Random amplified polymorphic DNA (RAPD) analysis of *Ornithobacterium rhinotracheale* strains isolated from chickens in Turkey

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ABSTRACT: Six field strains of *Ornithobacterium rhinotracheale* isolated from chickens in Elazig province located in the East of Turkey were typed by serotyping and random amplified polymorphic DNA assay using a random primer (OPG-11). Using the AGP test used for serotyping, serotype A was found to be the predominant serotype, only one strain was serotyped as serotype B. By RAPD assay, the tested ORT strains were found to have different RAPD profiles. In addition, the RAPD assay showed almost similar DNA profiles among the tested strains of the serotypes A, B, D and E. The strain of serotype C did give a different RAPD profile. Within strains of the same serotype (A), different profiles were found but the strain of serotype (B) had an identical profile as strains of serotype A. This study suggests that more genotypes of ORT strains are present within the same serotype and thus that no relationship exists between the RAPD pattern of ORT and their serotype.

Keywords: *Ornithobacterium rhinotracheale*; random amplified polymorphic DNA; chicken; typing

Ornithobacterium rhinotracheale (ORT) is a pleomorphic, rod shaped, gram-negative bacterium associated with respiratory disease, infecting turkeys and chickens and thus, causing significant economic losses (Tanyi et al., 1995; Roepke et al. 1998; Van Empel and Hafez, 1999; Lopes et al., 2000; Van Veen et al., 2000a,b). The worldwide spread of ORT within a relatively short time has encouraged interesting epidemiological investigations. Several studies have been performed in which properties of isolates from all over the world have been compared (Van Empel and Hafez, 1999). Strains could also be characterized by serotyping. So far, 18 serotypes of ORT could be discriminated (Van Empel, 1998; Hafez, 2002). Serotyping has revealed that the majority of isolates are of one serotype (A) and that 97% of strains belong to the four major serotypes A, B, D, and E (Van Empel and Hafez, 1999). They show a low diversity of serotype, especially those isolated from chickens (Van Empel et al., 1997). Epidemiological typing studies require the use of both phenotypic and genotypic markers and many methodological approaches have been developed (Chaslus-Dancla et al., 1996). Plasmid profiles and ribotyping have been largely used (Grimont and Grimont, 1986). As a fairly new tool, random amplification of polymorphic DNA (RAPD) technique has been introduced recently (Williams et al., 1990). This technique has succesfully been used in different studies of veterinary pathogenic strains (Leroy-Setrin et al., 1995; Chaslus-Dancla et al. 1996).

The aim of this study was to analyse the genetic variability among ORT strains from chickens and compare these strains with the serotype-specific ORT strains by using the RAPD assay with a random primer (OPG-11).

MATERIAL AND METHODS

Bacterial strains and antisera

Serotype-specific ORT strains of the serotypes A (B 3263/91), B (GGD 1261), C (ORV K91-201), D (ORV 94108 nr. 2) and E (O-95029 nr. 12229) and antisera against the serotypes A and B were obtained from P. van Empel, Intervet-International, Boxmeer, The Netherlands. In addition, 6 ORT field strains from chickens were used which were obtained from different flocks.

Antigen extraction

The heat-stable antigen extraction carried out according to the described by Hafez and Sting (1999).

AGP test

Petri dishes with 1.5% agar noble in 8.5% NaCl were used in accordance with a method described by Woernle (1966). A hexagonal pattern was cut into the agar layer that consisted of six wells (2 mm in diameter) located around a central well at a distance of approximately 5 mm. The peripheral wells were filled with the antigen extract and the central one with the antisera against a different serotype (A or B). The plates were incubated at room temperature and evaluated after 24, 48, and 72 hours.

DNA extraction

ORT strains were cultured on sheep blood agar at 37°C under micro-aerobic conditions. A few colonies from each culture were transferred into an Eppendorf tube containing 300 µl distilled water and the tubes were vortexed. Lysis was accomplished by the addition of 300 µl of TNES buffer (20mM Tris pH 8.0, 150mM NaCl, 10mM EDTA, 0.2% SDS) and 200 µg/ml Proteinase K. The lysis mixture was incubated at 37°C for 2 h and then boiled for 30 min. Bacterial DNA was extracted with use of phenol/chloroform-iso-amylalchohol. DNA was precipitated with ethanol and 0.3M sodium acetate at –20°C for one hour or overnight. The DNA pellet was washed with 300 µl of 90% and 70% ethanol, respectively, each step followed by 5 min centrifugation. The pellet was dried, and dissolved in 50 µl of distilled water, then used as template DNA for PCR.

Primer

A random OPG-11 primer (5'-TGCCCGTCGT-3') (Leroy-Setrin et al., 1998) was used at a concentration of 1 µM, according to the manufacturer's directions.

RAPD analysis

The RAPD reaction was performed in a 25 µl volume containing 2.5 µl 10× PCR buffer (750mM Tris-HCl, 200mM $(NH_4)_2SO_4$, 0.1% Tween 20), 3.5mM $MgCl_2$, 200µM deoxynucleoside triphosphates, 1.25 U of *Taq* DNA polymerase (Fermentas, Lithuania), 1µM of OPG-11 primer, 11 µl of dsH_2O (sterile distilled water) and 2.5 µl of template DNA. Reactions were amplified in a Touchdown Thermocycler (Hybaid, Middlesex, England). The amplification cycles were as follows; 50 cycles of 94°C for 30 s, 37°C for 1 min and 72°C for 1 min 30 s. A final step of extension was applied at 72°C for 10 min. The amplified DNA products were resolved by electrophoresis on a 2% agarose gel with Tris-borate EDTA buffer and stained with ethidium bromide, for 30 min and photographed under UV transillumination using Polaroid Gel Cam. A 100 bp DNA ladder (Promega, Maddison, USA) was used as a molecular weight marker on each gel. RAPD assays were performed at least three times each to check reproducibility.

RESULTS

Serotyping

Five of the field ORT strains were identified as serotype A and the sixth strain as serotype B.

RAPD results

The RAPD profiles of all ORT strains are shown in Figure 1. Using OPG-11 primer, the RAPD profiles of the serotype-specific ORT strains show high

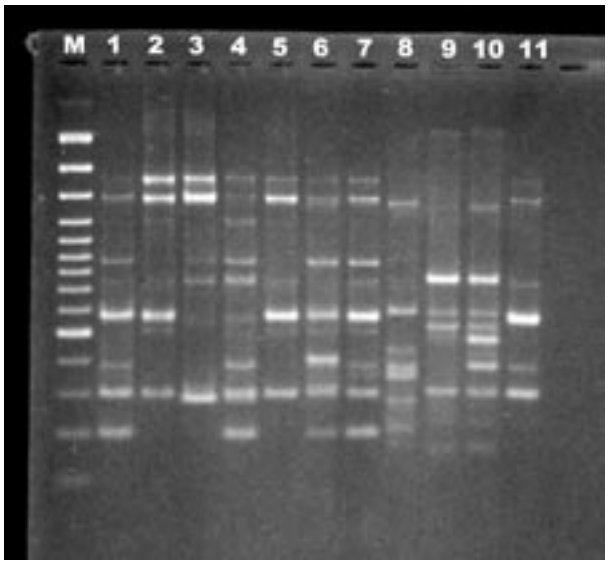

Figure 1. Results of RAPD analysis of ORT strains from chicken

Lane M – 100 bp DNA ladder; lane 1 – serotype A; lane 2 – serotype B; lane 3 – serotype C; lane 4 – serotype D; lane 5 – serotype E; lanes 6, 7, 8, 9, 10, 11 – ORT strains

similarity between the serotypes A and D (lanes 1 and 4) and between the serotypes B and E (lanes 2 and 5). Serotype C (lane 3) showed a RAPD profile differnt from all other tested strains. All six field strains showed different RAPD profiles. Three isolates (lanes 6, 7 and 11) showed high similarity with the serotype-specific strains of the serotypes A and D and two isolates (lanes 9 and 10) with the serotype-specific strain of serotype B. The field isolate in lane 8 showed a profile that was different from all others.

DISCUSSION

To date, apart from the studies by Leroy-Setrin et al. (1998) and Van Empel et al. (1998) no other studies related to RAPD analysis of ORT have been reported. This is the first announcement of RAPD analysis of ORT isolates from chickens in Turkey.

The AGP test with a heat-stable antigen extraction is considered a suitable method for serotyping ORT isolates (Hafez and Sting, 1999). In the present study, five of the field strains tested were identified as ORT serotype A and only one strain as serotype B. Scientists reported that serotype A was found to have the highest prevalence especially in chickens in other countries as well as in Turkey (Dudouyt et al., 1995; Van Empel et al., 1997; Turan and Ak, 2002). Erganis et al. (2002) reported that of the two strains isolated in Turkey, one was serotype B and the other could not be serotyped. Turkyilmaz (2001) suggested that three isolates were ORT serotype I. Turan and Ak (2002) showed that 90.9% were determined to be serotype A and one strain could not be serotyped. Our results are in agreement with the results reported in other countries and Turkey (Dudouyt et al., 1995; Van Empel et al., 1997; Erganis et al. 2002; Turan and Ak, 2002).

We used RAPD analysis to determine genetic heterogeneity of six ORT strains isolated from chicken. The method was succesfully applied to typing ORT strains. In a study in which 23 French strains isolated from various origins were investigated, three methods consisted of plasmid profiles, ribotyping based on the detection of the polymorphism of very conserved regions and RAPD assay tested to assess the genetic diversity of ORT (Leroy-Setrin et al., 1998). From these methods, plasmid profiling was not useful, ribotyping showed a low discriminatory power although different enzymes were used and the RAPD method gave a good level of discrimination and was relatively simple and easy to use for the typing of ORT compared to ribotyping. Among the 23 strains, only six were serotyped. For this particular reason, no relationship between serotypes and either ribotypes or RAPD types could be established. In the same study, different primers have been used for RAPD typing of ORT. Forty arbitrary primers from the G (OPG-1-OPG-20) and H (OPH-1-OPH-20) were all used and among them, the primers, OPG-11 and OPG-19 appeared to provide the best discrimination and among the 23 strains. Seven had identical RAPD types whatever the primers used (Leroy-Setrin et al., 1998). For these reasons, we used only one primer OPG-11 for RAPD typing to investigate genetic diversity among ORT isolates in this study.

Although we typed a small number of ORT strains by RAPD assay in this study, these results of RAPD analysis showed a high genetic diversity among ORT strains. The results are consistent with those found by Leroy-Setrin et al. (1998) who identified 16 different types among 23 ORT strains from various origins using RAPD. Van Empel (1998) even suggested that different subgenus do exist within the genus ORT since the differences found in his RAPD study of 56 strains of 12 different serotypes were high enough to justify that.

Both Leroy-Setrin et al. (1998) and Van Empel (1998) showed that no relationship between se-

rotypes and either ribotypes or RAPD types could be established. Also the present study showed no relationship between the RAPD pattern and serotype. However, it was noted that serotype A and D, B and E showed identical amplified patterns. This suggests that these serotypes may be sharing similar genomic sequences. Lawrence et al. (1993) suggested that the regions of amplified DNA in PCR were not serotype-specific. Serotype A strains belonged to RAPD types 6–10 and serotype B strain to RAPD type 11. Strains (No. 8, 9, 10) of the same serotype (A) were different profile although strain (No. 11) of serotype (B) had an identical profile. In addition, strains (No. 6, 7) of the same serotype (A) were identical profile.

Popp and Hafez (2001) analysed several ORT isolates by pulsed-field gel electrophoresis (PFGE). The restriction digest of genomic DNA of each isolate was carried out using the enzyme *Sal*I. The result of the genetic analyse showed that there was a considerable diversity of DNA fingerprint patterns. Each serotype of additional tested 17 standard strains (A–Q) showed a specific pattern. Also within serotype A isolates originated from German turkeys a wide variation was observed. On the other hand, serotype B isolates are identical. Comparing isolates from different countries high similarity within the isolates of the same serotype, despite the origin of the isolate (chicken/turkey), was observed. The primarily result suggests the existence of relationships between the geographic origin, the serotype and the DNA fingerprint pattern.

Also in this study the ORT field strains all show different DNA profile by RAPD indicating that the strains originate from different sources. More genotyping studies must be done with strains originated from different sources to prove this implication. In addition, this study showed that no relationship exists between RAPD pattern and serotype.

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