Ann Microbiol (2015) 65:1159–1163
DOI 10.1007/s13213-014-0883-2

SHORT COMMUNICATION

Growth in the presence of sucrose may decrease attachment of some oral bacteria to abiotic surfaces

Yi Wang · Sui M. Lee · Gary A. Dykes

Received: 4 December 2013 / Accepted: 18 March 2014 / Published online: 12 April 2014

Abstract Synthesis of cell-bound glucan from dietary sucrose by oral pathogenic bacteria may influence bacterial cell surface properties and colonization of surfaces. This study investigated the effects of the addition of 2 % sucrose to culture medium on cell surface properties (hydrophobicity, charge, and auto-aggregation) and colonization activities (attachment and biofilm formation) on three abiotic surfaces (hydroxyapatite, glass, and stainless steel) of two *Streptococcus mutans* strains, one *Streptococcus salivarius* strain, one *Streptococcus mitis* strain, and one *Actinomyces naeslundii* strain. The results showed that the additional sucrose reduced the hydrophobicity of three strains (44-62 %) and increased that of one strain (31 %). Cellular aggregation of one strain was decreased (13 %) and that of another increased (21 %). No change in the surface charge of strains was apparent. Additional sucrose also inhibited the attachment of three strains (0.6-1.3 log CFU cm^{-2}) and enhanced that of one strain (0.5-1.3 log CFU cm^{-2}) to glass and stainless steel. The attachment of two strains to hydroxyapatite was reduced (0.9-1.3 log CFU cm^{-2}). Biofilm formation by four strains was enhanced on all surfaces (0.4-1 log CFU cm^{-2}). No relationship between changes in cell surface properties and changes in colonization activities was apparent. Sucrose does not always enhance oral bacterial colonization of abiotic surfaces.

Keywords Bacterial colonization · Bacterial surface properties · Cell-bound glucan · Oral bacteria · Sucrose

Many oral bacteria can colonize tooth surfaces, resulting in the development of dental plaque and induction of dental caries (Weerkamp and Jacobs 1982; Kolenbrander and London 1993). It is well known that oral colonizers such as oral streptococci and *Actinomyces naeslundii* synthesize water-insoluble, adhesive glucan from dietary sucrose by the enzymatic action of glucosyl transferase (GTF) (Hamada et al. 1978; Sato et al. 1990; Ozek 2011) and form early plaque, which can be colonized by over 350 species of bacteria.

Glucan synthesis can occur on the surfaces of bacterial cells and form a layer of glucan film coating the cells (Hamada et al. 1978). This cell-bound glucan can induce bacterial cellular aggregation (Vickerman and Jones 1995), which enhances the ability of oral bacteria to adhere and grow on tooth surfaces (Kolenbrander and London 1993). This may help to explain the ability of oral bacteria to attach to various abiotic surfaces including glass, steel wire, hydroxyapatite, and extracted and artificial teeth (Slade 1976). Cell-bound glucan may also affect cell surface physicochemical properties, such as hydrophobicity and charge, and consequently influence bacterial colonization.

The role of sucrose in dental health has been studied *in vitro* or *in situ* in oral environments by many researchers with respect to the biochemistry of sucrose fermentation, mineral equilibrium, bacterial adhesion and biofilm formation (Cury et al. 2000; Aires et al. 2006; Paes Leme et al. 2006). The physicochemical effects of sucrose on the surface properties of oral bacteria and their colonization of abiotic surfaces has not been previously investigated.

In this study five strains of oral bacteria were grown in culture medium with or without 2 % sucrose. The effects of the addition of sucrose to the culture medium were investigated with respect to bacterial cell surface physicochemical properties (hydrophobicity, charge, and auto-aggregation) and two colonization activities (attachment and biofilm formation) on hydroxyapatite (a tooth model; Apella et al. 2008) and two other abiotic surfaces (glass and stainless steel) with different surface properties.

Y. Wang · S. M. Lee · G. A. Dykes (✉)
School of Science, Monash University, Jalan Lagoon Selatan, Bandar Sunway 46150, Selangor Darul Ehsan, Malaysia
e-mail: gary.dykes@monash.edu

Five bacterial strains of oral origin, namely *Streptococcus mutans* ATCC 25175, *Streptococcus mutans* ATCC 35668, *Streptococcus salivarius* ATCC 13419, *Streptococcus mitis* ATCC 49456, and *Actinomyces naeslundii* ATCC 51655, were obtained from the American Type Culture Collection (Manassas, USA) and used in this study. All bacteria were maintained on Mitis Salivarius Agar (MSA; Difco, USA) at 4 °C and grown in Tryptic Soy Broth (TSB; Merck, USA) with or without 2 % sucrose (Antonio et al. 2010) at 37 °C for 24 h (48 h for *A. naeslundii* ATCC 51655) with shaking at 150 rpm. Bacterial suspensions were prepared by centrifuging 20 ml of TSB cultures at 7669 g for 15 min. The pellets were washed with 150 mmol l^{-1} phosphate buffered saline (PBS; 2.7 mmol l^{-1} KCl, 10 mmol l^{-1} Na_2HPO_4, 17 mmol l^{-1} KH_2PO_4, and 137 mmol l^{-1} NaCl, pH 7.4; 1st BASE, Singapore) and resuspended in 20 ml PBS for all experiments.

The cell surface hydrophobicity was determined using the Bacterial Attachment to Hydrocarbon (BATH) method as previously described by Rosenberg et al. (1980) with slight modification. Bacterial suspensions were adjusted to an optical density (OD) of 1.0±0.2 at 550 nm. A 3 ml aliquot of each sample was mixed with 1 ml of hexane (Sigma-Aldrich, USA) and vortexed for 2 min. The mixture was allowed to separate for 1 h at 37 °C. The OD_{550} of the aqueous layer was measured before (A_0) and after (A) the addition of hexane. The cell surface hydrophobicity was expressed as % binding to hexane=$(1 - A/A_0)\times 100$ %.

Bacterial cell surface charge was measured as described by Bayoudh et al. (2009). A 1 ml aliquot of bacterial suspension at a cell density of 10^7 CFU ml^{-1} (pH 7.4) was measured for cell surface charge using a zetasizer (Nano ZS-ZEN3600; Malvern Instruments Ltd., UK). Cell surface charge was expressed as zeta potential (mV).

Auto-aggregation measurements were performed as described by Ellen and Balcerzak-Raczkowski (1977). A 1 ml aliquot of bacterial suspension was adjusted to an OD of 0.25± 0.05 at 600 nm prior to incubation at 37 °C for 6 h. The OD_{600} was measured before (A_i) and after (A_f) incubation. Aggregation percentage was expressed as % Auto-aggregation=$(1 - A_f / A_i)\times 100$ %.

Hydroxyapatite surfaces were prepared by coating glass slides with hydroxyapatite powder (Sigma-Aldrich) as previously described by Wang et al. (2013). Glass slides (75× 25 mm; Thermo Fisher Scientific, USA) were first coated with a bonding adhesive (60 % limestone, 30 % kaolin, 8 % ethylene glycol, 1 % SiO_2 and 1 % TiO_2, % w/w; Over Sea Plaster Supply and Construction Sdn. Bhd., Malaysia) mixed with distilled water at a ratio of 6:4 (w:v) and partially dried in air for 3 min. The slides were then coated with a paste consisting of 10 % cement powder (56 % CaO, 40 % SiO_2, 3 % $Al_2O_3 \cdot Fe_2O_3$, and 1 % MgO, % w/w; Over Sea Plaster Supply and Construction Sdn. Bhd., Malaysia), 40 % hydroxyapatite powder, and 50 % distilled water; the slides were then air-dried for 16 h.

Glass, stainless steel (75×25 mm; type 302, #4 finish), and hydroxyapatite slides were degreased by soaking in acetone for 30 min, rinsed in distilled water, and sterilized by autoclaving.

The attachment assays were carried out as previously described by Wang et al. (2013). A 20 ml bacterial PBS suspension containing 10^7 CFU ml^{-1} was incubated with a hard surface slide at 37 °C for 30 min without shaking. Bacterial cells were suspended in PBS and incubated for only 30 min to ensure that the cells did not grow during the incubation period. After incubation the slide was removed from the suspension, gently washed three times with PBS to remove loosely attached cells, and stained by 0.1 % w/v crystal violet (for glass; Sigma-Aldrich) or 0.1 % w/v acridine orange (for hydroxyapatite and stainless steel; Sigma-Aldrich). The attached cells were counted under a light or epifluorescence microscope (BX51; Olympus, Japan). A total of 50 fields were counted for each slide, and the number of attached cells was calculated and expressed as log CFU cm^{-2}. Attached cells of *A. naeslundii* ATCC 51655 were not counted under a microscope because they are morphologically indistinct and not countable (Coleman et al. 1969). They were, therefore, enumerated using a method adapted from Chia et al. (2011). After rinsing, slides with *A. naeslundii* ATCC 51655 cells attached were placed in a stomacher bag (Gossenlin, France) containing 50 ml of PBS and pummeled in a stomacher (400P, BigMixer®, France) for 10 min at a speed setting of 1 to remove the attached cells. The pummeled slides were stained and visualized under a microscope for 50 fields. No cells were observable; stomaching was, therefore, considered to be an effective means for the removal of attached cells. Aliquots of PBS were taken from the stomacher bags, serially diluted, spread-plated on Tryptic Soy Agar (TSA; Merck), and quantified after 48 h incubation at 37 °C. Chia et al. (2011) established that counts of attached bacteria obtained by the two methods (microscopic counting and stomaching) were not significantly different.

Biofilm formation assays were performed as described by Wang et al. (2013). A 0.1 ml aliquot of a 24 h TSB culture was inoculated into 20 ml of TSB with or without 2 % sucrose and incubated statically with a hard surface slide at 37 °C for 72 h to allow a biofilm growth on the slide. After incubation the slide was washed three times with PBS and placed in a Falcon tube (TPP®, Switzerland) containing 50 ml of PBS. The tube was sonicated for 10 min using a water bath sonicator (LC-130H; ELMA, Germany) at room temperature at a frequency of 35 kHz to detach the biofilm cells into the surrounding PBS. An aliquot of the PBS was serially diluted, spread-plated on TSA, and incubated at 37 °C for 48 h prior to enumeration.

All assays were carried out in triplicate with independently grown cultures. A Student's *t*-test was performed on all data

sets at a 95 % confidence level using SPSS software (PASW Statistics 18; SPSS Inc.).

The effects of sucrose on the cell surface properties of the five strains studied are shown in Fig. 1. It was found that all strains were hydrophobic (as defined by Gibbons and Etherden 1983), ranging from 60 to 90 % adhesion to the hydrocarbon. The addition of sucrose to the culture medium significantly reduced ($p<0.05$) the cell surface hydrophobicity (Fig. 1a) of the two *S. mutans* strains and the *S. salivarius* strain by 62, 52 and 44 %, respectively. The addition of sucrose significantly increased ($p<0.05$) the hydrophobicity of the *A. naeslundii* strain by 31 % but had no effect ($p>0.05$) on that of the *S. mitis* strain. All strains were negatively charged (Fig. 1b) with zeta potentials ranging from -4 to -12 mV. The net charges of the strains were not affected ($p>0.05$) by the addition of sucrose to the culture medium. All strains exhibited moderate to relatively high auto-aggregation (as defined by Ellen and Balcerzak-Raczkowski 1977) (Fig. 1c), ranging from 22 to 45 %. The addition of sucrose to the culture medium significantly reduced ($p<0.05$) the auto-aggregation of *S. mutans* ATCC 25175 by 13 %, significantly increased ($p<0.05$) that of the *S. mitis* strain by 21 %, and had no effect ($p>0.05$) on that of the other three strains.

The effects of cell-bound glucan on cell surface hydrophobicity might be due to the hydrophilic nature of glucan, which reduces the surface tension between cells and the aqueous medium, resulting in a reduced hydrophobicity (Van Oss et al. 1986). This was, however, not the case for the *S. mitis* and *A. naeslundii* strains, suggesting that cell surface hydrophobicity is not solely dependent on surface tension. Based on the assumption that glucan can affect surface tension, the reduced auto-aggregation seen in this study was expected. Vickerman and Jones (1995), on the other hand, indicated that synthesis of extracellular glucan enhances cellular aggregation of dental plaque-associated bacteria. The changes in auto-aggregation observed in this study, however, did not have a uniform pattern for all five strains. It was also found that glucan did not affect cell surface charge of the strains tested. Based on the soft particle theory (Ohshima 2009), which demonstrates that the zeta potential of a particle can be affected by a charged ion-penetrable layer (e.g. protein and carbohydrate) coated on its surface, it is reasonable to assume that the glucan film on the cell surfaces was ion-penetrable and did not carry a charge.

The effects of sucrose on the attachment of the five bacterial strains to hydroxyapatite, glass, and stainless steel are presented in Figs. 2a to c. The four streptococci strains attach to the hard surfaces in similar numbers to each other, ranging from 6.1 to 6.5 log CFU cm^{-2}. The *A. naeslundii* strain attached in significantly lower ($p<0.05$) numbers (~5.5 log CFU cm^{-2}) than the streptococci did. The addition of sucrose to the culture medium reduced ($p<0.05$) the attachment of the two *S. mutans* strains and the *S. mitis* strain by 0.6 to 1.3 log CFU cm^{-2} with respect to all of the three hard surfaces, except in the case of attachment of the *S. mitis* strain to hydroxyapatite ($p>0.05$). The addition of sucrose to the culture medium also enhanced ($p<0.05$) the attachment of the *A. naeslundii* strain to glass and stainless steel by 0.5 and 1.3 log CFU cm^{-2}, respectively. It had no effect ($p>0.05$) on the attachment of the *S. salivarius* strain for all surfaces.

It is generally agreed in the literature that cell-bound glucan enhances the level and strength of attachment of oral bacteria (Marshall et al. 1971; Vickerman and Jones 1995). The results in this study, however, showed the opposite for three strains. Mukasa and Slade (1973) indicated that the attachment of *S. mutans* to glass surfaces could only be increased by post-formed glucan but not pre-formed glucan. Clark and Gibbons (1977) reported a reduction of attachment to hydroxyapatite in an *S. mutans* strain grown in sucrose conditions, a finding which is in agreement with this study. This could be due to the inability of free streptococcal cells coated with glucan films to adopt the ideal steric arrangement and strongly interact with the substratum surface (Clark and Gibbons 1977) and that an 'active' form of glucan is required to enhance attachment (Mukasa and Slade 1973). Cell-bound glucan may also block the attachment sites on the cell surfaces and result in a reduced attachment (Vickerman and Jones 1995). *S. salivarius* may have behaved differently than the other strains due to its larger cell size as compared to other oral streptococci (Sherman et al. 1943). The *A. naeslundii* strain may have behaved differently to the other strains due to its clumped and filamentous morphology (Coleman et al. 1969) which may affect attachment. In addition, cell-bound glucan had a different effect on the attachment of the *S. mitis* and *A. naeslundii* strains to hydroxyapatite

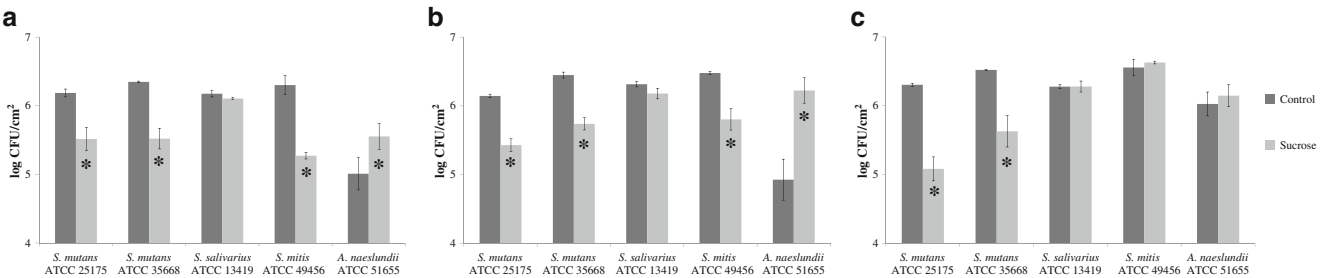

Fig. 1 The effects of sucrose on (**a**) surface hydrophobicity, (**b**) charge, and (**c**) auto-aggregation of five bacterial strains of oral origin. All results are presented in mean ± SD with n=3. Values labeled with the * symbol are significantly different from the control ($p<0.05$)

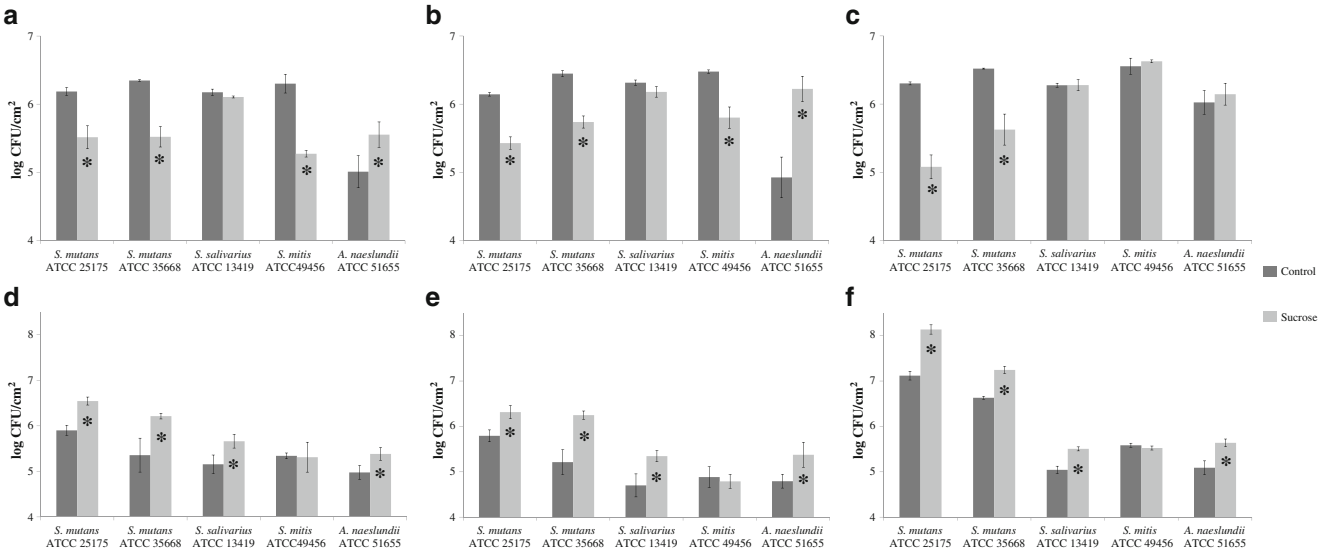

Fig. 2 The effects of sucrose on the colonization of abiotic surfaces by oral bacteria. Attachment (the upper row) and biofilm formation (the lower row) by five bacterial strains of oral origin on (**a**, **d**) glass, (**b**, **e**) stainless steel, and (**c**, **f**) hydroxyapatite. All results are presented as mean ± SD where n=3. Values labeled with the * symbol are significantly different from the control ($p<0.05$)

as compared to the other two abiotic surfaces. This may be due to the enlargement of cell size by the glucan film resulting in a different spatial arrangement of cells on hard surfaces displaying differences in roughness (Medilanski et al. 2002).

The effects of sucrose on biofilm formation by the five strains for hydroxyapatite, glass, and stainless steel are shown in Figs. 2d to e. Both *S. mutans* strains formed a significantly greater ($p<0.05$) amount of biofilm on hydroxyapatite (6.6 to 7.1 log CFU cm^{-2}) than on the other two surfaces tested (5.2 to 5.8 log CFU cm^{-2}). The biofilm formed by the two *S. mutans* strains on hydroxyapatite was also significantly greater ($p<0.05$) than the biofilm formed by the other strains on all surfaces (4.7 to 5.6 log CFU cm^{-2}). The addition of sucrose to the culture medium significantly enhanced ($p<0.05$) biofilm formed by the two *S. mutans* strains, the *S. salivarius* strain, and the *A. naeslundii* strain on all of the surfaces by 0.4 to 1 log CFU cm^{-2}. It had no effect ($p>0.05$) on biofilm formation by the *S. mitis* strain.

It was reported by many authors that cell-bound glucan enhanced biofilm formation by oral bacteria (Abbott et al. 1983; Lynch et al. 2007); the findings of this study concur. It was also apparent in the present study that the addition of sucrose enhanced biofilm formation by the two strains of *S. mutans* on all surfaces, but inhibited their attachment. This was probably because the glucan was pre-formed in the attachment assays, and therefore, reduced attachment as discussed above, while the glucan in the biofilm assays was formed after the cells attached to the surfaces. Post-formed glucan enhances biofilm formation due to its sticky nature (Jordan and Keyes 1966) that glued the cells to the hard surfaces. Bofilm formation by the *S. mitis* strain was not enhanced by glucan, and this could be due to the fact that the amount of glucan produced by *S. mitis* is approximately 10-fold lower as compared to other oral bacteria (Hamada et al. 1978). It was also apparent that the surface coverage (in log CFU cm^{-2}) of the biofilms was, in some cases, lower than those obtained from the attachment assays. This could be due to the different methods used to quantify attachment and biofilm formation. For example, sonication may not be able to break bacterial clumps detached from biofilms down into individual cells, which, in the subsequent plate counts, resulted in lower cell numbers than the actual cell numbers in the biofilms. Therefore, the results (in log CFU cm^{-2}) are directly comparable only when they were obtained by the same method, but not between the attachment assays and the biofilm assays.

This study found that the synthesis of cell-bound glucan can affect cell surface physical properties and colonization behavior on abiotic surfaces of some dental plaque-associated bacteria, but not all of them. There was, however, no clear relation between the changes in cell surface properties induced by sucrose and the changes in the colonization behavior. This suggests that the effects of glucan on bacterial attachment and biofilm formation were strain-dependent and were not purely due to the physical influence of glucan on the cells. The strain-specificity needs to be further confirmed by studies involving a larger diversity of oral strains and GTF-deficient mutants. This study also indicates that glucan on cell surfaces does not always enhance bacterial colonization. However, sucrose affects dental health not only at the physicochemical level but also at the molecular level. For example, sucrose can be fermented by oral bacteria into different organic acids which induce a low pH in oral environments that can demineralize enamel and dentin and trigger a shift in the balance of plaque microflora to a cariogenic one (Paes Leme et al. 2006). In addition, the role glucan plays in the structure of oral biofilm may also contribute to its cariogenicity, as

glucan increases the porosity of a biofilm and allows fermentable carbohydrates to diffuse into the interior of the biofilm, which in turn results in a lowered pH (Zero et al. 1986); and glucan promotes selective adherence of bacteria, especially that of cariogenic streptococci (Schilling and Bowen 1992), as shown in the present study. Sucrose has also been reported to reduce the concentrations of calcium, phosphorus, and fluoride in dental biofilms, which in turn may interfere with the mineral equilibrium between teeth and the oral environment (Paes Leme et al. 2006).

To carry out a more systematic study to verify these findings, saliva/saliva component conditioning film-coated hydroxyapatite surfaces should be used, and glucanhydrolase assays should be performed. A more extensive investigation into the role of glucan on the structure of biofilm matrices is also warranted based on the finding of the present study. In conclusion, the role that sucrose plays in surface attachment and biofilm formation by oral bacteria may be dependent on the species, the strain of bacteria, and the form of glucan synthesized. Consumption of dietary sucrose may, therefore, not always enhance the colonization of dental hard tissues by oral bacteria, but this finding needs to be further confirmed by *in vivo* studies.

Acknowledgements The authors acknowledge financial support for this study from Monash University, Malaysia.

References

Abbott A, Rutter PR, Berkeley RCW (1983) The influence of ionic strength, ph and a protein layer on the interaction between *Streptococcus mutans* and glass surfaces. J Gen Microbiol 129:439–445

Aires CP, Tabchoury CP, Del Bel Cury AA, Koo H, Cury JA (2006) Effect of sucrose concentration on dental biofilm formed *in situ* and on enamel demineralization. Caries Res 40:28–32

Antonio AG, Moraes RS, Perrone D, Maia LC, Santos KRN, Iório NLP, Farah A (2010) Species, roasting degree and decaffeination influence the antibacterial activity of coffee against *Streptococcus mutans*. Food Chem 118:782–788

Apella MC, Venegas SC, Rodenas LAG, Belsa MA, Morando PJ (2008) Synthetic hydroxyapatite as a surface model of dental enamel and dentine. J Argent Chem Soc 97:109–118

Bayoudh S, Othmane A, Mora L, Ouada HB (2009) Assessing bacterial adhesion using DLVO and XDLVO theories and the jet impingement technique. Colloid Surf B 73:1–9

Chia TWR, Nguyen VT, McMeekin T, Fegan N, Dykes GA (2011) Stochasticity of bacterial attachment and its predictability by the extended Derjaguin-Landau-Verwey-Overbeek theory. Appl Environ Microbiol 77:3757–3764

Clark WB, Gibbons RJ (1977) Influence of salivary components and extracellular polysaccharide synthesis from sucrose on the attachment of *Streptococcus mutans* 6715 to hydroxyapatite surfaces. Infect Immun 18:514–523

Coleman RM, Georg LK, Rozzell AR (1969) *Actinomyces naeslundii* as an agent of human actinomycosis. Appl Environ Microbiol 18:420–426

Cury JA, Rebelo MA, Del Bel Cury AA, Derbyshire MT, Tabchoury CP (2000) Biochemical composition and cariogenicity of dental plaque formed in the presence of sucrose or glucose and fructose. Caries Res 34:491–497

Ellen RP, Balcerzak-Raczkowski IB (1977) Interbacterial aggregation of *Actinomyces naeslundii* and dental plaque streptococci. J Periodontal Res 12:11–20

Gibbons RJ, Etherden I (1983) Comparative hydrophobicities of oral bacteria and their adherence to salivary pellicles. Infect Immun 41: 1190–1196

Hamada S, Tai S, Slade HD (1978) Binding of glucosyltransferase and glucan synthesis by *Streptococcus mutans* and other bacteria. Infect Immun 21:213–220

Jordan HV, Keyes PH (1966) *In vitro* methods for the study of plaque formation and carious lesions. Arch Oral Biol 11:793–802

Kolenbrander PE, London J (1993) Adhere today, here tomorrow: oral bacterial adherence. J Bacteriol 175:3247–3252

Lynch DJ, Fountain TL, Mazurkiewicz JE, Banas JA (2007) Glucan-binding proteins are essential for shaping *Streptococcus mutans* biofilm architecture. FEMS Microbiol Lett 268:158–165

Marshall KC, Stout R, Mitchell R (1971) Mechanism of the initial events in the sorption of marine bacteria to surfaces. J Gen Microbiol 68: 337–348

Medilanski E, Kaufmann K, Wick LY, Wanner O, Harms H (2002) Influence of the surface topography of stainless steel on bacterial adhesion. Biofouling 18:193–203

Mukasa H, Slade HD (1973) Mechanism of adherence of *Streptococcus mutans* to smooth surfaces I. roles of insoluble dextran-levan synthetase enzymes and cell wall polysaccharide antigen in plaque formation. Infect Immun 8:555–562

Ohshima H (2009) Theory of electrostatics and electrokinetics of soft particles. Sci Technol Adv Mater 10:063001

Ozek Y (2011) Characterization of a large amount of water-insoluble polysaccharide synthesis *Streptococcus salivarius*. Int J Oral-Med Sci 10:25–30

Paes Leme AF, Koo H, Bellato CM, Bedi G, Cury JA (2006) The role of sucrose in cariogenic dental biofilm formation-new insight. J Dent Res 85:878–887

Rosenberg M, Gutnick D, Rosenberg E (1980) Adherence of bacteria to hydrocarbons: a simple method for measuring cell surface hydrophobicity. FEMS Microbiol Lett 9:29–33

Sato S, Yoshimitsu-Narita A, Eifuku H, Inoue M (1990) Isolation and some properties of extracellular glucan-producing strains of human oral *Streptococcus salivarius*. Microbios 62:101–112

Sherman JM, Niven CF Jr, Smiley KL (1943) *Streptococcus salivarius* and other non-hemolytic streptococci of the human throat. J Bacteriol 45:249–263

Slade HD (1976) *In vitro* models for the study of adherence of oral streptococci. In: Bowen WH, Genco RJ, O'Brien TC (eds) Immunologic aspects of dental caries, a special supplement to immunology abstracts. Information Retrieval, Inc., Washington, D.C., pp 21–28

Van Oss CJ, Good RJ, Chaudhury MK (1986) The role of van der Waals forces and hydrogen bonds in 'hydrophobic interactions' between biopolymers and low energy surfaces. J Colloid Interface Sci 111: 378–390

Vickerman MM, Jones GW (1995) Sucrose-dependent accumulation of oral streptococci and their adhesion-defective mutants on saliva-coated hydroxyapatite. Oral Microbiol Immunol 10:175–182

Wang Y, Lee SM, Dykes GA (2013) Potential mechanism of the effects of tea extracts on the attachment, biofilm formation and cell size of *Streptococcus mutans*. Biofouling 29:307–318

Weerkamp AH, Jacobs T (1982) Cell wall-associated protein antigens of *Streptococcus salivarius*: purification, properties, and function in adherence. Infect Immun 38:233–242

Zero DT, van Houte J, Russo J (1986) The intra-oral effect on enamel demineralization of extracellular matrix material synthesized from sucrose by *Streptococcus mutans*. J Dent Res 65:918–923