Journal of Microbiological Methods 117 (2015) 95–99

Contents lists available at ScienceDirect

Journal of Microbiological Methods

journal homepage: www.elsevier.com/locate/jmicmeth

Simultaneous detection of 33 *Streptococcus suis* serotypes using the luminex xTAG® assay™

Xuemei Bai [a,1], Zhijie Liu [a,1], Shaobo Ji [a], Marcelo Gottschalk [b], Han Zheng [a,*], Jianguo Xu [a]

[a] State Key Laboratory for Infectious Disease Prevention and Control, Collaborative Innovation Center for Diagnosis and Treatment of Infectious Diseases, National Institute for Communicable Disease Control and Prevention, Chinese Center for Disease Control and Prevention, Changping, Beijing, China
[b] Groupe de Recherche sur les Maladies Infectieuses du Porc, Faculté de médecine vétérinaire, Université de Montréal, Québec, Canada

ARTICLE INFO

Article history:
Received 29 June 2015
Received in revised form 17 July 2015
Accepted 20 July 2015
Available online 22 July 2015

Keywords:
Streptococcus suis
Serotype
wzy
Luminex

ABSTRACT

We report the development and evaluation of a serotype-specific xTAG luminex assay (SSA) that allows detection of the 33 serotypes of *Streptococcus suis* (*S. suis*). This assay is based on *wzy* gene targets directly involved in the *cps* biosynthesis and can be completed 40 min post-PCR amplification. The assay correctly and specifically identified the serotype of all 209 isolates tested, in comparison with two serotyping multiplex PCR methods previously developed. The sensitivity was higher than that of the previously described methods. The SSA system described here provides an easy-to-use, high-throughput system for rapid detection of *S. suis* serotypes.

1. Introduction

Streptococcus suis (*S. suis*) is one of the most important swine pathogens worldwide and has significant impacts on the swine industry. Furthermore, it is an emerging zoonotic agent: humans can be infected when in close contact with pigs or following consumption of raw or undercooked pork products (Gottschalk et al., 2007; Wertheim et al., 2009). *S. suis* serotyping is a valuable phenotypic subtyping tool that is required for a better understanding of the epidemiology of this important zoonotic pathogen. Thirty-five serotypes (type 1 through 34, and 1/2) of *S. suis* have been identified based on antigenic differences in their capsular polysaccharide (CP) (Gottschalk et al., 1991a,b, Gottschalk et al., 1989; Perch et al., 1983). The CP synthesis (*cps*) genes are clustered in a single locus on the *S. suis* chromosome. The *cps* clusters for the 35 reference serotypes have been published (Okura et al., 2013). *S. suis* serotypes are routinely identified using the agglutination or co-agglutination tests with serotype-specific antisera. However, these techniques are laborious, time-consuming, and relatively expensive. The problems associated with the production of the antisera and testing have led to the development of different molecular approaches, of which multiplex PCR (mPCR) with amplification of serotype-specific *cps* genes are an attractive alternative to the existing serological tests. In fact, three mPCR assays were developed to identify *S. suis* serotypes (Kerdsin et al., 2014; Liu et al., 2013; Okura et al., 2014). However, two or three sets of mPCR amplification are needed when using these assays. Furthermore, the interpretation of the data may be difficult due to the similar sizes of the PCR products for some serotypes.

Here, we developed a 32-plex *S. suis* serotyping assay (SSA) that simultaneously detects 33 serotypes using the luminex xTAG universal array technology. The *Streptococcus orisratti* strains originally classified as the reference strains for *S. suis* serotypes 32 (strain EA1172.91) and 34 (strain 92-2742) were not included in the present study (Hill et al., 2005). Although additional serotypes 20, 22 and 26 have recently been suggested to belong to a bacterial species different from *S. suis* (Nomoto et al., 2015; Tien le et al., 2013), these serotypes are still commonly isolated from diseased animals and considered as *S. suis* by diagnostic laboratories (Gottschalk et al., 2013). For that reason, they have been included in the development of the present test. The approach used in this study combines amplification of the target genes using mPCR with 32 pairs of primers that have a unique 'TAG' sequence incorporated upstream of the primer and a biotinylated downstream primer with a multiplexed bead-based suspension array detection system. The method and sero-specific validation of the SSA are herein presented.

2. Materials and methods

2.1. Bacterial strains

Eighty-four serotypable *S. suis* strains, 19 other *Streptococcus* spp. strains and one *Klebsiella pneumoniae* strain from our previous study were used (Liu et al., 2013). One hundred-three additional *S. suis* field

* Corresponding author.
E-mail address: zhenghan@icdc.cn (H. Zheng).
[1] Those authors contribute to the manuscript equally.

http://dx.doi.org/10.1016/j.mimet.2015.07.018
0167-7012/

strains from clinically healthy pigs, 22 strains from diseased pigs, whose serotypes were determined by two previously developed mPCR (Liu et al., 2013; Okura et al., 2014), and 42 other non-*S. suis* strains were included in this study (Table 1). The strains were grown overnight on Columbia blood base agar (Oxoid, Hampshire, UK) at 37 °C and a single colony was used to inoculate 5 ml of Todd-Hewitt broth (THB, Oxoid Ltd., London, UK), incubated for 16 h at 37 °C with 5% CO_2. Chromosomal DNA was prepared from all isolates using the previously described method (Liu et al., 2013).

2.2. Primer design

We adapted and modified the primers from our previous study to increase the assay specificity (Liu et al., 2013). PCR primers were designed based on the *wzy* gene. Using the Primer-BLAST program (http://www.ncbi.nlm.nih.gov/tools/primer-blast/), primers were designed to have similar physical characteristics in order to allow simultaneous amplification using the same conditions during multiplex reactions. The lengths of the primers were 20 to 24 bp, their melting temperatures between 49.9 and 50.2 °C, and the expected amplicon sizes between 120 to 490 bp. A highly conserved region of *thrA* was used as internal control for identification of *S. suis* species (King et al., 2002). This gene was chosen over other described housekeeping genes (such as *gdh* or *recN*) (Tien le et al., 2013; Zheng et al., 2015) because primers giving a small amplicon (120 bp) could be designed. Small amplicons are needed to optimize positive-signal intensity and hybridization efficiency of the SSA system.

Table 1
List of bacteria used in this study.

Strains	Number of strains tested
Streptococcus suis	209
Streptococcus pneumoniae	7
Streptococcus agalactiae	1
Streptococcus orisratti	2
Streptococcus bovis	1
Streptococcus pyogenes	3
Streptococcus sanguis	1
Streptococcus oralis	1
Streptococcus lutetiensis	1
Streptococcus thermophilus	1
Streptococcus mutans	1
Streptococcus acidominimus	1
Klebsiella pneumoniae	1
Enterotoxigenic E. coli	1
Enteropathogenic E. coli	1
Enteroinvasive E. coli	1
Enterohemorrhagic E. coli	1
Enteroaggregative E. coli	1
Enterococcus faecium	1
Enterococcus faecalis	2
Enterobacter aerogenes	1
Enterobacter cloacae	1
Aeromonas veronii	1
Listeria monocytogenes	1
Bacillus cereus	1
Leptospira biflexa	1
Leptospira interrogans	1
Vibrio minicus	1
Vibrio fluvialis	1
Vibrio parahaemolyticus	1
Salmonella paratyphi A	1
Serratia marcescens	1
Neisseria meningitidis	3
Bordetella pertussis	1
Legionella pneumophila	1
Clostridium difficile	1
Salmonella enterica	2
Salmonella choleraesuis	1
Salmonella typhimurium	1
Salmonella gallinarum	1
Salmonella arizonae	1
Salmonella agona	1
Edwardsiella tarda	1
Staphylococcus aureus	1
Acinetobacter baumannii	1
Campylobacter jejuni	1
Helicobacter pylori	1
Enterobacter sakazakii	1
Bacillus anthracis	1
Brucella suis	1

The upstream primer of each pair was modified using an unique 24-base oligonucleotide 'TAG' sequence at the 5′ terminus and was used in conjunction with MagPlex-xTAG microspheres, which are superparamagnetic beads (6.5 μm in diameter) containing two internal fluorescent dyes and pre-coupled to the 24-base oligonucleotide 'anti-TAG' sequence on the beads. A 12-carbon amine containing group was incorporated between the 'TAG' sequence and primer. This spacer facilitates interaction with the target and reporter molecules needed in the assay. The downstream primer of each pair was biotinylated at the 5′ terminus, labeling the target strand for amplification and subsequent detection using the xTAG universal array system. The sequences and working concentrations of the primers are shown in Table 2. The primers were synthesized by Tsingke (Beijing, China) and dissolved in molecular analysis-grade water (TransGen Biotech, Beijing, China) to obtain 100 μM stock solutions.

2.3. SSA description and protocol

2.3.1. PCR amplification

mPCR was used to amplify *S. suis* serotype-specific *wzy* target sequences and incorporate the unique 'TAG' sequence and biotin label. The master mix was prepared by adding 0.2 μM or 0.3 μM of each of the xTAG and biotinylated primers to 10 μl 2 × EasyTaqTM PCR SuperMix (TaKaRa, Beijing, China) and molecular analysis-grade water (TransGen Biotech). An 18.5 μl volume of the master mix (reaction mix) was dispensed into a 200 μl strip cap tube or plate, and 1.5 μl of nucleic acid template was added to each reaction mixture, for a total volume of 20 μl. PCR amplification cycling parameters were 94 °C for 5 min; 30 cycles of 94 °C for 30 s, 58 °C for 30 s, and 72 °C for 30 s; followed by a final elongation step at 72 °C for 10 min.

2.3.2. Coupling of the PCR products

The amplified serotype-specific *wzy* sequences were labeled with the fluorescent reporter and captured by direct hybridization onto bead sets coupled with 'anti-TAG' sequences. Luminex MagPlex-xTAG microspheres (Luminex Corporation) were vortexed 30 s. A bead mixture was prepared by adding 2500 microspheres of each set per reaction. The diluted MagPlex-xTAG microsphere mixture was concentrated as to have 125 of each microsphere set per μl in 1 × Tm Hybridization Buffer (0.2 M NaCl, 0.1 M Tris, 0.08% Triton X-100, pH 8.0, filter sterilized). The reporter was prepared by diluting Streptavidin, R-Phycoerythrin Conjugate (SAPE, Invitrogen Corporation) to 10 μg/ml in 1 × Tm Hybridization Buffer. For each reaction, a volume of 20 μl of MagPlex-TAG microsphere mixture, 5 μl of each PCR reaction or of H_2O and 75 μl of reporter solution was dispensed in each well of a 100 μl strip cap tube (Ambion, ABI Corporation) and mixed by pipetting five times. The mixture was hybridized in a thermocycler for 30 min at 40 ° C.

2.3.3. Signal detection

Bead-PCR product-SAPE complexes were identified for each bead type and the fluorescence intensity was measured using a Bioplex 200 (Bio-Rad). Each serotype was attributed to a unique TAG sequence and designated bead. An analysis with the BioPlex 200 system was created using the Luminex xPONENT 3.1 software (Luminex Corporation). The sample size was set to 70 μl with a minimum of 100 beads per target analyzed. The bead type was set to MagPlex and the gate opening range was set at 7366 to 19262, while the plate heater was adjusted to 40 °C. The reactions were read by the instrument and the median fluorescence