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ORIGINAL ARTICLE

Morphological and proteomic analyses of the biofilms generated by *Streptococcus mutans* isolated from caries-active and caries-free adults

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biofilm;
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Abstract *Background/purpose:* Biofilm formation by *Streptococcus mutans* is a prerequisite for the development of caries. Different strains of *S. mutans* may differ in their capacity in biofilm formation and protein expression. The objective of this study was to investigate the morphological features and proteomes of biofilms of *S. mutans* clinical isolates.
Materials and methods: Clinical strains isolated from caries-active (SM 593) and caries-free (SM 18) adults were cultured on polystyrene sheets in tryptone–polypeptone–yeast extract medium. Biofilm formation and structure were assessed by confocal laser scanning microscopy and scanning electron microscopy. Proteins were extracted from SM 593 and SM 18 presented in biofilms and separated with two-dimensional gel electrophoresis, followed by peptide mass fingerprinting using matrix-assisted laser desorption time-of-flight mass spectrometry analysis.
Results: Initially detected 2 hours after incubation, biofilm formation reached its maximum level at 20 hours. The biofilm formed by SM 593 was thicker with a higher percentage of viable bacteria compared with that formed by SM 18. Hydrolase and pantothenate kinase were detected in the SM 593 biofilm only, whereas 6-pyruvoyl tetrahydropterin synthase and phosphoribosylglycinamide formyltransferase were expressed exclusively in the SM 18 biofilm. Expressions of D-alanyl-D-alanine carboxypeptidase and response regulator homolog of RumR were most greatly enhanced in the SM 593 and SM 18 biofilms, respectively.

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Conclusion: SM 593 exhibited greater biofilm-forming capacity compared to SM 18. SM 593 and SM 18 biofilms expressed specific proteins involved in nucleic acid metabolism and intermediary metabolism, respectively, which may account for the differences in their biofilm-forming abilities.

Introduction

One of the greatest challenges facing any pathogen attempting to cause dental caries is simply surviving the complex environment of the oral cavity. Considerable research has shown that the cells growing in biofilms are more antibiotic resistant and acid tolerant than their planktonic counterparts.[1—3] Dental biofilm formation is the prerequisite for bacteria to adhere and grow, and to withstand dynamic changes in oral cavity environment as well.[4] Polysaccharides produced by exoenzymes from *Streptococcus mutans* are the main constituents of the matrix of cariogenic plaque biofilms and are recognized as essential virulence factors associated with dental caries.[5] Among important virulence factors of this pathogen, the ability of *S. mutans* to form and sustain a polysaccharide-encased biofilm is vital not only to its survival and persistence in the oral cavity, but also for its pathogenicity.[6]

Although *S. mutans* are generally considered to be the principal etiological agent of dental caries,[7,8] they are widely distributed in both caries-active populations[9,10] and populations having no or low caries experience.[11,12] In order to find the possible explanation for their presence in caries-free individuals, several studies have been carried out to investigate the genetic heterogeneity among *S. mutans* strains.[13,14] However, the relationship between caries activity and the genetic diversity of *S. mutans* still remains controversial.[15,16]

Protein is the product of gene expression and is the final executor of function of gene. Surface-associated proteins play an important role in cariogenicity. Research has shown that biofilm regulatory protein A and glucan-binding proteins expressed by *S. mutans* are essential for survival of bacteria and biofilm formation within the host, and play a fundamental role in the interaction between the bacterial cell and its environment.[17,18] Our previous study demonstrated that, compared with those in planktonic status, clinical isolations of *S. mutans* in biofilms have higher expression of certain surface-associated proteins that are presumed to be essential for formation of biofilms.[19]

Based on the importance of biofilm-forming capability and surface-associated protein expression by *S. mutans* in the initiation and progression of caries, we hypothesized that differences exist between biofilm-forming capability and surface-associated protein expression by strains of *S. mutans* isolated from caries-active and caries-free individuals. To testify this, the process of biofilm formation by *S. mutans* strain was monitored with confocal laser scanning microscopy (CLSM) and scanning electron microscopy (SEM); expression of surface-associated proteins in bacteria was detected using two-dimensional gel electrophoresis (2-DE) followed by matrix-assisted laser desorption—ionization time-of-flight mass spectrometry (MALDI-TOF-MS).

Materials and methods

Bacterial strains and culture conditions

SM 593 was isolated from caries-active adults [the number of decayed and filled teeth (DFT) = 10, no missing tooth, 3 cavitated lesions]. SM 18 was isolated from caries-free adults [the number of decayed, missing, and filled teeth (DMFT) = 0] (both SM 593 and SM 18 were isolated in our previous study). The strains were stored at −80°C in basic growth medium containing 15% (v/v) glycerol. Bacteria were cultured anaerobically on tryptone—polypeptone—yeast extract (TPY) (Oxoid, Hampshire, England) at 37°C as described previously.[20] Cultivation of bacteria was performed in an anaerobic environment (80% N_2, 10% CO_2, and 10% H_2). Pure cultures of each test strain were obtained and suspended in fresh TPY to an optical density (OD) of 1.0 at 630 nm (approximately 10^8 cells/mL) for the following experiments.

Biofilm formation on polystyrene sheets and petri dishes

S. mutans biofilms were formed on sterile plastic sheets (1×1 mm^2) and plastic petri dishes (Dow Corning, Wiesbaden, Germany). In one group, the plastic sheets were immersed in plastic petri dishes containing 19 mL of TPY and 1 mL of bacterial suspension, as mentioned above. The plastic sheets were then incubated in an anaerobic chamber at 37°C for 2 hours, 4 hours, 6 hours, 12 hours, 20 hours, and 24 hours. For the other group, 19 mL of TPY and 1 mL of bacterial suspension were placed directly in plastic petri dishes without the plastic sheets and incubated in an anaerobic chamber at 37°C for 2 hours, 4 hours, 6 hours, 12 hours, 16 hours, and 20 hours.

Colony forming units assay

Biofilms were washed twice with 0.01M phosphate buffered saline (PBS, pH 7.4) at 2 hours, 4 hours, 6 hours, 12 hours, 16 hours, 20 hours, and 24 hours to remove excess medium and unattached cells. Then, 100 μL of PBS was added to each dish, and biofilm cells were scraped off the surface of the polystyrene petri dishes using a sterile cell scraper. The detached biofilm cells were serially diluted 10-fold and inoculated on TPY agar at 37°C for 24 hours in triplicate.