IN VITRO BACTERIAL PLAQUE SUPPRESSION AND RECOLONIZATION BY 
S. MUTANS AND S. SOBRINUS

Cássio Vicente Pereira; Luciano José Pereira; Reginaldo Bruno Gonçalves; José Francisco Höfling

1Departamento de Biologia Oral, Universidade Sagrado Coração, Bauru, SP, Brasil; 2Área de Diagnóstico Bucal, Universidade Vale do Rio Verde, Três Corações, MG, Brasil; 3Laboratório de Microbiologia e Imunologia, Faculdade de Odontologia de Piracicaba, Universidade Estadual de Campinas, Piracicaba, SP, Brasil

Submitted: April 27, 2004; Returned to authors for corrections: August 23, 2004; Approved: February 15, 2006

ABSTRACT

The in vitro study of the interactions between S. mutans and S. sobrinus is important to determine the role of these microorganisms in the formation of biofilms on dental structures and their potential to induce carious lesions. The objective of this research was to study the suppression of bacterial plaque formation and its recolonization by rifampycin-resistant S. mutans and streptomycin-resistant S. sobrinus. To study the competitive relationship between these species, previously standardized strains were incubated in media containing different fermentable carbohydrates. At determined time intervals, samples were collected from mixed cultures of S. mutans and S. sobrinus, diluted and plated on BHI-agar containing rifampycin or streptomycin to determine the number of viable cells of each species by counting colony-forming units. In order to study the bacterial colonization process and in vitro recolonization of bacterial plaque, three experiments were performed: I – co-cultivation of S. mutans and S. sobrinus; II – inoculation of bacterial plaque pre formed by S. sobrinus with S. mutans; and III - bacterial plaque pre formed by S. mutans dispersed and plated on BHI-agar containing streptomycin or rifampicin to determine the number of viable cells for each species. The results indicated a predominance of S. mutans in relation to S. sobrinus, demonstrating the capacity of S. mutans to inhibit plaque formation by S. sobrinus and recolonize the surfaces.

Key words: Streptococcus mutans, Streptococcus sobrinus, suppression

INTRODUCTION

Streptococci of the mutans group are closely associated with dental caries, mainly those involving smooth surfaces. Production of acid and extracellular polysaccharides due to hydrolysis of sucrose facilitates their adhesion to tooth surfaces (5,21).

In relation to the importance of the mouth microbiota in the decay process, S. mutans and S. sobrinus are the most frequent species from the mutans group encountered in saliva. Some authors have reported (1,8) that these bacterial species present a high caries-inducing potential, enhancing the infection process when combined. The beginning and development of caries are determined by a host-parasite relationship, which can be altered by several environmental factors as demonstrated by in vitro models simulating the in vivo phenomenon in the mouth (9,20). The in vitro studies of the bacterial relationship based on the action of these microorganisms in the plaque formation on tooth surfaces and their potential to induce caries are in agreement with several results reported in the literature (3,8-10,14,15).

The aim of the present study was to evaluate the suppression of bacterial plaque formation and recolonization by S. mutans and S. sobrinus, through in vitro tests using streptomycin- and rifampicin-resistant strains, cultivated separately and in combination.

*Corresponding Author. Mailing address: Laboratório de Microbiologia e Imunologia, Faculdade de Odontologia de Piracicaba, UNICAMP, Av. Limeira, 901, Areião, Caixa Postal 052, 13414-903, Piracicaba, SP, Brasil. Tel.: (+5519) 3412-5322. Fax: (+5519) 3412-5218. E-mail: microbiologia@fop.unicamp.br
MATERIALS AND METHODS

Suppression of bacterial plaque formation

Fresh culture samples of rifampicin-resistant *S. mutans* 32K and streptomycin-resistant *S. sobrinus* 6715 previously standardized by McFarland's scale (tube 2) in BHI medium were used. The samples were donated by the Microbiology Laboratory of Chiba University – Japan. A 0.2 mL sample from each culture was added to a tube containing 5 mL of BHI medium supplemented with 10% fermentable carbohydrate, either sucrose, glucose, fructose or glucose (5%) + fructose (5%), and incubated at 37°C for 24 h in a 10% CO2 atmosphere (10). After 3, 6, 12 and 24 h, 0.1 mL of the mixture was submitted to decimal dilution from 10−1 to 10−4 in sterile saline (0.9% NaCl), and plated in BHI-agar medium with rifampicin (0.1 mg/mL) or streptomycin (1.0 mg/mL). The plates were incubated for 24 h at 37°C and the number of colony-forming units for each species was determined.

In vitro bacterial plaque colonization and recolonization

Fresh cultures of rifampicin-resistant *S. mutans* 32K and streptomycin-resistant *S. sobrinus* 6715 were standardized by the McFarland scale (tube 2) in BHI medium and used in the in vitro study of bacterial plaque colonization and recolonization. The tubes containing BHI medium supplemented with 10% fermentable carbohydrates – sucrose, glucose, fructose or glucose (5%) + fructose (5%) – were inoculated with *S. mutans* and *S. sobrinus* samples. A capillary tube, with one flame-closed end, was introduced into the tube, and the other end was modified (19) to promote the development of bacterial plaque. To facilitate the evaluation of the interaction between these species and to determine the colonization and recolonization potential (10) the procedures were divided in three distinct experiments.

Experiment I – *S. mutans* and *S. sobrinus*, 0.2 mL each, were transferred to a tube containing 5.0 mL BHI medium with 10% fermentable carbohydrate – sucrose, glucose, fructose or glucose (5%) + fructose (5%) – and the capillary tube. The tubes were incubated for 48 h at 37°C in 10% CO2.

Experiment II – The capillary tube containing the bacterial plaque formed by *S. sobrinus* during incubation in BHI medium as described above was transferred to a new tube containing fresh culture medium supplemented with 10% fermentable carbohydrate [sucrose, glucose, fructose or glucose (5%) + fructose (5%)]. *S. mutans* was then added and the culture tubes incubated for 48 h at 37°C in 10% CO2.

Experiment III – The procedure was similar to Experiment II: the capillary tube containing the plaque formed by *S. mutans* was transferred to a new culture tube containing fresh medium supplemented with 10% fermentable carbohydrate [sucrose, glucose, fructose or glucose (5%) + fructose (5%)] and incubated further in the presence of added *S. sobrinus* for 48 h at 37°C in 10% CO2.

RESULTS

Fig. 1 shows that after 3h of incubation the number of *S. sobrinus* in the mixed colonies was greater than that of *S. mutans*. Afterwards, the number of *S. sobrinus* colonies decreased, and was lower than the number of CFU/mL of *S. mutans*. After 12 h of incubation, no viable *S. sobrinus* cells were detected.

As shown in Figs. 2, 3 and 4, the growth of *S. mutans* and *S. sobrinus* was similar regardless the substrate (glucose, fructose or glucose + fructose) added to the culture medium. The number of CFU/mL of *S. mutans* was greater than that of *S. sobrinus* for all incubation periods, in all culture media. After 12h of incubation, the samples obtained from the mixed culture of *S. mutans* and *S. sobrinus* in medium containing glucose, fructose or glucose + fructose, when plated on BHI – agar containing streptomycin, did not result in growth of *S. sobrinus*.

The growth interactions between *S. mutans* 32K and *S. sobrinus* 6715 in the in vitro colonization and recolonization process using media with different carbohydrate substrates added (Experiments I, II and III) are presented in Figs. 5, 6 and 7. According to these results, viable cells of *S. mutans* were shown to be present at the end of the incubation period while *S. sobrinus* decreased in number or was eliminated.

DISCUSSION

In the development of caries and formation of biofilms there are microbiotic interactions with the dental surface and among bacteria themselves and ecological changes caused by diet. Relevant physico-chemical characteristics and the composition of the plaque act together to initiate this pathology. These bacterial relationships that exist in the biofilm on the enamel surface are regulated by several factors such as the production of bacteriocins (antibacterial proteins that can interfere with the growth of other microorganisms, generally closely related bacteria). The bacteriocins produced by *S. mutans* are called mutacins, and these proteins are important for the establishment and balance of this species in dental plaque (7,15).

The results obtained from the bacterial suppression experiment shown in Figs. 1, 2 and 3 demonstrated similar behavior for *S. mutans* and *S. sobrinus* derived from mixed cultures during the incubation period, even though they showed a higher number of CFU/mL in medium with sucrose added when compared to the other carbohydrates. These data confirm other studies such as those of Ashley and Wilson (2), Brecc
et al. (4), Macpherson and Dawes (12), Gibbons and Van Houte (6) and Margolis et al. (13), which determined sucrose to be the main substrate of the streptococcus mutans group. After a short incubation period (3 h), the mixed culture in medium containing sucrose or glucose (10%), fructose (10%) or glucose (5%) + fructose (5%) showed a greater number of CFU/mL of S. sobrinus compared to S. mutans. After a period of 12 h, there was no detection of viable cells of S. sobrinus in any of the culture media tested. These results are in agreement with Ikeda et al. (10), who showed that S. sobrinus was eliminated in mixed in vitro cultures by bacteriocins produced by S. mutans.

Figure 1. Number of CFU/mL of S. mutans and S. sobrinus in the mixed culture in medium containing 10% sucrose, during incubation at 37°C.

Figure 2. Number of CFU/mL of S. mutans and S. sobrinus in the mixed culture in medium containing 10% glucose, during incubation at 37°C.

Figure 3. Number of CFU/mL of S. mutans and S. sobrinus in the mixed culture in medium containing 10% fructose, during incubation at 37°C.

Figure 4. Number of CFU/mL of S. mutans and S. sobrinus in the mixed culture in medium containing glucose (5%) + fructose (5%), during incubation at 37°C.

The data regarding bacterial plaque colonization and recolonization capacity of S. mutans and S. sobrinus strains are shown in Figs. 5, 6 and 7. These values indicate a higher number of viable cells of S. mutans in agreement with the results of Ikeda et al. (10) demonstrating the capacity of S. mutans to produce bacteriocins that inhibit the growth of other streptococcus of the mutans group and to recolonize the pre-formed bacterial plaque. These data suggest that S. mutans bacteriocins (mutacin) are able to eliminate sensitive plaque bacteria, resulting in greater colonization in vivo. Strains of the same S. mutans genotype show different mutacin profile,
Bacterial plaque by *S. mutans* and *S. sobrinus*

**Figure 5.** Number of CFU/mL X 10² obtained from the bacterial plaque formed by considered-culture of equal numbers of *S. mutans* and *S. sobrinus* in medium containing different substrates – sucrose (10%), glucose (10%), fructose (10%) and glucose (5%) + fructose (5%).

**Figure 6.** Number of CFU/mL X 10² obtained from the bacterial plaque, pre-formed by *S. sobrinus* and inoculated with *S. mutans* samples, cultivated in medium containing different substrates – sucrose (10%), glucose (10%), fructose (10%) and glucose (5%) + fructose (5%).

suggesting a high degree of interstrain diversity. Mutacin production seems to be of clinical importance in the colonization of *S. mutans* and is highly diversified in the *S. mutans* species (11-13).

In conclusion, *Streptococcus mutans* showed a higher number of cells after short or long incubation periods when cultivated with *Streptococcus sobrinus* indicating its inhibition and recolonization capacity *in vitro*.
RESUMO

Supressão e recolonização de placa bacteriana por S. mutans e S. sobrinus in vitro

O estudo in vitro das interações entre S. mutans e S. sobrinus pode ser importante na determinação do papel desses microrganismos na formação de biofilmes nas estruturas dentais e seu potencial em induzir lesões cariosas. O objetivo da presente pesquisa foi estudar a supressão da formação da placa dental e sua recolonização por S. mutans rifampicina-resistentes e S. sobrinus estreptomicina-resistentes in vitro. Para avaliar as relações de competitividade entre essas espécies, cepas que foram previamente padronizadas foram incubadas em meio de cultura contendo diferentes carboidratos fermentáveis. Em intervalos de tempo determinados,amostras de S. mutans e S. sobrinus foram coletadas a partir de culturas mistas, diluídas e semeadas em placas com meio BHI-ágardemiuviáveis de cada espécie por contagem de unidades formadoras de colônia. Para a avaliação da colonização bacteriana e recolonização da placa bacteriana in vitro, três experimentos foram realizados: I – co-cultivo de S. mutans e S. sobrinus; II – inoculação de S. mutans em placa bacteriana pré-formada por S. sobrinus; e III – placa bacteriana pré-formada por S. mutans dispersada e plaqueada em meio BHI-ágardemiuviáveis de S. mutans e S. sobrinus e estreptomicina-resistentes em meio BHI-ágardemiuviáveis de S. mutans e S. sobrinus e estreptomicina-resistentes. Os resultados indicaram uma predominância de S. mutans em relação ao S. sobrinus, demonstrando a capacidade do S. mutans em inibir a formação de placa por S. sobrinus e recolonizar a superfície dentária.

Palavras-chave: Streptococcus mutan, Streptococcus sobrinus, supressão

REFERENCES

Bacterial plaque by *S. mutans* and *S. sobrinus*


