Rev. Salud Anim. Vol. 35 No. 1 (2013): 59-63

SHORT COMMUNICATION

Evaluation of simplified DNA extraction methods for *Streptococcus suis* typing

Ivette Espinosa, M. Báez, María Irian Percedo, Siomara Martínez

Division of Molecular Biology, National Centre for Animal and Plant Health (CENSA), Apdo.10, San José de las Lajas, Mayabeque, Cuba. E-mail: espinosa@censa.edu.cu

ABSTRACT: *Streptococcus suis* is a gram-positive bacterium that causes serious diseases in pigs and in humans with occupational risk. The DNA extraction methods for amplification of gene fragments by PCR for typing *S.suis* may be complex, and expensive chemical reagents and time consuming. The aim of this study was to evaluate a method for the rapid release of the genomic DNA from *S.suis* colonies by using a physical method based on heating and freezing; in this case, temperatures of 100°C and 95°C were tested. The results showed that DNA extraction directly from colonies by heating at 100°C could be useful for an easy genotyping of *S.suis* strains in a short time, while 95°C was not sufficient for DNA release. The detection limit of the PCR assay using DNA obtained by chemical purification was 0.5ng; considering the size of *S.suis* genome, it is possible to estimate that an adequate amount of cells are in a single *S.suis* colony to ensure the sensitivity of the PCR assay.

Key words: *Streptococcus suis*, direct colony PCR.

Evaluación de métodos simples de extracción de ADN para la tipificación de *S. suis*

RESUMEN: *Streptococcus suis* es una bacteria grampositiva que causa serias enfermedades en cerdos y humanos con riesgo profesional. Los métodos de extracción de ADN para la amplificación de fragmentos de genes por PCR para la tipificación de *S.suis* pueden resultar complejos, consumir reactivos costosos y tiempo. El objetivo de este trabajo es la evaluación de un método físico para la extracción rápida del ADN, a partir de colonias mediante el calentamiento y la congelación, para lo cual se evaluaron dos temperaturas 100°C y 95°C. Los resultados mostraron que la extracción de ADN a partir de colonias a 100°C es válida para la genotipificación rápida de *S.suis* fácilmente en corto tiempo, mientras la temperatura de 95°C no fue suficiente para la liberación del ADN. El límite de detección del ensayo a partir de ADN genómico extraído por purificación química fue 0.5 ng; teniendo en cuenta el tamaño del genoma de *S. suis*. Es posible considerar que en una simple colonia de *S.suis* existe la suficiente cantidad de células para garantizar la sensibilidad del ensayo de PCR.

Palabras clave: *Streptococcus suis,* PCR directo de colonia.

Streptococcus suis is an important pathogen for pigs worldwide. This microorganism is associated with meningitis, arthritis, endocarditis, septicemia, pneumonia and sudden death in pigs during post-weaning and growing (1,2,3). *S. suis* is also associated with human infections, and is considered an occupational hazard for abattoir workers, meat workers and veterinarians (4,5,6,7). *S.suis* is a diverse species, approximately 33 serotypes of this entity have been described with differences in pathogenicity and geographic distribution, which can be detected by agglutination with the specific antiserum (8,9,10,11,12,13) and also by amplification of fragments of genes related to the capsule polysaccharide biogenesis (14,15). Serotype 2 strains are considered to be highly virulent based on European and Asian epidemiological studies or experimental infections (15,16).

Several molecular tests have been developed to detect *S. suis* species by means of regions conserved in all the capsular types. Okwumabua *et al.* (18) developed a PCR assay based on the *gdh* gene, which encodes the glutamate deshydrogenase, and Marois et al (19) developed a PCR system for *S. suis* detection by amplifying a fragment of RNAr16s.

Nucleic acid based tests are increasingly used in the bacteriological diagnosis for the speed, sensitivity and specificity, which exceed the benefits of the identification by biochemical tests (18). The isolation and purification of DNA is a key step for most protocols in molecular biological studies including PCR. The various methods proposed to extract and purify DNA from bacterial and yeast can be classified according to the system chosen to break the cells, including beadbeating, enzymatic cell wall lysis or cell permeabilization with chaotrophic agents; generally all the systems either are very time-consuming or they show poor release of DNA (20). The application of a direct PCR from colonies was first performed in rapid characterization studies of *Escherichia coli* strains transformed with plasmids (21). The DNA amplified directly from the colony has been sequenced with as satisfactory results as those obtained from DNA extracted by the conventional phenol-chloroform procedure (22, 23, 24). The aim of this study was to evaluate a method for the rapid release of the genomic DNA from *S.suis* colony by using a physical method based on heating and freezing; in this case, two heating temperatures, 100°C and 95°C, were tested for analyzing by polymerase chain reaction.

A total of 10 isolates of *S.suis* from lungs of pigs with respiratory disorders were cultured on Columbia agar base (Oxoid) supplemented with 5% sheep blood and they were identified with the following criteria: presence of pinpoint colonies with alpha-hemolysis, Gram-positive cocci, negative catalase test and biochemical tests API 20 STREP kit (BiomeÂrieux, Marcy-l'Etoile, France). The conventional phenol-chloroform DNA extraction, followed by ethanol precipitation according to the protocol reported by Douglas et al (25), from overnight broth cultures was used as the control.

For the rapid direct colony PCR, two protocols were followed. The first one consisted of lightly touching a colony of a culture on blood agar with a sterile pipette tip and placing of the collected material into a tube containing 50 µL nuclease-free water, then subjected to boiling at 100°C for five minutes and subsequently frozen at -20°C for 10 minutes, the mixture was centrifuged at 3000 g for 10 minutes. In the second procedure the colony was preheated at 95°C for 10 minutes in the thermal cycler and cooled. In both cases, 5 µL was used for PCR amplification. A colony from both 24 and 48 hour cultures were used. Finally, the mix was added into the two sets of samples separately.

The PCR with extracted genomic DNA as template of strains of *S. suis* was made as reported by Marois *et al.* (19). The primers amplify a fragment of 294 bp of 16S rRNA gene. For genoserotyping, specifically the gene fragment related to the biogenesis of the two capsular type (cps2), the conditions described by Smith et al (26) were followed.

Amplification was performed in a final volume of 25 µL containing 10 mM Tris-HCl, pH 8.3, 50 mM KCl, 3 mM $MgCl_2$; 0.1mg/mL BSA; 10 mM of each dNTP; 20 pmol of each primer and 1 µL of Amplicen (2µ/µL), CENSA, Cuba, and 2µl of template DNA, purified by the method of chemical analysis, was added. Different concentrations of the genomic DNA from one isolate were made to establish the detection limit of the assay, and 5 µL was used for PCR amplification for the DNA extracted from the colony.

All the isolates of *S.suis*, identified by morphological and biochemical criteria, amplified a fragment of 294 bp from the genomic DNA extracted by the method of chemical lyses. The amplification limit of the PCR corresponded to 0.5 ng of chromosomal DNA.

In both cases, colony from 24 and 48 hour cultures, the application of direct colony PCR was successful only when the samples were subjected to boiling at 100°C (Figure 1) but not when heating at 95°C, this latter condition was not sufficient for DNA release . Taking into account the detection limit detected from the genomic DNA (0.5 ng), considering the size reported for *S. suis* genomic DNA (2.14Mpb), assuming that the genome is of GC%=50, then it is possible to estimate a detection limit corresponding to about $2.1x10^4$cells (27). A single 24 hour colony grown on an agar plate contains the number of cells required for PCR amplification of fragment RNAr16s gene and locus fragment linked to biogenesis specific two capsular polysaccharide type.

DNA extraction from Gram-positive bacteria may be more complex than from Gram-negative bacteria and involves multiple steps such as cell wall treatment with enzymes or ionic detergents and cell lysis using mutanolysin and hyaluronidase. These methods are costly, time consuming and often lead to errors when processing a large number of samples (25, 26). For *S. suis* genotyping, methods based on chemical purification which include the use of proteinase K,

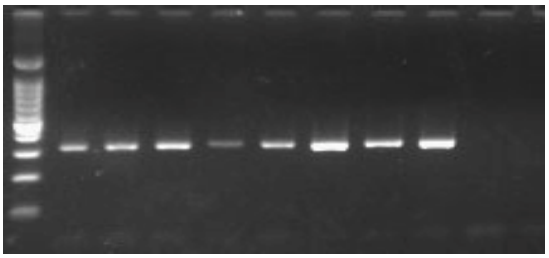

FIGURE 1. PCR products of fragment of RNAr16s from eigth *S.suis* isolates obtained from direct colony of 24 hours: Lane 1: molecular weight 50 PB Promega, lane: 2-9 PCR product of *S.suis,* lane 10: negative control./ *Productos de la reacción de amplificación del RNAr16S a partir de colonias de 24 horas de cultivos de 8 aislados de* ***S. suis****: Línea 1: marcador de peso molecular (50pb) líneas 2-9 amplicón del RNAr16s de 8 aislados de* ***S. suis*** *línea 10: control negativo.*

detergents such as Triton X-100, Nonidet P-40 and washing with phenol and chloroform are described (28). Trudy et al (29) reported the detection of genes in *S. pneumoniae* from a colony which was subjected to a chemical lysis solution and heated at 60ºC for one hour or at 95ºC for 5 minutes.

However, in recent years, several have been the reports on the use of PCR after the rapid extraction of DNA from the colony of Gram-positive bacterial entities. Boiling of the samples has been shown to be a simpler and more economical method for releasing DNA from bacteria (29). The rapid detection of *Staphylococcus aureus* resistant to methicillin was made from the colony DNA without the use of chemical reagents, but, despite the larger size of the single colony of *S. aureus*, 4 to 5 colonies were used,(29). Okwumabua *et al.* (18) used lysis by a boiling method for the PCR assay using *gdh* gene of *S. suis*. Briefly, a single colony of a bacterial isolate grown on sheep blood agar plate was suspended in 100 µl of water and heated at 100ºC for 20 min, followed by centrifugation for 2 min at 13 000g. However, in our study, the use of a singe colony of 24 hours heated at 100ºC for 5 min is enough for the application of the PCR test for genotyping *S.suis* using different genetic markers. This colony is touched with a simple sterile pipette tip; previously, and using the same pipette tip, this colony is placed on a sheep blood agar plate to be sub-cultured for other assays like antibiotic susceptibility and for ensuring the identity of this colony and its conservation.

Jose and Brahmadathan (28) developed a methodology for the typing of the group A of *Streptococcus* spp by PCR from colony where preheating at 95ºC for 2 minutes and then cooling was sufficient for DNA release. It should be noted that several colonies and not a single one were used in their work. However, our results showed that a colony of *S. suis* from a culture of 24 hours was enough for typing *S.suis*.

Although *S.suis* is a bacterium phenotypically well characterized, its identification in the laboratory may be complicated by the morphological and biochemical similarities with other members of this genus that may be present in the respiratory tract of pigs. Baele *et al.* (31) studied Gram-positive tonsillar and nasal microbiota in pigs of 2 and 6 weeks of age and identified the following species of *Streptococcus* spp: *S.suis*, *S. dysgalactiae*, *S. gallolyticus*, *S. bovis*, *S. agalactiae*, *S. cricetus*, *S. hyointestinalis*, *S. hyovaginalis*, *S. sanguinis*, *S. porcinus*, *S. pluranimalium*. *S.suis* was present in all the animals and at concentrations 10^2 to 10^7 ufc; however, none of the isolates corresponded to serotype 2. These data demonstrated the need for a rapid protocol for DNA genoserotyping *S. suis* from mixed primary cultures where other species may be present. The figure 2 shows the amplification products of a fragment of 656 bp of locus cps2j in isolates of *S.suis* directly from colony.

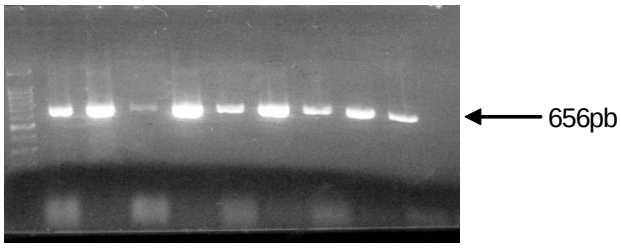

FIGURE 2. PCR products of fragment of cps 2 from nine *S. suis* isolates directly obtained from colony: Lane 1: molecular weight 1 KB Promega, lane: 2-10 PCR product of *S. suis,* lane 11: negative control./ *Productos de la reacción de amplificación del fragmento cps 2 a partir de colonias de 24 horas de cultivos de 9 aislados de* ***S. suis****: Línea 1: marcador de peso molecular (1 KB Promega) líneas 2-10 amplicón del cps de 9 aislados de* ***S. suis*** *línea 11: control negativo.*

It was demonstrated that *S.suis* cells from cultures could be used directly for PCR amplification of target DNA by heating at 100°C for 5 minutes and freezing for 10 minutes at -20°C for cell wall disruption and membrane denaturation; the DNA released was enough for amplification. Thus, these methods can not only replace more cumbersome and time-consuming cell lysate methods, but they also avoids the successive passes needed to obtain pure cultures for the application of biochemical tests and can be used for typing large number of strains in much less time.

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Recibido: 6-12-2012.
Aceptado: 25-1-2013.