* and *Escherichia coli*, respectively. Bacteria were aerobically cultured at 37°C unless otherwise stated. All reagents were purchased from Nacalai Tesque (Kyoto, Japan), FUJIFILM Wako Pure Chemical (Osaka, Japan) or Sigma-Aldrich (St Louis, MI) unless otherwise stated.

DNA and protein sequence analysis

The DNA was amplified using polymerase chain reaction (PCR) with an appropriate primer set (Table 2) (Solyman et al. 2013). KAPA Taq EXtra HotStart ReadyMix with dye (Kapa Biosystems, Wilmington, MA) and KAPA HiFi HotStart ReadyMix (Kapa Biosystems) were used for general PCR and cloning, respectively. DNAs were sequenced with the BigDye Terminator v3.1 cycle sequencing kit (Thermo Fisher Scientific, Inc., Waltham, MA) using a Model 3130 Genetic Analyzer (Thermo Fisher Scientific, Inc.), according to the manufacturer's instructions. The phylogeny was analyzed based on DNA sequences using MEGA 11 version 11.0.8 (Tamura et al. 2021). Allelic profile was determined in the PubMLST.org website (<https://pubmlst.org/>) (Jolley et al. 2018).

Protein sequences were analyzed using BLASTp at the NCBI and SignalP 5.0 (<https://services.healthtech.dtu.dk/service.php?SignalP-5.0>) (Almagro Armenteros et al. 2019). The protein structure was predicted using AlphaFold2 (<https://colab.research.google.com/github/sokrypton/ColabFold/blob/main/AlphaFold2.ipynb>) (Cramer 2021, Jumper et al. 2021), and the homologous structures were searched using the Dali server (<http://ekhidna2.biocenter.helsinki.fi/dali/>) (Holm 2020).

Canine serum

The sera obtained from 20 healthy dogs were collected. Moreover, dogs with nonseasonal chronic pruritus were diagnosed with AD using Prélaud's and Willemus' criteria in Fujimura Veterinary Hospital (Osaka, Japan) (DeBoer and Hillier 2001), with parasite infestation with fleas, demodex mite and sarcoptic mange; those with a fungal infection and those with pyoderma were excluded. The sera were collected from the AD dogs. Furthermore, immunized serum was obtained. A beagle dog (female, 4 years old) was subcutaneously injected with 100 µg bacterial protein mixed with 30 mg aluminum hydroxide (Sigma-Aldrich) on the dorsal part. After 2 weeks, 10 µg bacterial protein mixed with 30 mg aluminum

hydroxide was subcutaneously injected into the dorsal part. After 1 week, the serum was collected. In all the dogs, the presence and abundance of *S. pseudintermedius* on the normal/affected skin were not examined.

Extraction of bacterial proteins

After bacterial cultivation until an optical density of 0.8 at 600 nm (OD₆₀₀), the bacteria were washed with phosphate-buffered saline (PBS; 135 mM NaCl, 2.5 mM KCl, 8 mM Na₂HPO₄, 1.5 mM KH₂PO₄, pH 7.4) three times. The bacteria were suspended in a lysis solution (100 µg mL⁻¹ lysostaphin, 100 µg mL⁻¹ lysozyme, 50 mM Tris–HCl, 20 mM MgCl₂, 30% raffinose, pH 7.5) and were incubated (4 h, 37°C). After removal of debris by centrifugation (10 000 × g, 10 min, 4°C), urea was supplemented to the supernatant at 3 M. The protein concentration was measured using Bradford reagent (TaKaRa Bradford Protein Assay Kit; Takara Bio, Shiga, Japan).

Enzyme-linked immunosorbent assay

The wells of a microplate (Fluotrac 600 microtiter plates; Greiner Bio-One, Kremsmünster, Austria) were coated with 100 µL of bacterial proteins (10 µg mL⁻¹) or purified recombinant protein (1 µg mL⁻¹) suspended in carbonate–bicarbonate coating buffer (15 mM Na₂CO₃, 35 mM NaHCO₃, pH 9.6), at 4°C overnight. After washing with PBS supplemented with 0.05% Tween 20 (PBS-T), the wells were blocked with a 1× EzBlock Chemi (Atto Co., Tokyo, Japan). Serum was diluted 1:100 with 1× EzBlock Chemi supplemented with 0.05% Tween 20, and 100 µL of the diluted serum was supplemented to the well. After 2 h incubation at room temperature with shaking, the well was washed three times with PBS-T. The well was then incubated with 100 µL of goat anti-dog IgE antibody conjugated with horseradish peroxidase (HRP) (Bethyl Laboratories, Montgomery, TX), which was diluted 1:10 000 with 1× EzBlock Chemi supplemented with 0.05% Tween 20, for 1 h at room temperature with shaking. After washing with PBS-T three times, 100 µL of the 3-(p-hydroxyphenyl)propionic acid (HPPA) solution (20 mM HPPA, 3 mM sodium perborate tetrahydrate, 2 mM ethylenediaminetetraacetic acid (EDTA), 0.2 M Tris, 10 mM sodium acetate, pH 8.0) was added to each well and the plate was shaken for 30 min. The enzyme reaction was stopped by adding 100 µL of solution (0.2 M glycine, 0.15 M NaOH, pH 10.5). The fluorescence intensity was measured in fluorescence units (excitation 325 nm/emission 420 nm), with a microplate fluorescence reader (Powerscan MX; DS Pharma Biomedical, Osaka, Japan).

Sodium dodecyl sulfate–polyacrylamide gel electrophoresis (SDS–PAGE) and western blot

An equal volume of bacterial protein suspension (0.4 mg mL⁻¹) or purified protein suspension (0.05 mg mL⁻¹) was mixed with 2× sample buffer (0.125 M Tris–HCl, 4% SDS, 20% glycerol, 0.002% bromophenol blue, 10% 2-mercaptoethanol, pH 6.8). The sample (3 µL) was electrophoresed in 12.5% SDS–PAGE gels. If required, the proteins were stained with Coomassie brilliant blue R-250.

For the western blotting, the proteins were transferred to a polyvinylidene difluoride (PVDF) membrane (Amersham Hybond P western blotting membranes, PVDF; GE Healthcare, Chicago, IL), using blotting solution (192 mM glycine, 25 mM Tris, 20% methanol, pH 8.6). The membrane was blocked with 3% skim milk in PBS-T for 1 h at room temperature and then washed. All the membrane washes were performed with PBS-T for 5 min three times. The membrane was incubated with canine serum, diluted to 1:10 with 3% skim milk in PBS-T, for 2 h at room temperature. After washing, the membrane was incubated with goat anti-

Table 1. List of bacterial strains used in this study.

Bacteria	Comments
<i>Staphylococcus pseudintermedius</i> strain ^a :	
VDTSP1	Isolated from a 12 y.o. spayed female Miniature Dachshund dog
VDTSP2	Isolated from a 3 y.o. contraceptive female Toy Poodle dog
VDTSP3	Isolated from a 13 y.o. contraceptive female Border Collie dog
VDTSP4	Isolated from an 8 y.o. contraceptive female Miniature Dachshund dog
VDTSP5	Isolated from an 8 y.o. French Bulldog with unknown gender
VDTSP6	Isolated from a 13 y.o. male Labrador Retriever dog
VDTSP7	Isolated from a 7 y.o. castrated male Papillon dog
VDTSP8	Isolated from a 5 y.o. French Bulldog dog with unknown gender
VDT2SP1–45	These 45 strains were isolated from the affected skin sites in pyoderma or AD
<i>Escherichia coli</i> strain:	
DH5 α ^b	Used for cloning
NiCo21 (DE3) ^c	Used for protein expression

y.o., years old.

^aThese bacteria strains were isolated in this study.^bThe bacteria were purchased from Takara Bio (Shiga, Japan).^cThe bacteria were purchased from New England Biolabs (Ipswich, MA).**Table 2.** List of primers used in this study.

Primer	Sequence (5' to 3')	Comment
tuf forward	CAATGCCACAACTCG	These primers were used for bacterial phylogenetic analysis, which are generally used for multilocus sequence typing analysis in <i>S. pseudintermedius</i> .
tuf reverse	GCTTCAGCGTAGTCTA	
cpn60 forward	GCGACTGTACTTGACACAAGCA	
cpn60 reverse	AACTGCAACCGCTGTAAATG	Primers were used for cloning of the gene for DM13-domain-containing protein.
pta forward	GTGCGTATCGTATTACCAGAAGG	
pta reverse	GCAGAACCTTTTGTGAGAAGC	
purA forward	GATTACTTCCAAGGTATGTTT	
purA reverse	TCGATAGAGTTAATAGATAAGTC	
fdh forward	TGCGATAACAGGATGTGCTT	
fdh reverse	CTTCTCATGATTCACCGGC	
ack forward	CACCACTTCACAACCCAGCAAAC	
ack reverse	AACCTTCTAATACACGCGCACGCA	
sar forward	GGATTTAGTCCAGTTCAAAATTT	
sar reverse	GAACCATTCGCCCCATGAA	
DM13_F	TTAAGGTACCATGAACATGAAAATGGCATTAGTTTC	
DM13_R	TCATGAATTCTATTTAAGTTCCGCAGAACCAAA	
ImDoAgB_F	TTAAGGTACCATGAATCAAGTTACAAAAATTGCAATCG	
ImDoAgB_R	TCATGAATTCTTAACGACTCTCTTTTGCTTTAATC	
DM13_SQ_F	GAATTATTAATGAGTAGTTTATGTTAATTGCTGT	Primers were used to detect the gene for DM13-domain-containing protein in <i>S. pseudintermedius</i> .
DM13_SQ_R	GGATATAAAAACCTGATCCTAAGCG	

dog IgE antibody HRP conjugated (Bethyl Laboratories), diluted 1:10 000 with 3% skim milk in PBS-T, for 1 h at room temperature. Immunoblot signals were developed with an ECL Start Western Blotting Detection system (GE Healthcare), after washing the membrane. The signals were visualized using ImageQuant LAS 4000 mini (GE Healthcare).

Peptide mass fingerprinting

After excision of the targeted protein band from the SDS-PAGE gel stained with Coomassie brilliant blue R-250, the peptides

for mass spectrometry were prepared, as described elsewhere (Uchiyama et al. 2011). The peptide sample was analyzed using an LTQ XL mass spectrometer (Thermo Fisher Scientific, Inc.), which was equipped with liquid chromatography (Michrom BioResources, Inc., Auburn, CA), followed by a nanoelectrospray ion source (Thermo Fisher Scientific, Inc.). The mass (MS)/MS data were analyzed by MASCOT version 2.3.01 and SEQUEST, using Proteome Discoverer software version 1.2 (Thermo Fisher Scientific, Inc.). The in-house database was set up from protein sequences, which were derived from the complete genome sequences of *S. pseudintermedius* (Table S1, Supporting Information).

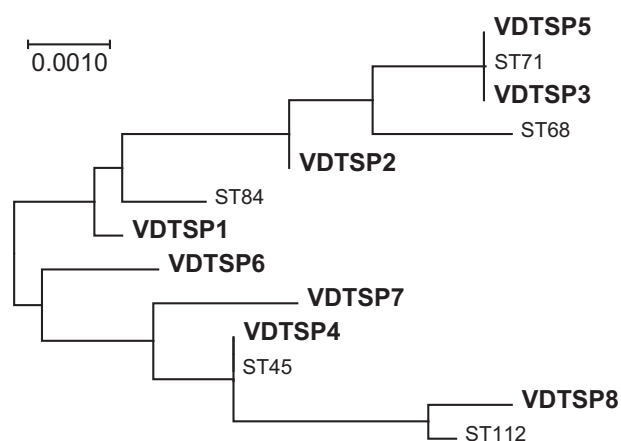


Figure 1. Phylogenetic analysis of the eight *S. pseudintermedius* strains used in this study. The partial sequences of housekeeping genes, including *tuf*, *cpn60*, *pta*, *purA*, *fdh*, *ack* and *sar* genes, were concatenated in order and analyzed using the maximum likelihood method. The allelic profiles of strains VDTSP2, VDTSP3, VDTSP4 and VDTSP5 were identified to be ST714, ST71, ST45 and ST71, respectively; the sequence types of VDTSP1, VDTSP6, VDTSP7 and VDTSP8 were registered as ST2265, ST2266, ST2267 and ST2268, respectively.

Cloning, protein expression and protein purification

The DNA of *S. pseudintermedius* strain VDTSP6 was amplified using PCR with a primer set (Table 2). The accurate sequence was transferred to a pCold GST plasmid (Takara Bio). The protein over-expression was made in 250 mL of culture broth, according to the manufacturer's instructions. After centrifugation ($10\,000 \times g$, 10 min, 4°C), the bacterial pellet resuspended in 25 mL of PBS was sonicated on ice. After centrifugation ($8000 \times g$, 30 min, 4°C), the supernatant was gently mixed with 200 μL of glutathione agarose resin for 1 h at 4°C . The resin was washed with 35 mL of PBS three times and was transferred into an open column. The proteins were eluted with 15 mM reduced glutathione in 50 mM Tris-HCl (pH 9.6). The elute was dialyzed against 50 mM Tris-HCl (pH 7.5) at 4°C for 1 h.

Animal ethics

The animal experiments conducted in this study were approved by the Ethics Committee of Azabu University (Nos 210407-4 and 210121-7).

Results and discussion

Examination of IgE-positive population in sera of AD in dogs

Eight strains of *S. pseudintermedius* from dogs with AD together with pyoderma were isolated, which were resistant to methicillin. The phylogenetic relationship of these strains was analyzed using the concatenated sequence of housekeeping gene sequences, such as *tuf*, *cpn60*, *pta*, *purA*, *fdh*, *ack* and *sar* genes. Because these housekeeping genes are used in multilocus sequence typing, sequence types of some major epidemic methicillin-resistant clones, such as ST45, ST68, ST71, ST84 and ST112, were included in the analysis (Rynhoud *et al.* 2021). As a result, these bacterial strains were phylogenetically different (Fig. 1).

Using the proteins extracted from these strains, antigen-down enzyme-linked immunosorbent assay (ELISA) to measure specific IgE was set up. Although urea-treated proteins can show different

Table 3. IgE reactivities specific to eight *S. pseudintermedius* strains.

<i>Staphylococcus pseudintermedius</i> strain	Positivity rate (%)
VDTSP1	61.7
VDTSP2	63.1
VDTSP3	9.9
VDTSP4	63.1
VDTSP5	9.9
VDTSP6	56.7
VDTSP7	44.0
VDTSP8	53.2

reactivity in ELISA, the urea-treated proteins still can be used as antigens in ELISA (Mine and Zhang 2002). In the ELISA, the bacterial proteins, which were treated with urea after protein extraction, were used as antigens because some bacterial proteins are processed after expression and secretion. In the ELISA to measure IgE reactivity specific to *S. pseudintermedius*, we set the cutoff value for IgE reactivity, which was mean + ($3 \times$ standard deviation) of the values of 20 healthy dogs in fluorescent units. When the IgE reactivity was higher than the cutoff value, the result was taken as a positive result for IgE reactivity.

The sera of 141 dogs with AD were analyzed. As a result, ~10–63% of the AD dogs had specific IgE to *S. pseudintermedius* (Fig. S1, Supporting Information; Table 3). The strains VDTSP3 and VDTSP5, which were genetically related to each other, showed positive IgE reactivity with ~10% of the AD dogs. The other strains showed positive IgE reactivity with 44–63% of the AD dogs. Observing the result, the strain variation in *S. pseudintermedius* was considered to lead to a difference in IgE reactivity among AD dogs.

Analysis of IgE-reactive molecules from *S. pseudintermedius*

Because of the serum availability of clinical specimens, the healthy dog was immunized with *S. pseudintermedius*, the proteins of *S. pseudintermedius* strain VDTSP6, which did not cause unusual behavior and obvious skin change. The immunized serum showed specific IgE reactivity to all eight strains of *S. pseudintermedius* (Fig. S1, Supporting Information). To examine the difference in IgE reactivities to *S. pseudintermedius* strains, the *S. pseudintermedius* was analyzed using western blot. After the protein separation of eight strains of *S. pseudintermedius* using SDS-PAGE (Fig. 2A), the IgE-reactive molecules were detected using western blot with the serum of the immunized dog. As a negative control, the pooled sera of five healthy dogs were used. As a result, observing the protein bands on the western blot, the tested strains showed different protein band patterns. However, four protein bands at ~47, 35, 24 and 15 kDa in common were observed, suggesting that more than four proteins were IgE reactive (Fig. 2B). Among the protein bands, the protein band at ~15 kDa was detected in most of the strains of bacterial proteins.

Staphylococcus pseudintermedius proteins at ~15 kDa were analyzed using peptide mass fingerprinting. The proteins of the VDTSP6 strain were analyzed using mass spectrometry. According to the analysis of mass spectrometric data against the in-house protein database of *S. pseudintermedius*, the initial candidate proteins were obtained. Among these candidates, the proteins other than 10–20 kDa and ribosomal proteins were removed. Subsequently, the proteins with predicted signal peptides were selected as potential candidates because the IgE-reactive molecules derived from *S. aureus* have been reported to be extracellular materi-

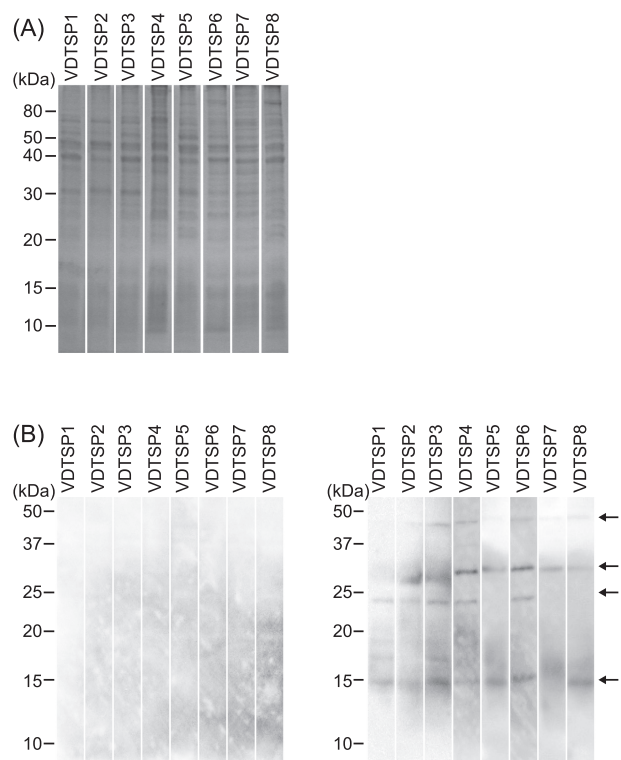


Figure 2. Analysis of *S. pseudintermedius* proteins using SDS-PAGE and western blot to detect IgE-reactive molecules, with proteins of the eight *S. pseudintermedius* strains. **(A)** SDS-PAGE. On the leftmost, the protein molecular weights are shown, for which XL-Ladder Broad protein marker (APRO Science, Tokushima, Japan) was used. **(B)** Western blot to detect IgE-reactive molecules of *S. pseudintermedius* strains. On the leftmost of both panels, the protein molecular weight standards are shown, for which Precision Plus Protein Dual Color Standards (Bio-Rad Laboratories, Hercules, CA) were used. The pooled sera of healthy dogs were used on the left panel; the serum of an immunized dog was used on the right panel. Arrows indicate the detected band on the right panel.

als (Hong et al. 2011, Nordengrun et al. 2018). As a result, two potential candidate proteins were assumed: DM13-domain-containing protein and immunodominant antigen B protein (Table S2, Supporting Information).

Identification of IgE-reactive molecule from *S. pseudintermedius*

To test the IgE reactivity to these molecules, we produced and purified the recombinant glutathione S-transferase (GST)-tagged proteins in *E. coli*: the recombinant GST protein (rGST) as a control, GST-fused DM13-domain-containing protein (rGST-DM13) and GST-fused immunodominant antigen B protein (rGST-IDA). These recombinant proteins contained the GST at the N-terminus. These recombinant proteins were separated by SDS-PAGE (Fig. 3A). First, the calculated molecular weight of rGST was 28.1 kDa, which was slightly smaller than the theoretical molecular weight at 30.9 kDa but almost corresponded to it. Second, the SDS-PAGE image of rGST-DM13 showed three upper thin faint bands at 48.4, 43.5 and 40.6 kDa, and one lower thick band at 39.8 kDa. The upper thin band was possibly the intact rGST-DM13 because the calculated molecular weight was similar to the theoretical molecular weight of 46.7 kDa. In addition, assuming that the upper thin band is rGST-DM13, the SDS-PAGE of rGST-DM13 was observed to be a protein ladder, which suggests that the rGST-DM13 might be degradable. Moreover, the rGST-IDA showed two major bands at 43.5 and

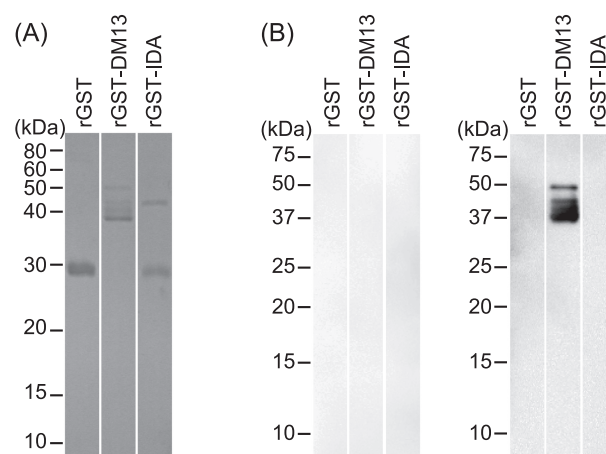


Figure 3. Analysis of IgE reactivity to three recombinant proteins, rGST, rGST-DM13 and rGST-IDA. **(A)** SDS-PAGE of the purified recombinant GST-tagged proteins. On the leftmost, the protein molecular weight standards are shown, for which XL-Ladder Broad protein marker (APRO Science) was used. The protein band is indicated by an arrow, which is shown on the right of each lane. **(B)** Western blot analysis to detect IgE reactivity to the recombinant proteins. On the leftmost of both panels, the protein molecular weight standards are shown, for which Precision Plus Protein Dual Color Standards (Bio-Rad Laboratories, Hercules, CA) were used. The pooled sera of healthy dogs were used on the left panel; the serum of an immunized dog was used on the right panel.

28.1 kDa, which were not homologous to the expected protein size of 49.3 kDa. From this observation, we considered that the intact rGST-IDA was not considered to be properly obtained in this protein expression system. However, because we believed that the higher band still contained the protein sequence of immunodominant antigen B protein, the produced rGST-IDA was used in the following experiment.

The IgE reactivity to these proteins was tested by western blot, using the sera of immunized and healthy dogs (Fig. 3B). As a result, when using the serum of the immunized dog, the rGST-DM13 alone showed the detected band, whereas rGST and rGST-IDA did not. When using sera of healthy dogs, no protein band was detected. Moreover, the IgE reactivities to the rGST and rGST-DM13 were also examined using ELISA (Fig. S2, Supporting Information). As a result, the IgE reactivity to rGST-DM13 was 31.9% (45/141), while that to rGST was 0.7% (1/141). Thus, the DM13-domain-containing protein of *S. pseudintermedius* was considered to be specifically reactive to the IgE in the dogs.

DM13-domain-containing protein in *S. pseudintermedius*

DM13-domain-containing protein has a β -strand-rich fold and might function as histidine kinase of the two-component system to sense the environmental change (Iyer et al. 2007). Because the DM13-domain-containing protein in *S. pseudintermedius* was predicted to have a signal peptide on the N-terminal end, it is assumed to be secreted to the extracellular milieu. Because the function of DM13-domain-containing protein in *S. pseudintermedius* was not clear, we attempted to predict the function of the protein by searching for proteins with similar structures based on the predicted structure with high accuracy. The best-predicted structure for the protein, excluding the 20 residues of the N-terminal signal sequence, was a typical β -sandwich structure in the high confidence (>89%) region (residues 50–150) (Fig. S3, Supporting Information). A similarity search based on this

β -sandwich structure identified 30 hits of bacterial extracellular structural proteins such as pili, cell surface proteins, flagella and cell-wall-anchored proteins (Table S3, Supporting Information) with z-scores of 2.0 or higher (out of 621 total hits). Together with the fact that this protein has a signal peptide, it might function as an extracellular structural component.

According to the BLASTp analysis, DM13-domain-containing protein found in this study was found majorly in *S. pseudintermedius*; the similar protein was not found in *S. aureus*. We investigated the frequency and similarity of DM13-domain-containing protein in *S. pseudintermedius*. We performed PCR for detection of the DM13-domain-containing protein gene among the eight *S. pseudintermedius* strains used in this study and another 45 *S. pseudintermedius* strains clinically isolated from dogs with pyoderma or AD. As a result, all the tested *S. pseudintermedius* strains were positive in the PCR screening. In addition, we investigated the genetic diversity of the gene for DM13-domain-containing protein in *S. pseudintermedius* strains. As a result, the DM13-domain-containing protein gene was highly conserved (Fig. S4, Supporting Information). Thus, the DM13-domain protein was considered to be adapted originally to *S. pseudintermedius*.

Staphylococci in AD among dogs and humans

Specific strains of *S. aureus* are likely to be related in AD patients (Ogonowska et al. 2020); various strains of *S. pseudintermedius* are present in AD dogs (Fazakerley et al. 2010). In this study, the strain variation in *S. pseudintermedius* was shown to be reflected in a difference in IgE reactivities. Moreover, many virulence and toxic factors of *S. aureus* have been reported to be IgE reactive in human AD (Nordengrun et al. 2018, Seiti Yamada Yoshikawa et al. 2019). In contrast, although some virulence and toxic molecules have been reported in *S. pseudintermedius*, such as exfoliative toxin B, enterotoxin, leukocidin and *S. pseudintermedius* surface protein L (Nishifuji et al. 2008, Abouelkhair et al. 2018, Phumthanakorn et al. 2018, Richards et al. 2018), the IgE reactivity to them is not known. In this study, the western blot analysis showed potential IgE-reactive proteins in *S. pseudintermedius*. Apart from the typical IgE-reactive molecules of *S. aureus*, the mass spectrometric analysis of *S. pseudintermedius* protein at ~15 kDa in this study did not predict any pathogenic and toxic factors, and the DM13-domain-containing protein was found to be specifically reactive to IgE, whose function is not known. Considering possible slight differences in interaction of staphylococci with host between dogs and humans, the interaction between *S. pseudintermedius* strains and AD dogs should be carefully investigated.

Supplementary data

Supplementary data are available at [FEMSLE](https://academic.oup.com/femsle/article/369/1/fnac019/6534253) online.

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Conflict of interest. None declared.

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