International Journal of Cosmetic Science, 2002, **24**, 225–234

Removal and prevention of dental plaque with D-tagatose

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Received 6 February 2002, Accepted 2 April 2002

Keywords: biofilm, dental plaque, D-tagatose, microbial coaggregation

Synopsis

Dental plaque develops when early bacterial colonizers adhere to the acquired pellicle (saliva-derived proteinous coating on the tooth surface) followed by adhesion of late interspecies colonizers to form this type of biofilm (coaggregation). In developing a D-tagatose-based toothpaste, we examined 15 oral isolates, including both early colonizers (*Streptococcus* and *Actinomyces*) and late colonizers (*Fusobacterium*, *Porphyromonas*, *Prevotella*, *Veillonella*, *Capnocytophaga*, and *Actinobacillus*), and tested them for their ability to coaggregate with each other. We then tested the ability of D-tagatose to reverse any such coaggregations. Coaggregation was examined visually and scored by using a system ranging from 0, for no visible coaggregation to 4, for maximum coaggregation. D-Tagatose, at a concentration of less than 750 mM, completely reversed the coaggregation of 17 (60%) of 28 strongly coaggregating pairs (coaggregation score = 2 or higher) tested. In contrast, D-sorbitol had little reversal effect. D-Tagatose-sensitive coaggregations were D-galactose-reversible as well. D-Tagatose acted on both early and late colonizers; both groups, especially the late colonizers, were frequently involved in periodontal diseases. Thus, D-tagatose has the potential for preventing and removing plaque development and for altering the subgingival microbiota. These effective qualities offer conservative control of gingival and periodontal disease.

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Résumé

La plaque dentaire se développe lorsque les premières colonizations bactériennes adhèrent à la pellicule acquise (revêtement protéique dérivé de la salive sur la surface dentaire), suivies par l'adhésion de colonisateurs multiespèces plus tardifs qui forment ce type de bio-film (coagrègation). Dans le développement d'un dentifrice à base de D- tagatose, nous avons examiné quinze isolats buccaux, incluant à la fois des colonisateurs précoces (Streptococcus et Actinomyces) et tardifs (Fusobacterium, Porphyromonas, Prevotella, Veillonella, Capnocytophaga et Actinobacillus) que nous avons testés dans leur capacité de se coagrèger mutuellement. Le pouvoir du D- tagatose d'inverser ces coagrègations fut alors testé. La coagrègation fut examinée visuellement puis scorée sur une échelle croissante, de 0 (pas d'agrègation visible) à 4 pour une agrégation maximale. Le D-tagatose, à une concentration inférieure à 750 mM, reversait complètement la coagrègation chez 17 (60%) des 28 paires coagrègées fortement (score 2 ou plus). Inversement, le D sorbitol montrait peu d'effet de reversions. Les coagègations sensibles au D tagatose étaient aussi réversibles par le D galactose. Le D tagatose agit à la fois sur les colonisateurs précoces et tardifs, ces deux groupes, principalement les tardifs, étant fréquemment impliqués dans les désordres du péri-odonte. Ainsi, le D tagatose possède le pouvoir de prévenir et éliminer le développement de la plaque dentaire et de modifier les microbiotypes sous

gingivaux. Ces qualités d'efficacité permettent un contrôle protecteur des désordres gingivaux et périodontaux.

Introduction

Over 500 different species of bacteria have been implicated in human dental plaque and caries [1]. Intra- and interspecies adhesion of bacteria to oral surfaces constitutes dental plaque, one of the major factors leading to periodontal diseases.

Streptococci and actinomyces are the major initial colonizers. Their adhesion to the pellicle of salivary glycoproteins on tooth surfaces is the first step in the formation of dental plaque [1]. Microorganisms that progressively accumulate thereafter, mostly Gram-negative anaerobic bacteria, in the gingival crevice area, are the late colonizers. They are believed to play a central role in the initiation and progression of periodontal diseases [2]. Stability of the plaque depends on interspecies coaggregation of bacteria. The bacterial species present in dental plaque are heterogeneous and their metabolisms change progressively as the clinical condition goes from normal health through gingivitis to advanced stages of periodontitis. Among all oral isolates, *Fusobacterium nucleatum* is the principal and most frequent cause of gingival inflammation. It is also the predominant pathogen in subsequent periodontal destruction [2–6]. *F. nucleatum* plays a central role by providing the physical bridges that mediate coaggregation of cells and promote anaerobic microenvironments. These microenvironments protect strict anaerobes within an aerobic atmosphere [1, 7]. *F. nucleatum* coaggregates with many putative periodontal pathogens, such as *Porphyromonas gingivalis, Actinobacillus actinomycetemcomitans, Prevotella intermedius* and certain species of *Treponema, Eubacterium,* and *Selenomonas* [8–15]. These periodonto-pathogens are also the prominent contributors to the formation of volatile sulphur compounds (VSC), the major components of halitosis [16–19]. Unlike *P. gingivalis*, usually absent in healthy gingival sulci [20, 21], *F. nucleatum* is one of the dominant species, not only in the lesions of periodontitis [20, 22, 23], but also in gingivitis lesions and healthy gingival sites [20, 21].

In vitro studies of several hundred pairs of oral bacteria revealed that coaggregation is the result of adhesion effected by specific interactions between complementary molecules on the surfaces of the participating bacteria [24]. However, the specific molecular mechanisms have been characterized for only a few. In many cases the coaggregation involves lectin–carbohydrate interaction whereby the sugar residues on one bacterium interact with a lectin on the surface of the other bacterium [12]. A number of simple and complex sugars and derivatives are now recognized to inhibit coaggregation: D-galactose, lactose, D-galactosamine, N-acetyl-D-galactosamine, methyl-α-D-galactoside, methyl-β-D-galactoside, D-fucose, sialic acid, and L-rhamnose [11, 15, 25–27].

Most lactose-reversible coaggregations are also D-galactose-reversible [11]. A comparison of the structures of the more coaggregation-inhibitory sugars to those of the less coaggregation-inhibitory sugars indicated that the D-galactose configuration of the hydroxyls (*cis* hydroxyls) on the C-3 and C-4 positions may be important for bacterial recognition [11]. Sugars with hydroxyls on the C-3 and C-4 positions in the *trans* configuration, such as L-rhamnose and N-acetyl-D-glucosamine, have no coaggregation-inhibitory effect, but 3, 4 *cis*-hydroxylated sugars that are methylated at either the C-1 or C-6 positions, such as methyl-D-galactoside and D-fucose, respectively, are inhibitory [11]. D-Tagatose is an isomer of D-galactose. Unlike D-galactose (an aldose), D-tagatose is a ketose. However, the configuration of the hydroxyls on the C-3 and C-4 positions is the same in both chemicals. Thus, it seemed plausible that the D-galactose-reversible coaggregations might also be D-tagatose-reversible. This study was undertaken to investigate the ability of D-tagatose to reverse interbacterial coaggregation.

Recently, D-tagatose was determined to be generally recognized as safe (GRAS) for use as a reduced-calorie bulk sweetener in foods and beverages, as an excipient in drugs, and as an ingredient in cosmetics [28]. The sweetener is now poised to enter such markets. In cosmetics, particularly toothpastes and mouthwashes, D-tagatose can greatly improve the taste [29]. However, the primary attribute of D-tagatose in these products is its excellent function as a humectant [30], which is essential for product performance and stability. Currently, D-sorbitol is very widely used as a humectant, either alone or in combination with other humectants, in toothpastes and mouthwashes. Therefore, this study compared the abilities of D-tagatose and D-sorbitol to reverse existing interbacterial coaggregations. It also compared the results with those achieved by parallel experiments with D-galactose, which is known to reverse bacterial coaggregations.

Materials and Methods

Bacterial Strains and Culture Conditions

The oral bacterial strains used in this study were *Streptococcus oralis* SO34, *Streptococcus oralis* C104, *Streptococcus mitis* J22, *Streptococcus morbillorum* PK509, *Actinomyces naeslundii* T14V, *Actinomyces naeslundii* PK29, *Actinomyces naeslundii* PK947, *Fusobacterium nucleatum* PK1594, *Porphyromonas gingivalis* PK1924, *Prevotella loescheii* PK1295, *Veillonella atypica* PK1910, *Capnocytophaga sputigena* 4, *Capnocytophaga ochracea* 25, *Capnocytophaga gingivalis* 27, and *Actinobacillus actinomycetemcomitans* JP2. They were originally isolated from human gingival crevices. The bacteria inocula, in the form of suspensions, were kept in cryogenic vials at −70 °C. They were revived by thawing at room temperature and inoculating into fresh media. Sterile procedures were used throughout.

The facultative anaerobes streptococci (*S. oralis* SO34, *S. oralis* C104, *S. mitis* J22, and *S. morbillorum* PK509) and actinomyces (*A. naeslundii* T14V, *A. naeslundii* PK29, and *A. naeslundii* PK947) were grown in brain–heart infusion broth (Difco 0037–17) in serum bottles (10 and 100 mL capacities) under an N_2 atmosphere (N_2 gassing for 5–10 min before autoclaving), and incubated at 37 °C.

F. nucleatum PK1594 was grown in brain–heart infusion broth (Difco 0037–17) supplemented with 0.25% NH_4 glutamate (Sigma G-1376). *P. gingivalis* PK1924 and *P. loescheii* PK1295 were grown in Todd Hewitt broth (Difco 0492–17) supplemented with 1 g L^{-1} yeast extract (Sigma Y-4000), 5 mg L^{-1} hemin (Sigma H-2250), and 1 mg L^{-1} vitamin K_1 (Sigma V-3501). A hemin stock (1 mg mL^{-1}) solution was prepared by dissolving 0.28 g KOH in 25 mL of 95% ethanol and adding 100 mg of hemin. The hemin was allowed to dissolve and was then brought up to 100 mL with deionized water. The solution was stored at 4 °C. A vitamin K_1 stock (1 mg mL^{-1}) solution was prepared in 100% ethanol and stored at 4 °C in a brown quartz vial. The vitamin K_1 was added to the broth after it was autoclaved. *V. atypica* PK1910 was grown in Schaedler broth (Difco 0534–17) supplemented with 0.1 M sodium lactate (Sigma L-1375). *C. sputigena* 4, *C. ochracea* 25, and *C. gingivalis* 27 were grown in Schaedler broth (Difco 0534–17) supplemented with 0.1% $NaHCO_3$. After autoclaving of the Schaedler broth, $NaHCO_3$ was added from a filter-sterilized (0.2 μm) 10% stock solution. *A. actinomycetemcomitans* JP2 was grown in Todd Hewitt broth (Difco 0492–17) supplemented with 2% yeast extract (Sigma Y-4000) and 0.1% $NaHCO_3$. After autoclaving of the broth, $NaHCO_3$ was added from a filter-sterilized (0.2 μm) 10% stock solution. All of these eight strict anaerobes were grown in serum bottles (10 and 100 mL capacities), incubated at 37 °C in 'prereduced' media (the media were gassed with N_2 for 5–10 min before autoclaving, and the headspace of the serum bottles was gassed with N_2 for 5–10 min immediately after inoculation). The N_2 was filtered through a 0.2-μm membrane filter to assure sterility.

The bacteria were serially subcultured from their −70 °C stocks two times in 10 mL capacity vials and maintained in a 37 °C incubator. Then, final cultures of 100 mL capacity were made and kept at 37 °C in a water-bath with a shaker run at 150 r.p.m. The cultures were harvested in the late exponential or early stationary growth phases and washed three times with coaggregation buffer, which consisted of: 0.1 mM $CaCl_2$, 0.1 mM $MgCl_2$, 0.02% NaN_3, and 0.15 M NaCl (dissolved in 1 mM Tris with pH adjusted to 8). The cultures were centrifuged at 7000 **g** for 10 min at room temperature. The cells were then washed and stored in coaggregation buffer at 4 °C, until used.

Coaggregation Assay

Cell suspensions of the pure species were adjusted to a cell density of 1 g L^{-1} (about 4–5 × 10^9 cells mL^{-1}). Equal volumes (0.15 mL) of two different bacterial suspensions were mixed in a 10 × 75-mm culture tube for at least 10 s on a Vortex mixer, allowed to stand at room temperature for 1–2 h, mixed again, and scored for coaggregation with potential partner strains by a visual assay [31]. Scores were: 0 = no visible aggregates in the cell suspension; 1 = small, uniform coaggregates in suspension; 2 = definite coaggregation, but with the suspension remaining turbid; 3 = large coaggregates which settled rapidly, but leaving some turbidity in the supernatant fluid; 4 = large coaggregates that settled quickly, leaving a clear supernatant fluid.

Coaggregation Dispersal

Using the visual assay procedure, we scored the ability of sugars (D-galactose, D-tagatose, and D-sorbitol) to reverse coaggregation of paired bacterial species. To an overnight-incubated suspension (0.3 mL) of coaggregated bacteria, 0.05–0.1 mL of either 1, 2 or 3 M sugar solution was added to give a final concen-

tration between 143 and 750 mM. After thorough mixing of the contents, the vial was allowed to stand at room temperature for 1–2 h, mixed again, and scored for coaggregation as described above. Deionized water was processed and scored as a control.

Results

The coaggregation reactions of the various strains of this study are given in Table I. The numbers are the scores for coaggregation achieved by the mixed pairs. Many pairs did not coaggregate, as seen by their 0 scores. Others produced strong coaggregations in which virtually all of the cells formed large flocculent coaggregates, leaving a clear supernatant (coaggregation = 4). Some of the coaggregations were reversed (asterisked coaggregation scores) by both D-galactose (final concentration of 143 mM) and D-tagatose (final concentration of 200–750 mM).

Seven of the strains tested were initial colonizers (four streptococci and three actinomyces). The coaggregations of certain streptococci and actinomyces have been delineated, and about 95% of each of the two bacterial types are represented by six streptococcal coaggregation groups (groups 1 through 6) and six actinomyces coaggregation groups (groups A through F) [32]. The findings of this study were consistent with those published [12], except that coaggregation of pairs of *A. naeslundii* PK947 with *S. morbillorum* PK509; *A. naeslundii* T14V with *S. oralis* SO34; and *A. naeslundii* T14V with *S. oralis* C104 [12, 31] were not seen. All lactose-reversible coaggregations [12] were shown to be D-galactose- and D-tagatose-reversible as well.

F. nucleatum PK1594 strongly coaggregated (score = 4) with all streptococci and actinomyces tested. Their coaggregations were not reversed completely by either D-galactose (250 mM) or D-tagatose (750 mM). These results are consistent with the findings by Kolenbrander *et al.* [13], that the coaggregations were strong (scores between 4 and 3) and were not completely reversed by lactose. *F. nucleatum* PK1594 strongly coaggregated with *P. gingivalis* PK1924 and *A. actinomycetemcomitans* JP2 and was reversed by D-galactose, a finding consistent with that published [11, 15, 33]. Unlike the report of Weiss *et al.* [33], we found that the coaggregation of *F. nucleatum* PK1594 with *V. atypica* PK1910 was D-galactose-reversible. Our finding seems consistent with other reports [7, 13] that showed coaggregation of *F. nucleatum* PK1594 with *V. atypica* PK1910 to be lactose-reversible. In our study, the coaggregation of *F. nucleatum* PK1594 was not seen with *P. loescheii* PK1295, nor with some capnocytophaga species including *C. sputigena* 4 and *C. gingivalis* 27. Coaggregations of *P. loescheii* PK1295 with streptococci listed in Table I have been reported to be lactose-reversible [12, 34], and have also been shown to be D-galactose-reversible. Table I shows that D-galactose-reversible coaggregations were found to be D-tagatose-reversible as well.

As mentioned previously, the coaggregations of the following pairs did not occur: *A. naeslundii* T14V with *S. oralis* SO34 or with *S. oralis* C104; *S. morbillorum* PK509 with *A. naeslundii* PK947; and *F. nucleatum* PK1594 with *P. loescheii* PK1295, or with *C. sputigena* 4 or with *C. gingivalis* 27. To check this result, we performed a duplicate experiment. Again, no coaggregations were seen. In a third experiment, when the Ca^{2+} concentration in the coaggregation buffer was increased to 1 mM, weak coaggregations of *A. naeslundii* T14V with *S. oralis* SO34; *F. nucleatum* PK1594 with *P. loescheii* PK1295; and *F. nucleatum* PK1594 with *C. gingivalis* 27 produced a score of 1. It is worth mentioning that, instead of incubating the bacteria under an anaerobic atmosphere containing H_2, CO_2, and N_2, as was done by other researchers [11, 12], we cultured the bacteria under an N_2 environment in our study. The difference in the culture technique may be responsible for the differences in coaggregations from those earlier reported.

A typical coaggregation reaction and its reversibility by simple sugars are illustrated in Fig. 1. The medium-to-long spindle-shaped rods with sharply pointed ends are *F. nucleatum* PK1594, which are 5–10 μm long (Image A). The small coccal cells are *P. gingivalis* PK1924 (Image B). After suspensions of the two cells were mixed, a strong coaggregation was found. Large coaggregates comprising a network of interacting fusobacteria and porphyromonads were formed (Image C). Fusobacteria are attached by porphyromonads when a proportion of 1 : 4 of fusobacteria to porphyromonads is used. At a ratio of 1 : 60 fusobacteria to porphyromonads, very little coaggregation was detectable. Microscopic viewing of the suspension revealed that porphyromonads lined up along the length of the fusobacterial cells (Image D). The corncob appearance of the individual paired cells within the coaggregate and extending from the coaggregate were obvious (Image C). The addition of D-galactose (143 mM final concentration) to the coaggregates (Image C) completely dissociated them into individual cells (Image E). Although there were still a few porphyromonads attached to the sur-

Table 1 Interspecies coaggregation and dispersal of oral bacteria

	SO34	C104	J22	PK509	T14V	PK29	PK947	PK1594	PK1924	PK1295	PK1910	Capno 4	Capno 25	Capno 27	JP2
Streptococcus oralis SO34		0	0	2*	0	4*	3*	4	0	4*	3*	0	0	0	0
Streptococcus oralis C104			0	3*	0	4*	2*	4	0	4*	3*	0	0	0	0
Streptococcus mitis J22				0	4	4*	2*	4	0	3*	2	0	0	0	0
Streptococcus morbillorum PK509					0	2*	0	4	0	2*	2*	0	0	0	0
Actinomyces naeslundii T14V						0	0	4	0	0	0	0	0	0	0
Actinomyces naeslundii PK29							0	4	0	0	1	0	0	0	0
Actinomyces naeslundii PK947								4	0	0	0	0	0	0	0
Fusobacterium nucleatum PK1594									4*	0	3*	0	1*	0	3*
Porphyromonas gingivalis PK1924										0	0	0	0	0	0
Prevotella loescheii PK1295											0	0	0	0	0
Veillonella atypica PK1910												0	0	0	0
Capnocytophaga sputigena Capno 4													0	0	0
Capnocytophaga ochracea Capno 25														0	0
Capnocytophaga gingivalis Capno 27															0
Actinobacillus actinomycetemcomitans JP2															

Each bacterial pair was visually examined for coaggregation and scored from 0 for no visible coaggregation to 4 for maximum coaggregation (see text).
*Coaggregation reversed by both D-galactose and D-tagatose.

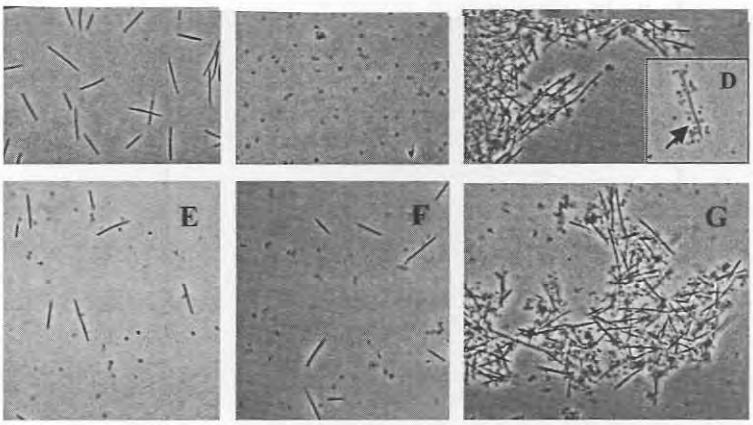

Figure 1 These are phase-contrast microphotographic images of coaggregation between *F. nucleatum* PK1594 and *P. gingivalis* PK1924. Images A and B show suspensions of *F. nucleatum* PK1594 and *P. gingivalis* PK1924, respectively. Image C shows clumping of coaggregates at a 1 : 4 proportion of fusobacteria to porphyromonads; most fusobacteria are coated with porphyromonads including on coaggregate extensions (see arrow), which gives a corncob appearance. Image D shows that, at a 1 : 60 proportion of fusobacteria to porphyromonads, the corncob morphology is clearly seen on a single fusobacterium (see arrow) which is surrounded by porphyromonads. Image E shows that the addition of D-galactose (143 mM final concentration) dissociates clumps into individual cells in the mixed cell suspension shown in C. Image F shows that D-tagatose also dissociates coaggregates into individual cells, but a final concentration of 400 mM is required. Image G shows that 400 mM final concentration of D-sorbitol does not dissociate the coaggregates into individual cells.

face of fusobacteria, no coaggregate was found by either visual or microscopic examination. D-Tagatose performed similarly (Image F), but a higher concentration was required (400 mM final concentration). D-Sorbitol at 400 mM did not significantly dissociate the existing coaggregates (Image G vs. Image C), with the coaggregation scores being reduced only from 4 to 3 (Table II).

The effects of D-galactose, D-tagatose, and D-sorbitol on interspecies coaggregates are shown in Table II. The study was focused on those strong coaggregations (scores of 2 or above) which were both D-galactose- and D-tagatose-reversible (see Table I). Deionized water was used as a control. D-Galactose completely dissociated the coaggregates (score = 0) or nearly completely (score = 1) at 143 mM. In general, all D-galactose-reversible coaggregations were also D-tagatose-sensitive, but a higher concentration of D-tagatose was required. At the same concentrations of D-tagatose, D-sorbitol had little or no effect on coaggregates, except on the coaggregates of *S. morbillorum* PK509 with *P. loescheii* PK1295 and with *V. atypica* PK1910, both of which pairs were also completely dissociated by both D-tagatose and D-galactose. The highest concentration used in this study was 750 mM. At this concentration, the reversal of the coaggregations of *P. loescheii* PK1295 with *S. oralis* C104 and with *S. mitis* J22 by D-tagatose or D-sorbitol was not complete (score = 0), nor even nearly complete (score = 1). For a few coaggregating pairs (asterisked in Table II), complete dissociation of existing coaggregates was not seen at 750 mM of D-tagatose or D-sorbitol, although the coaggregations were nearly reversed at lower concentrations.

For those interspecies coaggregates that were not completely dissociated by D-galactose at concentrations of 200–250 mM, neither D-tagatose nor D-sorbitol reversed these coaggregations at 750 mM, the highest concentration used in this study. An exception was the coaggregate of *V. atypica* PK1910 with *A. naeslundii* PK29, which was completely dissociated by 600-mM D-tagatose (Table III).

Among 28 strong coaggregating pairs (coaggregation score = 2 or above), the coaggregation of 17 (i.e. about 60%) pairs was reversed completely (score = 0) or near completely (score = 1) by D-tagatose at a concentration of 750 mM or below. D-Tagatose reversed the coaggregations involving both primary and late colonizers that were Gram-negative anaerobes frequently involved in periodontal diseases. Thus, D-tagatose has the potential for destabilizing plaque development and for altering the

Table II Comparison of reversibility among D-galactose, D-tagatose and D-sorbitol

Pairs	Water Score	D-Galactose Conc. (mM)	D-Galactose Score	D-Tagatose Conc. (mM)	D-Tagatose Score	D-Sorbitol Conc. (mM)	D-Sorbitol Score
PK509/PK1295	2	143	0	600	0	600	0
PK509/PK1910	2	143	0	400	0	400	0
PK29/SO34	4	143	1	750	1	750	4
PK29/C104	4	143	1	750	1	750	4
PK29/J22	4	143	1	750	1	750	4
PK29/PK509	2	143	0	200	0	200	2
PK947/SO34	3	143	0	200	0	200	2
PK947/C104	2	143	0	200	0	200	1
PK947/J22	2	143	0	200	0	200	2
PK1594/PK1924	4	143	0	400	0	400	3
PK1594/PK1910	3	143	0	200	0	200	1
PK1594/JP2	3	143	1	600	0	600	2
PK509/SO34*	2	143	0	400	1	400	1
PK509/C104	3	143	0	750	1	750	1
PK1295/SO34	4	143	0	750	1	750	1
PK1295/C104	4	143	1	750	2	750	2
PK1295/J22	4	143	0	750	2	750	2
PK1910/SO34*	3	143	1	200	1	200	1
PK1910/C104*	3	143	0	200	1	200	1

Each bacterial pair was visually examined for coaggregation and scored from 0 for no visible coaggregation to 4 for maximum coaggregation (see the text). The highest sugar concentration used was 750 mM. The bacterial strains used were *Streptococcus oralis* SO34, *Streptococcus oralis* C104, *Streptococcus mitis* J22, *Streptococcus morbillorum* PK509, *Actinomyces naeslundii* PK29, *Actinomyces naeslundii* PK947, *Fusobacterium nucleatum* PK1594, *Porphyromonas gingivalis* PK1924, *Prevotella loescheii* PK1295, *Veillonella atypica* PK1910, and *Actinobacillus actinomycetemcomitans* JP2.

*At concentrations up to 750 mM, complete reversal (score = 0) for these species was not seen for D-tagatose or D-sorbitol.

Table III Effects of D-tagatose and D-sorbitol on coaggregations not D-galactose-reversible

Pairs	Water Score	D-Galactose Conc. (mM)	D-Galactose Score	D-Tagatose Conc. (mM)	D-Tagatose Score	D-Sorbitol Conc. (mM)	D-Sorbitol Score
PK1594/SO34	4	250	3	750	3	750	3
PK1594/C104	4	250	3	750	3	750	3
PK1594/J22	4	250	2	750	2	750	2
PK1594/PK509	4	250	3	750	3	750	3
PK1594/T14V	4	250	3	750	3	750	3
PK1594/PK29	4	250	3	750	3	750	3
PK1594/PK947	4	250	3	750	3	750	3
T14V/J22	4	200	4	750	4	750	4
PK1910/J22	2	200	2	750	2	750	2
PK1910/PK29	1	200	1	600	0	600	1

Each bacterial pair was visually examined for coaggregation and scored from 0 for no visible coaggregation to 4 for maximum coaggregation (see text). The highest sugar concentration used was 750 mM. The bacterial strains used were *Streptococcus oralis* SO34, *Streptococcus oralis* C104, *Streptococcus mitis* J22, *Streptococcus morbillorum* PK509, *Actinomyces naeslundii* T14V, *Actinomyces naeslundii* PK29, *Actinomyces naeslundii* PK947, *Fusobacterium nucleatum* PK1594, and *Veillonella atypica* PK1910.

subgingival microbiota, resulting in conservative control of gingival and periodontal disease.

Discussion

All D-galactose-reversible coaggregations tested were also D-tagatose-sensitive (Table II). One out of the 10 D-galactose-irreversible coaggregations tested was D-tagatose-sensitive (Table III). On the other hand, D-sorbitol produced little effect on the coaggregating pairs tested. The similar structure of D-tagatose to D-galactose probably accounts for D-tagatose's action. That conclusion is consistent with the suggestion proposed by Kolenbrander and Andersen [11] that the D-galactose configurations of the hydroxyls (*cis* hydroxyls) on C-3 and C-4 are important for bacterial recognition. D-Sorbitol's C-3 and C-4 hydroxyls are in the *trans* configuration. Unlike D-galactose and D-tagatose that exist in both linear form and ring configuration, D-sorbitol only exists in linear form. These structural differences may be the reason that D-sorbitol has little effect on those D-galactose-reversible coaggregations.

Attempts to control plaque through antiadhesion mechanisms have involved non-stereospecific inhibition of bacterial attachment to teeth, usually with compositions containing surface-active polymers [35, 36]. With respect to blocking stereospecific interactions that mediate oral bacterial adherence, the use of mono- and oligosaccharides and their derivatives has been described as inhibitory of lectin-mediated adhesion [37–43]. Among those lectin–carbohydrate interactions, D-galactose-binding lectin was identified [44]. It was shown in this study that the D-galactose-reversible coaggregations tested were also D-tagatose-sensitive, probably because both sugars exist in ring and in linear forms; their C-3 and C-4 hydroxyls are in the *cis* configuration. The affinity of D-tagatose to D-galactose-binding lectin was weaker than that of D-galactose and thus a higher concentration of D-tagatose was required. D-Tagatose is low in caloric value, 1.5 kcal g^{-1}, has a taste profile virtually indistinguishable from that of sucrose, and has 92% of sucrose's sweetness intensity [29, 45]. D-Tagatose has been shown to have sufficient humectancy for use in toothpastes, as the sole humectant, or in combination with other humectant ingredients [30]. Also, an economical process for the manufacture of D-tagatose has been developed [46, 47]. Unlike all other antiplaque agents proposed [37–43], D-tagatose, because of its relatively low cost, can be used in toothpaste or mouthwash at high concentrations to increase its antiplaque efficacy. D-Tagatose in toothpaste and mouthwash can also provide a good humectant and a low calorie sweetener with sucrose-like taste. This newly discovered antiplaque property places D-tagatose in an advantageous position for use as a combined humectant and sweetener in toothpaste and mouthwash when compared with the ingredients currently used for such purposes.

Dental plaque, which is composed of bacteria biofilm, not only contributes to tooth discoloration, but, most importantly, is considered to be the major source of pathogens that cause gingival irritation and subsequent periodontal disease [2]. In general, a microorganism cannot be an effective pathogen unless it adheres to, and subsequently reproduces itself, within a host. Adherence is especially important in the early events of bacterial infection. Prevention and reversal of existing coaggregation (or adhesion) by using D-tagatose-containing toothpaste and mouthwash seems to offer a conservative control of such infections. Current study further suggests that periodontal diseases may trigger blood clots which can contribute to heart attacks or strokes [48]. Once plaque bacteria enter the bloodstream through ulcerations in the gums, they appear to cause clots that then impede blood flow. The adhesions among bacteria and between bacteria and blood cells may be the mechanism. Hence, reducing the bacterial flora of the plaque is expected to reduce the risk of such diseases. The sensitivity to D-tagatose of Gram-negative bacteria, such as *F. nucleatum*, *P. gingivalis* and *A. actinomycetemcomitans* tested in this study, is of particular interest since such bacteria appear to play a central role in the initiation and progression of periodontal disease [2]. These anaerobic periodonto-pathogens are also the prominent contributors to the formation of volatile sulphur compounds (VSC), the major components of halitosis [16–19]. Consequently, the use of D-tagatose in toothpaste and mouthwash as a combination low-calorie sweetener, bulking agent, and humectant can provide numerous other beneficial effects, including whitening of teeth and prevention of plaque, halitosis, gingival irritation, periodontal disease, and even heart attack and stroke.

Conclusions

Coaggregations among oral bacteria are mediated by complementary surface structures such as lectin-carbohydrate on the partner cells. Many coaggregations are D-tagatose-sensitive. Among those are early colonizers, such as streptococci and actinomyces,

and late colonizers, such as fusobacteria, porphyromonads and actinobacilli. In contrast, D-sorbitol, a humectant and low-calorie sugar substitute now used widely, has little effect on the coaggregating pairs tested. D-Tagatose, with its taste profile virtually indistinguishable from that of sucrose and with 92% of sucrose's sweetness intensity, has already shown promise as a sweetener, a humectant, a bulking agent, a synergizer, and a flavour enhancer [29, 30, 45, 49]. The research results reported herein indicate that the regular use of dental care products that contain D-tagatose as humectant and sweetener, with or without other antiplaque agents, provides control of plaque development and stability. The multifunctional properties of D-tagatose place it in a strong position for use in food and cosmetic products. We postulate that the regular use of D-tagatose-containing dental care products can change the ecology of the subgingival plaque, resulting in a microbiota in which the frequency and number of periodontal pathogens are significantly reduced. Together with the other benefits described herein, this innovative application of D-tagatose has profound implications for improved dental and oral health.

Acknowledgements

The bacterial strains analysed in this study were a gift from P. E. Kolenbrander, Ph.D., National Institute of Dental Research, Bethesda, Maryland. We thank R. N. Andersen and P. E. Kolenbrander for their help during the course of this study.

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