# Comparison of REP-, BOX-, ERIC- and $(GTG)_5$-PCR for molecular discrimination of *Salmonella* serotypes

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## Summary

Four repetitive-element polymerase chain reaction (rep-PCR) techniques (primersets ERIC1R, ERIC2 and REP1R, REP2I and primers BOXA1R and $(GTG)_5$) were evaluated for the discrimination of *Salmonella* isolates at serotype level. On the basis of number, even distribution over the whole fingerprints and clarity of bands in the fingerprints, the ERIC primerset and the $(GTG)_5$ primer were chosen to use in the following experiments. For these two primer(set)s, the reproducibility was tested on different lysates of selected *Salmonella* serotypes in the same PCR and using different PCR runs. The reproducibility between different PCR runs was poor, but high within the same PCR run. Furthermore, more than 50 different serotypes and some isolates which were not-typable by serotyping were fingerprinted to test the typability and the discriminatory power of the selected primer(set)s. All strains were typable by the ERIC primerset and the $(GTG)_5$ primer and generated unique fingerprints, except for some strains with incomplete antigenic codes. Finally, genetically different strains belonging to different serotypes were fingerprinted to examine the stability of the rep-PCR within serotypes. This experiment showed that one serotype does not always correlate to only one ERIC or $(GTG)_5$ fingerprint, but that the fingerprint heterogeneity within a serotype was limited. In epidemiological studies, ERIC- and/or $(GTG)_5$-PCR can be used to limit the number of strains that has to be serotyped.

## Introduction

*Salmonella enterica* is one of the major causes of human gastroenteritis worldwide. The internationally used characterizing method for *Salmonella* is serotyping the isolates according to the Kauffmann-White scheme (Popoff and Le Minor, 1997). Each *Salmonella* serotype is characterized by the combined expression of particular lipopolysaccharides or O antigens and flagellar proteins or H-antigens. Currently, more than 2500 serotypes are recognized (Popoff, 2004). Serotyping is in most countries restricted to national reference laboratories to which clinical and food microbiology laboratories send their *Salmonella* isolates. Serotyping, especially of less abundant types, is expensive and time consuming. PCR based molecular techniques are easy to perform and rapid. Repetitive-element polymerase chain reaction (rep-PCR) uses primers complementary to naturally occurring, highly conserved, repetitive DNA sequences. These non-coding sequences are in multiple copies present in the genomes of most Gram-negative and several Gram-positive bacteria Examples of these repetitive elements are the repetitive extragenic palindromic (REP) sequences, the enterobacterial repetitive intergenic consensus (ERIC) sequences, the BOX sequences and the polytrinucleotide $(GTG)_5$ sequence (Lupski and Weinstock, 1992).

The purpose of this study was to evaluate the different rep-PCR techniques for the discrimination of *Salmonella* isolates. Selected rep-PCR techniques were further evaluated for the discriminating power and the stability within several serotypes.

## Materials and methods

**Salmonella isolates.** The *Salmonella* isolates were isolated from the following sources: human faeces, poultry meat and eggs, pigeons, cattle, swine, deer, reptiles, water, farm environment and farm equipment. They were serotyped at the Belgian Salmonella reference laboratories according to the Kauffman-White scheme (Popoff and Le Minor, 1997).

**DNA isolation.** The bacteria were grown overnight on Tryptone Soya Agar plates at 37°C. The cells were harvested and resuspended in 300 µl 0.05M NaOH, 0.125% SDS (w/v) and heated at 90°C for 17 min.

**PCR.** PCR amplifications were performed in a Perkin Elmer 9700. The sequence of the used primers and the amplification protocols were as described by Versalovic *et al*. (1994). The reaction mix was made as described by Rademaker and de Bruijn (1997) but Tween20 (0.5%) and gelatin (0.01%) were added. The PCR products were size separated in a 1.5 % agarose gel in 1 x TBE at 120 V for 4h. The gels were stained with ethidiumbromide and digitally captured under UV light. The gel images were visually compared and analysed with GelCompar version 3.0 (Applied Maths, Kortrijk, Belgium).

**Experiment 1.** *Salmonella* strains belonging to different serotypes were typed with the primersets 1/ ERIC1R and ERIC2, or 2/ REP1R and REP2I and the primers 3/ BOXA1R or 4/ $(GTG)_5$. The best primer(set)s were selected on the basis of the number, distribution and clarity of bands in the obtained fingerprints.

**Experiment 2.** Different lysates were made on separate days, starting from different bacterial cultures of some selected serotypes. The reproducibility was evaluated on the different lysates and using different PCR runs on the same thermal cycler.

**Experiment 3.** More than 50 different serotypes and some isolates which were not typable by serotyping were fingerprinted to test the typability and the discriminatory power of the selected primers.

**Experiment 4.** Strains belonging to different serotypes isolated from different origin and genetically different, were fingerprinted to examine the stability of the rep-PCR with the selected primer(set)s; if different strains of the same serotype result in the same fingerprint. The genetic diversity of the strains had been tested by Pulsed Field Gel Electrophoresis using *Xba*I as restriction enzyme (Botteldoorn *et al*., 2004).

## Results and discussion

**Experiment 1.** On the basis of the number, clarity, even distribution of bands over the whole fingerprints and discriminatory power, the ERIC primerset and the primer $(GTG)_5$ were chosen to use in the following experiments.

**Experiment 2.** The serotypes clustered together within its serotype with a minimum similarity coefficient of 74% for the ERIC primerset and 83% for the $(GTG)_5$ primer. The low similarity coefficients were the result of different PCR runs. Within one PCR run, the minimum similarity coefficient between the three different lysates was 95% for the ERIC primerset, and 94% for the $(GTG)_5$ primer with a few exceptions. These exceptions were due to overall weaker patterns, locally weaker bands or normalisation errors. Based on these results, the delineation levels for serotype discrimination for the following experiments was set on 95% for the ERIC primerset and 94% for the $(GTG)_5$ primer. Since the reproducibility was poor between different PCR runs but high within the same PCR run, the following experiments were done within one PCR run.

**Experiment 3.** All strains, including the isolates which were not typable by serotyping, generated fingerprints both with the ERIC primerset as with the $(GTG)_5$ primer. At a delineation level of 95% for the ERIC primerset, all serotypes had unique fingerprints with the exception of three pairs of two strains. At a delineation level of 94% for the $(GTG)_5$ primer, all strains had unique fingerprints with the exception of 9 strains. These exceptions were strains, which were not-typable by serotyping or strains with incomplete antigenic codes. The not-typable strains clustered together with other (typable) serotypes and the strains with incomplete antigenic codes clustered together with strains with similar and complete antigenic codes. This clearly shows that rep-PCR can reveal additional information when serotyping is not possible or when some somatic or flagellar factors were not expressed during serotyping.

**Experiment 4.** This stability experiment showed that not all isolates from the same serotype had the same fingerprint. However, the isolates from the same serotype still clustered together at a similarity coefficient of 85% or higher. The fingerprint heterogeneity within a serotype seems to be limited to the absence or presence of mostly one and sometimes two bands for a primer(set) or to differences in intensities of some bands.

## Conclusion

In certain epidemiological studies, ERIC-PCR and/or $(GTG)_5$-PCR can be used to limit the number of strains that has to be classically serotyped, although it is only useful with isolates analyzed in one PCR run. An alternative is to include in the new PCR run some isolates (e.g. from each cluster one) that were already processed in a previous PCR run. The advantages for this technique is that only one isolate of each cluster has to be sent to the national reference laboratories for serotyping, that non-typable strains can be molecular serotyped on the basis of the relationship to known serotypes and that serotyping errors can be revealed. The reproducibility of isolates in one PCR run, the discriminatory power and the stability of the fingerprint is for both primers very similar, so both primers are equally able to discriminate *Salmonella* serotypes.

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