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Development of an appropriate PCR system for the reclassification of *Streptococcus suis*

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ABSTRACT

Thirty-five serotypes of *Streptococcus suis* (serotypes 1–34 and serotype 1/2) have so far been described on the basis of their polysaccharide capsular antigens. However, in the last decade, some serotype reference strains have been reexamined for their taxonomic status, and the reference strains of serotypes 20, 22, 26, 32, 33, and 34 may be different from taxon *S. suis*. In the present study, we developed a novel PCR method targeting the recombination/repair protein (*recN*) gene of *S. suis*, designated *recN* PCR, which corresponds to the current reclassification of this bacterium. We compared its specificity with other PCR methods for *S. suis*, and the results obtained confirmed its specificity. In addition, the detection limits of *recN* PCR were similar among all the reference strains of authentic *S. suis*, indicating that the *recN* PCR gave reliable results against bacterial strains and isolates used in this study. Therefore, *recN* PCR described in the present study will be a useful tool for the identification of authentic *S. suis*, and can also be used in epidemiological studies on this bacterium.

1. Introduction

Streptococcus suis is a Gram-positive coccus that causes a wide range of porcine diseases, including meningitis, septicemia, and endocarditis, and is recognized as an important pathogen that has been responsible for severe economic losses in the swine industry worldwide (Staats et al., 1997; Fittipaldi et al., 2012). *S. suis* can also affect humans who are in close contact with infected pigs or swine products (Ye et al., 2006; Mai et al., 2008; Takamatsu et al., 2008).

Thirty-five serotypes of *S. suis* (serotypes 1–34 and serotype 1/2) have so far been described on the basis of their polysaccharide capsular antigens (Fittipaldi et al., 2012). However, phylogenetic analyses of the 16S rRNA and chaperonin-60 (*cpn*60) genes showed that the reference strains of serotypes 32 and 34 should have been *S. orisratti* (Hill et al., 2005). More recently, sequence analyses of genes encoding manganese-dependent superoxide dismutase (*sodA*) and the recombination/repair protein (*recN*) indicated that the reference strains of serotypes 20, 22, 26, and 33 should be taxonomically removed from *S. suis* (Le et al., 2013). Strains of these serotypes have rarely been isolated from diseased animals (Wei et al., 2009; Gottschalk et al., 2013).

Okwumabua et al. (2003) developed a PCR method that targeted *gdh* encoding glutamate dehydrogenase, designated *gdh* PCR. Marois et al. (2004) also developed a PCR method targeting the 16S rRNA gene, designated 16S rRNA gene PCR. These methods have widely been used in clinical practice and laboratories; however, these were designed to detect all 35 serotypes of *S. suis*, even those that should not have been included (i.e., serotypes 20, 22, 26, 32, 33, and 34). Therefore, a new PCR assay is needed that corresponds to the current reclassification of *S. suis*.

The *recN* gene has been shown to have a lower degree of similarity at the species level and higher divergence value at the subspecies level than other housekeeping genes (Glazunova et al., 2010). In accordance with these findings, Le et al. (2013) reported that *recN* sequence analysis revealed complete concordance with the DNA-DNA reassociation results. These results suggested that the *recN* gene could be a suitable target for the rapid detection of *S. suis*.

We here describe a novel PCR method that targets the *recN* gene of *S. suis*, designated *recN* PCR, and evaluated its potential as an identification system based on a current taxonomical study.

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2. Material and methods

2.1. Bacterial strains and culture conditions

The *S. suis* strains and isolates used in this study are listed in Table 1.

All the *S. suis* strains and isolates, except for type and reference strains, were examined by either *gdh* PCR or 16S rRNA gene PCR, as well as serotyping with specific antisera, and were confirmed to be *S. suis*. Serotyping of *S. suis* was performed by the coagglutination test and capsular reaction test, as described previously (Higgins and Gottschalk, 1990), using commercial antisera (Statens Serum Institut, Copenhagen, Denmark). Additional strains and isolates of streptococcal species and other species that can cause diseases in pigs are listed in Table 2.

Those of streptococcal species include several taxonomically close relatives on the basis of *recN* sequences (i.e., *S. acidominimus*, *S. gallinaceus*, *S. minor*, and *S. ovis*) (Glazunova et al., 2010).

All streptococcal species, *Escherichia coli*, and *Salmonella enterica* subsp. *enterica* serovar Choleraesuis were cultured in Todd-Hewitt (TH) agar (Becton Dickinson, Sparks, MD). *Actinobacillus pleuropneumoniae*, *Bordetella bronchiseptica*, and *Staphylococcus hyicus* were cultured in Trypto soya agar base (Nissui, Tokyo, Japan) with 5% sheep blood. *Haemophilus parasuis* was cultured in chocolate blood agar prepared with 5% horse blood and Trypto soya agar base (Nissui).

Table 1
S. suis strains and isolates used in the present study, and results of *recN* PCR.

Strains	Serotype	Source	*recN* PCR
Type strain and serotype reference strains			
NCTC 10237 (=5428)	1	Diseased pig	+
NCTC 10234 T (=S735)	2	Diseased pig	+
4961	3	Diseased pig	+
6407	4	Diseased pig	+
11538	5	Diseased pig	+
2524	6	Diseased pig	+
8074	7	Diseased pig	+
14636	8	Diseased pig	+
22083	9	Diseased pig	+
4417	10	Diseased pig	+
12814	11	Diseased pig	+
8830	12	Diseased pig	+
10581	13	Diseased pig	+
13730	14	Diseased human	+
NCTC 10446	15	Diseased pig	+
2726	16	Diseased pig	+
93A	17	Clinically healthy pig	+
NT77	18	Clinically healthy pig	+
42A	19	Clinically healthy pig	+
86-5192	20	Diseased calf	-
14A	21	Clinically healthy pig	+
88-1861	22	Diseased pig	-
89-2479	23	Diseased pig	+
88-5299A	24	Diseased pig	+
89-3576-3	25	Diseased pig	+
89-4109-1	26	Diseased pig	-
89-5259	27	Diseased pig	+
89-590	28	Diseased pig	+
92-1191	29	Diseased pig	+
92-1400	30	Diseased pig	+
92-4172	31	Diseased calf	+
EA1172.91	32	Diseased pig	-
EA1832.92	33	Diseased lamb	-
92-2742	34	Diseased pig	-
2651	1/2	Diseased pig	+
Representative strains, human isolate, and porcine isolates			
P1/7	2	Diseased pig	+
89-1591	2	Diseased pig	+
HUT-1	2	Diseased human	+
GUT-49, 57	1	Diseased pigs	+
GUT-6, 11, 12, 13, 17, 18, 19, 20, 21, 25, 26, 40, 41, 42, 43, 45, 46, 47, 48, 51, 59, 60, 61, 62, 66, 69, 70, 72, 85, 92, 95, 102, 111, 116, 120, 123, 127, 131, 132, 136, 138, 143, 150, 151	2	Diseased pigs	+
GUT-14, 22, 23, 24, 31, 44, 55, 74, 84, 88, 89, 91, 96, 105, 106, 107, 114, 119, 124, 129, 130, 141, 144, 145, 148, 155, 162	3	Diseased pigs	+
GUT-1, 2, 3, 4, 16, 30, 34, 37, 50, 73, 86, 87, 90, 118, 134, 137, 146, 158	4	Diseased pigs	+
GUT-58	5	Diseased pig	+
GUT-8, 9, 10, 15, 28, 29, 52, 53, 78, 98, 115, 157	7	Diseased pigs	+
GUT-27, 99, 104	8	Diseased pigs	+
GUT-7, 63, 77, 128, SUT-3, 354, 376, 403, 405	9	Diseased pigs and clinically healthy pigs	+
GUT-156, SUT-288	11	Diseased pig and clinically healthy pig	+
SUT-38	12	Clinically healthy pig	+
GUT-32, SUT-290, 298, 300, 314, 333	15	Diseased pig and clinically healthy pigs	+
SUT-246, 268, 269	16	Clinically healthy pigs	+
SUT-283, 286, FUT-29	20	Clinically healthy pigs and pork	-
GUT-182, SUT-380	22	Diseased pig and clinically healthy pig	-
GUT-33, SUT-436	25	Diseased pig and clinically healthy pig	+
GUT-35, SUT-10	31	Diseased pig and clinically healthy pig	+
GUT-183, 184, 185, 186, 187, 188, 189, 190, 191, 192, 193	33	Diseased calves	-