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Virulence-associated genes and molecular typing of *Streptococcus uberis* associated with bovine mastitis in northern Thailand

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Abstract: *Streptococcus uberis* is a major bovine mastitis pathogen. Several studies have revealed a persistence of specific strains of *S. uberis* with enhanced virulence or transmissibility. We aimed to investigate the presence of *S. uberis* virulence-associated genes including plasminogen activator (*pauA*), glyceraldehyde-3-phosphate dehydrogenase (*gapC*), oligopeptide permease (*oppF*), metal transporter uberis A (*mtuA*), hyaluronic acid capsules (*hasA*, *hasB*, *hasC*), lactoferrin binding protein (*lbp*), adhesion protein (*sua*), and CAMP factor (*cfu*) as well as the genetic profiles using pulsed-field gel electrophoresis (PFGE) among isolates from cases of clinical mastitis, subclinical mastitis, and intramammary infection with low somatic cell count in dairy cows. A total of 642 milk samples from 178 milking cows of 53 farms in Chiang Mai, Thailand, were collected and cultured. Eighty-eight *S. uberis* isolates were identified. The most common pattern of virulence-associated genes was *hasA+hasB+hasC+sua+gapC+lbp+pauA+oppF+mtuA* (34.1%, 30/88). PFGE was performed with 71 isolates, which could be classified into 56 pulsotypes. *S. uberis* isolates tended to be clustered by farms regardless of the severity of infection. Therefore, molecular epidemiological investigation can be useful in developing an effective control strategy for bovine mastitis caused by *S. uberis* in dairy farms.

Key words: *Streptococcus uberis*, bovine mastitis, molecular typing, virulence factor

1. Introduction

Streptococcus uberis has been reported to be a major cause of bovine mastitis in many countries throughout the world (1,2), including Thailand (3). *S. uberis* is associated with subclinical and clinical intramammary infection (IMI) at any period of lactation (4) and in pregnant heifers (5). The major source of infection of this microorganism originates from the cow's environment, including body sites, manure, pastures, and bedding materials (6); consequently, the eradication of this species is particularly problematic.

In spite of the environmental nature of its transmission, a number of observations indicating contagious or cow-to-cow transmission of *S. uberis* have been reported based on molecular approaches. Molecular studies have shown evidence for direct transmission and the predominant strains in some herds (7,8). These observations suggest the existence of some *S. uberis* strains that are hypervirulent and hypertransmissible between cows.

Understanding of the pathogenesis and virulence factors required for IMI by *S. uberis* is not well established. Various virulence factors such as hyaluronic acid capsules (HasA, HasB, HasC) (9), plasminogen activator proteins (PauA and PauB) (10,11) and streptokinase (12), lactoferrin binding proteins (Lbp) (13), adhesion protein (SUAM) (14), CAMP factor (15), a surface dehydrogenase protein (GapC) (16), protein involved in the active transport of solutes essential for growth in milk (OppF) (17), and a lipoprotein receptor antigen responsible for acquisition of manganese during growth in milk (MtuA) (18) have been suggested to be associated with pathogenesis of IMI caused by *S. uberis*. The objective of the current study was to investigate genotypic variations and distribution of virulence-associated genes of *S. uberis* isolated from milk samples in northern Thailand.

2. Materials and methods

2.1. Sources of milk samples

Milk samples of mastitis cases submitted for culture at the Satellite Animal Hospital, Faculty of Veterinary Medicine, Chiang Mai University, Thailand, from July 2012 to June 2013 were included in the study. Veterinarians were requested to perform examinations of cows' udders and

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collect all four quarter milk samples from each cow with mastitis. Nonproductive udders or udders that the farmers ignored for routine milking were not examined and were excluded from the study. Quarter milk samples were subjected to measurements for milk somatic cell counts (SCCs) using the Somacount 150 (Bentley Instrument, Chaska, MN, USA). If multiple sets of milk samples from the same cow were submitted for culture more than once within a 1-month period, only the first set of quarter milk samples of the particular cow were included in the study. From these criteria, we included 642 quarter milk samples of 178 milking cows from 53 farms. Fifty-two farms were small-holder dairy farms with 10 to 50 milking cows using bucket-type machine milking. One studied farm was a big dairy farm with approximately 500 milking cows using a pipe-line milking system.

2.2. Severity of infection

All quarter milk samples were classified for severity of infection using the following criteria:

- Clinical mastitis: abnormal milk appearance with/without other clinical signs such as udder swelling, firmness, or fever.
- Subclinical mastitis: normal milk appearance with SCC of ≥250,000 cells/mL.
- IMI with low SCC: normal milk appearance with SCC of <250,000 cells/mL.

2.3. Microbiological culture

Ten microliters of milk were cultured on blood agar with 5% bovine blood. Only samples presenting more than 10 pure colonies of bacteria were used for further diagnosis (19). Streptococcal isolates were identified based on colonial appearance, Gram stain reaction, and catalase test (19). Presumptive identification was performed based on CAMP test and the utilization of sugar including esculin, inulin, salicin, raffinose, and hippurate. The selected colonies were frozen at –20 °C in brain-heart infusion broth (BHIB) containing 20% glycerol for further characterization.

2.4. Identification of *S. uberis*

Genomic DNA was extracted from all streptococcal isolates using the GF-1 Bacterial DNA Extraction Kit (Vivantis Technologies, Selangor Darul Ehsan, Malaysia). All streptococcal isolates were identified using conventional PCR, which is specific to amplify a region in the 16S rRNA gene of *S. uberis* (20) using primers as shown in Table 1. For PCR, the reaction mixture (25 µL) contained 1 µL of primers (10 pmol/L); 12.5 µL of 2X Taq Master Mix containing 1.25 U of Taq DNA polymerase, 1X

Table 1. PCR primers, annealing temperature, and expected PCR products for *Streptococcus uberis* strains.

Target gene	Primer sequence (5'-3')	Annealing temperature	Product size (bp)	References
16S rRNA	CGCATGACAATAGGGTACA GCCTTTAACTTCAGACTTATCA	58 °C	445	(20)
hasA	GAAAGGTCTGATGCTGATG TCATCCCCTATGCTTACAG	58 °C	319	(32)
hasB	TCTAGACGCCGATCAAGC TGAATTCCTATGCGTCGATC	58 °C	532	(32)
hasC	TGCTTGGTGACGATTTGATG GTCCAATGATAGCAAGGTCAC	58 °C	225	(32)
gapC	GCTCCTGGTGGAGATGATGT GTCACCAGTGTAAGCGTGGA	55 °C	200	(27)
lbp	TGACCGAAGAACGTGATG AGAGACCACTTGCCACTA	55 °C	570	(26)
pauA	TTCACTGCTGTTACATAACTTTGTG CCTTTGAAAGTGATGCTCGTG	55 °C	976	(26)
oppF	GGCCTAACCAAAACGAAACA GGCTCTGGAATTGCTGAAAG	54 °C	419	(17)
sua	ACGCAAGGTGCTCAAGAGTT TGAACAAGCGATTCGTCAAG	58 °C	776	(27)
mtuA	GAACCGCTACCTGAGGATGT ATTTGATCCGGTGTTCCTTC	54 °C	500	Accession No. AJ539135
cfu	ATCCATTAAAGGGCAAGTCG TTGGTCAACTTGTGCAACTG	54 °C	259	Accession No. U34322

ViBuffer A, 0.2 mM dNTPs, and 1.5 mM $MgCl_2$ (Vivantis Technologies); 5.5 µL of DNase-free water; and 5 µL of DNA template (50–100 ng/µL). The tubes were placed in a thermal cycler with program as follows: initial denaturation at 94 °C for 60 s, 30 cycles of denaturation at 94 °C for 60 s and annealing at 58 °C for 90 s and extension at 72 °C for 90 s. The presence of PCR products was determined by electrophoresis of the reaction product in 2% agarose gel and staining with ethidium bromide to visualize the PCR products.

2.5. PCR amplifications of virulence-associated genes

Regions in virulence-associated genes including the *pauA*, *gapC*, *oppF*, *mtuA*, *hasA*, *hasB*, *hasC*, *lbp*, *sua*, and *cfu* genes were amplified using 3 multiplex PCRs: 1) *hasA*, *hasB*, *hasC*, and *sua*; 2) *gapC*, *lbp*, and *pauA*; and 3) *oppF*, *mtuA*, and *cfu*. The multiplex PCRs were previously optimized for the detection of each set of genes. Details of the primer sequences are shown in Table 1. To amplify the genes, 25 µL of PCR reaction mixtures as described in the previous section were used. The reactions were carried out in a thermal cycler. The PCR products were revealed on 2% agarose gel at 110 V for 1.5 h.

2.6. Pulsed-field gel electrophoresis (PFGE) analysis

PFGE was adapted from the methods previously described (8). Frozen isolates were regrown on BHIB at 37 °C overnight and adjusted for turbidity to reach OD_{610} of 1.5. Isolates that failed to regrow were not further examined for PFGE. Two hundred microliters of the broth culture were centrifuged at 13,000 × *g* for 5 min. The cell pellet was resuspended in 240 µL of cell suspension buffer (10 mM Tris-HCl [pH 7.2], 20 mM NaCl, 50 mM EDTA). Cells were lysed using 60 µL of lysozyme (10 mg/mL) at 37 °C for 10 min. The cell suspension was mixed with an equal volume of 1% pulse-field certified agarose in agarose buffer (10 mM Tris-HCl [pH 7.2], 0.1 mM EDTA) and loaded into the plug molds. The plugs were subsequently incubated in 500 µL of PK-PK buffer (1% sarkosyl, 50 mg/mL proteinase K, 250 mM EDTA [pH 9]) at 50 °C for 2 h. The plugs were washed 3 times using wash buffer (10 mM Tris-HCl [pH 7.6], 0.1 mM EDTA) and digested with *Sma*I (10 U) at 25 °C overnight. After digestion, the plugs were loaded into a 1% agarose gel. DNA fragments were separated using a contour-clamped homogeneous electric field device (CHEF DRII, Bio-Rad, Hercules, CA, USA) with pulse times of 5.3 s to 34.9 s at 6 V/cm for 20 h. Gels were stained with ethidium bromide and visualized under UV light.

All PFGE patterns were interpreted according to the criteria described by Tenover et al. (21) and analyzed using BioNumerics version 7.1 software (Applied Maths BVBA, Kortrijk, Belgium). Similarity of the band patterns was calculated using Pearson's correlation coefficient and then clustered using the dendrogram generated by the unweighted pair group of arithmetic mean (UPGMA) method. Simpson's index values of diversity (D) with 95% confidence intervals (95% CI) of the PFGE techniques were determined as described previously (22).

2.7. Statistical analysis

Distributions of virulence-associated genes were presented as percentages (%). Associations of patterns of virulence-associated genes to severity of *S. uberis* infection were determined using Fisher's exact test and the Z-test in R statistical software (23) at the statistical significance of $P < 0.05$.

3. Results

Among the 642 milk samples, 89, 321, and 232 samples were from cows with clinical mastitis, subclinical mastitis, and milk with low SCC, respectively. Only 58.43% (52/89) of samples from cases of clinical mastitis, 58.26% (187/321) of samples from cases of subclinical mastitis, and 16.38% (38/232) of samples from milk with low SCC were cultivable for pathogens. A total of 88 *Streptococcus uberis* isolates were cultured from 88 quarter milk samples of 62 milking cows from 24 farms. Most *S. uberis* specimens were isolated from cases of subclinical mastitis (73%, 64/88). Only 14 *S. uberis* specimens were isolated from cases of clinical mastitis, and 10 isolates from cases of IMI with low SCC (Table 2).

3.1. Distribution of virulence genes

Three multiplex PCRs were performed to detect the presence of 10 virulence genes among 88 *S. uberis* isolates (Figures 1 and 2). All isolates carried at least 1 virulence gene. The most prevalent virulence-associated gene was *oppF* (94.32%), followed by *hasC* (92.05%) and *mtuA* (92.05%), as shown in Table 3. The *hasA* and *hasB* genes were always present together. There was no significant association between the presence of each virulence gene and the severity of infection. Moreover, the rate of the presence of *hasA* and *hasB* genes among *S. uberis* isolated from clinical mastitis cases was higher than, but not significantly different from, those among isolates from cases of IMI with low SCC (78.57% vs. 40%; $P = 0.051$). Most isolates carried 9 out of 10 virulence genes (34.1%, 30/88) with the pattern of *hasA+hasB+hasC+sua+gapC+lbp+pauA+oppF+mtuA*. The pattern of these 9 virulence genes was observed at the highest frequencies among *S. uberis* isolates from clinical (64.29%) and subclinical mastitis (28.13%) cases, which was significantly associated with severity of infection ($P = 0.045$), as presented in Table 3. However, statistical significance was not observed when isolates presenting ≥9 virulence genes were considered for statistical analysis.

3.2. PFGE

PFGE was performed with only 71 *S. uberis* isolates due to no growth of 17 isolates when frozen isolates were

Table 2. Proportions of individual virulence genes of *Streptococcus uberis* isolates associated with clinical mastitis, subclinical mastitis, and intramammary infection (IMI) with low somatic cell count (SCC) in dairy cows in Chiang Mai.

Virulence genes	Positive proportion (%)	Severity of infection		
		Clinical mastitis (n = 14)	Subclinical mastitis (n = 64)	IMI with low SCC (n = 10)
hasA	49/88 (55.68%)	11	34	4
hasB	49/88 (55.68%)	11	34	4
hasC	81/88 (92.05%)	14	58	9
sua	80/88 (90.91%)	12	59	9
gapC	80/88 (90.91%)	14	58	8
lbp	69/88 (78.41%)	13	49	7
pauA	66/88 (75.00%)	13	46	7
oppF	83/88 (94.32%)	14	59	10
mtuA	81/88 (92.05%)	13	58	10
cfu	31/83 (35.23%)	3	25	3

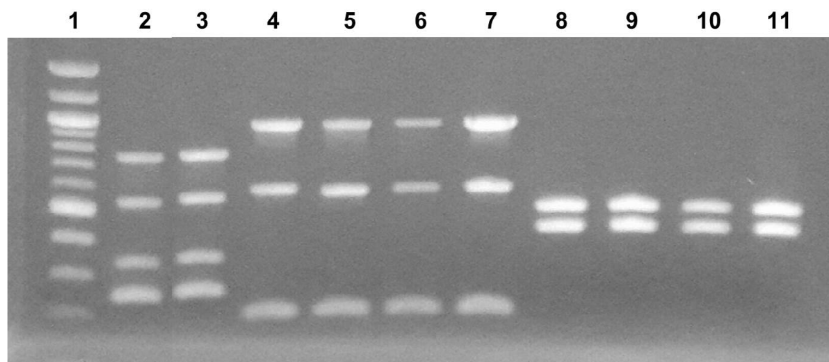

Figure 1. PCR products of virulence genes of *Streptococcus uberis* using three multiplex PCRs. Lane 1: 100-bp molecular ruler; lanes 2 and 3: PCR products of *sua* (776 bp), *hasB* (532 bp), *hasA* (319 bp), and *hasC* (225 bp); lanes 4 to 7: PCR products of *pauA* (976 bp), *lbp* (570 bp), and *gapC* (200 bp); lanes 8 to 11: PCR products of *mtuA* (500 bp) and *oppF* (419 bp).

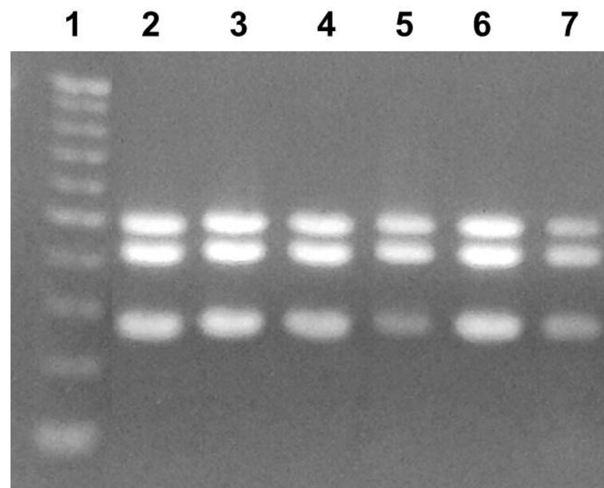

Figure 2. PCR products of virulence genes of *Streptococcus uberis* using multiplex PCR to amplify *mtuA*, *oppF*, and *cfu* genes. Lane 1: 100-bp molecular ruler, lanes 2 to 7: PCR products of *mtuA* (500 bp), *oppF* (419 bp), and *cfu* (259 bp).

Table 3. Distribution of *Streptococcus uberis* isolates presenting different patterns of virulence-associated genes among cows with different levels of severity of intramammary infection (IMI).

Virulence-associated gene patterns	Severity of infection		
	Clinical mastitis (n = 14)	Subclinical mastitis (n = 64)	IMI with low SCC (n = 10)
hasA+hasB+hasC+sua+gapC+lbp+pauA+oppF+mtuA+cfu	0	7 (10.94%)	1 (10%)
hasA+hasB+hasC+sua+gapC+lbp+pauA+oppF+mtuA	9 (64.29%)	18 (28.13%)	3 (30%)
hasC+sua+gapC+lbp+pauA+oppF+mtuA+cfu	2 (14.29%)	14 (21.88%)	2 (20%)
hasA+hasB+hasC+sua+gapC+pauA+oppF+mtuA	0	1 (1.56%)	0
hasA+hasB+hasC+gapC+lbp+pauA+oppF+mtuA	0	1 (1.56%)	0
hasC+sua+gapC+lbp+pauA+oppF+mtuA	1 (7.14%)	4 (6.25%)	1 (10%)
hasA+hasB+hasC+sua+gapC+oppF+mtuA	0	2 (3.13%)	0
hasA+hasB+hasC+gapC+oppF+mtuA+cfu	1 (7.14%)	0	0
hasC+sua+gapC+lbp+oppF+mtuA+cfu	0	2 (3.13%)	0
hasA+hasB+hasC+sua+oppF+mtuA+cfu	0	1 (1.56%)	0
hasA+hasB+hasC+gapC+lbp+pauA+oppF	1 (7.14%)	0	0
sua+gapC+lbp+pauA+oppF+mtuA+cfu	0	1 (1.56%)	0
hasA+hasB+hasC+sua+oppF+mtuA	0	3 (4.69%)	0
hasA+hasB+hasC+gapC+oppF+mtuA	0	1 (1.56%)	0
hasC+sua+gapC+lbp+oppF+mtuA	0	1 (1.56%)	0
hasC+sua+gapC+oppF+mtuA	0	1 (1.56%)	1 (10%)
hasC+sua+gapC+lbp+oppF	0	1 (1.56%)	0
hasC+sua+oppF+mtuA	0	1 (1.56%)	1 (10%)
sua+gapC	0	1 (1.56%)	0
oppF+mtuA	0	0	1 (10%)
gapC	0	3 (4.69%)	0
sua	0	1 (1.56%)	0

regrown on blood agar and BHIB. These 71 isolates were cultured from 71 quarter milk samples of 52 milking cows from 20 farms. PFGE analysis using *SmaI* endonuclease generated 56 distinct electrophoretic profiles composed of 6–14 polymorphic bands ranging from 50 to 580 kb. PFGE typing revealed a genetically diverse population with a Dice similarity index ranging from 40% to 100%. Simpson's index of diversity (D) of the performed PFGE was 0.993 (0.988–0.998). When the threshold of the Dice similarity index at 80% was used to cluster similar PFGE band patterns, 32 groups of similar-PFGE patterns were generated. Groups of two or more *S. uberis* isolates with similar PFGE patterns were clustered into 16 clusters: cluster I to XVI (Figure 3). Farms with high number of clusters included farm B (5 clusters), farm Q (3 clusters), and farm U (3 clusters). All isolates from farm F (2 isolates) and farm V (7 isolates) were clustered into clusters XVI and VII, respectively. Eleven clusters contained 2 or more *S. uberis* isolates from 1 farm, including clusters II, III, IV, V, VII, IX, X, XII, XIII, XIV, and XV. There were 5 clusters consisting of *S. uberis* isolates from mixed farms, including clusters I, VI, VIII, XI, and XVI. Regarding Tenover's criteria (21), PFGE patterns of *S. uberis* isolates in clusters III, VI, IX, XIII, XIV, and XVI were considered indistinguishable, whereas those of isolates in clusters I, II, V, X, XII, and XV were considered closely related and those of isolates in clusters IV, VII, VIII, and XI were considered possibly related. There were 7 clusters of *S. uberis* isolates that shared identical patterns of virulence genes within each cluster, including cluster III, IV, V, IX, X, XIV, and XV.

4. Discussion

In the present study, the majority of *S. uberis* specimens were isolated from subclinical mastitis cases. Subclinical mastitis caused by *S. uberis* can be mostly found prior to drying off and around parturition, whereas clinical cases of *S. uberis* IMI are frequently observed in the first months of lactation (24). Without any diagnosis and treatment, long-term subclinical infection of *S. uberis* can cause significant losses of milk production to dairy farms. Identification of *S. uberis* is therefore crucial in improving milk production of dairy herds.

S. uberis is generally identified on the basis of colony morphology on blood agar and esculin hydrolysis. However, an additional subsequent technique is usually required to confirm its identification. We used a species-

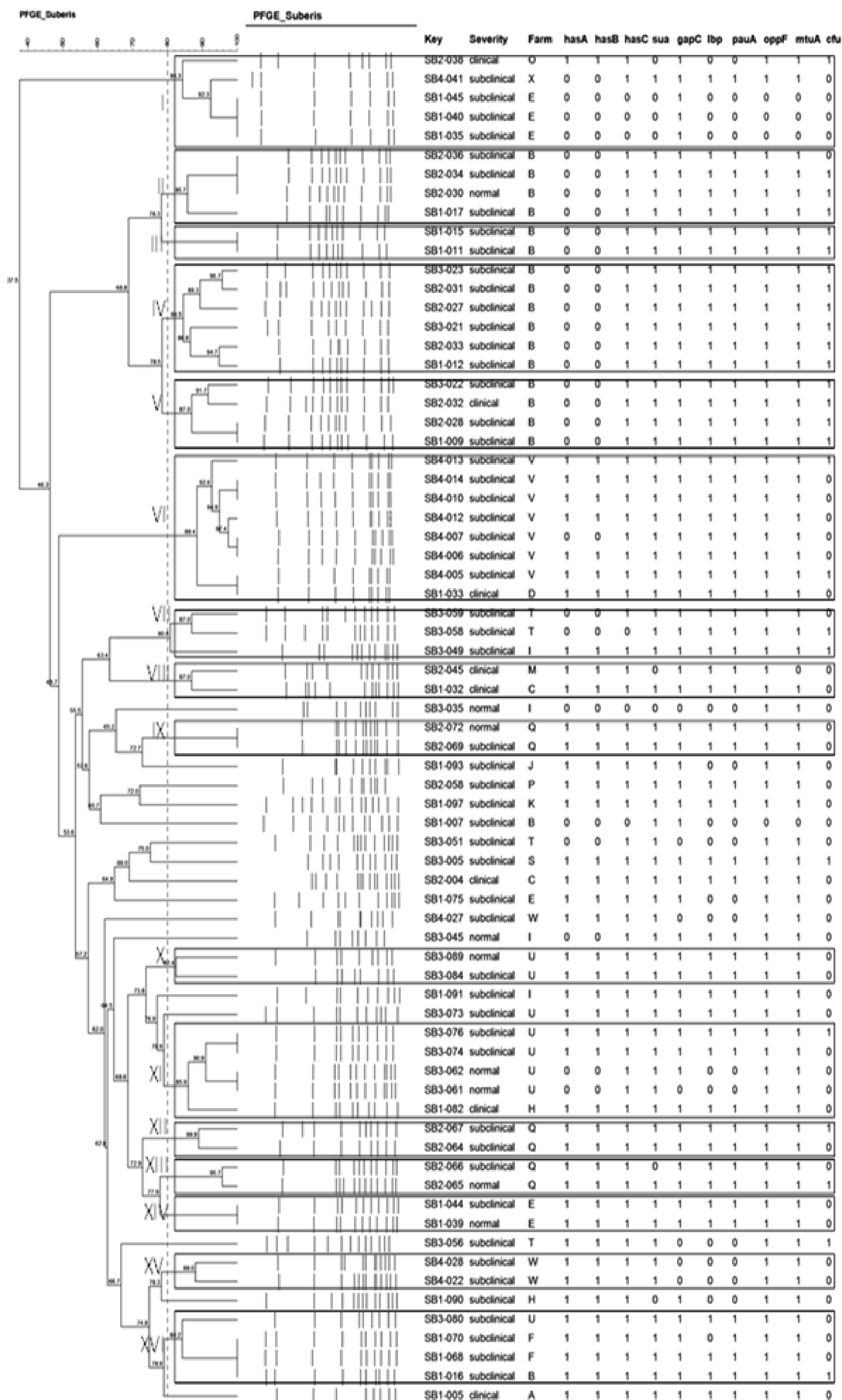

Figure 3. Dendrogram showing genetic relatedness of the 71 *S. uberis* isolates determined by the unweighted pair group of arithmetic mean (UPGMA) method for PFGE patterns. The dashed line represents the threshold of 80% Dice similarity index. Roman numerals and boxes denote clusters of isolates sharing band similarities above the threshold.

specific PCR technique targeting the 16S rRNA gene developed by Hassan et al. (20) for *S. uberis* identification. This technique can rapidly provide unambiguous results to detect *S. uberis* and can distinguish *S. uberis* from another phenotypically identical species, *S. parauberis* (20). This PCR protocol has been widely used to identify *S. uberis* isolated from mastitis cases prior to further genotyping methods (25,26).

We found that the virulence gene pattern of *hasA+has B+hasC+sua+gapC+lbp+pauA+oppF+mtuA* was the most prevalent pattern among our *S. uberis* collection. Similarly, in a previous study by Reinoso et al., the virulence gene-pattern of *hasA/B+hasC+sua+gapC+pauA+oppF+cfu* was the most common pattern among *S. uberis* in Argentina (27). However, Reinoso et al. reported a high frequency of the *cfu* gene and a low frequency of the *lbp* gene (27), while we reported a low and high prevalence of the *cfu* and *lbp* genes, respectively. In the present study, we could not find any significant association of each virulence-associated gene and disease status. A number of studies successfully reported a significant association of the presence of the *hasA* gene with particular strains of *S. uberis* that were identified to be related to subclinical and clinical bovine mastitis in the United Kingdom (28), New Zealand (29), and Australia (26). Even though we could not find any statistically significant association, the rate of *hasA/B* carriage among *S. uberis* isolated from clinical mastitis cases was higher than that among isolates from cases of IMI with low SCC (78.57% vs. 40%; P = 0.051). The limitation of our findings could be the limited number of isolates from cases of clinical mastitis and IMI with low SCC. Moreover, it should be considered that detection of virulence genes performed in the current study was based on the successful amplification of PCR reaction. Confirmation of the presence or absence of virulence genes in the bacterial genome was not determined. Therefore, results from this study only provide a screening survey of the distribution of virulence-associated genes among *S. uberis* isolates. A further investigation with a larger sample size and a definitive confirmation of gene carriage should be performed in order to confirm the relationships among virulence genes among *S. uberis* isolates in Thailand.

Virulence genes most commonly detected among our *S. uberis* collection included *oppF*, *hasC*, and *mtuA*. Oligopeptide permease, encoded by the *oppF* gene, is essential for the utilization of amino acids for bacterial growth in milk (17). Frequencies of the *oppF* gene detected from *S. uberis* have been reported differently in many regions, which could range from 0% in the United States and the Netherlands (30) to 64.1% in Argentina (27). The *hasC* gene was the second most common virulence gene detected in this study. Ward et al. (31) suggested that the *hasC* gene is a common virulence gene of *S. uberis*, and it is not associated with capsule production of *S. uberis*. In addition, they discussed that the hyaluronic acid capsule of *S. uberis* plays only a minor role in the early stages of IMI. Field et al. (32) also stated that noncapsulated strains of *S. uberis* are still able to resist phagocytosis by neutrophils and cause mastitis in dairy cows. However, its role in the pathogenesis of IMI and the reason for its highly frequent detection has not clearly been described. In addition, metal transporter uberis A (MtuA), a lipoprotein receptor antigen (LraI), has been identified to be necessary for the growth of *S. uberis* in milk and for IMI in dairy cows (18). To our knowledge, we first describe the distribution of the *mtuA* gene from field isolates of *S. uberis*.

The spreading of *S. uberis* from cow to cow or from a common environmental source within the farm might have been the mode of transmission, which can be confirmed by the presence of identical genotypes of *S. uberis* originating from different cows (8,25). We demonstrated 12 identical PFGE patterns among the examined isolates in this study (Figure 3). Most identical PFGE patterns were shared between isolates from the same farms. Moreover, some common PFGE patterns were observed among isolates from cows in different farms. The explanation for this finding is unclear, but it is probably due to the importation of mastitic cows from one farm to another farm. Infected cows might carry and introduce some different clones of *S. uberis* into the new herd. However, information on the cows' origins in the studied farms was not available in this study and has to be further investigated.

Even though our findings revealed numerous genotypes based on PFGE profiles, *S. uberis* specimens included in the study tended to be clustered and genetically related within farms. Evidence supporting this finding can be clearly observed from PFGE profiles among *S. uberis* isolates of farms V, U, and B, sharing 88.4%, 73.6%, and 68.8% similarity, respectively (Figure 3). In addition to the presence of identical genotypes within a farm, the presence of a number of genetically related strains is evidence supporting the contagious nature of *S. uberis*, as has been described in several studies in other counties (7,8). Our finding suggests that there are predominant strains of *S. uberis* that can mutate and spread within the farms of northern Thailand. Therefore, strategies for controlling contagious mastitis in dairy farms may be useful for controlling outbreaks of IMI caused by *S. uberis* in this region.

Our dataset demonstrated that isolates within the same PFGE cluster usually present identical profiles of detected virulence genes, as shown in clusters III, IV, V, IX, X, XIV, and XV (Figure 3). On the other hand, some isolates that were apparently clustered by PFGE may have different patterns of virulence genes, as illustrated in clusters VI, VII, VIII, XI, XII, XIII, and XVI (Figure 3). Similar to

this finding, Reinoso et al. previously demonstrated a variation of virulence gene profiles among *S. uberis* isolates presenting identical PFGE patterns (30). This finding suggests that interpretation of genetic relatedness using only DNA banding pattern-based typing methods, such as PFGE, should be cautious, and genetically related isolates may not necessarily have identical functional genotypes or phenotypes.

In conclusion, our findings reveal that several virulence-associated genes, particularly *hasA+hasB+has C+sua+gapC+lbp+pauA+oppF+mtuA*, were commonly present among *S. uberis* associated with bovine mastitis in northern Thailand. Contagious mastitis caused by *S. uberis* was observed in dairy farms in this region. Therefore, molecular typing can be useful in developing an effective control strategy for bovine mastitis caused by *S. uberis* in dairy farms.

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