PLASMID PROFILE OF SOME GRAM POSITIVE STRAINS ISOLATES FROM DOG'S ORAL CAVITY

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Summary

Some virulence factors can be mediated by extrachromosomal DNA elements carrying genes able to synthesis substances destroying host tissues. In this study, the plasmid profile of some Gram positive strains isolates from dog's oral cavity were evaluated. Plasmids were classified into two groups according to size.

Staphylococci and streptococci are Gram positive bacteria, aerobe, facultative anaerobe, non-sporeforming, usually immobile and are indigenous of dog oral cavity. These organisms are commonly recovered from different skin, upper respiratory and lower urogenital system infections in humans animals both (2, 3).

Some virulence factors can be mediated by extrachromosomal DNA elements carrying genes able to synthesis substances destroying host tissues. The presence of plasmids in genus *Staphylococcus* and *Streptococcus* has been reporter in literature. It is known that genus *Micrococcus* strains carrying plasmids.

The aim of these researches was to evaluate the plasmid profile of some Gram positive strains isolates from dog's oral cavity.

Material and methods

1. Bacterial isolating and identification.

There are study the next bacterial strains: one strain from *Staphylococcus simulans, Staphylococcus sciuri, Staphylococcus lentus, Micrococcus luteus, Streptococcus uberis, Gardnerella vaginalis* each one, and two strains from *Aerococcus viridans.*

From healthy dogs were isolated strains of *Staphylococcus simulans, Staphylococcus sciuri*, *Streptococcus uberis* and *Aerococcus viridans. Staphylococcus lentus, Micrococcus luteus* and *Gardnerella vaginalis* were isolated from dogs with periodontal disease. Identification of the bacteria was performed with API 20 Strep (bio Merieux, France) and API 20 Staph (bio Merieux, France). After identification the strains were stored in BHI broth and glycerol 40%, at - 40°C.

2. Plasmids DNA extraction.

Plasmid DNA extraction was performed using a Takahashi and Nagano method, modified by using DNA Minipreps Wizard Plus kit (Promega).

This technique evidentiate plasmids with size between 2 and 190 kb.

The bacterial walls were lyses by adding of a lizozim solution. Plasmid DNA was analyzed by electrophoresis in 0,8% agarose gel the ethidium bromide 0,5 µg/ml was added. The electrophoresis was performed with Bioblock Consort E425 at 120V, 60 mA, one hour (4, 5).

Next *E. coli* strains were used as reference plasmids or DNA standards: R 112 (100 kb), RSa (35kb), V 517 (55 kb, 7,4 kb, 5,7 kb, 5,2 kb, 4,0 kb, 3,1 kb, 2,8 kb, 2,2 kb), R 55 (165 kb), R 135 kb) INRA Tours France. There were used Herolab Easy RH and Image 2PC software.

Results and discussions

The strains were classified in two categories: strains harboring plasmids and strains neharboring plasmids. Strains (*Staphylococcus lentus, Micrococcus luteus, Gardnerella vaginalis*) harboring plasmids were recovered from dog with 3-rd stage of periodontal disease (fig. 1).

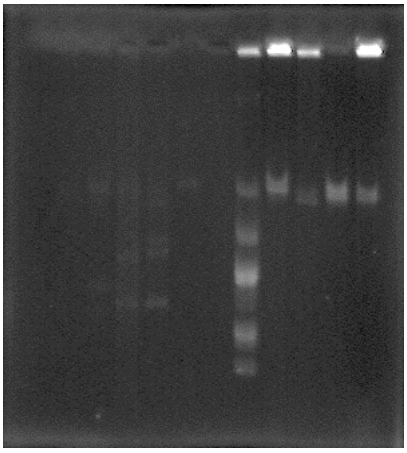

Fig. 1. Plasmid profile – 8 canine strains – line 1 *Staphylococcus simulans*; line 2 *Staphylococcus sciuri*; line 3 *Gardnerella vaginalis*; line 4 *Micrococcus luteus*; line 5 Staphylococcus lentus; line 6 *Streptococcus uberis*; line 7 *Aerococcus viridans*; V517; R 112; Rsa; R 55; R135

Strains isolated from healthy dogs: *Staphylococcus simulans, Staphylococcus sciuri, Streptococcus uberis* and *Aerococcus viridans* not presented DNA plasmid. From table 1 it could be observed that 3 (37.5%) out of 8 studied strains presented DNA plasmid bands. Plasmid is not detected in 62.5% (5 strains). The plasmid molecular weight were between 4.64 kb and 14.11 kb. This

weight was characteristically from small size plasmids. It is known that high size plasmids are presents in virulent strains.

We believe that aerobe Gram positive bacteria have a minor role in periodontal disease in dog.

Anaerobe Gram negative bacteria *Porphyromonas spp., Prevotella spp., Fusobacterium spp.* and *Bacteroides fragilis* group are very important role in gingivitis and periodontitis dog (1).

Table 1

The plasmid presence in Gram positive strains from dogs with periodontal disease and healthy dogs

Genus and species	Plasmids	
	No. plasmids	Weight (kb)
Staphylococcus lentus	4	12.84 kb. 8.69 kb. 7.72 kb. 4.75 kb
Micrococcus luteus	3	14.11 kb. 7.28 kb. 4.64 kb
Gardnerella vaginalis	1	5.48 kb
Staphylococcus simulans	-	-
Staphylococcus sciuri	-	-
Streptococcus uberis	-	-
Aerococcus viridans	-	-
Aerococcus viridans	-	-

Conclusions

Our results indicate the need of a better plasmid characterization of the oral cavity bacteria strains and determining the role of these elements could play in periodontal disease.

Plasmids were observed in 3 out of 8 strains. This result indicates that in periodontal pockets ecosystem it could be present strains high weight plasmids (100 kb) responsible for the lesions from periodontal disease.

We considers that is necessary an extensive study about plasmid profile and the role of these plasmids from the mostly indigenous oral flora from dogs and others species.

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