RAPID TYPING OF STAPHYLOCOCCUS AUREUS STRAINS OF BOVINE MILK ORIGIN BY USING RANDOM AMPLIFIED POLYMORPHIC DNA ASSAY

M. Parthiban, P. Ramadass, W. Manohar Paul and A. Mahalinga Nainar
Department of Animal Biotechnology
Madras Veterinary College, Chennai-600 007, India

ABSTRACT

Conventional typing techniques were found to be poor in discriminating different S. aureus strains. In this study, a rapid, technically simple RAPD assay was employed to type the S. aureus strains. DNA fragment patterns obtained using this assay gave good level of discrimination to type the S. aureus strains.

INTRODUCTION

Staphylococcal mastitis an important herd problem in dairy farms causes clinical, sub-clinical and chronic mastitis leading to gangrenous condition in dairy cattle. The contaminated milk may be a source of food-borne intoxication in man. A wide range of antibiotic resistance and differences in antibiotic susceptibility within Staphylococcus aureus strains may be the cause for failure of successful treatment of Staphylococcus mastitis. A variety of typing techniques are available to isolate and characterize Staphylococcus strains with the most common technique being bacteriophage typing (Pitt, 1994). However, some strains may be nontypeable by this method. To overcome this limitation, more recently DNA based typing techniques were employed. In the present study, PCR protocols were employed to derive random amplified polymorphic DNA (RAPD) fingerprints in order to develop a fast and reliable technique for typing of different S. aureus strains.

MATERIAL AND METHODS

Sample collection: A total of 123 milk samples were collected in a sterile container from cattle suffering from mastitis in and around Chennai, Tamil Nadu, India.

Isolation and identification of bacterial strains: All S. aureus strains were isolated and identified by routine laboratory procedures. Gram-positive and catalase positive cocci were confirmed to be S. aureus by using staphy-test kit (Medispan Diagnostics, India). The organisms were further confirmed as S. aureus by the tube coagulase test.

DNA isolation: The liquid cultures of bacteria were pelleted by centrifugation at 3000 rpm for 15 min and resuspended in 100 μl of phosphate buffered saline. DNA was isolated using proteinase K digestion followed by phenol-chloroform extraction method described by Sambrook et al. (1989).

The DNA pellet was dissolved in 50 μl of TE buffer (100 mM Tris; 1mM EDTA) containing 20 μl of RNase (10 mg/ml). The final concentration of DNA samples were measured at OD 260 nm and 280 nm using the spectrophotometer (Shimadzu, Japan) and checked in 0.8% agarose gel electrophoresis.

Primer selection: Random Amplified polymorphic DNA typing was performed using three arbitrary primer individually
which has already been used by Tambic 
et al. (1997) for typing of non-phage 
typeable methicillin resistant S. aureus.

<table>
<thead>
<tr>
<th>Primer</th>
<th>Sequence</th>
<th>Size</th>
</tr>
</thead>
<tbody>
<tr>
<td>P1</td>
<td>5' TACACCCGTCAACATTGAGG - 3'</td>
<td>20 mer</td>
</tr>
<tr>
<td>P2</td>
<td>5' ACA ACTGCTC - 3'</td>
<td>10 mer</td>
</tr>
<tr>
<td>P3</td>
<td>5' AGCAGCTGTC - 3'</td>
<td>10 mer</td>
</tr>
</tbody>
</table>

RAPD analysis: The staphylococcal DNA samples were diluted 10 fold and heated to 100°C for 5 min for efficient denaturation. The PCR reagents were supplied by the manufacturer (Finnzyme, Finland). The reaction mixture consists of 5μl of 10x reaction buffer, containing MgCl₂, 1.5Mm MgCl₂, 200μM of nucleotide mix containing all 4 dNTPs, 500 nM primer, 50ng of template DNA and 2 units of Taq DNA polymerase and the mixture was made up to 50μl with nuclease free water.

The RAPD cycling parameters were 94°C for 5 sec, 34°C for 30 sec and 72°C for 1 min for 35 cycles with a final extension at 72°C for 5 min. Reactions were carried out in a thermal cycler (DNA engine, MJ Research, USA). The products were electrophoresed in 1.5% agarose gel in 1 x TBE (Tris Borate EDTA) buffer along with DNA double digest molecular weight marker (Finnzyme, Finland). The gel run for 5-6 hrs at 70V to allow the complete separation of bands. DNA profiles were visualized under U.V. transilluminator (Fotodyne, USA) after ethidium bromide staining.

RESULTS AND DISCUSSION

Out of 123 samples tested, 38% cases were culturally positive for S. aureus. The main factors affecting the clarity of DNA profiles like MgCl₂ conc, primer concentration and annealing temperature were optimised by conducting preliminary experiments. The PCR reaction buffer containing MgCl₂ was used in this experiment. In addition, 1.5 Mm MgCl₂ was also included in the reaction mixture along with 500nM primer yielded a more number of visible bands in DNA fingerprints without any background. Three arbitrary primers were assessed individually for their suitability for use in a RAPD typing. The primer P2 resulted in no amplification for all the S. aureus strains and primer P3 gave amplification for few strains. However, best amplification was obtained using the primer P1.

The molecular weights of DNA bands obtained from each strain were estimated using ultra sum gel documentation system TUI 6000 model, U.S.A. Based on number of common bands and molecular weights, these strains were grouped into six different types (Table-1).

<table>
<thead>
<tr>
<th>Group</th>
<th>Group 2</th>
<th>Group 3</th>
<th>Group 4</th>
<th>Group 5</th>
<th>Group 6</th>
</tr>
</thead>
<tbody>
<tr>
<td>Molecular</td>
<td>836</td>
<td>861</td>
<td>861</td>
<td>861</td>
<td>770</td>
</tr>
<tr>
<td>Weight</td>
<td>761</td>
<td>578</td>
<td>779</td>
<td>669</td>
<td>658</td>
</tr>
<tr>
<td>Expressed in</td>
<td>676</td>
<td>528</td>
<td>640</td>
<td>553</td>
<td>571</td>
</tr>
<tr>
<td>bp</td>
<td>465</td>
<td>578</td>
<td>391</td>
<td>484</td>
<td>484</td>
</tr>
<tr>
<td>Group in which the</td>
<td>2,7,9</td>
<td>3,8</td>
<td>4,5</td>
<td>11,12</td>
<td>6,10</td>
</tr>
<tr>
<td>S. aureus strains belongs</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Different DNA fragment patterns were obtained using RAPD method clearly indicating suitability of RAPD assay to differentiate strains of S. aureus in mastitis cases. The traditional phenotypic methods like antibiogram may be poorly discriminatory as reported by Blane et al. (1994) and phage typing may not be suitable for typing all strains.
of Staphylococcus as reported by Tambic et al. (1997). Recently, DNA based typing methods were employed. The consistent results may not be obtained using plasmid profile study, as some bacterial strains may spontaneously lose plasmid or acquire new plasmids. Similarly, restriction enzyme analysis of genomic DNA has frequently been used to type S.aureus strains whereas the discriminatory power of this technique depends on the type of restriction enzymes used (Richardson et al. 1994). PCR-RFLP assay was employed to type S.aureus (Van Belkum et al., 1995). The designing of primer for PCR followed by suitable restriction enzyme for differentiation of strains are the major constraints in this assay. In case of RAPD only arbitrary primers are used so this assay requires no prior knowledge of the genome.

Hence in the present study, a rapid and technically simple RAPD assay was employed to type the S.aureus strains. Good reproducibility of RAPD has been well observed when emphasis was given for suitable PCR temperature profiles and MgCl₂ salt concentration. In conclusion, RAPD method was found to be a rapid and simple, and gave good level of discrimination to characterize different S.aureus isolates.

REFERENCES