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Reclassification of Phenotypically Identified *Staphylococcus intermedius* Strains▽

Takashi Sasaki,[1] Ken Kikuchi,[1]* Yoshikazu Tanaka,[2] Namiko Takahashi,[1] Shinichi Kamata,[2] and Keiichi Hiramatsu[1]

Department of Infection Control Science, Faculty of Medicine, Juntendo University, Tokyo 113-8421, Japan,[1] *and Department of Veterinary Hygiene, Veterinary School, Nippon Veterinary and Life Science University, 1-7-1 Kyounan, Musashino, Tokyo 180-8602, Japan*[2]

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To reclassify phenotypically identified *Staphylococcus intermedius* strains, which might include true *S. intermedius* strains and novel species such as *Staphylococcus pseudintermedius* and *Staphylococcus delphini*, we analyzed molecular phylogenies and phenotypic characteristics of 117 *S. intermedius* group (SIG) strains tentatively identified as being *S. intermedius* by the Rapid ID32 Staph assay. From phylogenetic analyses of *sodA* and *hsp60* sequences, the SIG strains were divided into three clusters, which belonged to *S. pseudintermedius* LMG 22219^T, *S. intermedius* ATCC 29663^T, and *S. delphini* LMG 22190^T. All the SIG strains from dogs, cats, and humans were identified as being *S. pseudintermedius*. The wild pigeon strains, except one, were identified as being *S. intermedius*, and strains from all domestic pigeons, one wild pigeon, horses, and a mink were identified as being *S. delphini*. In addition, a phylogenetic analysis of *nuc* genes revealed that *S. delphini* strains were divided into two clusters: one was the cluster (*S. delphini* group A) that belonged to *S. delphini* LMG 22190^T, and the other was the cluster (*S. delphini* group B) that was more related to *S. pseudintermedius* LMG 22219^T than *S. delphini* LMG 22190^T. The DNA-DNA hybridization results showed that *S. delphini* group B strains were distinguished from *S. delphini* group A, *S. intermedius*, and *S. pseudintermedius* strains. *S. intermedius* is distinguishable from *S. pseudintermedius* or *S. delphini* by positive arginine dihydrolase and acid production from β-gentiobiose and D-mannitol. However, phenotypical characteristics to differentiate *S. delphini* group A, *S. delphini* group B, and *S. pseudintermedius* were not found. In conclusion, SIG strains were reclassified into four clusters with three established and one probably novel species.

In 1976, *Staphylococcus intermedius* was previously described as being a new species isolated from pigeons, dogs, mink, and horses (14). The majority of coagulase-positive staphylococci in animals such as dogs and pigeons have been identified as being *S. intermedius* strains (3, 10–12, 19, 26, 35). This species has been recognized to constitute normal skin flora of various animal species and to occasionally cause a variety of infections in dogs and cats (3, 19).

There have been six species of coagulase-positive staphylococci other than *Staphylococcus aureus*, *S. intermedius*, *S. schleiferi* subsp. *coagulans*, *S. hyicus*, *S. lutrae*, *S. delphini*, and *S. pseudintermedius* (6, 9). Sequence similarities of 16S rRNA genes among these four species except *S. hyicus* and *S. lutrae* are >99% identical (6, 30). In addition, it is very difficult to differentiate between *S. intermedius*, *S. delphini*, and *S. pseudintermedius* phenotypically, and commercial kits are not available for the identification of *S. pseudintermedius* and *S. delphini* (6, 34). *S. delphini* was isolated from dolphins in 1988, and *S. pseudintermedius* was isolated from a cat, a dog, a horse, and a parrot in 2005 as a new species (6, 34). Since these two species were closely related to *S. intermedius*, the concise identification of *S. intermedius* has been in a confused state. Since some researchers indicated that *S. intermedius* strains isolated from different sources could be genotypically or phenotypically distinct from one another (1, 2, 5, 10–12, 24, 36), it is possible that previously reported "*S. intermedius*" strains with heterogeneous biotypes might have included *S. delphini* and *S. pseudintermedius* (1–3, 5, 10–12, 14, 28, 33, 36). We have recently reported that molecular phylogenetic analysis based on partial *sodA* gene sequences and *hsp60* gene sequences is sufficiently discriminative for *S. intermedius*, *S. pseudintermedius*, and *S. delphini* (28).

Thermonuclease has been used for species identification of *S. aureus* as one of the key characteristics (4, 13, 38). Recently, Becker et al. (2) described PCR of the thermonuclease gene (*nuc*) for species identification of *S. intermedius* that could differentiate it from *S. hyicus* and *S. schleiferi*. However, even using that method, there were some *nuc*-negative isolates from horses and camels (2).

In the present study, we reclassified phenotypically identified *S. intermedius* strains from various origins using molecular phylogenetic analyses based on partial sequences of *sodA* and *hsp60* genes, which exhibited higher divergences than 16S rRNA genes in staphylococci and *nuc* genes encoding thermonuclease (8, 29). In addition, to distinguish them, we searched for biochemical reactions including some commercial identification kits for staphylococci.

MATERIALS AND METHODS

Bacterial strains. We selected 117 *S. intermedius* strains, which were derived from animals for which the isolation of *S. intermedius* had previously been reported (2, 5, 6, 10–12, 14, 36) and tentatively identified by Rapid ID32 Staph

* Corresponding author. Mailing address: Department of Infection Control Science, Faculty of Medicine, Juntendo University, 2-1-1 Hongo, Bunkyo-ku, Tokyo 113-8421, Japan. Phone: 81-3-3813-3111, ext. 3822. Fax: 81-3-3816-2782. E-mail: kikuti@med.juntendo.ac.jp.

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TABLE 1. SIG strains used in the present study[a]

Host species	No. of strains	Clinical status	Source	Strain(s)	Source or reference
Dog	78	Pyoderma	Wound pus from skin	NVAU 02002, NVAU 02003, NVAU 02004, NVAU 02005, NVAU 02006, NVAU 02007, NVAU 02008, NVAU 02009, NVAU 02010, NVAU 02012, NVAU 02013, NVAU 02014, NVAU 02015, NVAU 02016, NVAU 02017, NVAU 02019, NVAU 02020, NVAU 02021, NVAU 02022, NVAU 02023, NVAU 02024, NVAU 02026, NVAU 02027, NVAU 02028, NVAU 02029, NVAU 02030, NVAU 02031, NVAU 02032, NVAU 02033, NVAU 02034, NVAU 02035, NVAU 02036, NVAU 02037, NVAU 02038, NVAU 02039, NVAU 02040, NVAU 02041, NVAU 02042, NVAU 02043, NVAU 02044, NVAU 02045, NVAU 02046, NVAU 02047, NVAU 02048, NVAU 02049, NVAU 02050, NVAU 02051, NVAU 02052, NVAU 02053, NVAU 02054, NVAU 02055, NVAU 02056, NVAU 02057, NVAU 02058, NVAU 02059, NVAU 02060, NVAU 02062	This study
				S. intermedius CCUG 50815, *S. intermedius* CCUG 51770	CCUG
		Noninfection inpatients	Nares	NVAU 06002, NVAU 06013, NVAU 06016, NVAU 06017, NVAU 06018, NVAU 06021, NVAU 06022, NVAU 06029, NVAU 06031, NVAU 06033, NVAU 06034, NVAU 06037, NVAU 06038, NVAU 06039, NVAU 06043, NVAU 06044, NVAU 06045, NVAU 06046	28
		Unknown	Unknown	*S. pseudintermedius* CCUG 32915	CCUG
Cat	3	Pyoderma	Wound pus from skin	NVAU 02082, NVAU 02083	This study
		Unknown	Lung tissue	*S. pseudintermedius* LMG 22219^T	6
Human	2	Infection of mastoid cavity	Wound pus	TW 6698	18
		Unknown	Unknown	*S. pseudintermedius* CCUG 46004	CCUG
Pigeon	26	Wild, healthy	Nares	P-2, P-4A, P-6A, P-9B, P-45A, P-46A, P-50, P-52B, P-53, P-54A, P-66A, P-69A	This study
		Domestic, healthy	Nares	P-13B, P-14A, P-18A, P-19A, P-21B, P-22A, P-26, P-27B, P-28A, P-30A, P-36B, P-37A, P-39B	
		Unknown	Nares	*S. intermedius* ATCC 29663^T	ATCC
Horse	6	Domestic, healthy	Nares	h-2C, h-4A, h-5D, h-6C, h-7C, h-9D	This study
Mink	1	Healthy	Nares	*S. intermedius* CCUG 51769	CCUG
Dolphin	1	Unknown	Unknown	*S. delphini* LMG 22190^T	34

[a] ATCC, American Type Culture Collection; CCUG, Culture Collection University Göteborg; LMG, Bacteria Collection Labolatorium Voor Microbiologie Universiteit Gent.

(bioMérieux, Marcy-l'Etoile, France) from our coagulase-positive staphylococcus collections from various animal sources, which we designated the *S. intermedius* group (SIG) (Table 1). These strains were stored in 10% skim milk at −85°C until use and maintained on Trypticase soy agar II with 5% sheep blood (BD Japan Co., Ltd., Tokyo, Japan).

Phenotypic characterization. Coagulase was determined by tube coagulase test using rabbit serum (Denka Seiken Co., Ltd., Tokyo, Japan) for all strains tested. Acid production from carbohydrates was detected under aerobic conditions, except D-mannitol, using a broth microplate method (16). Each carbohydrate of D-galactose, D-mannitol (in anaerobic culture for 20 h), arbutin, α-lactose, D-mannose, β-gentiobiose, and D-turanose was added in purple broth base (24 g/liter; Difco, MD) and thioglycolate medium without glucose or indicator (12 g/liter; Difco) at a final concentration of 0.5% (wt/vol). Each plate was incubated at 37°C and was evaluated at 20 and 48 h. MIC testing for polymyxin B using Etest (AB Biodisk, Solna, Sweden) was performed. An acetoin production (Vogues-Proskauer) test was carried out according to a method reported previously (9).

Thermonuclease production was detected by the metachromatic agar diffusion method using DNA and *o*-toluidine blue by using a modified method described

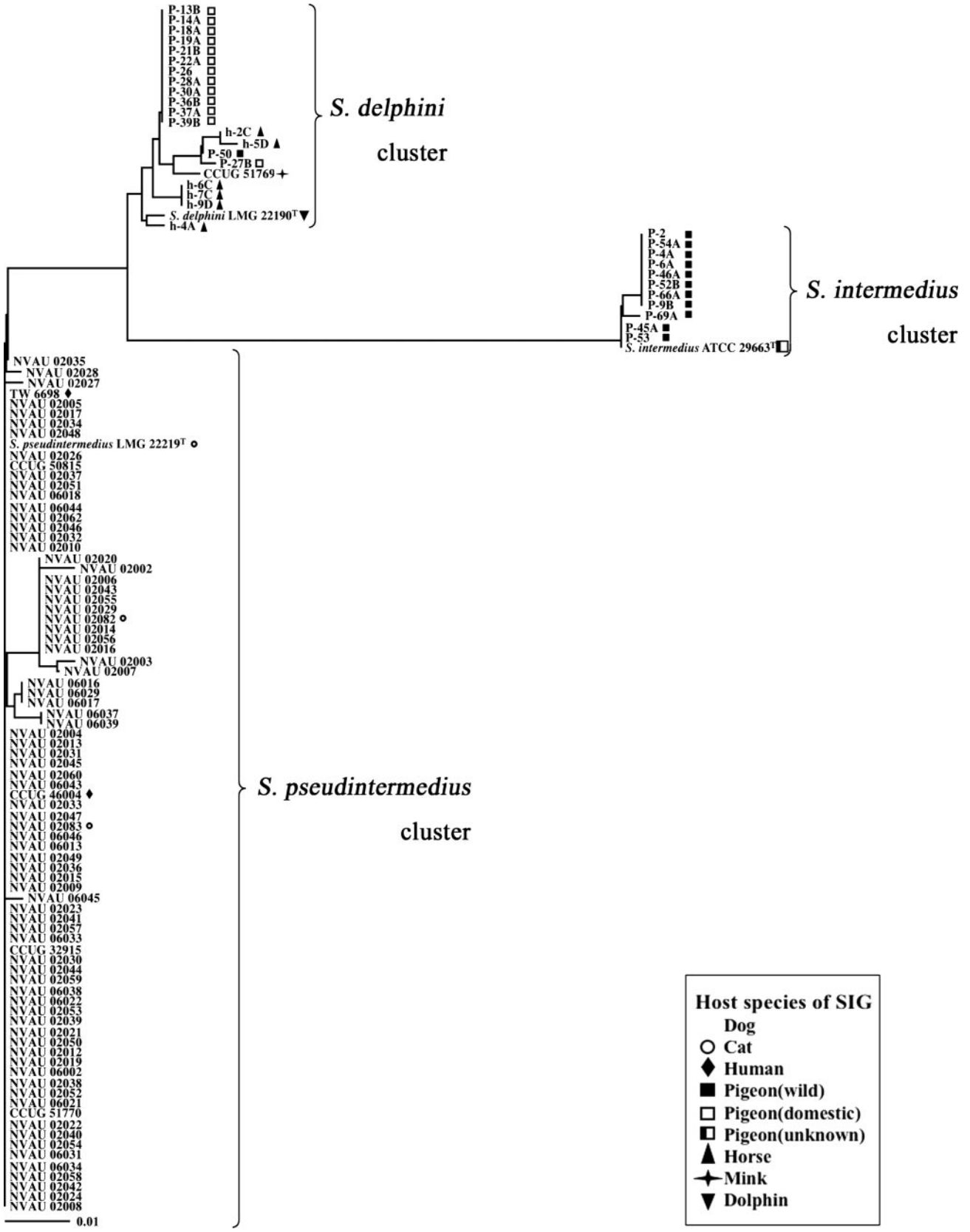

FIG. 1. Phylogenetic tree (unrooted) based on partial *sodA* gene sequences of SIG strains used in the present study. The tree was constructed by the neighbor-joining method using CLUSTAL X. It is unknown whether *S. intermedius* ATCC 29663^T is derived from a wild or domestic pigeon.

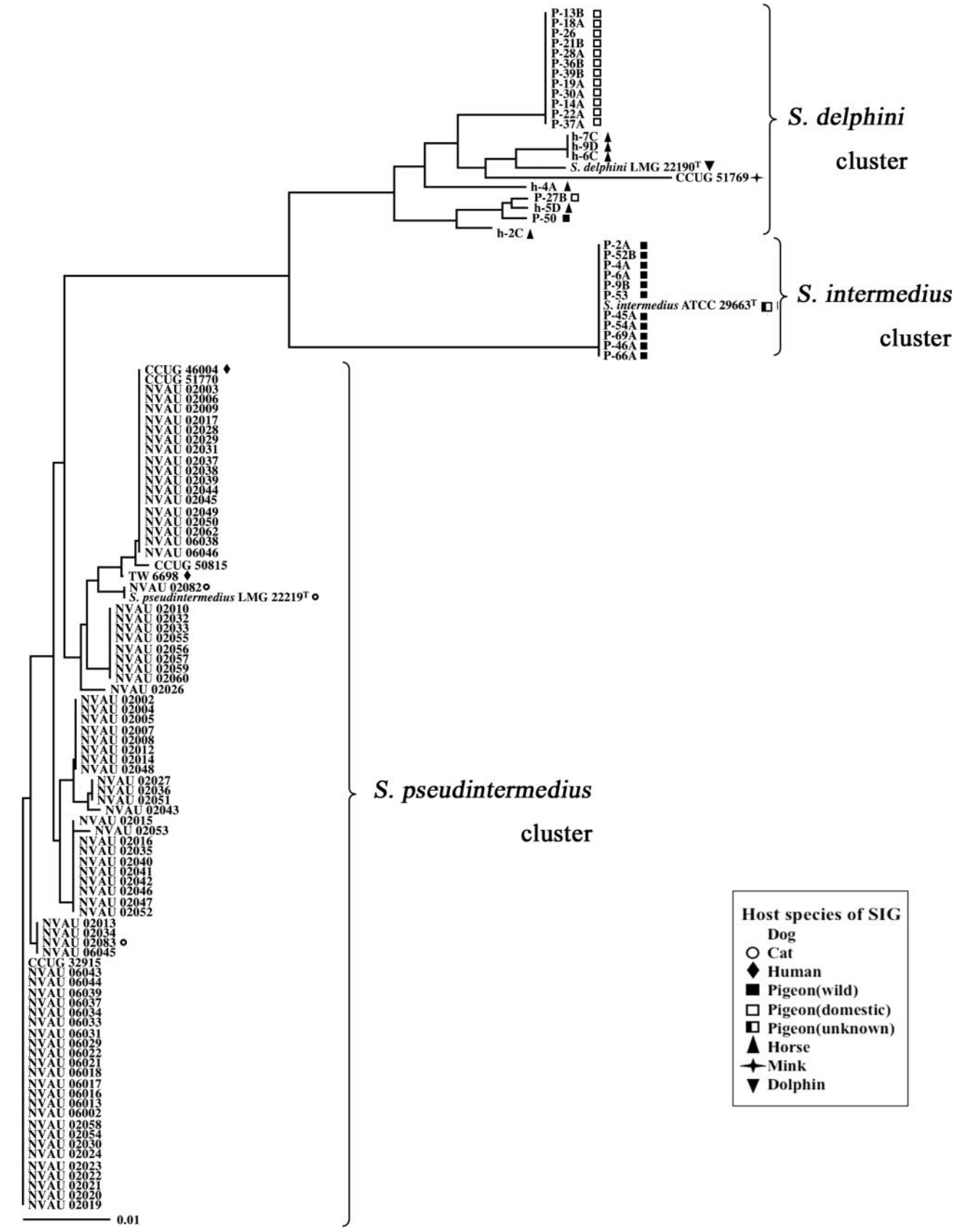

FIG. 2. Phylogenetic tree (unrooted) based on partial *hsp60* gene sequences of SIG strains used in the present study. The tree was constructed by the neighbor-joining method using CLUSTAL X. It is unknown whether *S. intermedius* ATCC 29663^T is derived from a wild or domestic pigeon.

TABLE 2. Phenotypic and genetic characterization of *nuc* among SIG strains

Characteristic	Rate (%) of positive strains or range of sequence similarity (%)			
	S. pseudintermedius (n = 83)	*S. intermedius* (n = 12)	*S. delphini*	
			Group A (n = 17)	Group B (n = 5)
Phenotype (thermonuclease production)	100	67	88	60
Detection by PCR1	100	100	0	100
Detection by PCR2	0	0	100	0
Sequence similarity against *S. pseudintermedius* LMG 22219^T	98.8–100	85.6–87.2	79.2–80.3	95.9–96.1

previously (23), which was conducted as follows: culture supernatants in brain heart infusion broth (Difco) were filtrated and heated (at 100°C for 15 min), and disks with the filtrate were applied to DNase test agar (Becton Dickinson Co., Ltd., France) with *o*-toluidine blue. Agars were incubated at 37°C for 12 h.

DNA extraction. A single colony was suspended to a McFarland standard of 1.0 in 100 μl of TE buffer (20 mM Tris, 2 mM EDTA [pH 7.5]) with 10 U of achromopeptidase (Wako Chemical Co., Ltd., Osaka, Japan), and the suspension was incubated at 55°C for 10 min. After centrifugation at 18,500 × *g* for 5 min, the supernatants were used as crude DNA extracts for PCR (17). Purified DNA extracts for DNA-DNA hybridization were made according to methods reported previously (7).

Genetic identification of the SIG. Direct sequencing of *sodA* and *hsp60* genes was performed for the differentiation of SIG strains as described previously (21, 22, 25, 27). Multiple alignment was carried out by using the CLUSTAL X program (32). The construction of the unrooted phylogenetic tree was performed by the neighbor-joining method (27).

Genetic characterization of the thermonuclease (*nuc*) gene. Detection of the *nuc* gene, encoding thermonuclease, was carried out for SIG strains by PCR (PCR1) as previously described (2). PCR products were sequenced directly. Another PCR (PCR2) was conducted for PCR1-negative strains. The primer set of Nuc-dF (5′-GAA GGC ATA TTG TAG AAC AA-3′) and Nuc-eR (5′-NCK YTC CCA DAT RTT NAR YTK-3′) was designed to amplify the conserved region of the *nuc* gene by multiple alignment of amino acid sequences of the staphylococcal *nuc* gene from those of PCR1-positive strains and the staphylococcal species whose genomes had previously been wholly sequenced through the GenBank database. The size of PCR products was 724 bp including the coding sequence of the *nuc* gene. The reaction mixture for the PCR consisted of 2 μl of DNA extract in a total volume of 50 μl composed of 2 U of Ex *Taq* (Takara Shuzo Co., Ltd., Kyoto, Japan), 10 pmol of each primer, 0.2 mM deoxynucleoside triphosphate mixture, 1.5 mM $MgCl_2$, and 1× reaction buffer (Takara Shuzo Co., Ltd.). Reaction mixtures were thermally cycled once at 95°C for 5 min; 35 times at 95°C for 1 min, 44°C for 1 min, and 72°C for 1 min; and then once at 72°C for 7 min. These PCR products were also sequenced directly.

DNA-DNA hybridization. DNA-DNA hybridization was performed by microplate methods using a Benchmark Plus microplate reader (Bio-Rad, Tokyo, Japan) (7). Hybridization was performed at 45°C for 2 h in a hybridization mixture containing 2× SSC (1× SSC is 0.15 M NaCl plus 0.015 M sodium citrate), 5× Denhardt solution, 3% dextran sulfate, 50% formamide, 200 ng/ml denatured herring DNA, and 1,500 ng/ml biotinylated probe DNA.

RESULTS

Molecular phylogenetic analysis of *sodA* and *hsp60* gene sequences among SIG strains. As shown in Fig. 1 and 2, all 78 strains from dogs were very close to *S. pseudintermedius* LMG 22219^T, with high sequence homology of both the *sodA* (98.8 to 100%) and *hsp60* (98.7 to 99.5%) genes. All the strains from cats and humans also belonged to the same cluster. Thus, these 83 strains were identified as being *S. pseudintermedius* strains. All the 11 wild pigeon-derived strains except strain P-50 showed high sequence similarities to both the *sodA* (99.7 to 100%) and *hsp60* (100%) genes of *S. intermedius* ATCC 29663^T. Consequently, these strains were identified as being *S. intermedius* strains. On the other hand, strains from 13 domestic pigeons, 1 wild pigeon (P-50), 6 horses, and a mink showed close sequence similarities to *sodA* (97.7 to 99.3%) of *S. delphini* LMG22190^T. However, unlike the *S. intermedius* and *S. pseudintermedius* clusters, *hsp60* sequences among these strains were more divergent, and similarities to *S. delphini* LMG22190^T were 95.7 to 98.9%.

Detection and phylogenetic characterization of *nuc* genes among SIG strains. For a better differentiation of *S. pseudintermedius*, *S. intermedius*, and *S. delphini*, we carried out two *nuc* PCRs and direct sequencing of the *nuc* genes (Table 2).

PCR1 could detect predictive fragments among all the *S. pseudintermedius* and *S. intermedius* strains and 5 of 22 *S. delphini* strains. The remaining *S. delphini* strains were not amplified by PCR1 in spite of the ability to produce thermonuclease. PCR2 successfully amplified predictive fragments with 17 PCR1-negative strains.

In the phylogenetic tree of the *nuc* gene, SIG strains were classified into four clusters (Fig. 3). *S. intermedius* and *S. pseudintermedius* strains constituted a single cluster that confirmed their *sodA* and *hsp60* phylogeny. Interestingly, *S. delphini* strains were divided into two clusters: one (*S. delphini* group A) was similar to *S. delphini* LMG22190 ^{T}, and the other (*S. delphini* group B) was closer to the *S. pseudintermedius* cluster than *S. delphini* LMG22190^T.

DNA-DNA hybridization among SIG strains. To elucidate the taxonomic position of *S. delphini* groups A and B, We performed DNA-DNA hybridizations among SIG strains (Table 3). Given a threshold level of 70% DNA-DNA relatedness for the definition of a bacterial species (37), our reclassification based on phylogenetic analysis of the *nuc* gene corresponded to the species distinction. Among *S. delphini* group strains and those of the other clusters, *S. delphini* group A had the highest level (77%) of DNA similarity to *S. delphini* LMG22190^T. On the other hand, *S. delphini* group B strains showed less than 70% similarities with those of any clusters. DNA similarities of greater than 70% were observed among *S. delphini* groups A and B, respectively (data not shown). These results suggest that *S. delphini* group B constitutes a novel species.

Phenotypic characteristics among SIG strains. To search for phenotypic characteristics that distinguish the genetically distinct SIG strains, we compared their biochemical characteristics. As shown in Table 4, *S. intermedius* could be differentiated from the other SIG strains by 100% negative arginine dihydrolase and 100% positive acid production from β-gentiobiose aerobically and D-mannitol anaerobically. There was no obvious difference in biochemical reactions among *S. pseudintermedius*, *S. delphini* group A, and *S. delphini* group B. There

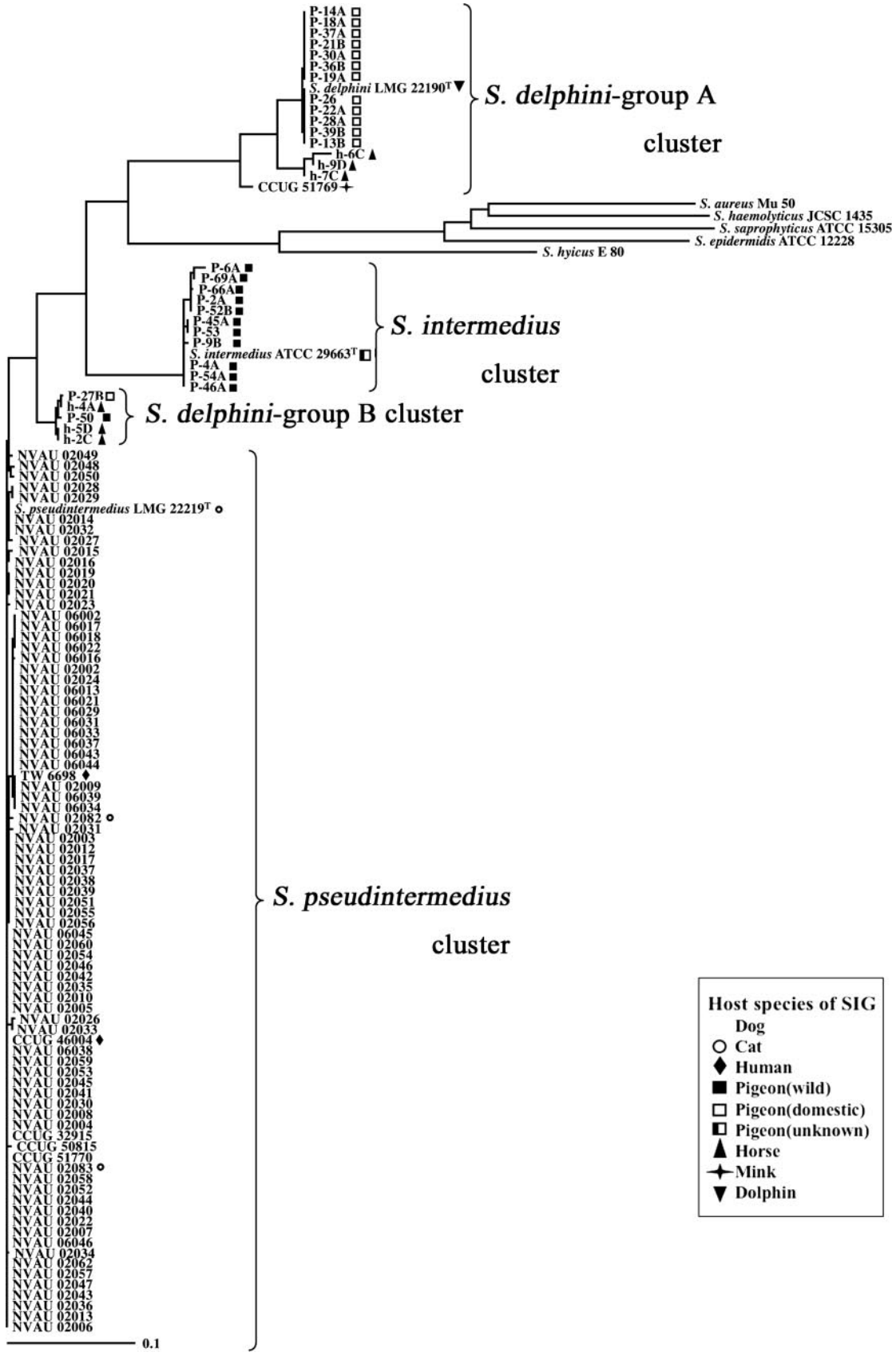

FIG. 3. Phylogenetic tree (unrooted) based on partial *nuc* gene sequences of SIG strains used in the present study. The tree was constructed by the neighbor-joining method using CLUSTAL X. It is unknown whether *S. intermedius* ATCC 29663^T is derived from a wild or domestic pigeon.

TABLE 3. DNA-DNA hybridization among SIG strains

Strain	% DNA similarities with biotin-labeled DNA from:				
			S. delphini		
	S. pseudintermedius LMG 22219^T	*S. intermedius* ATCC 29663^T	LMG 22190^T	Group A (h-6C)	Group B (P-27B)
S. pseudintermedius LMG 22219^T	100				
S. intermedius ATCC 29663^T	29	100			
S. delphini LMG 22190^T	46	28	100		
S. delphini group A (h-6C)	50	28	74	100	
S. delphini group B (P-27B)	45	40	61	57	100

were some variations in the abilities of acid production from D-turanose: *S. pseudintermedius* and *S. delphini* group B showed weak or delayed reaction, *S. delphini* group A strains were negative, and *S. pseudintermedius* and *S. delphini* group A strains did not use β-gentiobiose, whereas about 60% of the *S. delphini* group B strains fermented β-gentiobiose. In standard acetoin production tests (9), 66.3% (55/83) of *S. pseudintermedius* strains were positive. The abilities were not as strong as

TABLE 4. Phenotypic characteristics of SIG strains

Test and characteristic	% Positive strains			
			S. delphini	
	S. pseudintermedius (n = 83)	*S. intermedius* (n = 12)	Group A (n = 17)	Group B (n = 5)
ID 32 Staph				
Urease	100	100	88	100
Arginine dihydrolase	100	0	100	80
Ornithine dihydrolase	0	0	0	0
Esculin hydrolysis	0	0	0	0
Acid production from:				
Glucose	100	100	100	100
Fructose	100	100	100	100
D-Mannose	100	100	100	80
D-Maltose	91	91	94	100
Lactose	100	73	94	40
Trehalose	100	100	94	80
D-Mannitol	46	100	71	60
Raffinose	0	0	0	40
D-Ribose	100	100	100	100
D-Cellobiose	0	0	0	20
Saccharose	100	100	59[a]	80
N-Acetylglucosamine	100	18[a]	100	100
D-Turanose	0[a]	0	0	0[a]
L-Arabinose	0	0	0	20
Nitrate reduction	100	100	100	100
Acetoin (Vogues-Proskauer)	10	0	0	20
β-Galactosidase	100	100	100	100
Arginine arylamidase	0	0	0	0
Alkaline phosphatase	99	100	100	100
Pyrrolidonyl arylamidase	100	100	100	100
Novobiocin resistance	0	0	18	60
β-Glucuronidase	0	0	0	0
Acid production from:				
D-Mannitol (anaerobically)	0	100	0	0
D-Galactose	92	0[a]	94	100
Arbutin	0	0[a]	0	0
β-Gentiobiose	0	100	0	60
D-Turanose (48 h)	17[a]	0	0	40[a]
Acetoin production (Vogues-Proskauer)	66	0	5	0
MIC of polymixin B (Etest)				
Range (mg/ml)	16–32	8–12	12–32	24
Geometric mean (mg/ml)	25	11	22	24

[a] Many of the strains showed a weak reaction and were designated as being negative.

with *S. aureus*, and most of them were not detected by the Rapid ID32 Staph test. *S. intermedius* and *S. delphini* group A (except for *S. delphini* LMG22190^T) and group B strains were negative for this characteristic.

All the *S. pseudintermedius* strains produced thermonuclease; the level of production was stronger than that for the other species. Although 66% (8/12) of *S. intermedius* strains showed a positive reaction, the activities were weaker than those of *S. pseudintermedius*. All *S. delphini* group A strains except *S. delphini* LMG22190^T and strain CCUG 51769 showed a positive reaction.

DISCUSSION

Our results suggest that the majority of SIG strains isolated from commensal flora or infection sites in dogs are reclassified as being *S. pseudintermedius* strains. In fact, *S. intermedius* CCUG 50815 and *S. intermedius* CCUG 51770 (both of dog origin) obtained from the Culture Collections of the University Göteborg (CCUG) were also identified as being *S. pseudintermedius* strains by molecular phylogenetic analyses. Previously, we also reported that *S. pseudintermedius* was a common species among methicillin-resistant coagulase-positive staphylococci in dogs (28).

Van Hoovels et al. reported the first case of *S. pseudintermedius* infection in a human (33). In the present study, we also identified two strains from humans as being *S. pseudintermedius* strains, one of which (strain TW 6698) had been identified as being *S. intermedius* before our reclassification (18). Although there have been reports that *S. intermedius* strains were isolated from human infection or food poisoning from dog origins (15, 18, 33), there is another possibility, that these isolates were *S. pseudintermedius* isolates and not *S. intermedius* or *S. delphini* isolates.

In pigeons, SIG strains were isolated from the nares of wild and domestic species in the present study: the former was *S. intermedius*, and the latter was *S. delphini* group A. Distinct SIG species might be distributed to different host habitats in pigeons.

Some reports suggested previously that SIG strains from horses were heterogenous (2, 5, 36). In the present study, both *S. delphini* group A and group B strains were isolated from horses. Becker et al. also previously reported three different groups from horses using a PCR-DNA enzyme immunoassay of the *nuc* gene (2). With respect to *nuc* sequences in our results, these three groups might be *S. pseudintermedius* and *S. delphini* groups A and B, respectively.

SIG strains from minks have been reported to be distinct from those from dogs by pulsed-field gel electrophoresis analyses and by their lack of thermonuclease production (5, 6, 36). In the present study, all SIG strains from dogs were identified as being *S. pseudintermedius* strains and were positive for thermonuclease production. These results suggest that SIG strains from minks are different from *S. pseudintermedius* strains. In fact, strain CCUG 51769, from a mink, was identified as belonging to *S. delphini* group A in the present study.

Devriese et al. previously described *S. pseudintermedius* as being a novel species and reported that this species could be discriminated from *S. intermedius* or *S. delphini* by some biochemical characteristics (6). For example, those authors reported that *S. delphini* differed from *S. pseudintermedius* in its negative acidification of trehalose and thermonuclease production. However, some *S. delphini* strains, which had been included in the species *S. intermedius*, conventionally identified and reclassified in our study, showed a positive reaction in these tests. Furthermore, in acetoin production tests, the type strain of *S. delphini*, which had been reported to be negative previously (6, 9), showed a positive result by the standard method (9). These characteristics are not available to differentiate between *S. pseudintermedius* and *S. delphini*. In the present study, *S. intermedius* is easily distinguishable among SIG strains using some phenotypical properties such as positive arginine dihydrolase and acid production from β-gentiobiose and D-mannitol (anaerobically for 20 h). However, there is no "gold standard" to differentiate phenotypically among *S. pseudintermedius* and *S. delphini* groups A and B, although host animal species, acid production from β-gentiobiose and D-turanose (48 h), acetoin production (Vogues-Proskauer) by the standard method, and thermonuclease production might be useful to some extent. Finally, to discriminate between these species correctly, there is no method except PCR and sequencing of the *nuc* gene.

Whether or not they had thermonuclease activity, all the SIG strains had the *nuc* gene. All the whole-genome-sequenced staphylococci, *S. epidermidis*, *S. saprophyticus*, and *S. haemolyticus*, possess *nuc*-related genes with 40 to 60% putative amino acid sequence homologies to SIG thermonuclease (20, 31, 39). Although thermonuclease activity is not detected in *S. epidermidis*, *S. saprophyticus*, and *S. haemolyticus* (4, 9), these strains still keep this gene. In the present study, we detected the *nuc* gene from *S. delphini*, which was recognized as a species that is negative for thermonuclease activity (9, 34). These results suggest that the *nuc* gene is ubiquitous in the genus *Staphylococcus*. Differences in thermonuclease activities among staphylococcal species might be affected by differences in the heat stability of nuclease or expression levels. Gudding previously reported that the heat stability of *S. intermedius* nuclease showed lower levels than those of *S. aureus* and *S. hyicus* (13). However, the conservation and genetic diversities of *nuc* genes means that it could be used as another molecular evolutionary tool in staphylococci. Interestingly, *nuc* sequences in *S. delphini* group B strains were more closely related to those of *S. pseudintermedius* than *S. delphini* group A. The phylogenetic position of *nuc* gene in *S. delphini* group B was quite different from that of *sodA* or *hsp60* genes. The *nuc* genes of *S. delphini* group B might have proceeded to a unique evolutionary path among staphylococci.

In conclusion, phenotypically identified *S. intermedius* strains were reclassified into at least four clusters, *S. intermedius*, *S. pseudintermedius*, and *S. delphini* groups A and B. *S. delphini* group B might be a novel species. Our study provides important information on the phylogenetic reclassification of staphylococcal species by molecular methods.

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