



Revista Brasileira de Ciência Avícola

ISSN: 1516-635X

revista@facta.org.br

Fundação APINCO de Ciência e Tecnologia  
Avícolas  
Brasil

Baratto, CM; Gelinski, JMLN; Bernardi, AZ; Marafon, A; Braun, F  
Potential Use of Molecular-Typing Methods for the Identification and Characterization of Salmonella  
Enterica Serotypes Isolated in the Poultry Production Chain  
Revista Brasileira de Ciência Avícola, vol. 14, núm. 3, julio-septiembre, 2012, pp. 173-179  
Fundação APINCO de Ciência e Tecnologia Avícolas  
Campinas, SP, Brasil

Available in: <http://www.redalyc.org/articulo.oa?id=179724984003>

- How to cite
- Complete issue
- More information about this article
- Journal's homepage in redalyc.org

redalyc.org

Scientific Information System  
Network of Scientific Journals from Latin America, the Caribbean, Spain and Portugal  
Non-profit academic project, developed under the open access initiative



## **Potential Use of Molecular-Typing Methods for the Identification and Characterization of *Salmonella* Enterica Serotypes Isolated in the Poultry Production Chain**

### ■ Author(s)

Baratto CM  
Gelinski JMLN  
Bernardi AZ  
Marafon A  
Braun F

### ■ Mail Address

Corresponding author: César Milton Baratto.  
Universidade do Oeste de Santa Catarina –  
Molecular Biology Laboratory - building K.  
198, Paese Street. CEP.89560-000 Videira-  
SC-Brazil. E-mail: cesar.baratto@unoesc.  
edu.br  
Phone number: +55 49 35334479 Fax: +55  
49 35334422

### ■ Keywords

Molecular analysis, *Salmonella*, serotypes,  
typing.

### ABSTRACT

*Salmonella* is widespread in nature and can be found in all links of the poultry production chain. Due to its high impact on meat processing, techniques for the rapid detection and reproducible characterization of *Salmonella* serotypes in foods are needed. The present study investigated the potential of molecular profiling to identify and differentiate 15 *Salmonella* serotypes isolated from the poultry production chain, based on 5 primers by random amplified polymorphic DNA (RAPD), enterobacterial repetitive intergenic consensus (ERIC-PCR), amplification of rDNA internal spacer analysis (RISA), and amplified ribosomal DNA restriction analysis (ARDRA) of 16S-23S rRNA internal spacer region (ISR) cleaved with Alu I and Hha I restriction enzymes. Three isolates of each serotype were analyzed for the identification of similar and different profiles. Dendrograms were constructed from molecular profiles using the UPGMA method (unweighted pair-group method for the arithmetic averages) and the software program WinBoot. The present study indicates the usefulness of RISA and ARDRA of the 16S-23S rRNA intergenic spacer region (ISR) for systematic, epidemiological, and diagnostic purposes. Since these techniques can be used for the differentiation of serotypes, they are highly promising for the characterization of *Salmonella* serotypes and intra-serotypes. Data indicate that these techniques may be used to produce more consistent, reliable, and reproducible results in the identification and epidemiological study (traceability) of *Salmonella* in the poultry industry.

### INTRODUCTION

Salmonellosis is one of the most common infectious diseases in the world and affects both animals and humans. *Salmonella* infections may cause gastroenteritis, involving an abrupt onset of nausea, fever, vomiting, and diarrhea, with several virulence factors involved (Baumler *et al.*, 1998; Schaechter *et al.*, 2001). Some outbreaks affecting the population are caused by specific *Salmonella* serovars (serotypes), and the difficulty in detecting carriers makes these a potential source of contamination, particularly due to detection limitations of culture techniques, which are also time-consuming (Robinson *et al.*, 1995; Aspinall *et al.*, 1992).

Moreover, about 2,600 *Salmonella* serovars are known and new serovars, which may potentially be foodborne pathogens, have been discovered (Guibourdenche *et al.*, 2010). This high number and wide diversity of serovars causes the nomenclature of the *Salmonella* genus, species and serovars to be very complex (Smith *et al.*, 2011).

There is a high incidence of *S. Enteritidis* in broiler breeder (57.5%) and broiler flocks (84.0%), and it is the serovar most frequently responsible for foodborne outbreaks and sporadic cases of salmonellosis in humans (Kanashiro *et al.*, 2005).



When dealing with the epidemiology of *Salmonella* infections, determining how humans acquire the infection is essential (Dhillon *et al.*, 2001). Therefore, when monitoring the health quality of poultry meat used for human consumption, it is important to identify which *Salmonella* serotypes are present in the production chain. Aiming at developing a more robust assessment of intraspecific diversity within *Salmonella* species using genetic markers, some techniques based on PCR were devised (Tindall *et al.*, 2005; Wang *et al.*, 2009).

Among the methods used to identify *Salmonella* isolates are random amplified polymorphic DNA (RAPD) analysis (Betancor *et al.*, 2004; Smith *et al.*, 2011), which is based on the amplification of repetitive elements present in several copies in the chromosome; enterobacterial repetitive intergenic consensus (ERIC-PCR) (Rasschaert *et al.*, 2005; Anderson *et al.*, 2010); repetitive extragenic palindrome (REP-PCR) sequences (Merino *et al.*, 2003; Woo & Lee, 2006); *Salmonella enteritidis* repetitive extragenic (SERE) sequences (Rajashekara *et al.*, 1998); and BOX elements (Woo & Lee, 2006; Suh & Song, 2006). Previous studies have shown PCR fingerprinting techniques to be strain specific and highly useful in *Salmonella* strain typing (Woo & Lee, 2006; Suh & Song, 2006; Merino *et al.*, 2003; Smith *et al.*, 2011; Kumao *et al.*, 2002).

Another PCR-based method that enables the study of the biodiversity of *Salmonella* isolates is amplification followed by product separation of the spacer between the 16S and 23S rRNA genes (intergenic spacer region - ISR) (Baudart *et al.*, 2000; Luz *et al.*, 1998; Lagatolla *et al.*, 1996). This method, identified by the acronym RISA (rDNA internal spacer analysis), provides products with a highly variable size because of its hypervariable nature (García-Martínez *et al.*, 1999).

In addition, the amplification of this region followed by restriction digestion and analyses of its products is another potential use of ISR. This technique, called ARDRA (amplified ribosomal DNA restriction analysis), is easy, fast and accurate to identify and characterize *Lactobacillus* sp. and *Bradyrhizobium* sp. isolates (Moreira *et al.*, 2005; Han *et al.*, 2005; Mohania *et al.*, 2008; Vinuesa *et al.*, 1998). However, despite its great potential, ISR-ARDRA has not yet been used for the identification or characterization of *Salmonella* serotypes.

The purpose of the present study was to evaluate the usefulness and the potential application of different techniques based on PCR analysis (RAPD, ERIC, RISA and ARDRA) for the differentiation of 15 *Salmonella*

*enterica* subsp. *enterica* serotypes isolated from the poultry meat production chain.

## MATERIAL AND METHODS

### Salmonella strains

The *Salmonella* isolates used in this study were obtained from poultry production chain environments, including hatcheries, broiler breeder farms, broiler farms and slaughterhouses located in the states of Santa Catarina and Rio Grande do Sul, between 2006 and 2010 (data not shown). *Salmonella* sp. were isolated and identified following the recommendations of Oliveira *et al.* (2006). The colonies suspected of *Salmonella* were collected for presumptive identification by biochemistry tests and the positive isolates were submitted to serologic tests using polyvalent serum against *Salmonella* O antigens. The positive isolates were submitted to reference official laboratories (Oswaldo Cruz Institute Foundation; FIOCRUZ, Rio de Janeiro, Brazil) for complete identification and serotyping. A total of 15 different serotypes of *Salmonella enterica* subsp. *enterica* were chosen for the study and isolated during that period, including *S. Infantis*, *S. Tennessee*, *S. Bredeney*, *S. Schwarzengrund*, *S. Ohio*, *S. Montevideo*, *S. Newport*, *S. Sandiego*, *S. Panama*, *S. Hadar*, *S. Rissen*, *S. Anatum*, *S. Muenchen*, *S. Typhimurium* and *S. Saintpaul*. Three isolates of each serotype were randomly selected and analyzed for the determination of common profiles.

### DNA extraction and characterization by RAPD

For the molecular characterization of *Salmonella* serotypes, DNA extractions were performed using the extraction kit Whatman FTA® Classic Card (Whatman, USA), as specified by the manufacturer.

The RAPD profiles of *Salmonella* isolates were generated from five different primers: P1254 (5'-CCGCAGCCAA-3'), 784 (5'-GCGGAAATAG-3'), 23L (5'-CCGAAGCTGC-3'), OPA-4 (5'-AATCGGGCTG-3') and OPB-15 (5'-CCAGGGTGTT-3') (Woo & Lee, 2006; Malorny *et al.*, 2001). The PCR mixture contained 2 mM MgCl<sub>2</sub>, 0.25 mM dNTP, 2.5 U of *Taq* polymerase (Invitrogen), PCR buffer (Invitrogen), 50 pmol of primer and DNA template. Amplification was performed as previously described with minor modifications (Betancor *et al.*, 2004), using the following program: 4 cycles of 94°C for 4 min, 37°C for 4 min and 72°C for 4 min; then 35 cycles of 94°C for 30 s, 37°C for 1 min, and 72°C for 2 min, followed by final 10 min at 72°C



with PCR thermal cycler HBSP02110 (Thermo Electron Corp.). The PCR products (15 µl of each sample) were loaded on 1.5% agarose gel with 0.5 µg/ml of ethidium bromide in the electrophoresis tank, using 0.5 X TBE buffer, at 3 Vcm<sup>-1</sup> for 2 hours. A DNA molecular weight marker, 100-bp DNA ladder (Ludwig Biotech, Brazil), was used as standard. Gels were observed under UV light and a digital image was captured (Photo Capt Software version 12.5 for Windows - Vilber Lourmat) for analysis. In order to confirm the reproducibility of the method, each experiment was repeated three times.

### ERIC-PCR analysis

For the amplification of ERIC motifs, the PCR mixture and reaction programs were utilized according to similar conditions reported on previous studies (Suh & Song, 2006), using 1R (5'-ATGTAAGCTCCTGGGGATTCAC-3') and 2R (5'-AAGTAAGTGACTGGGGTGAGCG-3') primers. The gel and digital image were produced as previously described.

### Molecular analysis by RISA and ARDRA methods

The amplification of the 16S-23S rRNA intergenic spacer region (ISR) was performed using a 50 µL mix (10X PCR buffer with MgCl<sub>2</sub> 1.5mM, 0.25mM dNTP, 2.5U of Taq polymerase (Invitrogen), 50 pmol of each primer and DNA template) and the following universal primers: P1 (5'-TTGTACACACCGCCCGTCA-5') and P2 (5'-GGTACTTAGATGTTTCAGTTC-3') (Lagatolla *et al.*, 1996). Samples were submitted to the following program: 35 cycles of 94°C for 1 min, 53°C for 1 min and 72°C for 1 min and 30 sec, and the reaction products were analyzed by electrophoresis as described. For the ARDRA method, 14 microliters of the amplification product of each *Salmonella* isolate were digested with restriction endonucleases. Ten units of enzymes - Alu I and Hha I (Invitrogen) - were added to each reaction and the mixture was incubated at 37°C for 2 hours. The restriction fragments were separated by electrophoresis on 3% agarose gel at 2 Vcm<sup>-1</sup> for 4 hours.

### Phylogenetic data analysis

Dendrograms were constructed by UPGMA (unweighted pair-group method for the arithmetic average) based on Jaccard's similarity coefficient from a matrix based on the binary code of molecular profile data for bootstrapping. Each phenogram was reconstructed 2000 times by repeated sampling with

replacement using computer program WinBoot (Yap & Nelson, 1996).

## RESULTS AND DISCUSSION

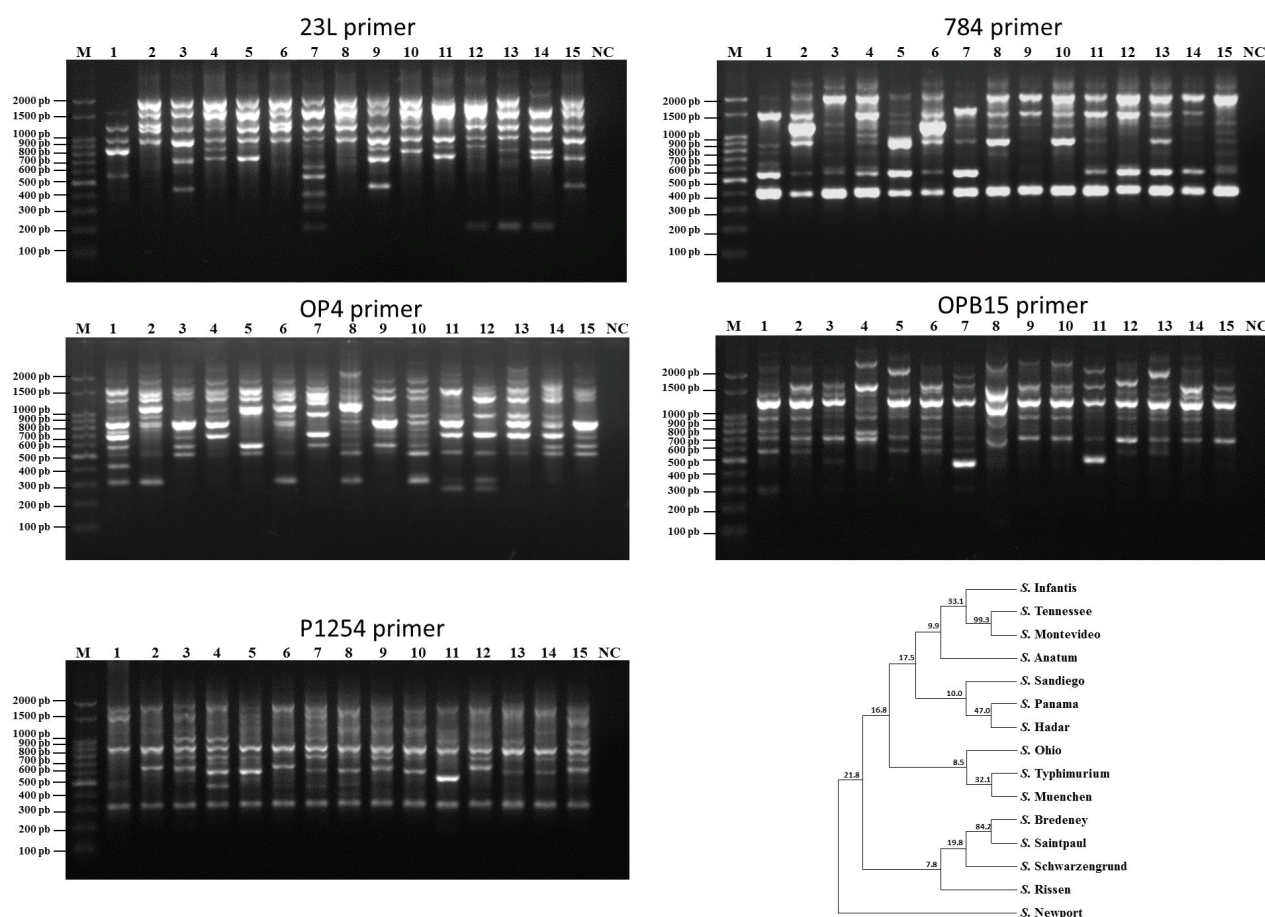
A total of 15 different serotypes of *Salmonella enterica* subsp. *enterica* isolated from the poultry production chain were typed using different molecular methods for the purpose of obtaining a common molecular profile. Three isolates of each serotype were used.

Salmonellosis is a worldwide problem in the poultry industry, affecting both animal and public health. Studies in several regions of Brazil have shown that *S. Enteritidis*, *S. Typhimurium*, *S. Panama*, *S. Newport*, *S. Infantis*, *S. Senftenberg*, *S. Heidelberg*, *S. Saintpaul*, *S. Indiana*, *S. Agona*, and *S. London* are the most common *Salmonella* serovars found in poultry commercial breeders and broiler flocks (Ribeiro *et al.*, 2006; Oliveira *et al.*, 2006; Kanashiro *et al.*, 2005). As expected, these serovars were found in the present study and were submitted to molecular analyses. However, the most prevalent *Salmonella* serotypes causing human toxic infections are *S. Enteritidis* and *S. Typhimurium* (Robeson *et al.*, 2008; Oliveira *et al.*, 2006).

Because of the need of detecting and identifying infections to prevent disease and their dissemination, methods are required for the epidemiological study of salmonellosis (Dhillon *et al.*, 2001). The genetic characteristics of *Salmonella* serotypes must be accurately and objectively analyzed using an efficient, reliable, and discriminatory genetic analysis method (Christensen *et al.*, 1998). Thus far, several genetic analysis methods for the genotyping of *Salmonella* serotypes have become available (Lagatolla *et al.*, 1996; Baudart *et al.*, 2000).

Because of it is easy to use and it has discriminatory power, RAPD-PCR has become an important tool to fingerprint bacteria involved in disease outbreaks and to determine the sources, vectors and vehicles of transmission (Betancor *et al.*, 2004). For RAPD analysis, five known primers used for *Salmonella* characterization were selected, as well as the best simplification conditions to differentiate each serotype based on their DNA amplification profiles. The use of RAPD enabled the differentiation of most genotypes (Betancor *et al.*, 2000). Primers P1254, 784, 23L, OPA-4 and OPB-15 were effective for the differentiation of most *Salmonella* serotypes isolated from the broiler production chain (Figure 1).





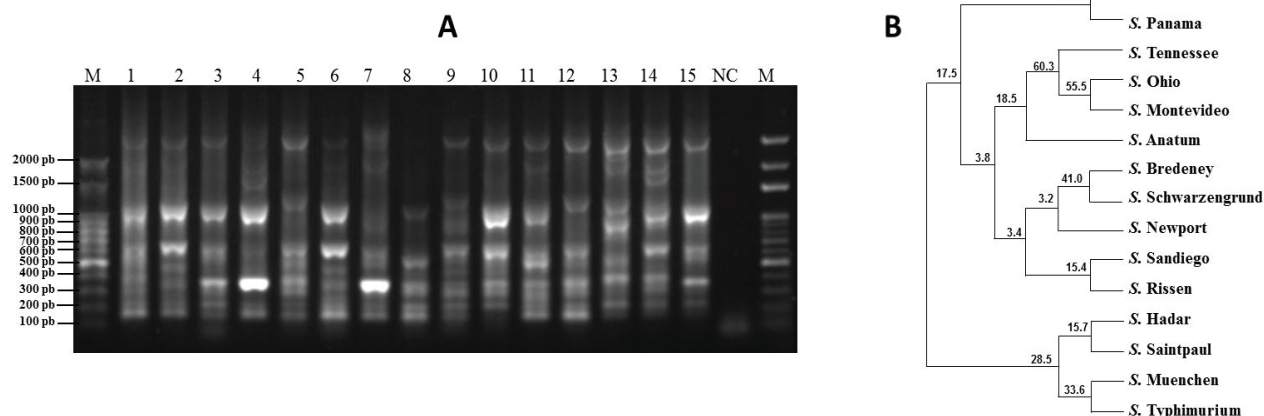
**Figure 1** – Representative molecular profiles of 15 *Salmonella* serotypes obtained by RAPD. Electrophoretic analysis of amplification profile of *Salmonella* isolates obtained by RAPD using 5 primers (identified over each gel). Where: M identifies the molecular weight marker 100 bp DNA ladder, NC - negative control and the lines 1 – 15, respectively, *Salmonella* serotypes *S. Infantis*, *S. Tennessee*, *S. Bredeney*, *S. Schwarzengrund*, *S. Ohio*, *S. Montevideo*, *S. Newport*, *S. Sandiego*, *S. Panama*, *S. Hadar*, *S. Rissen*, *S. Anatum*, *S. Muenchen*, *S. Typhimurium*, *S. Saintpaul*. Below and right: Dendrogram constructed by UPGMA using Jaccard's coefficient based on RAPD patterns. The values found in the groups indicate the percentage recorded for a particular branch.

DNA fragments ranging from approximately 2.5 to 0.3 kbp were generated, providing the molecular profile from which a dendrogram was constructed using UPGMA by profile numerical analysis to group isolates by similarity. This approach allowed molecular analyses because, due to the high number of molecular markers developed over the last decades, it has been difficult to associate genetic diversity or molecular profiles with classification and phylogeny, especially of closely-related *Salmonella* isolates (Yap & Nelson, 1996; Baudart *et al.*, 2000).

Similarly to the RAPD results, ERIC-PCR results (Figure 2A) provided evidences of clear molecular diversity among the *Salmonella* serotypes isolated in this study. As suggested earlier, the molecular profile obtained by ERIC, associated with the dendrogram (Figure 2B), proved to be a convenient method for fingerprinting and therefore, a good typing tool.

According to Rasschaert *et al.* (2005), it is possible to use the ERIC1R-ERIC2 primer set to differentiate *Salmonella* serotypes and to improve the reproducibility and resolving power of the method by using appropriate annealing temperatures in order to obtain a correlation between the molecular pattern (footprinting) and the specific serotype.

The results obtained by RISA (rDNA internal spacer analysis) demonstrated that 16S-23S rRNA intergenic spacer regions (ISR) are highly polymorphic in *Salmonella* isolates, and therefore, it is possible to use this method to detect variability. Amplification generated DNA fragments of approximately 500 to 1000 pb, and it was possible to differentiate most of the isolates analyzed (Figure 3A-RISA). The high polymorphism of the DNA spacer in the regions between 16S and 23S is due to the fact that *sp.*, as well as *S. enterica*, has seven ribosomal operons, and they are usually not identical (intercistronic heterogeneity) (García-Martínez *et al.*,



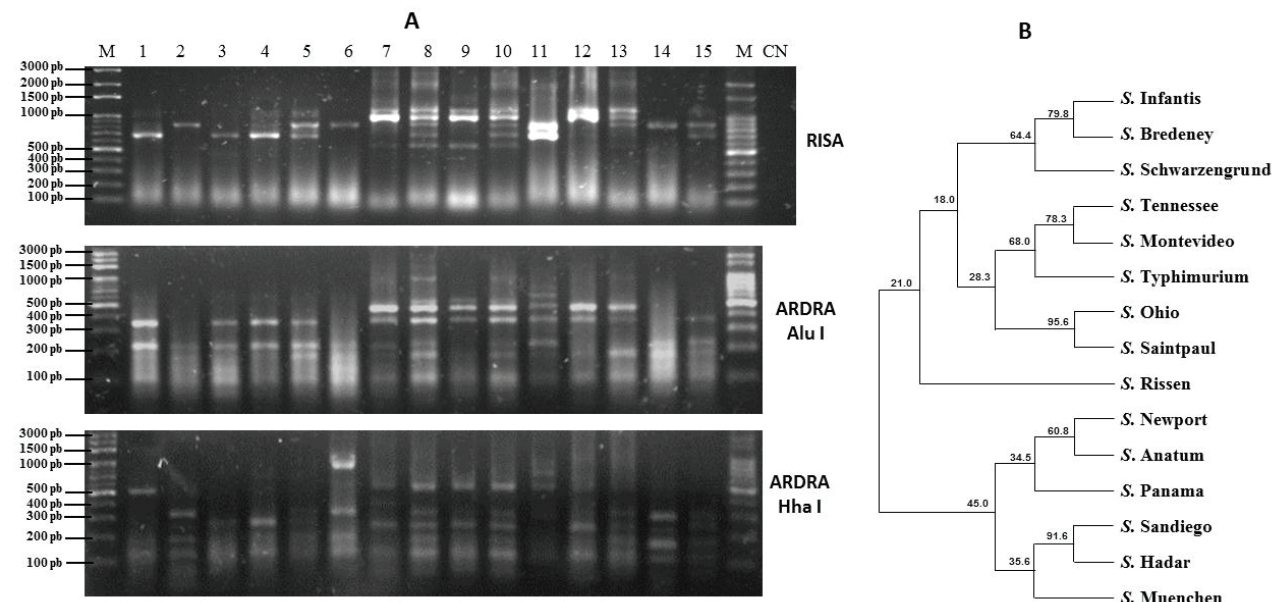
**Figure 2** – Molecular profile of 15 *Salmonella* serotypes obtained by ERIC-PCR. (A) Electrophoretic analysis of amplification profile of *Salmonella* isolates obtained by ERIC. M - molecular weight marker 100 bp DNA ladder and NC - negative control. Lines 1 to 15, respectively, *Salmonella* serotypes sequence as described in the key to Figure 1. (B) Dendrogram constructed by UPGMA using Jaccard's coefficient based on ERIC patterns in Panel A. The values found in the groups indicate the percentage recorded for a particular branch.

1999; Jensen *et al.*, 1993; Thomson *et al.*, 1998). In consequence, spacer region polymorphisms have been useful in the identification of *Listeria*, *Staphylococcus*, and *Salmonella*, as well as the identification of *Salmonella* serotypes (Jensen *et al.*, 1993), and the intraserovar or subtyping discrimination of *Salmonella* (Baudart *et al.*, 2000; Lagatolla *et al.*, 2006 ). From the results obtained, most of the examined serotypes could be differentiated by their profile. The length and sequence polymorphisms present in the PCR product

can therefore be used for the recognition of genotypic diversity.

The most direct and rapid method to visualize the polymorphic character of 16S-23S rRNA ISR is PCR amplification of the spacer regions with the use of primers from highly-conserved flanking sequences (García-Martínez *et al.*, 1999).

A second approach is to use the PCR product digested with a restriction enzyme (amplified ribosomal DNA restriction analysis - ARDRA), and have



**Figure 3** – Molecular profile of 15 *Salmonella* serotypes obtained by RISA and ARDRA. (A) Electrophoretic analysis of the amplification of the 16S-23S rRNA intergenic spacer region (RISA) by PCR and electrophoretic analysis of the restriction profile (ARDRA - Alu I and Hha I) of amplified fragments of 16S-23S rRNA intergenic spacer region (ISR). M - molecular weight marker 100 bp DNA ladder and NC - negative control. Lines 1 to 15, respectively, *Salmonella* serotypes sequence as described in the key to Figure 1. (B) Dendrogram constructed by UPGMA using Jaccard's coefficient based on RISA and ARDRA (Alu I and Hha I) patterns of the Panel A. The values found in the groups indicate the percentage recorded for a particular branch.



the resulting fragments resolved electrophoretically (Moreira *et al.*, 2005). If the PCR product contains the restriction endonuclease recognition sequence at unique or different locations, then the resultant fragment size pattern can indicate a particular profile.

In this study, the results of 16S-23S rRNA ISR – ARDRA using Alu I and Hha I restriction enzymes for the 15 serotypes analyzed showed very complex molecular profile patterns (Figure 3A-ARDRA), and no common patterns among the three isolates tested of most serotypes (data not shown). Profiles were differentiated according to band number and position, as suggested by Betancor *et al.* (2000). The number of bands per profile varied, and most fragments were below 500 pb. Additional information may be inherent in the polymorphic character of the amplified and digested products. This indicates the great potential of the technique for footprinting.

However, as those fragments were very small, possibly having small differences in length (few pb), they were not perceptible when the products were separated by agarose gel electrophoresis. In these cases the molecular profile may be obtained by other means, such as thermal gradient gel electrophoresis (TGGE) analysis (Yasuda & Shiaris, 2005), denaturing gradient gel electrophoresis (DGGE) (Anderson *et al.*, 2010), or on 4-8 % polyacrylamide gel (Jensen *et al.*, 1993; Baudart *et al.*, 2000), although these methods are more expensive and time-consuming.

A dendrogram was constructed by UPGMA from the molecular profiles obtained using RISA and ARDRA (Figure 3B). The clusters generated were different from those obtained by RAPD (Figure 1) and ERIC (Figure 2B), but the highest numbers on a branch, from the high bootstrap values, indicated the percentage that reflected on the concordant structure with which the nodes were supported, meaning that data were more robust than in the other dendrograms.

The 16S-23S rRNA ISR (intergenic sequence region) is a hypervariable region, which is useful for the fine discrimination of operational taxonomic units, but limited work has been found focusing on *Salmonella* or other enteric pathogens. However, despite the advantages of the ISR-ARDRA method and its usefulness in the characterization of others organisms (Jensen *et al.*, 1993; Luz *et al.*, 1998), it had not been used for the identification of serotypes or the analysis of isolates, possibly due to the difficulty in visualizing the results, whereas rDNA internal spacer analysis (RISA), RAPD, repetitive extragenic palindromic-PCR (REP-PCR) and ERIC are more common (Rasschaert *et al.*, 2005; Woo & Lee, 2006).

In conclusion, the main *Salmonella* serotypes found in poultry products to be sources of infection may be identified by molecular techniques such as RAPD and ERIC. Depending on the conditions of the reactions, these methods may also be used for footprinting analyses. On the other hand, RISA and ARDRA techniques, especially the 16S-23S rRNA intergenic spacer regions, are primarily intended for intra-serotype characterization, that is, the differentiation of *Salmonella* strains at the intra-serovar level. Moreover, the use of a complementary method is essential for obtaining more reliable and accurate results both in serotype determination and isolate characterization. Methods should be combined as needed. Therefore, the new approach to the ISR-ARDRA technique will possibly have important practical implications for the epidemiological analysis of *Salmonella*. The method has proven to be an important tool for *Salmonella* fingerprinting and was highly discriminatory among *Salmonella* isolates; therefore, it may potentially detect wider variability among fingerprint profiles.

## ACKNOWLEDGEMENTS

This study was funded by CNPq (Conselho Nacional de Desenvolvimento Científico e Tecnológico) and FAPESC (Fundação de Amparo à Pesquisa e Inovação do Estado de Santa Catarina) grants.

## REFERENCES

- Anderson P N, Hume ME, Byrd JA, Hernandez C, Stevens SM, Stringfellow K, Caldwell DJ. Evaluation of repetitive extragenic palindromic-polymerase chain reaction and denatured gradient gel electrophoresis in identifying *Salmonella* serotypes isolated from processed turkeys. *Poultry Science* 2010; 89(6):1293-1300.
- Aspinall ST, Hindle MA, Hutchinson DN. Improved isolation of salmonellae from faeces using a semisolid Rappaport-Vassiliadis medium. *European Journal of Clinical Microbiology & Infectious Diseases* 1992; 11(10):936-939.
- Baudart J, Lemarchand K, Brisabois A, Lebaron P. Diversity of *Salmonella* strains isolated from the aquatic environment as determined by serotyping and amplification of the ribosomal DNA spacer regions. *Applied and Environmental Microbiology* 2000; 66(4):1544-1552.
- Baumler AJ, Tsolis RM, Ficht TA, Adams LG. Evolution of Host Adaptation in *Salmonella enterica*. *Infection and Immunity* 1998; 66(10):4579-4587.
- Betancor L, Schelotto F, Martinez A, Pereira M, Algorta G, Rodríguez M A, Vignoli R, Chabalgoity JA. Random Amplified Polymorphic DNA and phenotyping analysis of *Salmonella enterica* serovar enteritidis isolates collected from humans and poultry in Uruguay from 1995 to 2002. *Journal of Clinical Microbiology* 2004; 42(3):1155-1162.
- Christensen H, Nordentoft S, Olsen JE. Phylogenetic relationships of *Salmonella* based on rRNA sequences. *International Journal of Systematic Bacteriology* 1998; 48:605-610.





- Dhillon AS, Shivaprasad HL, Roy P, Alisantosa B, Schaberg D, Johnson S. Pathogenicity of environmental origin *Salmonella* in specific pathogen-free chicks. *Poultry Science* 2001; 80:1323-1328.
- García-Martínez J, Acinas SG, Antón AI, Rodríguez-Valera F. Use of the 16S–23S ribosomal genes spacer region in studies of prokaryotic diversity. *Journal of Microbiological Methods* 1999; 36(1-2):55-64.
- Guibourdenche M, Roggentin P, Mikoleit M, Fields PI, Bockemühl J, Grimont PA, Weill FX.
- Supplement 2003-2007 (No. 47) to the White-Kauffmann-Le Minor scheme. *Research in Microbiology* 2010;161(1):26-29.
- Han KS, Kim Y, Choi S, Oh S, Park S, Kim SH, Whang KY. Rapid identification of *Lactobacillus acidophilus* by restriction analysis of the 16S-23S rRNA intergenic spacer region and flanking 23S rRNA gene. *Biotechnology Letters* 2005;27(16):1183-1188.
- Kanashiro AMI, Stoppa GFZ, Cardoso ALSP, Tessari ENC, Castro AGM. Serovars of *Salmonella* spp isolated from broiler chickens and commercial breeders in diverse regions in Brazil from July 1997 to December 2004. *Brazilian Journal of Poultry Science* 2005;7(3):195-198.
- Kumao T, Ba-Thein W, Hayashi H. Molecular subtyping methods for detection of *Salmonella enterica* Serovar Oranienburg outbreaks. *Journal of Clinical Microbiology* 2002;40(6):2057-2061.
- Lagatolla C, Dolzani L, Tonin E, Lavenia A, Di Michele M, Tommasini T, Monti-Bragadin C. PCR ribotyping for characterizing *Salmonella* isolates of different serotypes. *Journal of Clinical Microbiology* 1996;34(10):2440–2443.
- Luz SP, Rodríguez-Valera F, Lan R, Reeves PR. Variation of the ribosomal operon 16S-23s gene spacer region in representatives of *Salmonella enterica* subspecies. *Journal of Bacteriology* 1998; 180(8):2144-2151.
- Merino LA, Ronconi MC, Navia MM, Ruiz J, Sierra JM, Cech NB, Lodeiro NS, Vila J. Analysis of the clonal relationship among clinical isolates of *Salmonella enterica* serovar Infantis by different typing methods. *Revista do Instituto de Medicina Tropical de São Paulo* 2003;45(3):119-123.
- Mohania D, Nagpal R, Kumar M, Bhardwaj A, Yadav M, Jain S, Marotta F, Singh V, Parkash O, Yadav H. Molecular approaches for identification and characterization of lactic acid bacteria. *Journal of Digestive Diseases* 2008;9(4):190-198.
- Moreira JL, Mota RM, Horta MF, Teixeira SM, Neumann E, Nicoli JR, Nunes AC. Identification to the species level of *Lactobacillus* isolated in probiotic prospecting studies of human, animal or food origin by 16S-23S rRNA restriction profiling. *BMC Microbiology* 2005;23:5-15.
- Oliveira GH, Berchieri Jr A, Montassier HJ. Chicken Serologic Response to *Salmonella enterica* Serotype Typhimurium assessed by Elisa. *Brazilian Journal of Poultry Science* 2006;8(1):51-54.
- Rajashekara G, Koeuth T, Neville S, Back A, Nagaraja KV, Lupski JR, Kapur V. SERE, a widely dispersed bacterial repetitive DNA element. *Journal of Medical Microbiology* 1998;47:489-497.
- Rasschaert G, Houf K, Imberechts H, Grijspeerdt K, De Zutter L, Heyndrickx M. Comparison of five repetitive-sequence-based PCR typing methods for molecular discrimination of *Salmonella enterica* isolates. *Journal of Clinical Microbiology* 2005;43(8):3615–3623.
- Ribeiro SAM, Galletti MCM, Orsi MA, Ferrati AR, Mendonça AO, Doretto Jr L, Camillo SCA, Reischak D. Incidence of *Salmonella* in imported day-old ducklings. Brazil, 1998-2003. *Brazilian Journal of Poultry Science* 2006;8(1):39-43.
- Robeson J, Retamales J, Borie C. Genomic variants of bacteriophages against *Salmonella enterica* serovar Enteritidis with potential application in the poultry industry. *Brazilian Journal of Poultry Science* 2008;10(3):173-178.
- Robinson A, Mccarter YS, Tetreault J. Comparison of Crystal Enteric/Nonfermenter system, API 20E system, and Vitek Automicrobic System for identification of gram-negative bacilli. *Journal of Clinical Microbiology* 1995;33(2):364-370.
- Schaechter M, and the view from here group. *Escherichia coli* and *Salmonella* 2000: the view from here. *Microbiology and Molecular Biology Reviews* 2001;65(1):119-130.
- Smith SI, Fowora MA, Goodluck HA, Nwaokorie FO, Aboaba OO, Opere B. Molecular typing of *Salmonella* spp isolated from food handlers and animals in Nigeria. *International Journal of Molecular Epidemiology and Genetics* 2011;2(1):73-77.
- Suh DK, Song JC. Analysis of *Salmonella enterica* serotype Enteritidis isolated from human and chickens by repetitive sequence-PCR fingerprinting, antibiotic resistance and plasmid profiles. *Journal of Veterinary Science* 2006;7(1):37-41.
- Tindall BJ, Grimont PAD, Garrity GM, Euzé JP. Nomenclature and taxonomy of the genus *Salmonella*. *International Journal of Systematic and Evolutionary Microbiology* 2005;55:521-524.
- Thomson NR, Clayton DJ, Windhorst D, Vernikos G, Davidson S, Churcher C, et al. Comparative genome analysis of *Salmonella* Enteritidis PT4 and *Salmonella* Gallinarum 287/91 provides insights into evolutionary and host adaptation pathways. *Genome Research* .2008;18(10):1624-37. Epub 2008 Jun 26.
- Vinuesa P, Rademaker JLW, Bruijn FJ, Werner, D. Genotypic characterization of *Bradyrhizobium* strains nodulating endemic woody legumes of the Canary Islands by PCR-restriction fragment length polymorphism analysis of genes encoding 16S rRNA (16S rDNA) and 16S-23S rDNA Intergenic spacers, repetitive extragenic palindromic PCR genomic fingerprinting, and partial 16S rDNA sequencing. *Applied Environmental Microbiology* 1998;64(6):2096-2104.
- Wang M, Cao B, Gao Q, Sun Y, Liu P, Feng L, Wang L. Detection of *Enterobacter sakazakii* and other pathogens associated with infant formula powder by use of a DNA microarray. *Journal of Clinical Microbiology* 2009;47(10):3178-3184.
- Woo Y-K, Lee S-H. Genetic Diversity of Multi-resistant *Salmonella enterica* serotype Typhimurium isolates from animals and humans. *The Journal of Microbiology* 2006;44(1):106-112.
- Yap IV, Nelson RJ. Winboot: a program for performing bootstrap analysis of binary data to determine the confidence limits of UPGMA-based dendrograms. Philippines: International Rice Research Institute; 1996.
- Yasuda M, Shiaris MP. Differentiation of bacterial strains by thermal gradient gel electrophoresis using non-GC-Clamped PCR primers for the 16S–23S rDNA intergenic spacer region. *FEMS Microbiology Letters* 2005 243:235-242.