CHAPTER 5

RESULTS

5.1 Identification of *Streptococcus suis*

A total of 304 streptococcal-positive hemocultures were collected from hospitalized patients. One-hundred and thirty-five samples were from Maharaj Nakorn Chiang Mai Hospital. One-hundred and sixty-seven samples were from Lamphun Hospital and two samples were from Doi Saket Hospital and Sanpatong Hospital (Table 8). All hemoculture specimens were primarily screened by Gram-staining and identified as suspected streptococci infection as shown by Gram-positive cocci in chain. Hemocultures that were negative for coccal or oval shaped bacteria arranged in pair or chain were excluded from this study.

All streptococcal-positive hemocultures were observed as small colonies with alpha, beta or gamma-hemolysis. For Gram's stain, all specimens selected were shown as gram-positive coccoid and ovoid arranged in pairs and chains. Biochemical reactions were different for each species of Streptococci-isolated. The prevalence of *S. suis* detected by culture and biochemical method was 11.5% (35 of 304) (Table 9). Fourteen isolates identified from Maharaj Nakorn Chiang Mai Hospital, H15/52 MNCH, H23/52 MNCH, H85/53 MNCH, H131/53 MNCH, H132/53 MNCH, H153/53 MNCH, H156/53 MNCH, H219/53 MNCH, H290/54 MNCH, H301/54 MNCH, H302/54 MNCH, H303/54 MNCH, H304/54 MNCH and H307/54 MNCH were detected as *S. suis*. From Lamphun Hospital, 20 isolates of *S. suis* were H26/52 LPH, H29/52 LPH, H110/53 LPH, H148/53 LPH, H150/53 LPH, H155/53 LPH,

H163/53 LPH, H179/53 LPH, H186/53 LPH, H187/53 LPH, H193/53 LPH, H194/53 LPH, H203/53 LPH, H210/53 LPH, H218/53 LPH, H240/53 LPH, H244/54 LPH, H252/54 LPH, H272/54 LPH, H286/54 LPH. Another *S. suis* isolate was H291/54 SPT from Sanpatong Hospital and none of *S. suis* isolate was found from Doi Saket Hospital (Table 8). *S. suis* colonies were small grayish colonies with alpha-hemolytic zone on blood agar (Figure 4 (A)). By Gram's stain finding, *S. suis*-suspected isolates were appeared as gram-positive cocci and oval in pairs and short chains (Figure 4 (B)). The biochemical reactions of 35 *S. suis* isolates were shown in Appendix A. They were negative for bile esculin hydrolysed and acid production from mannitol and sorbitol and positive for esculin hydrolysed and acid production from trehalose, raffinose and lactose (Figure 4 (C)). Moreover, these isolates were observed optochin resistant (Figure 4 (D)).

Table 8 Specimen collection and *S. suis* positive samples

Source	**Number of streptococcal positive hemocultures**	**Number of *S. suis* positive samples**
MNCH	135	14
LPH	167	20
DSK	1	0
SPT	1	1
Total	304	35

MNCH: Maharaj Nakorn Chiang Mai Hospital

LPH: Lamphun Hospital

DSK: Doi Saket Hospital

SPT: Sanpatong Hospital

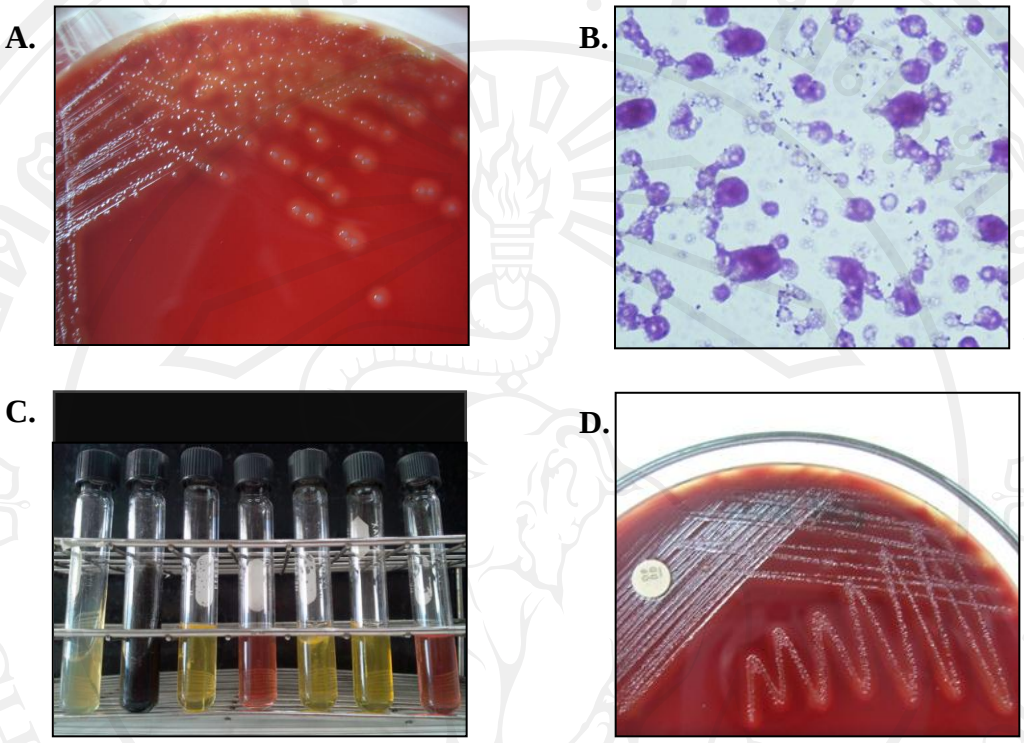


Figure 4 Identification of *S. suis* by culture and biochemical methods. Colony morphology: small grayish colony with alpha (α)-hemolytic zone (A); Gram's stain of patient hemoculture: gram positive coccoid and ovoid occurred in pairs or short chains (B); Biochemical reactions: positive for esculin, trehalose and raffinose and negative for bile esculin, mannitol, and sorbitol (C); Optochin disc diffusion test: optochin resistant (D)

5.2 Detection of *S. suis* by polymerase chain reaction

Conditions for PCR targeted to 16S rRNA gene and *gdh* gene were optimized for investigating optimal temperature of *S. suis* detection. Maximun temperature of PCR targeted to 16S rRNA gene was 63.5 degree Celsius and 56.0 degree Celsius for PCR targeted to *gdh* gene (Data not shown). Thus, these optimal temperatures were selected to detect *S. suis* throughout the study.

DNA of 304 streptococcal-positive hemocultures were extracted and diluted with sterile distilled water as 1:10, 1:100 and 1:1,000. Detection of *S. suis* by using PCR targeted to 16S rRNA gene showed that 15.1% (46 of 304) of samples were positive, which appeared as 294 bp of PCR product (Table 9 and Figure 5 (A)) whereas, PCR targeted to *gdh* gene was positive with 11.5% (35 of 304) samples which appeared as 566 bp of PCR product (Table 9 and Figure 5 (B)). Of these, 35 samples that could be detected as *S. suis* by both PCR at DNA dilution of 1:10, 1:100 and 1:1,000 were 5, 18 and 12 samples, respectively. None of positive band to 16S rRNA gene and *gdh* gene appeared in 269 hemoculture specimens (Table 10). Interestingly, different results were found by PCR detection based on 16S rRNA gene and *gdh* gene. However, the results of *S. suis* detection using PCR targeted to *gdh* gene was corresponding to detection by culture and biochemical test. Eleven hemoculture specimens, H24/52 MNCH, H32/52 LPH, H37/52 LPH, H66/53 MNCH, H84/53 MNCH, H103/53 MNCH, H106/53 MNCH, H137/53 MNCH, H151/53 DSK, H197/53 LPH and H205/53 LPH were false positive that cause by PCR targeted to 16S rRNA gene (Table 10). As in Figure 7, 294 bp PCR products of 16S rRNA gene were detected from H24/52 MNCH, H103/53 MNCH, H106/53 MNCH and H137/53

MNCH. All these 11 isolates were further confirmed by sequencing of 16S rRNA gene to be *S. bovis* (8 isolates) and *E. faecalis* (3 isolates).

Additionally, DNA-extracted from colonies was adjusted to range of 100 to 200 ng for *S. suis* detection using PCR. The similar results of PCR amplification using DNA-extracted from colonies were in comparison to DNA-extracted from hemoculture specimens. *S. suis* detected by PCR targeted to 16S rRNA gene and *gdh* gene were 15.1% (46 of 304) and 11.5% (35 of 304), respectively. Specific band of PCR products were shown in Figure 6.

Athough, 11 non-*S. suis* isolates were identified by *gdh* gene and culture method, they were detected as *S. suis* by PCR targeted to 16S rRNA gene. Their biochemical reactions were all similar to *S. suis* except bile esculin. They showed optochin resistant, positive reaction for esculin, trehalose and raffinose and negative reaction for mannitol and sorbitol. All 11 isolates showed positive reaction for bile esculin test (Table 11). Moreover, biochemical reactions including 6.5% NaCl, Urea, Motility, Inulin, Arginine, VP, PYR and L-Arabinose were used to identify these strain. Of these, 8 isolates, H24/52 MNCH, H66/53 MNCH, H84/53 MNCH, H103/53 MNCH, H106/53 MNCH, H137/53 MNCH, H197/53 LPH and H205/53 LPH were identified as *S. bovis* biotype II/2 (*S. gallolyticus* subsp. pasteurianus), which were positive for bile esculin, esculin, trehalose, raffinose, VP and negative for 6.5%NaCl, mannitol, sorbitol, urea, motility, inulin, arginine, PYR and L-Arabinose. Three isolates; H32/52 LPH, H37/52 LPH and H151/53 DSK, were identified as *E. faecalis*, which were positive for bile esculin, esculin, 6.5% NaCl, trehalose, sorbitol, VP, arginine, PYR, and negative for raffinose, urea, motility, inulin and L-Arabinose.

Additionally, PCR targeted to *sod*A gene that encoded Manganese-dependent superoxide dismutase was used to further identified non-*S. suis* isolates. This primer set provides useful information for differentiation of species of the so-called *S. bovis*-*S. equinus* group. Thus, 8 isolates of *S. bovis* biotype II/2 were confirmed by using PCR targeted to *sod*A gene. The 408 bp PCR products of *sod*A gene were detected in these isolates as shown in Figure 8.

Table 9 Detection of *S. suis* from 304 streptococcal positive hemocultures by PCR targeted to 16S rRNA gene, PCR targeted to *gdh* gene and culture

Method	**No. positive**	**% positive**	**Sensitivity Value**	**Specificity Value**
PCR targeted to 16S rRNA	46	15.1	100%	95.9%
PCR targeted to *gdh*	35	11.5	100%	100%
Culture	35	11.5	100%	100%

Table 10 Comparison of *S. suis* detection by PCR targeted to 16S rRNA gene, PCR targeted to *gdh* gene and culture

Method		**Culture**		**Total**
		+	-	
PCR targeted to 16S rRNA	+	35	11	46
	-	0	258	258
Total		35	269	304
PCR targeted to *gdh*	+	35	0	35
	-	0	269	269
Total		35	269	304

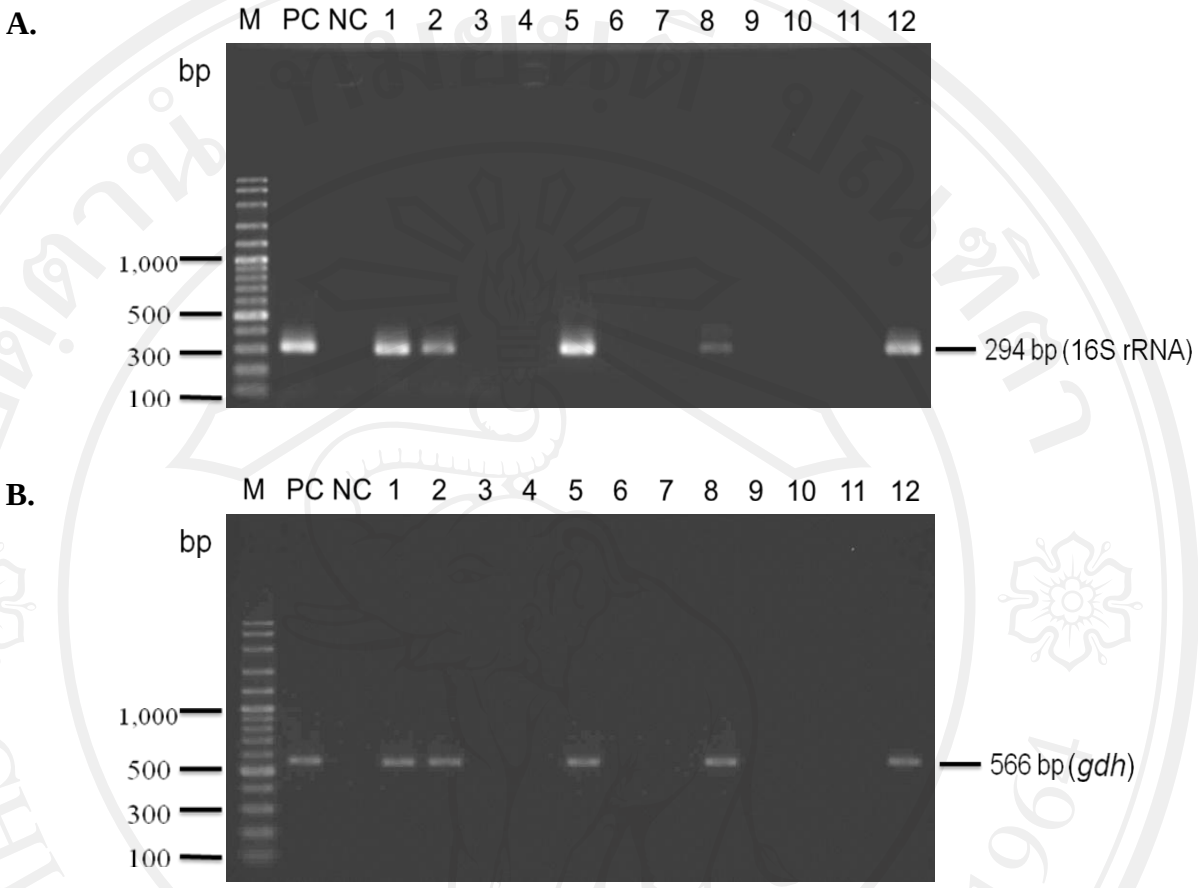


Figure 5 Detection of *S. suis* in hemoculture specimens using PCR targeted to 16S rRNA gene (A) and PCR targeted to *gdh* gene (B) (lane 1 to 12). Lanes: M, 100 bp molecular weight marker; PC, positive control (*S. suis* P1/7); NC, negative control; 1, H291/54 SPT; 2, H290/54 LPH; 3, H288/54 LPH; 4, H287/54 LPH; 5, H286/54 LPH; 6, H274/54 LPH; 7, H273/54 LPH; 8, H272/54 LPH; 9, H247/54 LPH; 10, H246/54 LPH; 11, H245/54 LPH; 12, H244/54 LPH.

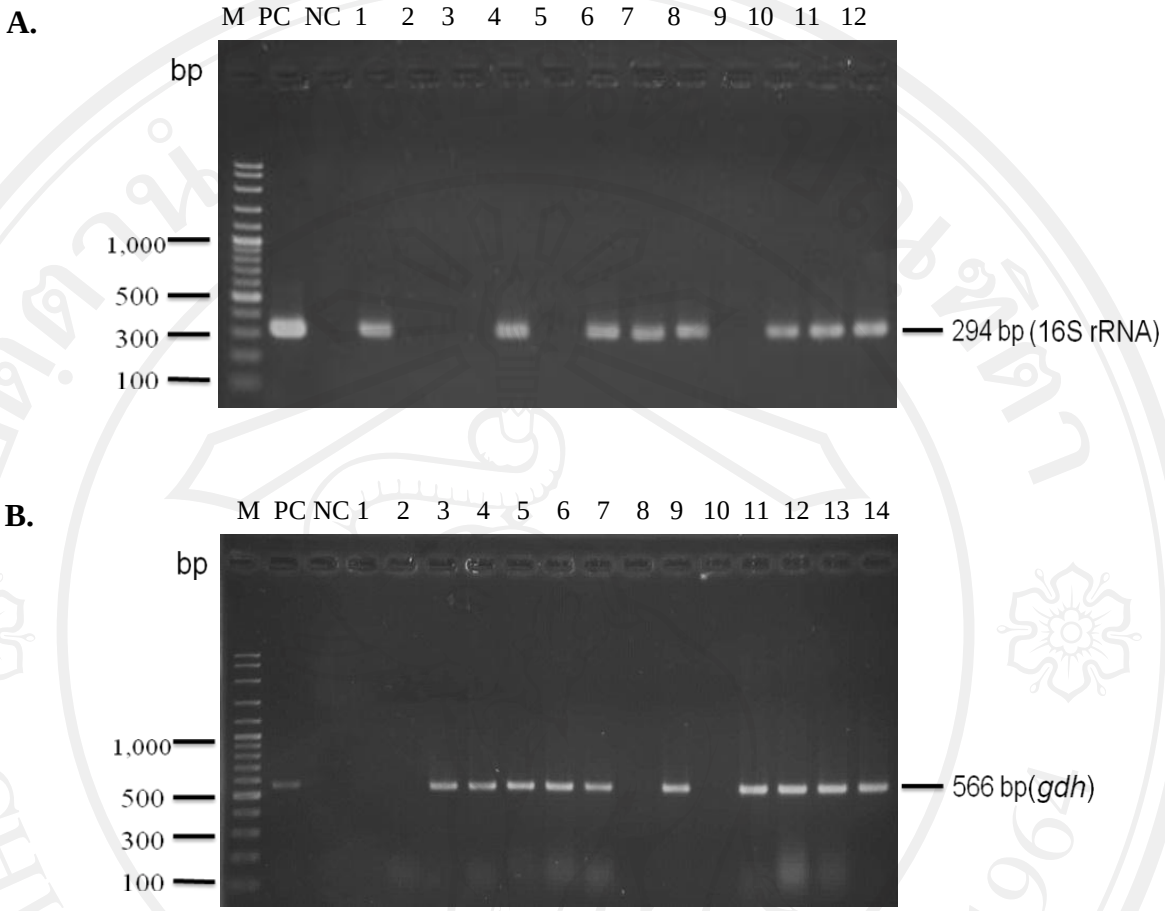


Figure 6 Detection of *S. suis* in clinical isolates using PCR targeted to 16S rRNA gene (A) and PCR targeted to *gdh* gene (B) (lane 1 to 14). Lanes: M, 100 bp molecular weight marker; PC, positive control (*S. suis* P1/7); NC, negative control. (A) Lanes: 1, H163/53 LPH; 2, H164/53 LPH; 3, H165/53 MNCH; 4, H179/53 LPH; 5, H180/53 LPH; 6, H186/53 LPH; 7, H187/53 LPH; 8, H188/53 LPH; 9, H189/53 LPH; 10, H203/53 LPH; 11, H210/53 LPH; 12, H218/53 LPH. (B) Lanes: 1, H17/52 LPH; 2, H22/52 LPH; 3, H163/53 LPH; 4, H179/53 LPH; 5, H186/53 LPH; 6, H187/53 LPH; 7, H188/53 LPH; 8, H193/53 LPH; 9, H194/53 LPH; 10, H197/53 LPH; 11, H203/53 LPH; 12, H210/53 LPH; 13, H218/53 LPH; 14, H219/53 MNCH.

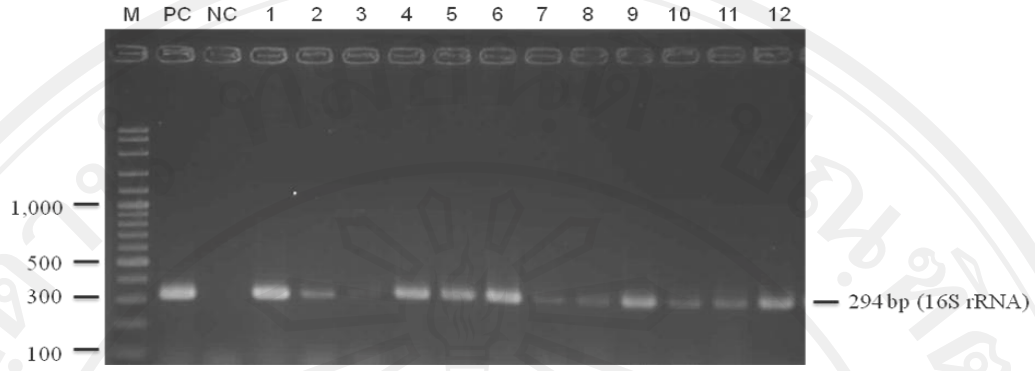


Figure 7 Detection of non-*S. suis* in hemoculture specimens using PCR targeted to 16S rRNA gene (lane 1 to 12). Lanes: M, 100 bp molecular weight marker; PC, positive control (*S. suis* P1/7); NC, negative control; 1, H15/52 MNCH; 2, H23/52 MNCH; 4, H26/52 LPH; 5, H29/52 LPH; 6, H85/53 MNCH; 9, H131/53 MNCH; 10, H132/53 MNCH; 12, H148/53 LPH were *S. suis*. Lanes: 3, H24/52 MNCH; 7, H103/53 MNCH; 8, H106/53 MNCH; 11, H137/53 MNCH were *S. bovis.*

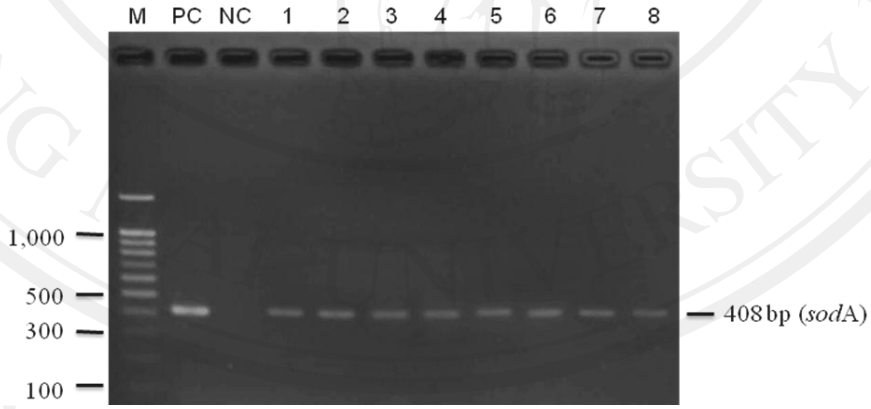


Figure 8 Identification of *S. bovis* in clinical isolates using PCR targeted to *sod*A gene (lane 1 to 8). Lanes: M, 100 bp molecular weight marker; PC, positive control (*S. bovis* clinical isolate); NC, negative control; 1, H24/52 MNCH; 2, H66/53 MNCH; 3, H84/53 MNCH; 4, H103/53 MNCH; 5, H106/53 MNCH; 6, H137/53 MNCH; 7, H197/53 LPH; 8, H205/53 LPH.

Table 11 Biochemical reactions of 11 non-*S. suis* isolates

Clinical isolates (n=11)	Biochemical reactions*								Identification**
	BE	ESC	TRE	MAN	RAF	LAC	SOR	OP	
H24/52 MNCH	+	+	+	-	+	+	-	R	*S. bovis* biotype II/2
H32/52 LPH	+	+	+	-	+	+	-	R	*E. faecalis*
H37/52 LPH	+	+	+	-	+	+	-	R	*E. faecalis*
H66/53 MNCH	+	+	+	-	+	+	-	R	*S. bovis* biotype II/2
H84/53 MNCH	+	+	+	-	+	+	-	R	*S. bovis* biotype II/2
H103/53 MNCH	+	+	+	-	+	+	-	R	*S. bovis* biotype II/2
H106/53 MNCH	+	+	+	-	+	+	-	R	*S. bovis* biotype II/2
H137/53 MNCH	+	+	+	-	+	+	-	R	*S. bovis* biotype II/2
H151/53 DSK	+	+	+	-	+	+	-	R	*E. faecalis*
H197/53 LPH	+	+	+	-	+	+	-	R	*S. bovis* biotype II/2
H205/53 LPH	+	+	+	-	+	+	-	R	*S. bovis* biotype II/2

*BE, Bile Esculin; ESC; Esculin; TRE, Trehalose; MAN, Mannitol; RAF, Raffinose; LAC, Lactose; SOR, Sorbitol; OP, Optochin; R,Resistance

**Identify by Department of Medical Sciences, Ministry of Public Health. *S. bovis* biotype II/2: *S. gallolyticus* subsp. pasteurianus or *S.* pasteurianus. Biochemical reactions: Bile esculin, Esculin, 6.5%NaCl, Trehalose, Mannitol, Raffinose, Sorbitol, Urea, Motility, Inulin, Arginine, VP, PYR and L-Arabinose

5.3 Serotyping of *S. suis* by polymerase chain reaction

Thirty-five *S. suis* isolates were detected by using PCR based on 16S rRNA gene and *gdh* gene. Serotyping of these *S. suis* isolates was done by using PCR based on *cps* gene. Duplex PCR was used to classify *S. suis* serotype 1 or 14 and serotype 2 or 1/2 with *cps*1 and *cps*2 specific primers, respectively. As shown in Figure 9, based on the sizes of PCR products by comparing with the *S. suis* P1/7 (PC1) and the *S. suis* MNCM07 (PC2), 515 bp belonged to *S. suis* serotype 2 or 1/2 and 217 bp belonged to *S. suis* serotype 1 or 14. All these 35 *S. suis* isolates, 34 (97.1%) isolates were identified as serotype 2 or 1/2 and the remaining 1 isolate (2.9%), H29/52 LPH was identified as serotype 1 or 14 (Table 12).

To compare with serotyping by duplex PCR, PCR targeted to *cps*1 gene was used to detect *S. suis* serotype 1 or 14. The expected PCR product size of *S. suis* serotype 1 or 14 was 637 bp. As shown in Figure 10 (A), 1 isolate of *S. suis* serotype 1 or 14 (H29/52 LPH) was detected. The prevalence of *S. suis* serotype 1 or 14 was 2.9% (1 of 35) (Table 12). Additionally, PCR targeted to *cps*2 gene was used to compare with serotyping by duplex PCR. The expected PCR product size of *S. suis* serotype 2 or 1/2 was 459 bp. As shown in Figure 10 (B), 34 isolates of *S. suis* serotype 2 or 1/2 were detected. The prevalence of *S. suis* serotype 2 or 1/2 was 97.1% (34 of 35) (Table 12). The result from PCR revealed that the common serotype of *S. suis* (34 of 35; 97.1%) in the present study belonged to serotype 2 or 1/2.

An example of sample identified as serotype 2 was H26/52 LPH, which showed positive band for serotype 2 or 1/2 by duplex PCR (Figure 9: lane 2) and monoplex PCR targeted to *cps*2 (Figure 10 (B): lane 2), but none of band detected for serotype 1 or 14 by monoplex PCR targeted to *cps*1 (Figure 10 (A): lane 2). The only sample identified as serotype 14 was H29/52 LPH, which showed positive band for serotype 1 or 14 by duplex PCR (Figure 9: lane 3) and monoplex PCR targeted to *cps*1 (Figure 10 (A): lane 1), but none of band detected for serotype 2 or 1/2 by monoplex PCR targeted to *cps*2 (Figure 10 (B): lane 3).

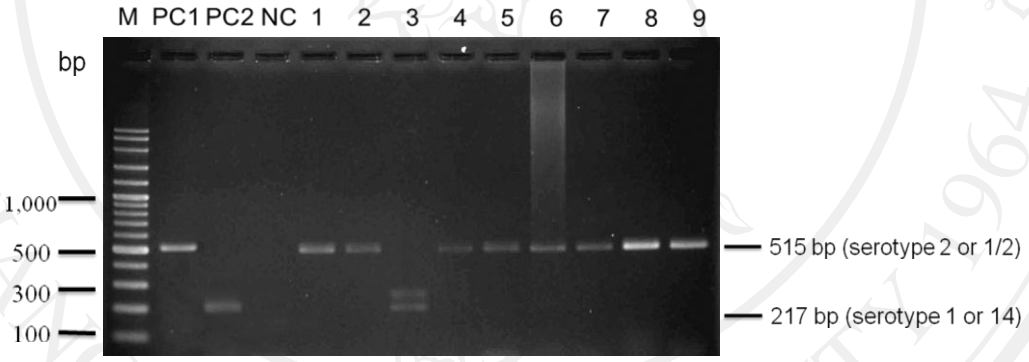
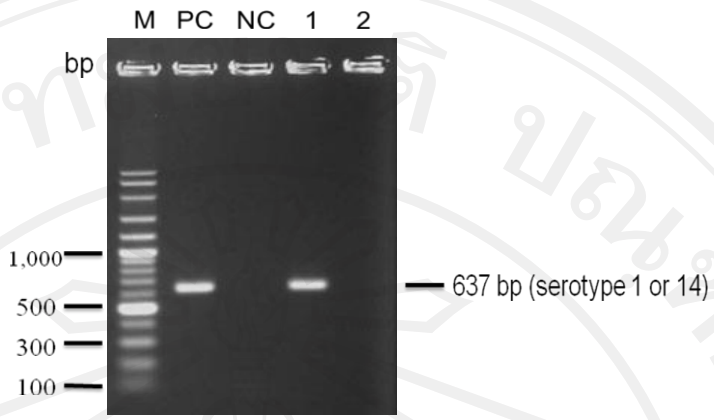


Figure 9 Serotyping by duplex PCR of *cps*1 and *cps*2 genes. Lanes: M, 100 bp molecular weight marker; PC1, positive control for serotype 2 or 1/2 (*S. suis* P1/7); PC2, positive control for serotype 1 or 14 (*S. suis* MNCM 07); NC, negative control. Lanes: 1, H23/52 MNCH; 2, H26/52 LPH; 3, H29/52 LPH; 4, H153/53 MNCH; 5, H155/53 LPH; 6, H163/53 LPH; 7, H193/53 LPH; 8, H210/53 LPH; 9, H218/53 LPH.

A.



B.

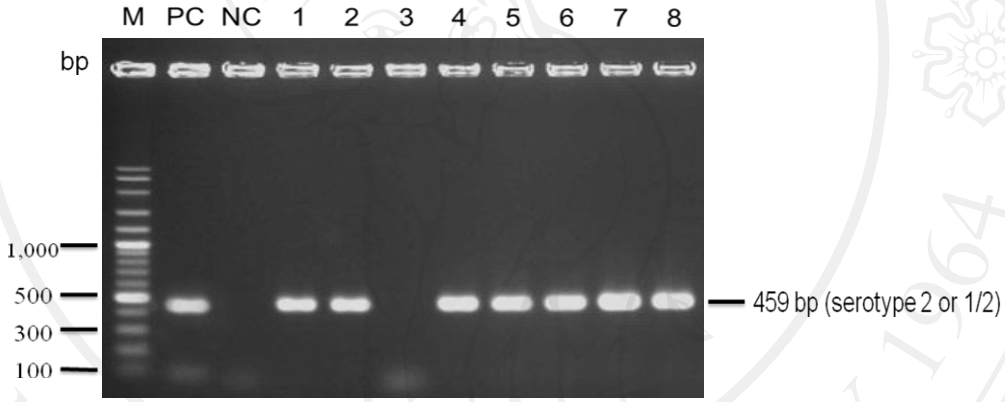


Figure 10 Serotyping by monoplex PCR targeted to *cps*1 (A) and *cps*2 (B). (A) Lanes: M, 100 bp molecular weight marker; PC, positive control for serotype 1 or 14 (*S. suis* MNCM 07); NC, negative control; 1, H29/52 LPH; 2, H26/52 LPH (*S. suis* serotype 2 or 1/2). (B) Lanes: M, 100 bp molecular weight marker; PC, positive control for serotype 2 or 1/2 (*S. suis* P1/7); NC, negative control; 1, H23/52 MNCH; 2, H26/52 LPH; 4, H153/53 MNCH; 5, H155/53 LPH; 6, H163/53 LPH; 7, H193/53 LPH; 8, H210/53 LPH.

5.4 Serotyping of *S. suis* by coagglutination test

All 35 *S. suis* isolates were further characterized for serotype 2 or 1/2 and serotype 14 by coagglutination test. All of these isolates were agglutinated with anti-serotype 2 antiserum, except 3 isolates of H29/52 LPH, H110/53 LPH and H153/53 MNCH. However, H110/53 LPH and H153/53 MNCH were positive to *S. suis* serotype 2 or 1/2 using both duplex PCR and monoplex PCR. Thus, all *S. suis* isolates were repeated following by culture in THB. After overnight incubation, 1 drop of culture supernatant was tested with anti-serotype 2 antiserum. Thirty-four isolates were agglutinated with this test, but none of reaction to sample number H29/52 LPH. Later, the isolate of H29/52 LPH was typed using coagglutination test and was agglutinated with anti-serotype 14 antiserum.

The data of this present study indicated that 34 of 35 (97.1%) *S. suis* isolates were serotype 2 or 1/2 and 1 of 35 (2.9%) isolate was serotype 14. This result was corresponded to serotyping using duplex PCR and monoplex PCR as shown in Table 12.

Table 12 Serotyping of *S. suis* using duplex PCR, monoplex PCR and coagglutination test

Method	Positive isolates (%)
Duplex PCR	
- detect serotype 1 or 14	1 (2.9)*
- detect serotype 2 or 1/2	34 (97.1)
Monoplex PCR	
- detect serotype 1 or 14	1 (2.9)*
- detect serotype 2 or 1/2	34 (97.1)
Coagglutination	
- detect serotype 14	1 (2.9)
- detect serotype 2 or ½	34 (97.1)

* One isolate was identified as serotype 1 or 14 by duplex PCR and monoplex PCR and was finally confirmed as serotype 14 by coagglutination test.

5.5 Sensitivity of PCR condition

For *S. suis* detection in hemoculture, sensitivity of PCR condition to detect *S. suis* was evaluated. Bacterial strains used were *S. suis* P1/7 and *S. suis* MNCM07. Viable count in a range of dilution 10^{-6} to dilution 10^{-10} of *S. suis* P1/7 were >300, 288, 23, 1 and 0 colonies, respectively, while viable count in a range of dilution 10^{-6} to dilution 10^{-10} of *S. suis* MNCM07 were >300, 102, 11, 0 and 0 colonies, respectively (Appendix B). Of these, hemoculture of each dilutions were DNA-extracted and PCR was performed. By using PCR targeted to 16S rRNA, PCR product of *S. suis* P1/7 and *S. suis* MNCM07 were appeared at the minimum concentration of dilution 10^{-8}, which comparable to PCR targeted to *gdh*. DNA extracted from hemoculture dilution 10^{-9} and 10^{-10} could not be amplified by both

PCR as well as negative control as shown in Figure 11. Therefore, the cells limitation of *S. suis* P1/7 and *S. suis* MNCM07 were 4.6×10^{2} cells/ml (at dilution 10^{-8}) and 2.2×10^{2} cells/ml (at dilution 10^{-8}) for PCR targeted to 16S rRNA, respectively, as well as for PCR targeted to *gdh*.

Moreover, the minimum DNA detection level of *S. suis* was evaluated. DNA of *S. suis* P1/7 and *S. suis* MNCM07 were 10-fold diluted and the least DNA concentration used in the tests was 0.1 fg. The minimum DNA concentration of *S. suis* P1/7 and *S. suis* MNCM07 detected by PCR targeted to 16S rRNA was 100 fg, whereas PCR targeted to *gdh* was 100 pg as shown in Figure 12. Statistic analysis of sensitivity values of PCR targeted to 16S rRNA gene and *gdh* gene compared with culture method were evaluated. Both PCR had 100% sensitivity to detect *S. suis* as shown in Table 9. This high percentage of sensitivity suggested that PCR targeted to 16S rRNA gene and *gdh* gene were sensitive to identify *S. suis*.

5.6 Specificity of PCR condition

For the specificity test, *S. pneumoniae*, *S. bovis*, Viridans streptococci, *S. pyogenes*, *E. faecalis*, *S. aureus* and *E. coli* were examined for the presence of 294 and 566 bp specific fragments corresponding to 16S rRNA gene and *gdh* gene of *S. suis*, respectively. Universal primers target the conserved region of 16S rRNA gene in bacteria and amplify the target in parts were used as condition control for monitoring false negative. Viable count of these organisms was performed in a range of dilution 10^{-4} to dilution 10^{-9}. Viable count in a range of dilution 10^{-4} to dilution 10^{-8} of *S. pyogenes* and *S. aureus* were >300, 110, 12, 0 and 0 colonies and >300, 44, 0, 0 and 0 colonies, respectively. For *S. pneumoniae*, *S. bovis*, Viridans streptococci, *E. faecalis*, and *E. coli* could count in a range of dilution 10^{-5} to dilution 10^{-9}. Colony

count of *S. pneumoniae* were >300, 64, 6, 0 and 0 colonies, *S. bovis* were >300, 123, 8, 0 and 0 colonies, Viridans streptococci were >300, 92, 9, 0 and 0 colonies, *E. faecalis* were >300, 104, 22, 1 and 0 colonies, and *E. coli* were >300, 108, 12, 1 and 0 colonies (Appendix B).

As shown in Figure 13, the lane of positive control was appeared 3 bands of 996 bp corresponding to bacterial 16S rRNA gene, 294 bp corresponding to 16S rRNA gene of *S. suis* and non specific band that was between 100 and 200 bp. The 996 bp corresponding to bacterial 16S rRNA gene was detected from various bacterial species in a first 2 dilutions and none of them showed the 294 bp PCR products in this study.

Additionally, DNA was extracted from colonies of *S. pneumoniae*, *S. bovis*, Viridans streptococci, *S. pyogenes*, *E. faecalis*, *S. aureus* and *E. coli* and 100 ng of DNA was evaluated by PCR. By using PCR targeted to *S. suis* 16S rRNA gene, PCR products of these bacteria were not detected (Figure 14). However, 996 bp corresponding to bacterial 16S rRNA gene was detected in this experiment. None of PCR product was detected by PCR targeted to *gdh* gene (Figure 14). Thus, the 16S rRNA primers was specific to *S. suis* as well as *gdh* primers. Comparison of specificity value of PCR targeted to 16S rRNA gene and *gdh* gene to the culture method were evaluated. PCR targeted to *gdh* gene was 100% specificity value and was 95.9% specificity value of PCR targeted to 16S rRNA gene as shown in Table 9.

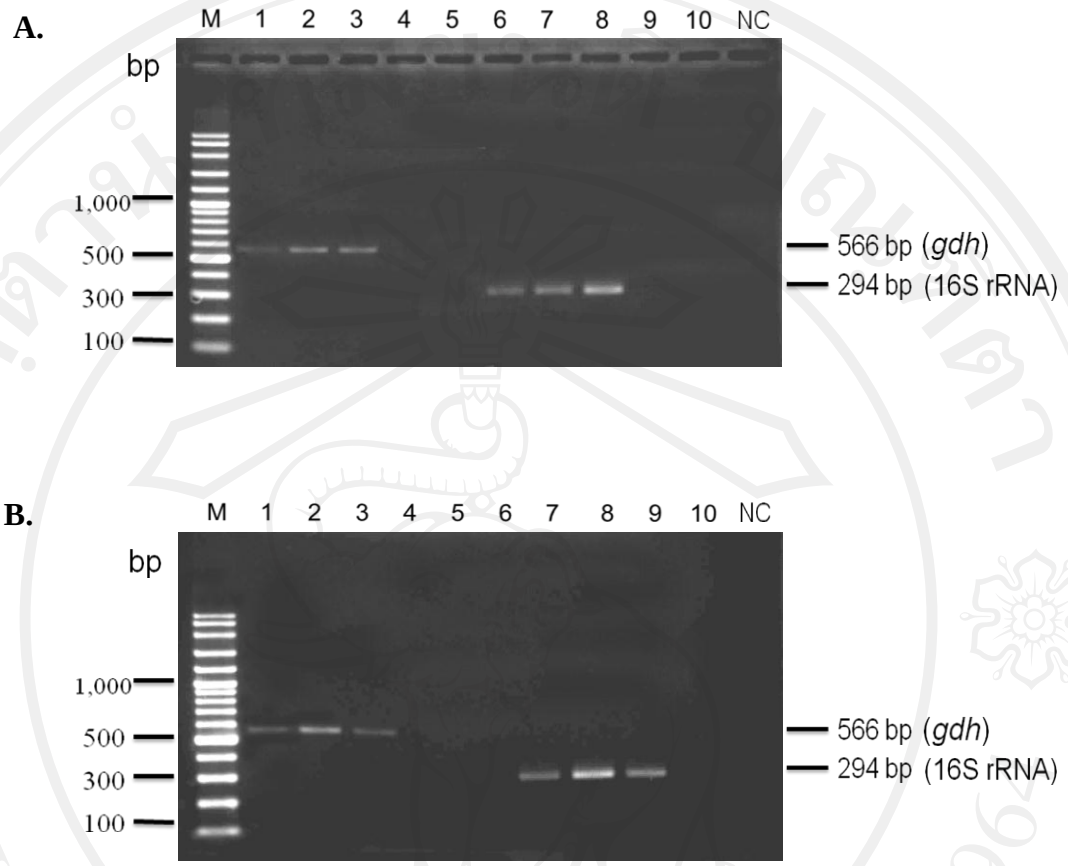


Figure 11 Sensitivity of PCR to detect *S. suis* P1/7 (serotype 2) and *S. suis* MNCM07 (serotype 14) in hemoculture. The upper figure (A) for *S. suis* P1/7 and the lower figure (B) for *S. suis* MNCM07. Lanes: M, 100 bp molecular weight marker; 1-5, DNA dilution products at 10^{-6} to 10^{-10} of *gdh* gene; 6-10, DNA dilution products at 10^{-6} to 10^{-10} of 16S rRNA gene. NC, is a negative control.

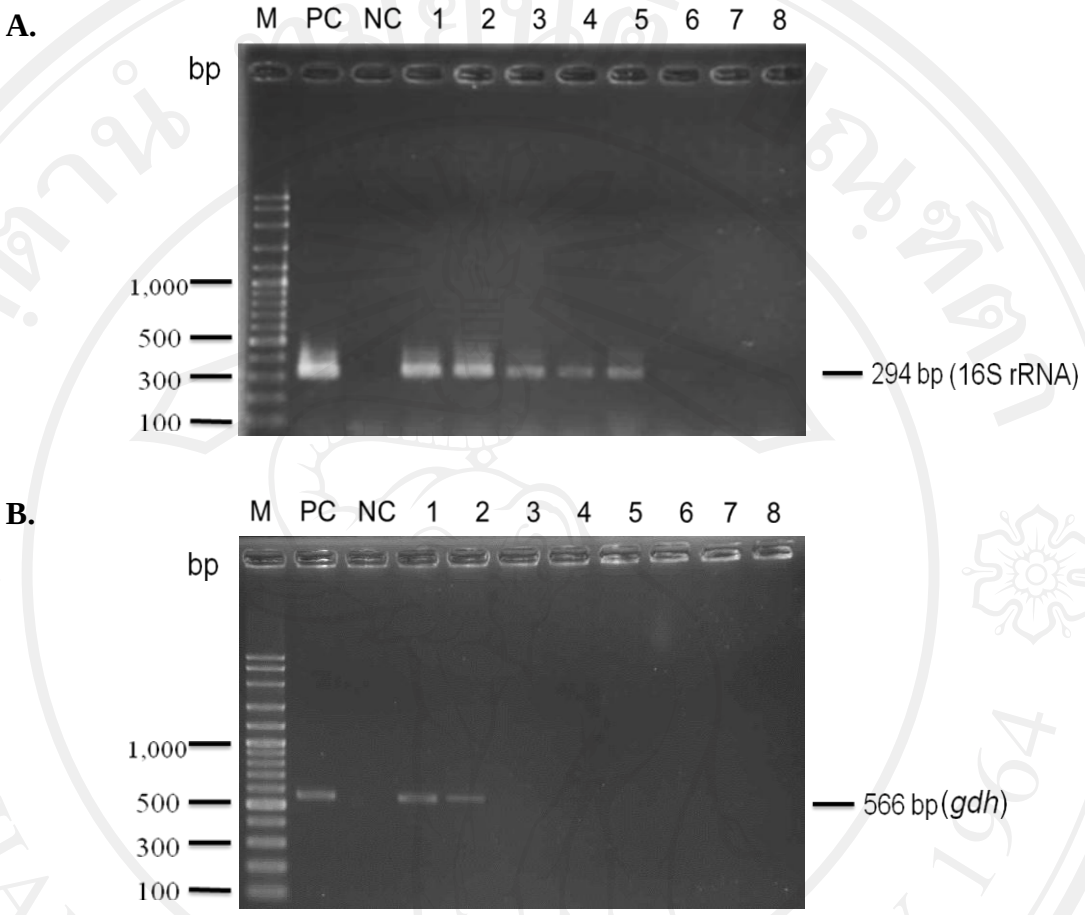


Figure 12 Sensitivity of PCR to detect *S. suis* from DNA-extracted. Agarose gel demonstrates the PCR product sizes of *S. suis* P1/7 by using PCR targeted to 16S rRNA gene (A) and PCR targeted to *gdh* gene (B). Lanes: M, 100 bp molecular weight marker; PC, positive control (100 ng of *S. suis* P1/7 reference strain); NC, negative control; 1 to 8, DNA-diluted products at 1 ng, 100 pg, 10 pg, 1 pg, 100 fg, 10 fg, 1 fg, and 0.1 fg.

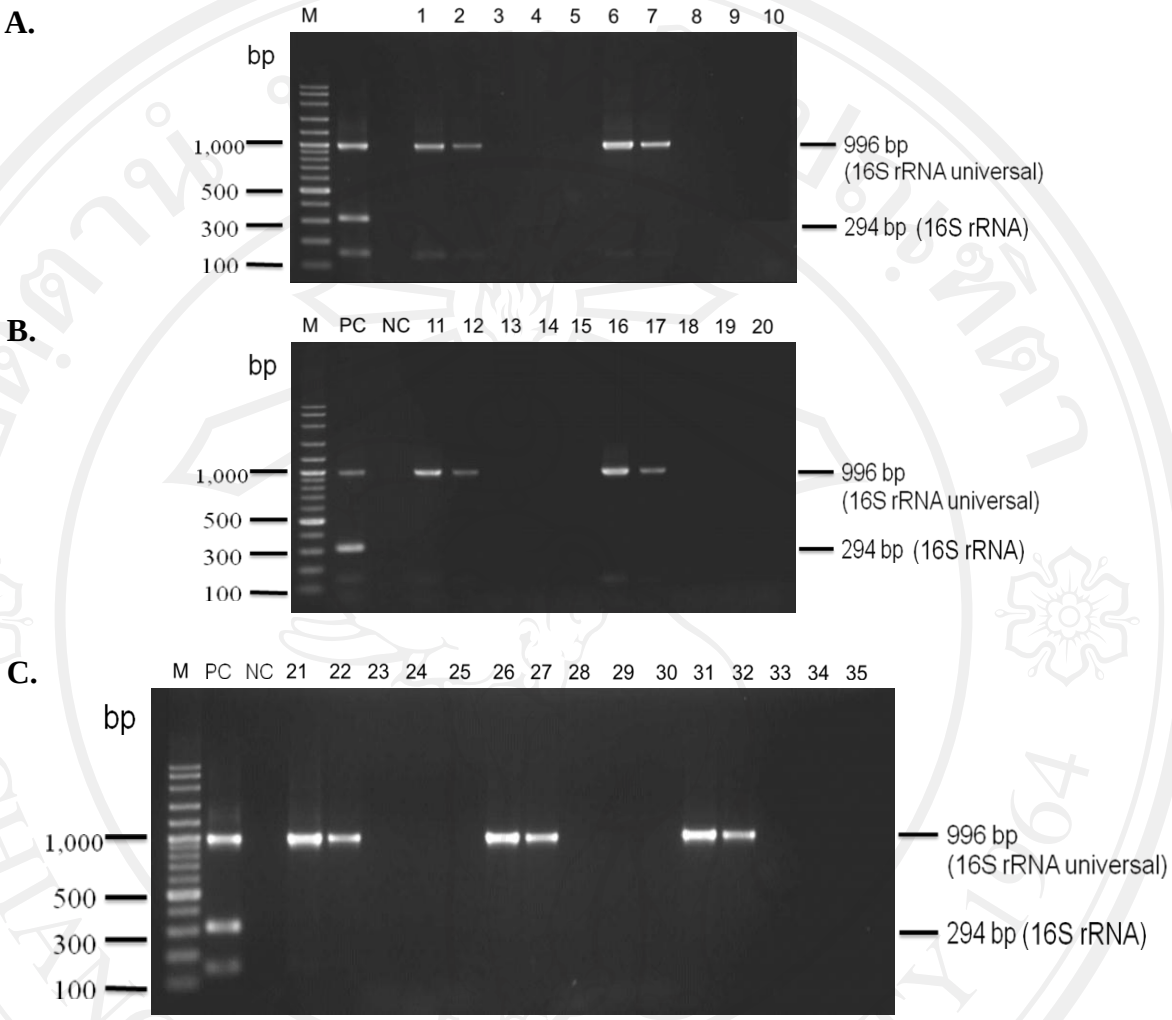


Figure 13 Specificity of PCR condition to detect *S. suis* in hemoculture. Lanes: M, 100 bp molecular weight marker; PC, is a positive control (*S. suis* P1/7); NC, is a negative control. *S. pneumoniae* (A; Lane 1-5, DNA dilution products at 10^{-6} to 10^{-10}), Viridans streptococci (A; Lane 6-10, DNA dilution products at 10^{-5} to 10^{-9}), *S. pyogenes* (B; Lane 11-15, DNA dilution products at 10^{-4} to 10^{-8}), *S. bovis* (B; Lane 16-20, DNA dilution products at 10^{-5} to 10^{-9}), *E. faecalis* (C; Lane 21-25, DNA dilution products at 10^{-5} to 10^{-9}), *S. aureus* (ATCC 25923) (C; Lane 26-30, DNA dilution products at 10^{-4} to 10^{-8}), and *E. coli* (ATCC 25922) (C; Lane 31-35, DNA dilution products at 10^{-5} to 10^{-9}).

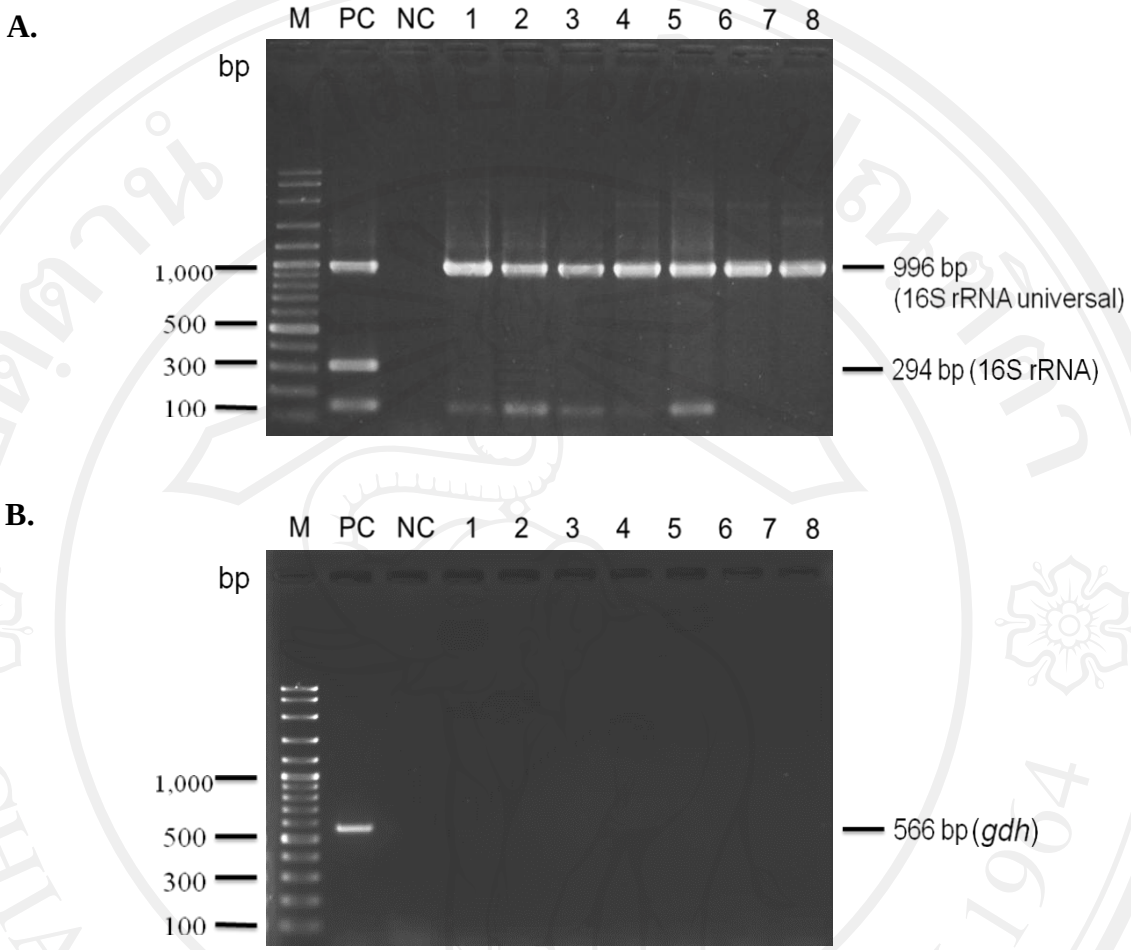


Figure 14 Specificity of PCR condition to detect *S. suis* from DNA-extracted. Agarose gel demonstrates the PCR product sizes of *S. suis* P1/7 by using PCR targeted to 16S rRNA gene (A) and PCR targeted to *gdh* gene (B). Lanes: M, 100 bp molecular weight marker; PC, positive control (100 ng of *S. suis* P1/7); NC, negative control; 1, *S. pneumoniae*; 2, Viridans streptococci; 3, *S. bovis*; 4, *S. pyogenes*; 5, *E. faecalis*; 6, *S. aureus* (ATCC 25923); 7, *E. coli* (ATCC 25922).

5.7 Nucleotide sequence analysis

5.7.1 Nucleotide alignment analysis of *S. suis*

Six isolates of *S. suis*, H23/52 MNCH, H29/52 LPH, H85/53 MNCH, H131/53 MNCH, H148/53 LPH and H156/53 MNCH were randomly selected and found to share great homology of 16S rRNA nucleotide sequence identity (99%) with those of the published *S. suis* reference strains (Appendix C). Moreover, 2 isolates of *S. suis*, H23/52 MNCH and H29/52 LPH were shared 99% and 100% homology of gdh nucleotide sequence identity with those of the published *S. suis* reference strains (Appendix C).

5.7.2 Nucleotide alignment analysis of *S. bovis*

The DNA of 8 *S. bovis* biotype II/2 isolates, H24/52 MNCH, H66/53 MNCH, H84/53 MNCH, H103/53 MNCH, H106/53 MNCH, H137/53 MNCH, H197/53 LPH and H205/53 LPH were amplified using PCR targeted to 16S rRNA gene of *S. suis*. Thus, their PCR products were purified and subjected to nucleotide sequencing and shared 98% to 100% of 16S rRNA nucleotide sequence identity with those of the published *S. suis* reference strains (Table 13 and Appendix C). However, they also shared a homology (range of 97% to 99%) with partial sequence of 16S ribosomal RNA gene of uncultured bacterium clone. In addition, PCR products of sample number H24/52 MNCH, H84/53 MNCH and H103/53 MNCH were selected to amplify with PCR targeted to *sod*A gene and purified, subjected to nucleotide sequencing. Their sequences showed the range of 99% to 100% of *sod*A nucleotide sequence identity with those of the published *S. pasteurianus* (*S. bovis* biotype II/2) isolates reference strains (Appendix C).

Table 13 Nucleotide sequence analysis against a nucleotide database (NCBI) of 16S rRNA gene of 11 non-*S. suis* isolates

Clinical isolates (n=11)	**Identities (%)***
H24/52 MNCH	98
H32/52 LPH	100
H37/52 LPH	99
H66/53 MNCH	99
H84/53 MNCH	98
H103/53 MNCH	99
H106/53 MNCH	99
H137/53 MNCH	99
H151/53 DSK	99
H197/53 LPH	99
H205/53 LPH	99

*Max identity of sequence in database.