

GENOTIPIC DIFFERENTIATION OF BACTERIAL STRAINS ISOLATES FROM ORAL CAVITY FROM DOGS WITH PERIODONTAL DISEASE BY RAPID (RANDOM AMPLIFICATION OF POLYMORPHIC DNA)

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Summary

RAPD (Random Amplification of Polymorphic DNA) is a technique for rapidly detecting genomic polymorphism, utilizing a single short oligonucleotide primer of arbitrary sequence in a polymerase chain reaction (PCR). RAPD analysis has been used in numerous application including detection of strain diversity, gene mapping, epidemiology, population analysis and the demonstration of phylogenetic and taxonomic relationships (1, 3, 4). RAPD analysis it enables the quick detection of polymorphism at a number of different loci using only nanogram quantities of genomic DNA. The amount of genomic DNA in a RAPD reaction can vary from as little as 1 ng to as much as 100ng (1, 2).

The aim of this paper was to evidentiare genomic diversity of the Gram positive and Gram negative bacteria isolates from dog's periodontal disease.

Materials and methods

Bacterial strains

A total of 19 strains isolates and identification at Institute Pasteur Bucharest, between May 2006 and June 2006 was evaluated. Bacteria were obtained from dogs with periodontitis and gingivitis.

In table 1 it presents the isolates used in this study. The strains were identified by conventional tests and API 20 E, API 20 NE, API 20 Staph, API 20 Strep (bio Merieux, France).

Table 1

Strains isolates from oral cavity from dogs with periodontal disease processing by RAPD

Strains Gram negative	Strains Gram positive
<i>Escherichia coli</i>	Staphylococcus simulans
<i>Pseudomonas aeruginosa</i>	Staphylococcus sciuri
<i>Burkholderia cepacia</i>	<i>Staphylococcus lentus</i>
Moraxella canis	Micrococcus luteus
<i>Morganella spp.1</i>	Aerococcus viridans 1
<i>Morganella spp. 2</i>	<i>Aerococcus viridans 2</i>
<i>Providencia spp.</i>	Gardnerella vaginalis 1
Bergeyella zoohelcum	Gardnerella vaginalis 2
<i>Alcaligenes spp.</i>	Streptococcus uberis
	Leuconostoc spp.

DNA isolation

Bacterial DNA was isolated using a Germani method (1995).

RAPD fingerprinting

To evaluate bacterial DNA for species identification, RAPD fingerprinting was performed by Ready To Go RAPD Analysis Beads (Amersham Biosciences).

In table 2 were presented the primers contained by Ready To Go RAPD Analysis Beads (Amersham Biosciences). In this study we used only primer 1.

Table 2**Primers used for RAPD (Amersham Biosciences)**

Primer	Sequence (5' → 3')	%GC
Primer 1	GGTGCGGGAA	70
Primer 2	GTTTCGCTCC	60
Primer 3	GTAGACCCGT	60
Primer 4	AAGAGCCCGT	60
Primer 5	AACGCGCAAC	60
Primer 6	CCCGTCAGCA	70

RAPD was done in a total volume of 25 µg. Amplification was performed by Gene Amp PCR System 9600, Perkin Elmer and cycle using the following profile:

- 1 cycle 94°, 5';
- 44 cycles: 94° 40", 35° 1', 72° 2', 60-35° 50", 35-72° 130";
- 1 cycle 72°, 10'.

6 hours and half were necessary for amplification.

Amplified products were electrophoresed in 1.5% agarose with TBE 1X buffer. Gels were run at 120V, 60 mA for 1.5 h and stained with ethidium bromide 2µg/ml (Sigma). The DNA standard used was PCR marker (Sigma, 50-2000pb). The DNA was visualized by transillumination Herolab Easy RH and Image 2 WinPC. There were use Un Scan IT (Gel Software v.5.1.1998, Silk Sci. Co) and Treecon (Treecon for Windows v.1.3b. 1997) software's (3).

Results and discussions

RAPD screening molecular with primer 1 (table 2) at nine Gram negative isolates from dogs demonstrate that these are genotypical different. The same was observed at studied Gram positive isolates.

The results are presents in picture 1 and 2. It could be observed that Gram positive isolates from dogs with gingivitis and periodontitis presents more genotypes. The strains from genus *Aerococcus spp.* present the lower degree of dissimilarity. This demonstrate once again that oral microflora from dogs are genetically very complex and dynamic.

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Fig. 1. Strains Gram positive. RAPD results. Horizontal electrophoresis. Line 1 – *S. simulans*; line 2 – *Aerococcus viridans*; line 3 – *Aerococcus viridans*; line 4 – *S. sciuri*; line 5 – *S. lentus*; line 6 - DNA marker (Sigma).

Fig. 2. Strains Gram negative. RAPD results. Horizontal electrophoresis. Line 1 - *Pseudomonas aeruginosa*; line 2 - *Burkholderia cepacia*; line 3 – *E. coli*; line 4 – *Moraxella canis*; line 5 –DNA marker (Sigma).

Conclusions

It could be observed great genetic diversity of strains isolated from gingivitis and periodontitis dogs.

RAPD analysis is a technique for rapidly detecting genomic polymorphism and is used in molecular epidemiology. It could be observed that primer 1 has induced different profiles in all strains belonging bacterial genus studied.

References

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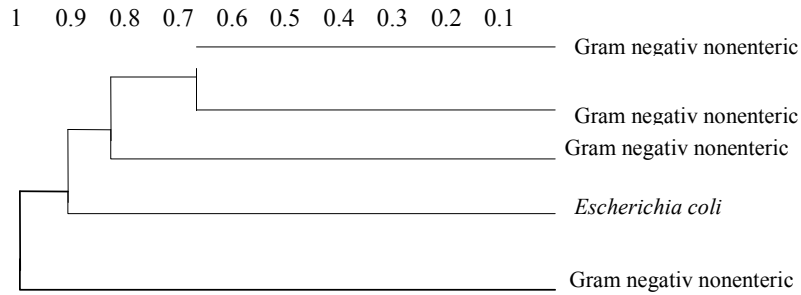
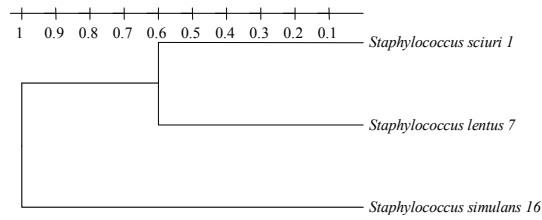


Fig.3. *S. sciuri*, *S. lentus*, *S. simulans*, *E.coli* and 4 strains Gram-negative de canine origin. Dendrogram realised by RAPD.

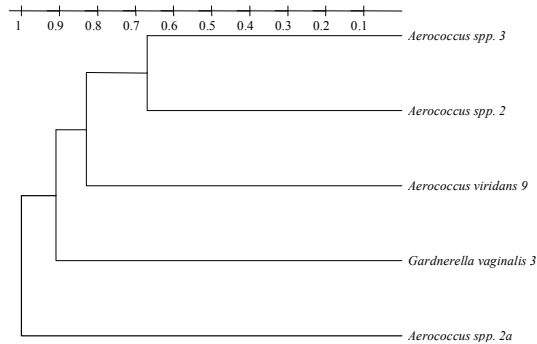


Fig.4. *Aerococcus spp* and one strains *Gardnerella vaginalis* canine origin. Dendrogram realised by fingerprinting RAPD