ARCHIVES OF ORAL BIOLOGY 58 (2013) 317–323

Available online at www.sciencedirect.com

SciVerse ScienceDirect

journal homepage: http://www.elsevier.com/locate/aob

Assessing the oral microbiota of healthy and alcohol-treated rats using whole-genome DNA probes from human bacteria

Zaher Jabbour [a], *Cássio do Nascimento* [c], *Bruna Gabriela dos Santos Kotake* [b], *Michel El-Hakim* [c], *Janet E. Henderson* [d], *Rubens Ferreira de Albuquerque Junior* [a,b,*]

[a] *Division of Restorative Dentistry, Faculty of Dentistry, McGill University, Montreal, QC, Canada*
[b] *Department of Dental Materials and Prosthodontics, Faculty of Dentistry of Ribeirão Preto, University of São Paulo, Brazil*
[c] *Division of Oral and Maxillofacial Surgery, Faculty of Dentistry, McGill University, Montreal, QC, Canada*
[d] *Division of Orthopedic Surgery, Faculty of Medicine, McGill University, Montreal, QC, Canada*

ARTICLE INFO

Article history:
Accepted 31 July 2012

Keywords:
DNA checkerboard hybridization
Rats
Alcohol
Oral bacteria

ABSTRACT

Objective: This study aimed to evaluate the capacity of whole-genome DNA probes prepared from human oral bacteria to cross-react with bacteria from the oral cavity of rats, and to assess the influence of alcohol ingestion on the animals' oral biofilm.

Design: Twenty four mature Wistar rats were equally divided in two groups. One group (control) was fed balanced diet of rat pellets and water. The alcohol-treated group (AT) received the same diet and 20% ethanol solution. Upon euthanasia after 30 days, bacterial samples from the oral biofilm covering the animals' teeth were collected using microbrushes. Bacteria identification and quantification were performed using the DNA checkerboard hybridization method with 33 probes prepared from human oral bacteria. Signals corresponding to bacterial genome counts and percentages were compared using a Mann–Whitney *U* test with a significance level <0.05.

Results: Cross-reaction for all targeted species, except *Streptococcus mutans* and *Streptococcus mitis*-like species, occurred in the control group. *Escherichia coli*, *Pseudomonas aeruginosa*, *Porphyromonas endodontalis*, and *Veillonella parvula*-like species only produced detectable signals in the AT group. Significantly more signals were detected in the control group compared to the AT group ($p = 0.001$). The percentage of *E. coli*-like species was highest in both groups.

Conclusions: Whole-genome DNA probes prepared from human oral bacteria can cross-react with rats' oral bacterial species. Alcohol consumption is associated with lower levels and diversity of bacterial species in the oral cavity of rats.

1. Introduction

The use of genetic molecular methods for identification and quantification of oral bacterial is increasing.[1–3] These methods are considered faster and offer higher accuracy compared to traditional bacterial culture methods.[4] The DNA checkerboard hybridization method was well described in 1994 by Socransky.[5] It uses whole-genome DNA probes to allow the simultaneous screening of up to 45 bacterial species in 28 samples.[6]

Alcohol consumption has well documented effects on the oral cavity in humans. It was directly linked to pharyngeal cancer, presence of fewer teeth, periodontal disease, horizontal

* *Corresponding author at*: Division of Restorative Dentistry, Faculty of Dentistry, McGill University, 3640 University Street, Montreal, Quebec H3A 2B2, Canada. Tel.: +1 514 398 7203x00090; fax: +1 514 398 8900.
E-mail addresses: rubens.albuquerque@mcgill.ca, ralbuque@forp.usp.br (Albuquerque Junior, RF).
0003–9969/$ – see front matter
http://dx.doi.org/10.1016/j.archoralbio.2012.07.017

bone loss and higher prevalence of caries.[7–11] The influence of alcohol ingestion on the oral microbiota is not fully described in the literature.[12] It has been reported that alcohol might decrease the salivary flow leading to an increase in the concentration and number of gram-positive bacteria,[13] and that production of acetaldehyde from oral yeasts and bacteria may result in inhibition of certain species.[14] However, information about the effect of alcohol ingestion on oral bacteria remains scarce and limited to a small number of species isolated in culture media.[15] More analyses using molecular methods are still lacking.

Similarities in the oral microbiota between humans and other mammalians were reported previously.[16,17] Gram-positive cocci, such as *Streptococcus*, *Staphylococcus* and *Enterococcus*, and gram-positive rods, such as *Lactobacillus*, were found in the oral cavity of humans and rats.[18] Considering handling and culturing difficulties associated with traditional methods, particularly in relation to fastidious and anaerobic-strict species, and the high sensitivity of molecular methods, it would be beneficial to verify the applicability of molecular methods in animals. More recently, DNA probes prepared from human bacteria were found to cross-react with DNA from bacteria of the oral cavity of dogs and rats with ligature-induced periodontitis.[19,20] However, these probes failed to produce detectable signals for samples taken from healthy sites of rats' mouth.[19] As a result, it is still unclear whether the DNA hybridization method could be used to detect bacteria from the oral cavity of healthy rats. Therefore, the aim of this study was to evaluate the potential of whole-genome DNA probes prepared from human oral bacteria to cross-react with oral bacterial species from healthy rats, and to assess the influence of alcohol consumption on their oral microbiota using thee DNA checkerboard hybridization method.

Table 1 – Human DNA bacterial species used to prepare probes for cross-reaction with bacterial species from the oral cavity of rats.

Species	Reference
Escherichia coli	ATCC 53323
Streptococcus mitis	ATCC 49456
Streptococcus gordonii	ATCC 10558
Pseudomonas aeruginosa	ATCC 27853
Neisseria mucosa	ATCC 25996
Parvimonas micra	ATCC 33270
Porphyromonas endodontalis	ATCC 35406
Lactobacillus casei	ATCC 393
Fusobacterium periodonticum	ATCC 33693
Fusobacterium nucleatum	ATCC 25586
Staphylococcus pasteuri	ATCC 51129
Veillonella parvula	ATCC 10790
Tannerella forsythia	ATCC 43037
Treponema denticola	ATCC 35405
Solobacterium moorei	CCUG 39336
Streptococcus parasanguinis	ATCC 15911
Streptococcus salivarius	ATCC 25975
Streptococcus sobrinus	ATCC 27352
Streptococcus sanguinis	ATCC 10556
Streptococcus oralis	ATCC 35037
Streptococcus mutans	ATCC 25175
Streptococcus constellatus	ATCC 27823
Staphylococcus aureus	ATCC 25923
Pseudomonas putida	ATCC 12633
Prevotella intermedia	ATCC 25611
Prevotella melaninogenica	ATCC 25845
Porphyromonas gingivalis	ATCC 33277
Eikenella corrodens	ATCC 23834
Enterococcus faecalis	ATCC 51299
Capnocytophaga gingivalis	ATCC 33624
Bacteroides fragilis	ATCC 25285
Aggregatibacter actinomycetemcomitans a	ATCC 29523
Aggregatibacter actinomycetencomitans b	ATCC 29522

2. Methods

2.1. Animals

Twenty four mature Wistar rats were housed for 30 days in groups of six per cage, kept in a temperature-controlled room (23–25 °C), with a light/dark cycle of 12/12 h and free access to food and water. Animals were divided in two groups: one group was fed regular solid diet of rat pellets and water (control group). Rats in the alcohol-treated group (AT group) received the same diet and 20% ethanol solution.[15] The study was approved by the Animal Ethics Committee at University São Paulo. After euthanasia by CO_2, samples of oral biofilm were collected by rubbing a sterile microbrush on the surface of the teeth for 30 s. The use of microbrush was shown to be a suitable method for microbial biofilm collection.[21] Standard-sized microbrush tips are capable to absorb a volume of about 6 μL. Each sample was placed in individual microtubes containing 150 μL of TE (10 mM Tris–HCl, 1 mM EDTA pH 7.6), followed by the addition of 150 μL of 0.5 M sodium hydroxide. Samples were boiled for 5 min for DNA denaturation and then neutralized using 800 μL of ammonium acetate. The tubes were stored at 4 °C until laboratory processing.

2.2. Microbiological evaluation

Bacterial hybridization was performed using the DNA–DNA checkerboard method, according to do Nascimento et al.[22,23] Validation of the method in humans is well reported.[6] Thirty three probes prepared from bacteria whole-genome DNA taken from human oral cavity were tested (Table 1). Bacterial species selection was based on the relevance to the human oral health. Following DNA denaturation, biofilm samples were individually inserted in the extended slots of the MiniSlot 30 apparatus (Immunetics, MA, USA) and fixed on the hybridization membrane (Hybond N+, Amersham Biosciences, Buckinghamshire, UK) using vacuum and baked during 2 h at 80 °C. Next, samples were pre-hybridized using oven shaker (Amersham Biosciences) to control for temperature and humidity. Labelled human probes (Amersham Gene Images AlkPhos Direct Labelling and Detection System, GE Healthcare, Buckinghamshire, UK) were applied on the membrane using Miniblotter 45 device (Immunetics, Immunetics, Cambridge, MA, USA). Chemoluminescent signals from the DNA hybridization process were registered onto a Hyperfilm (Amersham Biosciences), digitized and quantified using Image Quant TL software (GE Healthcare, UK).