Microbial Pathogenesis 71-72 (2014) 32–36

Contents lists available at ScienceDirect

Microbial Pathogenesis

journal homepage: www.elsevier.com/locate/micpath

Molecular detection of bacteria associated to dental caries in 4–12-year-old Tunisian children

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ARTICLE INFO

Article history:
Received 8 April 2014
Received in revised form
20 April 2014
Accepted 23 April 2014
Available online 9 May 2014

Keywords:
PCR
Genomic DNA
Oral bacteria
Streptococcus mutans
Dental caries
Children

ABSTRACT

The occurrence of several microbial species in the oral cavity of 4–12-year-old Tunisian children was investigated. Samples were taken from 158 children (81 caries actives and 77 caries free). Genomic DNA was extracted and analyzed for the presence of 17 microbial species using a polymerase chain reaction assay.

All samples were positive for at least one of the target microbial strains. *Streptococcus mutans* was the most prevalent species (76.5%) detected in genomic DNA collected from carious lesions. Other prevalent species were *Candida* spp (63%), *Streptococcus salivarius* (59%) and *Streptococcus oralis* (42%). The frequency of *Lactobacillus acidophilus*, *Lactobacillus plantarum*, and *Lactobacillus casei*-group in caries lesions was 29.5%, 34.5% and 22% respectively. Pathogenic bacteria such as *Staphylococcus aureus* was found in 28.5% of carious lesion samples compared to 15.5% in the control.

Frequency of *Porphyromonas endodontali*, *Actinomyces radicidentis* and *Treponema denticola* recovery did not differ significantly between origins of samples.

PCR analysis of genomic DNA detect various oral bacteria that differ between caries actives and caries-free children. In addition, the association of same aciduric bacteria (*S. mutans*, *S. salivarius*, *L. acidophilus*) and caries formation was noticed.

1. Introduction

Dental caries is a multifactorial disease, which is characterized by a local destruction of the tooth [1]. *Streptococcus mutans* and *Lactobacilli* are the leading cause of dental caries [2]. Over 700 bacterial species colonize the human oral cavity [3] including *Streptococcus salivarius*, *S. mutans* and *Streptococcus sobrinus* [4]. Mutans streptococci (MS), a group of cariogenic bacteria, can survive in acidic pH [5], attach to the tooth surface, form dental biofilm [6] and are involved in the initiation of dental caries [7]. Oral streptococci have long been considered as significant pathogenic agents in dental caries. Their clinical importance includes its contribution in infective endocarditis, bacterial pneumonia [8] and as a reservoir of antimicrobial resistance genes [9,10].

It has been reported that the severity of periodontal disease is related to the presence of *Porphyromonas gingivalis*, *Tannerella forsythia* and *Treponema denticola* [11]. In addition, *Actinomyces* may be involved in the initiation of dental caries [12] and *P. gingivalis* is associated with chronic periodontitis [13].

Although oral cavity contain essentially various streptococci strains, it was of interest to investigate the occurrence of the other bacteria such as *Lactobacillus*, *Treponema*, *Staphylococcus*, *Enterococcus*,… Several study reported the detection of these bacteria in the oral cavity [14,15]. Many fastidious microorganisms are still difficult to identify with conventional methods. PCR assays have been used elsewhere to detect the major oral bacteria [16–18].

As no data are available on molecular detection of bacteria associated to dental caries in Tunisian children, this work was carried out to obtain primary idea on oral bacterium and its probably role in caries development. This paper analyzed the occurrence of several oral microorganism in carious lesions and supragingival plaque samples of Tunisian 4–12 year old children using specific PCR method.

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http://dx.doi.org/10.1016/j.micpath.2014.04.008
0882-4010/

2. Subjects and methods

2.1. Subjects

The study was done on 158 children, 81 caries actives (36 males and 45 females) and 77 caries free (46 males and 31 females) from dental clinic of Monastir, Tunisia. All clinical procedures were approved by the Ethical Committee of the Faculty of Medicine, Monastir University, Tunisia. A detailed medical and dental history was obtained from each parent. Written informed consent was obtained from the parents of all participants. The criteria for inclusion were: no systemic disease, no antibiotic treatment during the 4 weeks previous to sampling, had no orthodontic appliances, no use of mouth rinses or any other preventive measure.

The age group selected for the present study was about 4–12 years. The wide age range of children (4–12 year old) included in this study was selected so that we could examine a spectrum of different dentitions, which may influence oral colonization by cariogenic bacteria. A dental examination was conducted in each child, and caries status was recorded based on the World Health Organization (WHO) criteria [19].

Samples were taken from the oral cavity of each patient with a sterile swab: one from carious lesions, one from supragingival plaque for all the children with dental caries and only one from supragingival plaque for the control one. Each swab was transferred in glass tube containing 2 ml sterile brain heart infusion (BHI) medium and incubated during 24 h at 37 °C.

2.2. Detection by PCR of oral bacteria

One ml of bacterial culture was introduced in an Eppendorph tube, total genomic DNA was extracted using a Wizard Genomic Purification Kit (Promega, Lyon, France). The concentration of purified DNA was adjusted at 50 ng/μl using spectroscopy (Ultraspec 2100 pro; Amersham Biosciences Europe GmbH, France). The presence of 17 oral bacteria was investigated by polymerase chain reaction (PCR) using specific forward and reverse primers as listed in Table 1. The PCR mixture (25 μl) contained 1 mM forward and reverse primers (Table 1), dNTP mix (100 mM each of dATP, dCTP, dGTP and dTTP), 1 U of GO *Taq* DNA polymerase (Promega), 5 μl green Go *Taq* buffer (5X), and DNA template. PCR products (5 μl) were analyzed on 2% (wt/v) agarose gel stained with ethidium bromide (0.5 μg/μl), and visualized under ultraviolet transillumination and photographed using Syngene apparatus (Biorad, USA).

2.3. Statistical analysis

Statistical analysis was performed on SPSS v.17.0 statistics software. The Fisher's Exact test was used to assess inter-group significance. Statistical significance was set at $P < 0.05$.

3. Results

DNA detection of common oral bacteria known to cause dental caries has been performed. Among the 158 children, there were 81 caries actives (36 males and 45 females) and 77 caries free (46 males and 31 females). The mean age for the carious group was 8.14 ± 2.51 years and that for the control group was 8.32 ± 2.42 years. PCR analysis resulted in a single band of the predicted size. All samples were positive for at least one target microbial species. With regard to gender there was not significant difference between categories of samples.

Table 2 and Table 3 summarises the frequencies of bacteria detected in the genomic DNA extracted from carious lesions and supragingival plaque obtained from the oral cavity of 4–12 year old Tunisian children.

Table 1
Specific primers used to detect oral bacteria from metagenomic DNA.

Target strains	Primers	5′-3′	Product size (bp)	References
Streptococcus mutans	gtfDF	5′-GGCACCACAACATTGGGAAGCTCAGTT-3′	433	[20]
	gtfDR	5′-GGAATGGCCGCTAAGTCAACAGGAT-3′		
Streptococcus sobrinus	gtfTF	5′-GATGATTTGGCTCAGGATCAATCCTC-3′	328	
	gtfTR	5′-ACTGAGCCAGTAGTAGACTTGGCAACT-3′		
Streptococcus salivarius	gtfKF	5′-GTGTTGCCACATCTTCACTCGCTTCGG-3′-3′	544	
	gtfKR	5′-CGTTGATGTGCTTGAAAGGGCACCATT-3′		
Streptococcus sanguinis	gtfPF	5′-GGATAGTGGCTCAGGGCAGCCAGTT-3′	313	
	gtfPR	5′-GAACAGTTGCTGGACTTGCTTGTC-3′		
Streptococcus gordonii	gtfGF	5′-CTATGCGGATGATGCTAATCAAGTG-3′	440	
	gtfGR	5′-GGAGTCGCTATAATCTTGTCAGAAA-3′		
Streptococcus oralis	gtfRF	5′-TCCCGGTCAGCAAACTCCAGCC-3′	374	
	gtfRR	5′-GCAACCTTTGGATTTGCAAC-3′		
All Lactobacillus	IDL03R	5′-CCACCTTCCTCCGGTTTGTCA-3′	–	[21]
All Lactobacillus	IDL04F	5′-AGGGTGAAGTCGTAACAAGTAGCC-3′	–	
Lactobacillus casei-group	IDL11F	5′-TGGTCGGCAGAGTAACTGTTGTCG-3′	727	
Lactobacillus acidophilus	IDL22R	5′-AACTATCGCTTACGCTACCACTTTGC-3′	606	
Lactobacillus plantarum	IDL62R	5′-CTAGTGGTAACAGTTGATTAAAACTGC-3′	428	
Porphyromonas gingivalis	PgF	5′-AGGCAGCTTGCCATACTGCG-3′	404	[22]
	PgR	5′-ACTGTTAGCAACTACCGATGT-3′		
Porphyromonas endodontalis	PorpF	5′-GCTGCAGCTCAACTGTAG TC-3′	672	[23]
	PorpR	5′-CCGCTTCATGTCACCATGTC-3′		
Actinomyces radicidentis	ArF	5′-AGGCCTTATTGGCTTGGTTG-3′	550	[24]
	ArR	5′-CGGTCACTCACATGTCAAGC-3′		
Treponema denticola	denticoF	5′-TAATACCGAATGTGCTCATTTACAT-3′	316	[22]
	denticoR	5′-TCAAAGAAGCATTCCCTCTTCTTCTTA-3′		
Enterococcus faecalis	E1	5′-ATCAAGTACAGTTAGTCT-3′	941	[25]
	E2	5′-ACGATTCAAAGCTAACTG-3′		
Enterococcus faecium	EM1F	5′-TTGAGGCAGACCAGATTGACG-3′	658	[26]
	EM1R	5′-TATGACAGCGACTCCGATTCC-3′		
Staphylococcus aureus	Sa442F	5′-AATCTTTGTCGGTACACGATATTCTTCACG-3′	107	[27]
	Sa442F	5′-CGTAATGAGATTTCAGTAGATAATACAACA-3′		
Candida spp	Its86	5′-GTGAATCATCGAATCTTTGAAC-3′	310	[28]
	Its4	5′-TCCTCCGCTTATTGATATGC-3′		