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Association between biofilm-forming isolates of mutans streptococci and caries experience in adults

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ARTICLE INFO

Article history:
Accepted 17 May 2010

Keywords:
Caries experience
DMFT
Mutans streptococci
Streptococcus mutans
Cariogenicity
Biofilm
Biofilm-forming *Streptococcus mutans*

ABSTRACT

Objectives: Although it is still controversial, mutans streptococci (MS) have been typically considered the primary etiological agents of dental caries. Besides the acidogenic and aciduric properties, extracellular polysaccharide synthesis leading to biofilm formation from sugar constitutes one of the key virulence factors of MS. The aim of this study was to investigate whether biofilm formation by MS was associated to an increased experience of caries in young adults.

Methods: A cross sectional study with a total of 96 randomly selected patients aged 15–27 years old was carried out. DMFT was determined by clinical examination and bite-wing radiographs. A sample of stimulated saliva was obtained and seeded on agar plates to culture MS. Colonies with and without biofilm formation were identified and quantified.

Results: When the total MS count was considered, levels of MS were not associated with higher caries experience. 50% of the patients showed at least one biofilm-forming colony. Patients with biofilm-forming colonies showed significantly higher DMFT ($p < 0.001$) than individuals whose plates did not reveal the structure surrounding the colony, but only at the low and moderate MS count.

Conclusion: Biofilm formation in MS appears to be associated with higher caries experience in individuals with low counts of the cariogenic microorganism.

1. Introduction

Dental caries is currently considered as an ecological unbalance within the oral biofilm leading to the dissolution of the tooth hard tissues.[1] It has been traditionally thought that two species belonging to the streptococci group, *Streptococcus mutans* (*S. mutans*) and *Streptococcus sobrinus* (*S. sobrinus*), are the etiological responsible for the onset of dental decay.[2–4] Furthermore, an increased number of *S. mutans* is considered as a risk factor for the onset of caries.[5] A direct relation between high counts of *S. mutans* and increased caries rates has been widely regarded as truthful.[3,6] Conflicting evidence, however, has shown that high counts of *S. mutans* relate to a rather weak or no association with caries patterns.[7–9] In opposition with the assumed role of *S. mutans* in dental caries, similar bacterial counts have been obtained amongst children from various countries in Africa, Europe and North America with different caries rates and in populations radically different to each other in racial, cultural and social aspects.[10] In consequence, variation in the clinical caries pattern observed in those dissimilar populations cannot be explained only by the number of *S. mutans*.

While the relation between MS and dental caries remains debatable, the cariogenic potential of MS is indisputable. Most of the evidence on the role of the microorganism in dental decay has been gathered from animal and *in vitro* studies,

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0003–9969/$ – see front matter
doi:10.1016/j.archoralbio.2010.05.010

reviewed in Ref. [2]. MS are capable to ferment nutrients from diet to produce acids.[9] In particular, *S. mutans* is endowed with a noteworthy machinery to survive, proliferate and produce large amounts of acids in acidic environments.[11] *S. mutans* and *S. sobrinus* also possess glucosyltransferases, enzymes that enable the microorganisms to form a range of intra- and extracellular polymers from sugars. Furthermore, like most of the streptococci species, *S. mutans* has adhesins, such as the antigen I/II that allow the bacteria to adhere to the tooth structure and to other species within the biofilm.[12] Cariogenic capabilities of MS have served as rationale for the vast research on the microbiological traits of the microorganism.

An extracellular coating of molecules, usually formed by exopolysaccharides (EPS) or slime, can be found in many bacterial species. From sucrose, MS use glucosyltransferases to synthesize exoplysaccharides, which are implicated in the capability of MS to form biofilms.[13,14] Biofilm-forming cells of the Viridans group are linked to bacterial endocarditis.[15] The basis for this association is the enhanced adhesion provided by the exopolysaccharides to damaged heart valves. Since *S. mutans* has the distinctive ability to synthesize vast amounts of exopolysaccharides, e.g., mutan and glucan,[2] and thus, form biofilms, it is plausible to speculate that subjects with a high caries experience are colonized by MS more prone to form dense biofilms. Indeed, water-insoluble glucan formation was positively associated with higher caries experience,[16] suggesting that these polymers act as a critical virulence factor in caries onset by promoting bacterial adhesion to hard oral tissues. Furthermore, exopolysaccharides seem to be formed in response to environmental constrains and its production is key for the entire biofilm formation.[17] The aim of this study, therefore, was to determine whether biofilm formation by MS is associated with a higher caries experience in young adults.

2. Materials and methods

2.1. Subjects and clinical examinations

Ninety-six patients between 15 and 27 years old were considered in the study. Subjects attended the undergraduate dental clinic of the University of Talca seeking dental care. Patients were randomly chosen for the study amongst those in treatment by the students. Before the clinical examination, an informed consent was requested from the participants. Patients that had received previous hygiene instruction or were using a mouthrinse of any kind or received any antimicrobial treatment were excluded from the study. Age and gender distribution is presented in Table 1. By the time of the study, fluoride content of running water in the region was 0.08 ppm. 100% of the patients used fluoridated toothpaste at least once daily. Subjects were examined by means of a mouth mirror and a curved probe under the light of the dental unit. Teeth status assessment followed WHO criteria,[18] whereby a tooth (T) or its surface (S) were recorded as decayed (D), missed (M) and filled (F) to obtain the DMFT and the DMFS index. Bitewing radiographs for each subject were also used as a supplement to the clinical examination.

Table 1 – Patients distribution by age and gender.

Age	Male	%	Female	%	Total	%
15–19	16	16.7	14	14.6	30	31
20–24	18	18.8	26	27.1	44	46
25–28	4	4.17	18	18.8	22	23
Total	38	39.6	58	60.4	96	100

2.2. Saliva samples and microbiological identification

Patients were asked to chew on a piece of paraffin wax for 2 min, expectorate the saliva and collect it into sterile glass tubes. Samples were immediately brought to the microbiology laboratory for further processing. Saliva was diluted (1: 1000 (v/v)) in sterile distilled water and an aliquot (50 μL) of the dilution was manually seeded on agar plates of MS selective Trypticase–yeast–cysteine–sucrose–bacitracin (TYCSB) medium (Merck, Darmstadt, Germany), as described.[19] TYCSB agar plates modified by the addition of 20% sucrose (w/v) (Merck, Darmstadt, Germany) were anaerobically incubated at 37 °C for 48 h in anaerobic jars (Gen Box Anaer; bioMérieux, Marcy-l'Etoile, France), followed by phenotypical colony identification, as described elsewhere.[20] Colonies growing on the plates were counted adjusting for the dilutions to obtain the final number of colony forming units (cfu/mL). The levels of MS were divided into three categories: $<1 \times 10^5$, 1×10^5 to 1×10^6 and $>1 \times 10^6$ cfu/mL. Biofilm formation was verified by direct observation of the colonies.[17,21] A colony was identified as biofilm-forming when a clear mass of viscous material surrounding a typical MS colony (slime) was observed on the MS colonies growing on solid medium (Fig. 1). Colonies showing biofilm formation were confirmed by light microscopy. Participants were classified in biofilm-forming or non-biofilm-forming patients according to whether they formed at least one biofilm-positive colony.

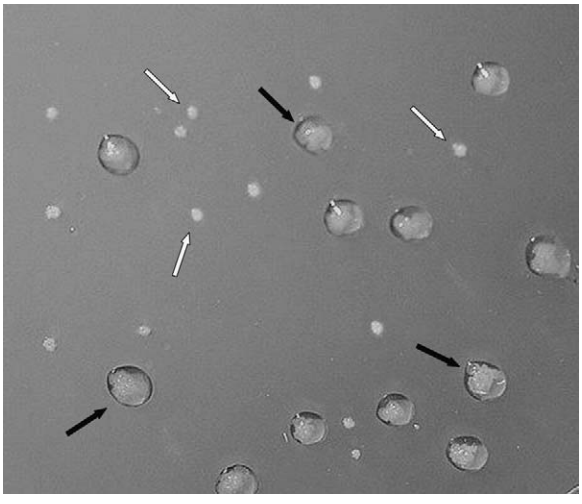

Fig. 1 – Biofilm formation by MS isolates. Saliva samples were plated on TYCSB for MS identification and counting. Biofilm-forming colonies were identified as having a gel-like transparent halo around the colony (black arrows). Non-biofilm-forming colonies lack a surrounding halo and look as sugar grain-like colonies (white arrows).