Randomly amplified polymorphic DNA (RAPD) typing of Salmonella Senftenberg in animal feed production.

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Summary: Randomly Amplified Polymorphic DNA (RAPD) was studied as genotyping method typing for a strain collection of Salmonella, belonging to the serotype Senftenberg. This collection consisted of 48 strains/isolates that were sampled in Sweden during 1995-96. In this study the aim was evaluate the usefulness of RAPD in terms of discriminatory power, reproducibility and typeability to compare it with other genotyping methods. By using Taq and Tth DNA polymerase in separate reactions the ability to increase discriminatory power and reproducibility was studied. When Tth DNA polymerase was used in the RAPD reaction brighter bands were obtained and the reproducibility was increased. The results suggest that RAPD can be used for rapid screening of a strain material and in combination with other more discriminating genotyping methods to more truly reflect the genetic diversity.

Key words: animal feed, DNA polymerase, genotyping, RAPD

Introduction: Salmonella spp. are some of the most serious contaminants of food products and thus is of major concern to the food industry. These bacteria are well-known agents for food-borne outbreaks of human gastroenteritis (salmonellosis) in Europe. Salmonella is transmitted to humans via animals infected by consuming contaminated feed (Hinton, 1988; Jones et al, 1982). The development of molecular markers as well as improved detection methods for DNA has been of great importance and will in the future help to prevent the spread of infections and provide data about the best choices for treatment (Ling et al, 1998). In this particular case the goal was to compare S. Senftenberg isolates from an animal feed factory in Sweden, where it had been established as a house flora, with other more or less related isolates. S. Senftenberg is considered to be more resistant towards acidification, heating, desiccation and irradiation compared to other serotypes (Liu et al, 1969), which makes the risks for sanitizing problems bigger. RAPD is both a less costly and more time-efficient method than several other genotyping methods, like ie Pulse-field gel electrophoresis (PFGE). One particular aim of this study was to see how well it could be discriminate between epidemiologically related strains at the subserotype level.

Material and methods: From the collection of strains of S. Senftenberg samples were streaked onto Tryptone Glucose Agar (TGE) plates, to check for their purity. One colony was transferred to buffered peptone water (BPW). DNA was subsequently isolated, using the Easy DNA™ Kit (Invitrogen). The DNA concentrations were measured using a fluorometer (Turner Designs). From each DNA preparation (1:10 dilution in ddH₂O) 5 ml was taken to RAPD analysis. The total volume was 25 ml in each reaction tube. The PCR reagents that were added to each sample contained 1 x PCR buffer for each enzyme, supplied with 1U of Taq or Tth DNA polymerase (Roche), 0.2 mM of each of the four nucleotides dATP, dCTP, dGTP and dTTP (Roche), 1.0 mM of primer S 1254, sequence (5’Æ 3’): CCG CAG CCAA (Scandinavian Gene Synthesis) and 2.5 mM of MgCl₂ (Roche). The temperature programme of the thermal cycler included: an initial denaturation step of five minutes at 94 °C, 45 cycles of denaturation (30 seconds at 94 °C), annealing (30 seconds at 30 or 36 °C) and extension (40 seconds at 72 °C) and a final extension step of seven minutes at 72 °C, followed by cooling to 4 °C. The amplification products were analysed by electrophoresis, using 1 % agarose gel. Cluster analysis by GelComparII was performed,
using the unweighted pair group method with arithmetic averages (UPGMA) for the Dice coefficient (band based) and Pearson correlation (curve based) method, respectively. To illustrate the reproducibility of fingerprints from single strains, five duplicates (from different gels) were used in a cluster analysis, where the average similarity coefficient from duplicates was calculated (see Figure 1). Finally, a numerical index of discrimination (D) (Hunter & Gaston, 1988) was calculated for RAPD analysis.

**Results:** Cluster analysis by GelComparII gave in all 9 RAPD types with Tth DNA polymerase, identified with at a similarity index of >80%. The average number of reproducible electrophoresis bands per strain was 12.0 for Taq and 12.8 for Tth DNA polymerase. No correlation was found between the DNA concentration of the sample preparations and the number of electrophoresis bands, both for the RAPD analysis with Taq and Tth DNA polymerase, respectively. In the calculation example of reproducibility for PCR duplicates (Figure 1) a similarity coefficient between 83 and 97% was obtained (average 88%). The index of discrimination was calculated for Tth DNA polymerase (D = 0.614). When both enzymes are used for this calculation, the index of discrimination will become higher.

**Discussion:** The result from the analyses with Tth DNA polymerase was that they generally gave more distinct RAPD fingerprints than those made with Taq DNA polymerase. The similarity level within the clusters in the dendrograms for Tth was generally higher than for Taq DNA polymerase, regardless of which clustering method that was used. In general it seemed that the use of Tth DNA polymerase increased stability of the PCR system. The RAPD fingerprints from the experiments showed that there are several demands that have to be met in order to make a correct statistical analysis by GelComparII. First the cluster analysis should preferably be based on a limited part of the gel. The part of the gels with the generally highest number of discriminating bands should be chosen, to make sure that the fingerprints are analysed in the most optimal way. To achieve the highest possible reproducibility the operations and equipment for RAPD analysis need to be well standardised. The results also showed that RAPD can be used for typing of strains within the serotype SSenftenberg.

**Acknowledgements:** This work was financially supported by the Swedish Agency for Innovation Systems (VINNOVA), and the Foundation of the Swedish Farmers Supply and Crop Marketing Cooperation (SL-stiftelsen).
SYBR Green Real-Time PCR for Salmonella
detection in meat products

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Summary: The objective of this study was to develop a SYBR Green Real-Time PCR method for detecting salmonellae in meat samples. The study was conducted both on S. Typhimurium experimentally and naturally contaminated meat samples analyzed in parallel with the standard cultural method (ISO 6579/2001). After the pre-enrichment phase, a boiling DNA extraction procedure combined with SYBR-Green I Real Time PCR, using primers Styinv-JHO-2, was developed. The specificity of the reaction was confirmed by the Melting Temperature (Tm), which was consistently specific for the amplicon obtained (S. Typhimurium Tm=77.33±0.058). The standard curve constructed using the mean threshold cycle (Ct) and various concentrations of S. Typhimurium (ranging from 10^3 to 10^8 cfu/ml) showed a good linearity (R²=0.9767) and a sensitivity limit of less than 10^3 cfu/ml. The comparison with the ISO method confirmed the effectiveness of the proposed method.

Keywords: rapid methods, pathogens detection, food.

Introduction: Salmonella continues to be one of the major causes of food poisoning in the western world. Different methods have been developed in order to reduce the time for the detection of the salmonella from food, since the ISO standard cultural method requires up of five days. Many of the PCR assays employ either visual scoring of ethidium bromide-stained agarose gels or post-PCR hybridisation-capture methods that are labour intensive, time consuming and difficult to automate. Recently, the use of double stranded DNA binding dye SYBR Green I for the detection of PCR product allows an early and simple approach to the Real-Time PCR and require less knowledge than classic Real-time PCR using fluorogenic oligoprobes (Hoorfar et al., 2000). The objective of the present study was to develop a SYBR Green I Real-Time PCR method for the detection of salmonellae in meat products. The experiments were conducted on S. Typhimurium experimentally and naturally contaminated meat samples and the results were compared to the standard cultural method.

Materials and methods: a sample was experimentally contaminated as follows: twenty-five grams of pork meat homogenized with 225 ml of buffered peptone water in a Stomacher were incubated at 37 °C for 24 h. The pre-enrichment broth was then divided into three aliquots: the first aliquot was used to confirm the absence of salmonellae by means of standard cultural method (ISO 6579/2001);