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Veterinary Microbiology 114 (2006) 345–351
veterinary microbiology
www.elsevier.com/locate/vetmic

Short communication

Improvement and validation of RAPD in combination with PFGE analysis of *Salmonella enterica* ssp. *enterica* serovar Senftenberg strains isolated from feed mills

Charlotta Löfström [a,b], John Eriksson [a], Anna Aspán [c], Per Häggblom [c], Anders Gunnarsson [c], Elisabeth Borch [d], Peter Rådström [a,*]

[a] *Applied Microbiology, Lund University, P.O. Box 124, SE-221 00 Lund, Sweden*
[b] *AnalyCen Nordic AB, P.O. Box 905, SE-531 19 Lidköping, Sweden*
[c] *National Veterinary Institute, SE-751 89 Uppsala, Sweden*
[d] *SIK, Ideon Research Park, SE-223 70 Lund, Sweden*

Received 19 October 2005; received in revised form 9 December 2005; accepted 13 December 2005

Abstract

In 1995 and 1996 a Swedish feed mill had problems due to a persistent contamination of *Salmonella enterica* spp. *enterica* serovar Senftenberg that was difficult to eliminate. Forty-eight strains isolated from the feed mill, together with unrelated strains included to evaluate the discriminatory power and reproducibility, were analysed by pulsed-field gel electrophoresis (PFGE). The source of contamination in the feed mill was identified and preventative measures were taken, that led to a resolution of the problem. A previously developed randomly amplified polymorphic DNA (RAPD) protocol was used, to evaluate a rapid and low-cost alternative to PFGE typing. The use of the alternative thermostable DNA polymerase *Tth* was shown to increase the reproducibility of the RAPD analysis. The reproducibility, in terms of Pearson's and Dice's similarity coefficients for duplicate runs, increased from 72.0 ± 16.9% and 72.3 ± 12.9% for *Taq* to 91.6 ± 7.5% and 90.9 ± 5.3% for the fingerprints obtained for the RAPD method employing *Tth* DNA polymerase. Simpson's index of diversity was calculated and found to be 0.580 for RAPD and 0.896 for PFGE. All of the seven RAPD types could be subdivided into one or more PFGE types, whereas none of the 22 PFGE types was divided into more than one RAPD type. RAPD provides a simple, rapid and powerful screening method that can be used to initially select isolates for further analysis by PFGE.

Keywords: Animal feed; Genotyping; Pulsed-field gel electrophoresis; Randomly amplified polymorphic DNA; *Salmonella*

Salmonella is a predominantly zoonotic bacterium of substantial importance. There is evidence that *Salmonella* can be transmitted to humans via animals infected by consuming contaminated feed (Jones

* Corresponding author. Tel.: +46 46 222 3412; fax: +46 46 222 4203.
E-mail address: Peter.Radstrom@tmb.lth.se (P. Rådström).

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doi:10.1016/j.vetmic.2005.12.015

et al., 1982; Hinton, 1988) and, therefore, the control of *Salmonella* in feed production is essential to ensure safety of the food supply. *Salmonella* Senftenberg is one of the most important feed-related serovars, and it has frequently been isolated from feed ingredients, feed products and environmental samples from feed factories (Bailey et al., 2001; Nesse et al., 2003). *S.* Senftenberg has caused several food-borne outbreaks of salmonellosis (L'Ecuyer et al., 1996; Rushdy et al., 1998; Mohle-Boetani et al., 2001). Furthermore, *S.* Senftenberg has been suggested to be more resistant to stress such as acidification, heating, desiccation and irradiation than other serovars (Liu et al., 1969). This might contribute to the establishment of a long-lasting flora of *S.* Senftenberg in a feed mill environment.

Hazard analysis critical control point systems can be used to limit the spread of *Salmonella* in feed production and to eliminate sources of contamination (Malmqvist et al., 1995). For efficient identification of these sources, there is a need for fast and reliable detection (Löfström et al., 2004) and typing methods with the ability to discriminate between strains. A number of DNA-based typing methods for *Salmonella* spp. have been developed, including amplified fragment length polymorphism (AFLP) (Aarts et al., 1998) pulsed-field gel electrophoresis (PFGE) (Liebana et al., 2001), randomly amplified polymorphic DNA (RAPD) (Hilton et al., 1997; Laconcha et al., 1998; Soto et al., 1999; De Cesare et al., 2001), and ribotyping (De Cesare et al., 2001). PFGE is considered to be the "gold standard" for epidemiological genotyping and is used today worldwide to trace outbreaks (Fisher, 1999; Swaminathan et al., 2001).

In 1995 and 1996 a Swedish feed mill was affected by a persistent contamination of *S.* Senftenberg, which was difficult to eliminate. In this present study, 48 strains of *S.* Senftenberg were included (Table 1), of which 17 originated from the feed mill and the rest were collected during 1995 and 1996 by the National Veterinary Institute (SVA) in Uppsala, Sweden. To try to trace the emission pathway, a subtyping study of the strains isolated from the feed mill was made using PFGE. All *S.* Senftenberg strains sent to the SVA during the same period of time were also analysed, to provide reference material. In order to assess the use of other more rapid subtyping methods for *S.* Senftenberg in feed, a previous RAPD protocol (Eriksson et al., 2005) was used. The influence of two different DNA polymerases on the RAPD results was, furthermore, investigated in an attempt to improve the reproducibility of the method. The RAPD method was evaluated on the 48 *S.* Senftenberg strains previously analysed by PFGE.

The strains collected during 1995–1996 were kept frozen at −70 °C in serum broth with 15% of glycerol. *Salmonella* cells used for RAPD analysis were grown on tryptone glucose extract (TGE, Merck, Darmstadt, Germany) agar plates and incubated at 30 °C for 24 h. From each plate one colony was transferred to a sterile Eppendorf tube with 1 ml sterile buffered peptone water (BPW, Lab 46, Lab M, Bury, England). The tubes were incubated in a water bath at 28 °C for 24 h and then centrifuged in a table centrifuge (Hermle Z 160 M, Hermle Labortechnik, Wehingen, Germany) at 14,000 rpm for 3 min. The supernatant from each tube was removed and the pellet was resuspended in 200 μl 0.9% (w/v) NaCl. DNA isolation and purification were performed according to Protocol #3 of the Easy DNA™ Kit (Invitrogen, Groningen, The Netherlands). The DNA concentrations were measured using a TD-700 Laboratory Fluorometer (Turner Designs, Sunnyvale, CA, USA) with the fluorescent ds-DNA binding dye PicoGreen® (Molecular Probes, Eugene, OR, USA). The DNA preparation from each strain was diluted 10 times with DNA-free double distilled water and then stored at −20 °C until required. The DNA concentration in the DNA preparations used for RAPD analysis was 970 ± 370 ng/ml.

The reaction mixture and conditions for RAPD analysis, including agarose gel electrophoresis have been described previously (Eriksson et al., 2005). One exception from this protocol was that when *Taq* DNA polymerase was used the annealing temperature was adjusted to 36 °C. DNA for PFGE analysis was prepared according to Christensen et al. (1994), with slight modifications (Eriksson et al., 2005).

The gel images from RAPD and PFGE were saved in TIFF format and then imported into the computer software GelCompar II (Version 3; Applied Maths, Sint-Martens-Latem, Belgium). Using the unweighted pair group method with arithmetic averages (UPGMA), cluster analysis was performed with the Dice (1945), or Pearson (1926) product moment correlation method. Simpson's index of diversity (Hunter and Gaston, 1988) was used for calculation of the discriminatory index of each genotyping method.