Short Communications

Strain Differentiation of *Salmonella* Bareilly by AP-PCR

M K Saxena\(^1\), V P Singh\(^1\), Anjani Saxena\(^2\),
B D Lakhchura\(^3\) and B Sharma\(^4\)

\(1\)Department of Bacteriology and Mycology, Indian Veterinary Research Institute, Izatnagar 243 122, India
\(2\)Department of Molecular Biology and Genetic Engineering,
College of Basic Sciences, G B Pant University of Agriculture and Technology, Pantnagar 263 145, India
\(3\)Division of Biochemistry, Indian Veterinary Research Institute, Izatnagar 243 122, India

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Several PCR based methods such as AP-PCR, ERIC, ERIC-PCR, REP-PCR and RAPD have been used for bacterial strain differentiation and identification. These methods are rapid, reliable and economical. In the present study, out of 10 strains of *Salmonella* Bareilly differentiated by AP-PCR, seven profiles were obtained. The study indicates that AP-PCR is an efficient tool for strain differentiation and can be used for molecular epidemiological studies. This is the first report in India in which AP-PCR has been used for molecular typing of *Salmonella* Bareilly.

Keywords: molecular typing, ribotyping, AP-PCR, ERIC-PCR, REP-PCR

Introduction

Salmonellosis, one of the important diseases of animals, causes high mortality and morbidity and thus has a high zoonotic index. Due to considerable variation among *Salmonella* strains, proper identification and differentiation is important for eradication of the disease. Several methods such as phage typing (Threlfall & Frost, 1990), biotyping (Christensen et al., 1992) and outer membrane protein profiles (Helmuth et al., 1985) have been widely used for typing of *Salmonella*. These conventional methods are, however, time consuming and often result in ambiguous identification. DNA based techniques (ribotyping & pulse field gel electrophoresis), though replacing the conventional methods but are technically more demanding and require sophisticated laboratory facilities. Several PCR based methods, used for identification of the organism, are faster, relatively simple and economical. AP-PCR, which involves use of arbitrary primer for analysis of variability in genome, has been used in *Salmonella* types (Fadl et al., 1995; Hilton & Penn, 1998; Kerounton et al., 1996; Guo et al., 1999; Radu et al., 2001). The present study was conducted with the objective to differentiate the isolates of *Salmonella* Bareilly by AP-PCR.

Ten isolates obtained from two different places and a reference strain obtained from National Salmonella Centre (IVRI), Izatnagar were used in this study (Table 1). The reference strain of *Salmonella* Bareilly was imported from Salmonella Reference Laboratory, Canada. The remaining strains were isolated from field samples and serologically types as *Salmonella* Bareilly on the basis of O and H antigen. These strains were tested for their growth and colony characters on Macconkey’s lactose agar and Brilliant green agar.

Single colony was inoculated in 5 ml of Luria Bertini broth and incubated for 18 hrs in a shaker incubator at 37°C. One ml of culture was centrifuged at 5,000 rpm for 10 min and pellet was suspended in 100 μl of sterilized distilled water. The bacterial suspension was kept at 100°C in water bath for 10 min and was immediately transferred to ice for 10 min. These treatments caused lysis of the cells. Lysate was centrifuged at 6,000 rpm for 10 min. The supernatant (10 μl) was used in 50 μl of PCR reaction mixture containing 200 μM dNTPs, 1 × PCR buffer, 10pmol primer (5’TGA GCATAG ACC TCA3‘), 3 units of Taq polymerase and 2.5 mM MgCl₂ (Hilton & Penn, 1998). Negative control did not contain the cell lysate. PCR was conducted in standard conditions with the following programme: initial denaturation at 94°C for 5 min followed by 40 cycles of denaturation at 94°C for 1 min; annealing at 35°C for 1 min; and extension at 72°C for 1 min followed by a final extension at 72°C for 1 min. Five μl of amplified product was loaded on 7.5% acrylamide gel with 1 μl of 5× loading dye [0.25% bromophenol blue, 0.25% xylenes cyanol, 40% (w/v) sucrose in water]. Gels were run at 1.5 volt/cm for 3 hrs and stained with ethidium bromide (0.5 μl/ml). Bands were visualized under UV transilluminator and photographed by gel

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\(4\)Author for correspondence:
Tel: 0581-2441638; Fax: 0581-2447284
E-mail: mumtesh@rediffmail.com
Table 1—AP-PCR isolates of *Salmonella* Bareilly

<table>
<thead>
<tr>
<th>No.</th>
<th>Serovar No.</th>
<th>Place of isolation</th>
<th>Host of isolation</th>
<th>AP-PCR profile</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>E90</td>
<td>Canada</td>
<td>Sheep</td>
<td>P1</td>
</tr>
<tr>
<td>2</td>
<td>E997</td>
<td>Pantnagar</td>
<td>Sheep</td>
<td>P2</td>
</tr>
<tr>
<td>3</td>
<td>E1324</td>
<td>Pantnagar</td>
<td>Sheep</td>
<td>P2</td>
</tr>
<tr>
<td>4</td>
<td>E1329</td>
<td>Pantnagar</td>
<td>Sheep</td>
<td>P3</td>
</tr>
<tr>
<td>5</td>
<td>E1322</td>
<td>Izatnagar</td>
<td>Chick</td>
<td>P4</td>
</tr>
<tr>
<td>6</td>
<td>E734</td>
<td>Izatnagar</td>
<td>Chick</td>
<td>P5</td>
</tr>
<tr>
<td>7</td>
<td>E733</td>
<td>Izatnagar</td>
<td>Chick</td>
<td>P4</td>
</tr>
<tr>
<td>8</td>
<td>E1317</td>
<td>Izatnagar</td>
<td>Chick</td>
<td>P4</td>
</tr>
<tr>
<td>9</td>
<td>E736</td>
<td>Izatnagar</td>
<td>Sheep</td>
<td>P6</td>
</tr>
<tr>
<td>10</td>
<td>E735</td>
<td>Pantnagar</td>
<td>Chick</td>
<td>P7</td>
</tr>
</tbody>
</table>


documentation system. Each sample was amplified thrice to check for artifacts.

All samples, except negative control, showed amplification. Out of ten strains studied, seven profiles were observed (Fig. 1). In amplified products, the size were ranged from 400 bp to 3.0 kb. In the reference strain E90, a different profile (P1), which was composed of 400, 550, 800, 980, 200 and 2,500 bp products, was observed. This profile was not shown by other field isolates. Profile P2 observed in E997 and E1324 consisted of 650, 800 and 980 bp products. Profile P3 contained an extra band of 600 bp in addition to other bands of profile P2. Profile P4, which was observed in E1322, E733 and E1317 strains, seems genetically closer to reference strain as in both these strains only band of 400 bp was absent, remaining profile were similar to P1. In E734, profile P5 was obtained and comprised bands of 680, 800 and 980 bp. In profile P6, three bands of size 550, 800 and 200 bp were observed. In P7, only two bands of 800 and 980 bp were observed. Band of 800 bp, which was observed in all the strains, may be *Salmonella* specific loci. The reference strain produced a profile, which was different from field isolates. The field isolates had also shown genetic variation among them. Only profiles P2 and P4 were repeated. So, it can be presumed that the AP-PCR can detect genetic variation efficiently. Reference strain E90 was isolated in Canada. The P4 profile was observed in three strains (E733, E1317 & E1322), which were isolated from chicks of same location (Izatnagar) and may be of same genetic makeup. Similar types of results were observed in case of E997 and E1324 strains isolated from sheep of same place (Pantnagar). Both the strains show P2 profile.

Earlier reports on AP-PCR also indicated that it could be used for strain differentiation of *Salmonella* (Guo et al, 1999). Radu et al (2001) reported ten different profiles out of ten strains studied. Hilton & Penn (1988) compared ribotyping and AP-PCR for molecular typing of *Salmonella* and reported that AP-PCR is more discriminative than ribotyping. Kerouanton et al (1996) compared zymotyping, ribotyping, enterobacterial repetitive intergeneric consensus PCR (ERIC-PCR) and repetitive extragenetic palindromic-PCR (REP-PCR) and found AP-PCR to be more discriminative in comparison to zymotyping, ERIC-PCR and REP-PCR.

On the basis of this study and the findings of other workers, it can be concluded that AP-PCR is a very efficient tool for molecular typing of *Salmonella* strains.

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References


