BINDING OF ORAL *VEILLONELLA* SPECIES TO SALIVA-COATED HYDROXYAPATITE

by

Sonya L. Wu

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David R. Avery

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Richard L. Gregory

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Brian J. Sanders

_____

Dennis E. Bowman

_____

Christopher V. Hughes

Chairman of the Committee

Date_____

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INTRODUCTION

Dental plaque is composed of a complex and dynamic microbial community. Two prominent bacterial genera found in plaque are *Veillonella* and *Streptococcus*. Certain species of *Streptococcus* (*gordonii, oralis and sanguis*) have previously been shown to be early colonizers of the salivary pellicle. *Veillonella* spp. have been described as later colonizers of plaque. Studies have shown that *Veillonella* coaggregate with *Streptococcus* spp. This thesis will investigate the ability of *Veillonella atypica* PK1910 to adhere to the salivary pellicle. The following review of the literature will be a discussion of the stages of plaque formation, the disease process in caries formation, and finally the genus, *Veillonella*. This discussion will introduce and develop the foci of this thesis. First, oral bacteria have well-established adherence mechanisms by which they colonize dental plaque. These mechanisms involve the recognition by specific adhesins of distinct receptors in salivary pellicle and on other bacteria. Secondly, since *Veillonella* spp. adhere to other bacteria, do they also bind directly to salivary pellicle? And thirdly, do they use similar mechanisms or is a distinct mechanism used for binding to the salivary pellicle?

REVIEW OF LITERATURE

Dental caries is a prevalent disease of the 20th century. Numerous archaeological reports have indicated that during antiquity carious lesions were infrequent in humans.[1,2] The frequency of caries was low as recently as the Saxon times in England.[3] Increases occurred during the Middle Ages with the largest increases having occurred since the middle of the 19th century.[4-6] Due to various preventive procedures, the prevalence of caries in children has decreased substantially since the early 1970s.[7-9] Susceptibility to caries is highest in childhood and adolescence since many teeth become carious soon after eruption.[10] In adulthood, caries occurs largely around the margins of old restorations or under leaking restorations.[11]

Dental caries is a chronic infectious disease in which the active agent or agents are members of the indigenous oral flora.[11] Caries is comprised of three interdependent parts. These parts include the bacterial agent, host, and the environment, and numerous interrelations among them.[12] The bacterial agent is composed of a population of cariogenic microorganisms. The host is the tooth which is susceptible to the development of carious lesions as well as the host response. The environment consists of nutrients that the bacterial agent can consume to produce demineralizing acids.

Control and prevention of caries has long been a goal of dental research. The role of dental plaque is well established in the initiation of carious formation and periodontal disease. Fundamental to that goal is an understanding of the complex microbial ecology of dental plaque. Teeth are very unique. They are a non-shedding surface that lies exposed to the external environment. As such, they undergo no metabolic turnover and are subject to dense microbial colonization or plaque formation.[13] Plaque is composed of approximately 10^8 bacteria/mg of plaque wet weight.[14] More than 700 strains representing 18 genera of bacteria have been reported in plaque.[15]

Plaque formation and bacterial adherence

The first step in plaque formation is development of the salivary pellicle. Saliva is rich in proteins and minerals, especially calcium and phosphate ions.[16] It provides a constant supply of ions to the enamel surface.[17] Saliva bathes the tooth. Specific proteins from saliva are adsorbed to the tooth surface and form the salivary pellicle or acquired pellicle.[18] The pellicle is composed of molecues that show a high selective absorptive capacity onto apatitic surfaces.[19] The thickness of the salivary pellicle ranges from 100 nm after two hours to about 400 nm after 24 to 48 hours.[20] Initially, the pellicle is predominantly bacteria-free.[17] Surface denaturation and acid precipitation cause the loss of solubility of the adsorbed proteins.[21] The salivary pellicle is the foundation of plaque, and is the initial

location of bacterial colonization (See Figure 1). The next step of plaque formation is initial attachment of bacteria. Initial attachment occurs when bacteria adhere to receptors in the salivary pellicle.

The salivary pellicle is first colonized by *Streptococcus sanguis, Streptococcus gordonii, Streptococcus oralis,* and *Streptococcus mitis.* These colonizations appear to occur by specific ionic,[22] hydrophobic[23] and lectinlike[24,25] interactions. Daily variations of microbial types and numbers occur in saliva[26] and may account, in part, for the variations observed in the microbial composition of early plaque.[26] Another early colonizer of the tooth surface is *Actinomyces viscosus.*[27] In a study by Nyvad and Fejerskov,[28] it was found that all individuals studied consistently showed the presence of a layer of densely packed gram-positive bacteria resembling *Actinomyces* close to the enamel surface. The bacteria maintain their metabolic activity by using the salivary proteins and carbohydrates as a substrate. The presence of other ions, e.g., iron, zinc and strontium in plaque fluid may also directly affect plaque metabolism.[29] Both bacterially produced and host-derived substances present in saliva have been recognized to influence bacterial attachment.[30,31] The role of extracellular glucans in sucrose-mediated attachment of *S. mutans* is well documented.[32]

Streptococcus mutans are not early colonizers of the tooth surface.[33] They synthesize extracellular adhesive water-insoluble glucans from sucrose by the combined action of at least two glucosyltransferases resulting in the adherence of cells to

tooth surfaces and subsequent cariogenic plaque formation.[34-39] In a report by Hiroi *et al.*,[40] it was proposed that glucan synthesis from sucrose may participate not only in cell-to-cell accumulation but also in initial cell-to-pellicle attachment. Schilling *et al.*[41] showed that strains of *S. mutans* bound specifically to glucans formed in experimental salivary pellicles suggesting an initial adherence to tooth surfaces by *S. mutans.*

Short-term experimental pellicles can be formed by exposure of cleaned teeth to the oral environment or by exposure of hydroxyapatite (HA) to saliva. Such pellicles formed *in vivo* and *in vitro* contain similar salivary macromolecules; they are also similar in amino acid and carbohydrate composition.[42-46] The adsorption of bacteria to saliva-coated spheroidal hydroxyapatite (SHA) surfaces and to teeth covered with an acquired salivary pellicle would therefore be expected to exhibit similar characteristics.[44,46] Many bacterial adhesion studies have used this SHA model to simulate the salivary pellicle. For example, Clark, Bammann and Gibbons[47] showed that *S. mutans, S. salivarius* and *A. naeslundii* adsorbed less to saliva-treated HA than to untreated HA and the opposite was true for *S. sanguis, S. mitis* and *A. viscosus.* The difference was due to whether the adsorbed salivary components provided fewer or greater numbers of adsorption sites for the bacteria. These adsorption sites serve as recognition molecules (receptors) for the complementary structures (adhesins) on bacteria.[48]

Many bacteria bind stereochemically to these complementary receptor molecules through adhesins that are proteinaceous ligands on the bacterial surface.[49] Adhesins are often associated with filamentous appendages known as fimbriae or pili; however, some are present in a non-appendage related form.[50] The adhesin-receptor interaction is similar to a lock and key mechanism on a molecular level, and is analagous to antibody-antigen or enzyme-substrate interations.[49] An individual bacterial cell can have several thousand adhesin sites comprising a number of different adhesin specificities that can interact with multiple receptor molecules to result in firm binding.[51,52] Components of oral secretions make up part of the acquired pellicle coating of teeth, where they function as receptors and mediate bacterial adhesion to the tooth surface. However, salivary components that represent soluble receptors may interact with bacterial adhesins and block their attachment to teeth and oral epithelal cells. This molecular mimicry of host tissue receptors contributes to the cleansing action of secretions.[49]

Recent studies concerning the receptors in the enamel pellicle or on oral epithelial cells have suggested that previously hidden segments of molecules may function as binding domains for some bacterial adhesins. Because these hidden segments, termed cryptitopes, are uniquely exposed on surface-associated molecules, they are not readily mimicked by related molecules in secretions.[53,54] The cryptitopes can become exposed as a result of conformational changes when molecules interact with

each other, or adsorb to surfaces.[49] Their apparent function as bacterial receptors enables a microorganism to effectively evade host defense cleaning mechanisms, and greatly facilitates microbial adhesion and colonization.[49]

In a study by Murray *et al.*,[55] the interactions between strains of streptococci and multiple salivary components, including low-molecular-weight salivary mucin, highly glycosylated proline-rich glycoproteins and alpha-amylase were examined. They concluded that these interactions might govern bacterial adherence and clearance within the oral cavity.

The next step in plaque formation is accumulation. This occurs by growth and interbacterial adherence. A form of interbacterial adherence is easily visualized in the laboratory and is known as coaggregation.[56] Mixed cell suspensions of coaggregating pairs form visibly discernable aggregates consisting of both cell types.[15]

Adhesins that recognize other oral bacteria have been identified on the cell surface of many oral bacteria.[57,58] Intergeneric coaggregation of the bacteria leads to further bacterial accumulation and, therefore, plaque formation. This adherence between bacterial pairs is a result of cell-to-cell recognition through the mechanism of adhesins and receptors. This leads to a myriad of thousands of coaggregating pairs of bacteria, which in total composition, is plaque.

The combined studies of coaggregation mediators and coaggregation mutants have clearly shown that bacterial cells express more than one mechanism of cell-to-cell recognition. Cells may express both proteinaceous adhesins and carbohydrate receptors for different adhesins found on partner cells. The adhesins may be borne on fimbriae or be part of the outer membrane of gram-negative cells. Mutants that are altered or lack one mechanism of coaggregation may still be capable of coaggregating with other partners by remaining mechanisms.[15] The early colonizers coaggregate with each other, and the late colonizers coaggregate with each other, but the early colonizers of a clean tooth surface do not necessarily coaggregate with bacteria that increase in numbers with advancing stages of periodontal disease or caries.[56,59]

Recently, it has become evident that a single adhesin may recognize receptors in pellicle and on other oral bacteria.[60,61] Ganeshkumar *et al.*[60] have shown that *S. sanguis* binds to saliva-coated hydroxyapatite through a specific adhesin. Furthermore, this adhesin also appears to mediate coaggregation with other oral bacteria.[61] Weiss *et al.*[57] identified a 75 kDa protein from *Prevotella loescheii* PK1295 that mediates a similar coaggregation with the same streptococci. This protein also recognizes receptors on erythrocytes.[62] Thus, it appears that coaggregation-mediating adhesins also recognize receptors on other surfaces.

The final stage of plaque formation is maturation. As the plaque accumulates and matures, the flora is occupied predominantly by gram-negative bacteria. Complex nutritional, spatial, and genetic relationships occur in this microbial community. Fluctuations in bacterial populations may be due to changes in available nutrients, enzymatic activities or host responses.

Dental Plaque and Disease

Dental plaque has been closely associated with periodontal disease. It has also been associated with enamel decalcification that may lead to the formation of the carious lesion. Products of plaque can produce an inflammatory response in adjacent soft tissue. The resulting plaque exudate provides fluids that encourage further plaque development and serve as a source for additional mineralization.[63] Plaque and its resulting products not only play a role in the development of dental caries, but in the formation of periodontal disease.

Numerous hypotheses regarding the formation, composition and effects of plaque have been postulated. The nonspecific plaque hypothesis assumes that there is no bacteriologic distinction between plaques associated with decay, periodontal disease or health.[14] Therefore, the same genera of bacteria should be found at all times in the oral cavity at comparable concentrations whether located at diseased or healthy sites. On the contrary, the specific plaque hypothesis indicates that specific odontopathic organisms form specific

plaques associated with decay, periodontal disease and health, respectively.[14] To test this hypothesis several types of studies have been conducted. Examples of these association, virulence, longitudinal, and response-to-treatment studies as related to *S. mutans* are described below.[14] Association studies look for a statistical difference between bacteria found in plaque removed from caries-active and caries-free teeth.[14] Virulence studies evaluate the organisms for their pathogenicity under different dietary and host conditions.[14] Examples of such studies have shown a definite connection between sucrose and dental caries both in humans and animals such as rats, hamsters and monkeys.[64] *S. mutans* has been shown to utilize sucrose faster and produce more lactic acid than any other bacteria found in carious sites.[65] These studies have indicated that sucrose has a close relationship with *S. mutans* and the formation of caries.[65] Longitudinal studies monitor the number of microorganisms or microbial proportions before, during, and after the carious lesion develops.[14] In the case of *S. mutans*, these studies would demonstrate whether the elevation in microbial proportions or numbers of *S. mutans* preceded or coincided with caries development rather than following it.[14] Longitudinal studies have shown that *S. mutans* numbers peak at the time that caries is diagnosed; however, some teeth become carious in the absence of high proportions of *S. mutans* possibly indicating that other bacteria such as lactobacilli can contribute to the carious process.[66] Response-to-treatment studies evaluate the bacterial flora after assumed successful treatment is applied.[14] Studies

have shown that reducing the amount of *S. mutans* in mothers with high levels of *S. mutans* by following a comprehensive treatment protocol including professional tooth cleaning, excavation of carious lesions, fluoride varnish, and discussions about the role of sucrose, reduced the incidence of *S. mutans* infection in their children as compared with children in a control group whose mothers were similarly infected but did not receive treatment.[67] Plaque related to dental caries is actively influenced by antimicrobial agents such as fluoride and the local effects of diet.[63]

The Formation of the Carious Lesion

The formation of the carious lesion occurs when plaque bacteria utilize carbohydrates in the diet to reduce the pH of the oral environment and keep the pH low enough to decalcify teeth.[63] Bacteria have been implicated as the cause of decay as early as the 19th century by Miller and associates.[68]

Many studies have positively correlated *S. mutans* with the carious lesion. Mutans streptococci, particularly *S. mutans*, are considered generally to be the prime etiological agents of human dental caries.[69] Tanzer[70] found a positive correlation between acidogenic plaque bacteria and caries activity in humans established only for the mutans streptococci and lactobacilli. In a study by Nyvad and Kilian,[71] the initial streptococcal microflora of caries-inactive individuals were colonized by significantly higher proportions of *S. sanguis*, whereas caries-active individuals had higher numbers of mutans streptococci

among the early colonizers on enamel. Loesche[13] has stated that of the 200 to 300 bacterial species that can be isolated from plaque, several clinical studies have indicated that only *S. mutans* and to a lesser degree *S. sobrinus* and *L. casei* can be consistently associated with dental decay. It has also been shown that lowering the sucrose level *in vivo* lowers the salivary numbers of Lactobacillus[72-74] and the plaque numbers of *S. mutans*,[75] *Veillonella* sp. and yeasts. For example, during the years of World War II, the decay rate declined among the European[76] and Japanese[77] populations since sucrose was restricted in the diet because of the war effort.

The early enamel lesion is characterized by an intact surface with subsurface enamel demineralization. The predecessor of this lesion is caused by the diffusion into the enamel of undissociated lactic acid produced by plaque microbes during a nutrient pulse. During this time, the pH for plaque isolated from caries-active individuals drops to 5.0 or below, whereas that of caries-free individuals drops to 6.0. Numerous studies have shown that cariogenic plaque is more acidogenic than plaque from noncariogenic sites.[65] Thus, lactic acid plays a critical role in initiating the carious lesion. Veillonellae utilize lactic acid and other end products of bacterial carbohydrate metabolism for their metabolic needs. Veillonellae are unable to use glucose to support growth. They can utilize lactate as carbon and an energy source for growth.[78]

Veillonellae

Veillonellae were first discovered by Veillon and Zuber in 1898. They are present in the mouth shortly after birth. By the age of 101 days, 75 percent of infants have veillonellae present in their oral cavity. By 1 year of age, following the colonization of *S. salivarius*, 100 percent of children have veillonellae present in their mouths.[79]

Veillonellae live in a flowing environment and have developed mechanisms to colonize the exposed surfaces[78] but can adhere to other species of bacteria that are early colonizers of these tissues.[80,81] The ability of veillonellae to use lactic acid produced by other oral bacteria from carbohydrate metabolism establishes its unique and essential link in the plaque food chain.[78] High numbers of veillonellae are found at all times in dental plaque, whether in healthy sites or diseased sites[82-84] such as the carious lesion or the periodontal lesion in both caries-free and caries-active individuals.[85,86]

Veillonellae are anaerobic gram-negative cocci and the most numerous anaerobes in human saliva. They constitute a major fraction of the total bacterial population on the epithelial surface of the human oral cavity and are found in high numbers in sub- and supragingival plaque, and on oral mucosal surfaces.[87] Veillonellae are found at concentrations of 1.7 - 6.9 x 10^7/ml of whole saliva,[88] and constitute from 5 percent[89] to about 16 percent[90] of the total cultivable anaerobic flora. *Veillonella parvula* is the predominant *Veillonella* species in human dental plaque and constitutes from 93 to 98 percent of

the total cultivable veillonellae in healthy subgingival sites.[82] *Veillonella atypica* and *Veillonella dispar* occur in low numbers and are present consistently in plaque, but are not associated with oral diseases.[78] *V. dispar* predominates on mucosal surfaces.[81]

It is known that *Veillonella* is a species of bacteria usually present in natural plaque. The rapid clearance of veillonellae from the tooth surface six hours after inoculation of germ-free animals with veillonellae can be related to either its inability to bind to saliva-coated HA, its sensitivity to oxygen or the lack of available lactic acid. In a study by McBride and van der Hoeven,[91] *Veillonella* strains that were capable of coaggregating with streptococci colonized the streptococcal plaque in higher numbers than did *Veillonella* strains that did not coaggregate with the streptococci. Mixtures of *S. mutans* and *Veillonella* (*alcalescens* V-1) *parvula* formed aggregates that were evident macroscopically 30 seconds after mixing.[91] In a study by Bladen *et al.* in 1970,[92] it was shown that *Veillonella* was unable to bind to nichrome steel wires but was able to adhere to the primary plaque formed on the wires by *Actinomyces viscosus* T6.

The primary habitats of oral veillonellae are the buccal mucosa, tongue and dental plaque. Hughes *et al.*[81] showed that 59 strains of *Veillonella dispar* isolated from the tongue coaggregated with *S. salivarius*, which is a predominant inhabitant of the tongue, and 58 of these strains did not coaggregate with bacteria that inhabit the subgingival flora such as *Actinomyces viscosus*, *Actinomyces naeslundii*, *Actinomyces*

israelii and *Streptococcus sanguis*. Conversely, the 20 strains of *Veillonella parvula* that predominated in subgingival plaque was found to coaggregate with species of *Actinomyces* and *Streptococcus* found in the subgingival flora and did not coaggregate with *S. salivarius*. *Veillonella atypica*, which can be found on mucosal surfaces and in plaque, can fall into either coaggregation group. Thus, it appears that veillonella coaggregation is species-, bacterial- and location-specific, and suggests that coaggregation is an important determinant of oral colonization by *Veillonella* spp.[81]

Although not associated with any oral diseases, veillonellae are present as a significant part of the plaque population in both healthy and diseased sites.[93-95] They are among the 10 most numerous species of subgingival plaque bacteria under all conditions,[82-84] including experimental gingivitis,[96] localized juvenile periodontitis,[82,97] adult chronic moderate periodontitis,[98] and rapidly progressing generalized periodontitis.[99] Cleaned tooth surfaces are first colonized by streptococci and actinomyces,[78] both of which are coaggregation partners of *V. parvula*, the predominant *Veillonella* species in subgingival plaque.[81] The bacterial population found in periodontally diseased sites is dominated by fusobacteria,[100] which are coaggregation partners of all three human *veillonella* species.[100]

Veillonella has unique physiologic properties. Unlike many oral bacteria such as *S. mutans*, it does not ferment carbohydrates. However, it grows well anaerobically on lactate,

pyruvate, malate and fumarate. In fact, Veillonellae utilize lactic acid and break it down into the weaker propionic and acetic acids. The main end product is proprionic acid followed in concentration by acetic acid, carbon dioxide and hydrogen. These weaker acids have a lower dissociation constant than lactate, pyruvate, malate and fumarate, which may reduce acid demineralization of enamel.[101]

Veillonellae play a principal role in lactate consumption in dental plaque because of its predominance among dental plaque bacteria[102] and ability to metabolize lactate.[103] This has led to the proposal that veillonellae participate in nutritional relationships with oral streptococci.[104,105] Relationships between veillonellae and other oral bacteria, especially streptococcal spp., based upon nutrition have been demonstrated both *in vivo*[104,105] and *in vitro*.[106]

Several studies have suggested a relationship between *Veillonella* spp. and dental caries. In a study of various salivary bacteria, Russell *et al.*[107] found that veillonellae were associated consistently with mutans streptococci alone and no other bacteria. Germ-free rats infected with *S. mutans* and *V. alcalescens* developed fewer caries than did animals monoinfected with *S. mutans*.[104] van der Hoeven[106] found that the dental plaque of gnotobiotic rats infected with *S. mutans* and veillonellae produced significantly less lactic acid than animals infected only with *S. mutans*. Interestingly, Orstavik and Brandtzaeg[108] found a significant negative correlation between titers of serum antibodies to veillonellae and dental caries

experience. Bowden *et al.*[109] suggested that a decreased number of *Veillonella* might be a high-risk condition of caries production brought on by the reduced ability to metabolize lactic acid produced by *S. mutans*. Finally, Kilian *et al.*[110] attributed the lack of caries activity in Tanzanian children to the high proportion of lactate metabolizing bacteria such as veillonellae.

In contrast, many other studies suggest veillonellae have no direct relationship to *S. mutans* or any anti-cariogenic effects. In a study of dental decay of children's molars shortly after eruption, *S. mutans* and lactobacilli were associated, whereas veillonellae, although consistently present, could not be linked to the development of decay.[85] Milnes and Bowden[111] found increased numbers of *Veillonella* at susceptible sites independent of lesion formation and concluded that this might be due to an increase in lactic acid in plaque. Boue *et al.*[112] found higher numbers of *Veillonella* spp. at developing carious lesions. Despite these conflicting data, it is generally accepted that *Veillonella* play a critical role in lactic acid utilization in dental plaque.

Recently, the adherence properties of *Veillonella* have been studied. Hughes *et al.*[81] described the coaggregation of *Veillonella* spp. and *S. gordonii* and *S. oralis*. No differences in veiilonella-streptococcal coaggregations were observed between the two streptococcal species. Two kinds of interactions were observed. The first type was inhibited by lactose and the second type was lactose-resistant. In each coaggregation type, a veillonella protein appeared to recognize a receptor on the

streptococcal cell surface. In a subsequent study, spontaneously occurring coaggregation-defective (COG-) mutants of *Veillonella atypica* strain PK1910 were isolated and studied.[113] Three classes of mutants were identified (See Figure 2). The first class, Class I mutants, failed to participate in lactose-inhibitable coaggregations with these streptococcal cells. The second class, Class II mutants, failed to coaggregate by the lactose-resistant interactions. The third class, Class III mutants, failed to coaggregate with any of these oral streptococci. Utilizing these mutants, Hughes *et al.*[58] identified a 45 kilodalton protein that appeared to mediate the lactose-inhibitable interactions of *V. atypica* PK1910 and these oral streptococci. The protein appeared to be absent in Class I and Class III mutants, but was present in the wild type and Class II mutants (See Figure 2).[61] Little information is available regarding the ability of veillonellae to bind directly to the salivary pellicle. A major focus of the present study will be investigating the ability of *V. atypica* PK1910 to bind to SHA. In addition, as mentioned earlier, it has recently become evident that a single adhesin may recognize receptors in pellicle and on other oral bacteria.[60,61] These earlier studies raise the question, does the 45 kDa adhesin from *V. atypica* PK1910 also mediate adherence to other oral surfaces besides oral streptococci? Specifically, does it recognize receptors on the saliva coated tooth surface? If so, then these molecules may play a critical role in colonization of dental plaque by *Veillonella* spp. By evaluating the adherence of *V. atypica* PK1910 and its three classes of coaggregation-

defective mutants to saliva-coated spheroidal hydroxyapatite beads, it will be possible to determine whether the 45-kDa adhesin plays a role in binding to the salivary pellicle.

METHODS AND MATERIALS

Growth of bacteria

1. Bacterial strains

Veillonella atypica PK1910 was originally isolated from dental plaque and has coaggregation properties characteristic of isolates from that site (Table I). The following strains represented the three mutant phenotypes:[113] 1) *V. atypica* PK2726 (Class I mutant; COG- with *S. oralis* 34 and *Streptococcus* SM PK509; does not express 45 kDa protein); 2) *V. atypica* PK2739 (Class II mutant; COG- with *S. gordonii* DL1 and *S. gordonii* PK488; expresses the 45 kDa protein); and 3) *V. atypica* PK2745 (Class III mutant; COG- with all streptococci; does not express the 45 kDa protein).

The following strains were utilized in the coaggregation assay to represent the six coaggregation groups of Streptococci: *S. gordonii* DL1, *S. gordonii* PK488, *S. oralis* H1, *S. oralis* 34, *S. oralis* J22, *S. oralis* C104 , and *Streptococcus SM* PK509.

2. Culture conditions

All veillonella strains were grown in modified Schaedler medium without glucose but supplemented with 0.1 M sodium lactate. All streptococcal strains were grown in Schaedler medium with 2 percent glucose. All were grown at 37°C under anaerobic conditions for 24 hours.

Radioactive labelling of bacteria

6 - [^{3}H] thymidine (2 Ci/mmol; Amersham Life Science Arlington Heights, VA) at 20 microcuries per ml of growth medium was used to label the veillonella and streptococcal cells. Culture tubes containing 10 ml of growth medium were used. After four to six cell doublings, cells were harvested and washed by centrifugation three times with coaggregation buffer (1 mM Tris buffer [pH 8.0], supplemented with 0.1 mM $CaCl_2$, 0.1 mM $MgCl_2$, 150 mM NaCl and 0.2 percent sodium azide), resuspended in coaggregation buffer to a cell concentration of 10^9 cells/ml and stored at 4°C. The radioactivity measurements were obtained by pipetting 100 microliters of sample into a scintillation vial, and adding 5 ml of scintillation counting solution for aqueous samples (Hydrofluor, National Diagnostics, Somerville, NJ). Radioactivity measurements were made by a scintillation counter (Tri-Carb 1900 TR Liquid Scintillation Analyzer Model B1900 Packard, Canberra Co./Packard Inst. Co. Meriden, CT).

Coaggregation assay

In order to confirm the coaggregation phenotype of each culture of *V. atypica* PK1910 and its coaggregation-defective mutant strains, the following coaggregation assay was used. Washed cell suspensions of each strain were adjusted to a density of 10^9 cells per ml (260 Klett units at 660 nm (red filter); Klett-Summerson, Inc. New York, NY). A visual assay was used to determine coaggregation with potential partner

strains.[114] A 0.1-ml cell suspension of each strain was added to culture tubes (10 by 75 mm), vortexed for 10 sec, rocked gently, and observed over an indirect light source illuminated magnifier no. 39-101; (Stocker and Yale, Inc., Beverly, MA) for formation of coaggregates. The assay was scored from 0 for no coaggregation to 4 for maximum coaggregation. A score of +4 was assigned when large coaggregates were observed immediately upon mixing, leaving a clear supernatant. A score of 0 was assigned when the mixture of cells remained as a homogeneous suspension. Reversal of coaggregation was determined after addition of lactose to a final concentration of 0.06 M and rescoring of each coaggregation pair.

Saliva-coated hydroxyapatite assay

1. Preparation of saliva

Whole saliva was collected and pooled from three laboratory personnel by use of a container chilled over ice. The saliva was clarified by centrifugation at 12,000 x *g* for 10 min. Samples of the saliva were frozen at -20°C prior to use. When ready to be used, the saliva was heated in a water bath at 60°C for 30 min.

2. Bacterial adsorption to hydroxyapatite

Bacterial binding to spheroidal hydroxyapatite (HA) was performed by the method of Clark, Bammann and Gibbons.[47] Spheroidal HA beads (BDH Biochemicals Ltd., Poole, England) with a diameter of 85 to 125 mm and an approximate surface

area of 0.27 cm2/mg were employed. HA beads (40 mg) were placed in polypropylene microtest tubes and washed in 1.0 ml distilled water. The beads were then allowed to equilibrate in 1.0 ml of coaggregation buffer overnight. Twenty microliters of 0.2 percent sodium azide was added for pretreatment of the HA to provide a final concentration of 0.04 percent in order to inhibit microbial growth. For the preparation of SHA, the beads were then incubated with 1.0 ml of clarified whole saliva for 24 hours at room temperature and rotated continuously. The beads were washed twice with coaggregation buffer and incubated with 0.5 ml of various concentrations of ^{3}H-labelled bacterial suspensions and 0.5 ml of coaggregation buffer. The mixtures were continuously inverted at room temperature for 90 min. After incubation, the mixtures were permitted to stand for 60 sec to allow the beads containing adsorbed bacteria to settle. The supernatant liquids, which contained unadsorbed organisms, were removed. The beads were then washed three times with 1 ml coaggregation buffer to further remove unadsorbed cells. The beads were rotated 30 sec between each wash. The beads were resuspended in 1 ml coaggregation buffer and transferred into scintillation vials. Five ml of scintillation cocktail was added to each scintillation vial. The cells were then counted by a scintillation counter. Known numbers of ^{3}H - labelled cells were counted in a comparable manner so that the counts per min could be related to bacterial cell number.

All assays were carried out in duplicate, and most experiments were performed at least twice.

All binding data are expressed as the ratio of bound counts per min to the input counts per min.

Effect of heat and protease on *V. atypica* PK1910 adsorption to hydroxyapatite

One ml of *V. atypica* PK1910 (1 x 10^9 cells/ml) labelled with 6-[^{3}H] thymidine was pretreated with 25 microliters of Protease K (20 mg/ml; Sigma St. Louis, MO) by incubating in a water bath at 60°C for 1 hr. Another 1 ml of *V. atypica* PK1910 (1 x 10^9 cells/ml) labelled with 6-[^{3}H] thymidine was pretreated by heating at 85°C for 30 min. The numbers (amount) of cells remaining bound to the SHA were determined as described above (Bacterial adsorption to hydroxyapatite).

Statistical analysis

All variances are reported as standard errors of the mean. Differences among means were analyzed for statistical significance by the t-test: paired two-sample for means and the t-test: two-sample assuming unequal variances. Significant differences between groups were defined as $p < 0.05$.

RESULTS

Binding of *V. atypica* PK1910 and Streptococcal strains to saliva-coated and uncoated hydroxyapatite beads.

The comparative data for *V. atypica* PK1910 and *S. gordonii* DL1, its binding to SHA and uncoated HA beads are presented in Figure 3. The *S. gordonii* served as a positive control for this study since it has been previously shown in other studies that *S. gordonii* DL1 binds well to SHA.[115] This experiment indicated that *V. atypica* PK1910, when compared to *S. gordonii* DL1, bound as well to SHA and equally poorly to HA.

Tables II and III present the data for these experiments. The data represent a proportion for the number of labelled cells bound to the hydroxyapatite beads of the actual number of labelled cells added per ml. Each ml contained approximately 1 x 10^9 cells.

The mean ratio of bound counts to input counts for the binding of *V. atypica* PK1910 to SHA (0.27 ± 0.05) was significantly greater ($p < 0.05$) than to HA beads (0.12 ± 0.01). This represented a two-fold increase in binding to SHA beads than to HA beads (Table II). In addition, the SHA and HA binding of *V. atypica* PK1910 was similar to the *S. gordonii* DL1 positive control. The mean ratio of bound to input counts for the binding of *S. gordonii* DL1 to SHA beads was 0.32 ± 0.06 in comparison to only 0.08 ± 0.01 for binding to HA beads

providing confirmation of the enhanced binding of *S. gordonii* to SHA (Table III).

Binding of the coaggregation-defective mutant strains of *V. atypica* PK1910 : *V. atypica* PK2726, *V. atypica* PK2739, and *V. atypica* PK2745 to saliva-coated and uncoated hydroxyapatite beads.

In order to evaluate how the coaggregation properties of a strain may effect its binding to SHA beads, the binding of the mutants to SHA was tested. The data for the binding of *V. atypica* PK2726, *V. atypica* PK2739 and *V. atypica* PK2745 to SHA and HA beads are presented in Figure 4. These data represent a proportion of the number of labelled cells bound to the hydroxyapatite beads to the actual number of labelled cells added per ml (Tables IV, V and VI). The statistical analyses of these data are presented in Table VII.

The data indicate that *V. atypica* PK2726 bound poorly to both SHA beads and HA beads, and there was no significant difference ($p > .05$) in the binding to SHA and HA (Table VII). *V. atypica* PK2726 was found to bind to SHA beads at a mean ratio of 0.023 ± 0.004 in comparison to $0.015 \pm .001$ to HA beads (refer to Table IV).

Binding data for *V. atypica* PK2739 is presented in Table V. *V. atypica* PK2739 also bound poorly to both SHA beads and HA beads, and there was no significant difference ($p > 0.05$) between the binding to SHA and HA (Table VII). *V. atypica* PK2739 bound to SHA at a mean ratio of 0.052 ± 0.014 in

comparison to a ratio of 0.026 ± 0.002 binding to uncoated HA beads.

Interestingly, the binding of *V. atypica* PK2745 to SHA was significantly lower ($p < 0.05$) than to uncoated HA beads (Tables VI and VII). As seen in Table VI, the binding of *V. atypica* PK2745 to SHA beads and to uncoated HA beads was unlike that seen in *V. atypica* PK2726 and *V. atypica* PK2739. The mean ratio of bound to input counts for the binding of *V. atypica* PK2745 to SHA beads was 0.012 ± 0.001 in comparison to 0.032 ± 0.004 for binding to uncoated HA beads.

Comparison of the binding of *V. atypica* PK1910 to saliva-coated hydroxyapatite beads with the binding of its coaggregation-defective mutant strains of *V. atypica* PK2726, *V. atypica* PK2739 and *V. atypica* PK2745 to saliva-coated hydroxyapatite beads

V atypica PK1910 bound much better to SHA than its coaggregation-defective mutant strains bound to SHA. When *V. atypica* PK1910 was statistically compared to each of the coaggregation-defective mutant strains individually, a significant difference ($p < 0.05$) was found. The comparisons were made of those data, which were collected on cells which bound to SHA beads only (refer to Figure 5).

The mean ratio of bound to input counts for the binding of *V. atypica* PK2745 to SHA beads was 0.012 ± 0.001, which was less than that for both *V. atypica* PK2726, which was 0.023 ± 0.004 and *V. atypica* PK2739, which was 0.052 ± 0.014. A

statistically significant difference was not found when comparing the binding of *V. atypica* PK2726 to SHA to the binding of *V. atypica* PK2739 to SHA. But, when comparing *V. atypica* PK2745 to both *V. atypica* PK2726 and *V. atypica* PK2739 respectively, a statistically significant difference ($p < 0.05$) was seen. *V. atypica* PK2726 and *V. atypica* PK2739 bound better to SHA than *V. atypica* PK2745 bound to SHA.

Effect of protease or heat treatment of *V. atypica* PK1910 on binding to saliva-coated hydroxyapatite beads

Traditionally, protease and heat treatments are used to distinguish protein factors from non-protein factors. Protease and heat treatments of certain bacterial strains have been demonstrated in previous studies to affect the ability of that strain, e.g., *V. atypica* PK1910, to coaggregate. In this study, protease and heat treatments were used to evaluate its effects on the binding to SHA beads with *V. atypica* PK1910.

A. Protease-treated *V. atypica* PK1910

Protease treatment was found to have an inhibitory effect on the binding of *V. atypica* PK1910 to SHA beads (Figure 6). *V. atypica* PK1910, which was treated with protease, bound poorly to SHA. The mean ratio of bound to input counts was 0.05 ± 0.0. Whereas, untreated *V. atypica* PK1910, bound to SHA at a mean ratio of 0.27 ± 0.05. This difference was statistically significant ($p < 0.05$) (Table VIII).

B. Heat-treated *V. atypica* PK1910

Results showed that the binding of heat-treated *V. atypica* PK1910 to SHA beads was significantly greater than untreated cells ($p < 0.05$; Table VIII and Figure 6). Interestingly, the *V. atypica* PK1910 treated by heat exhibited increased binding to SHA beads compared to the untreated *V. atypica* PK1910. The mean ratio of the bound to input counts for the binding of *V. atypica* PK1910 to SHA beads was 0.64 ± 0.04 .

FIGURES AND TABLES

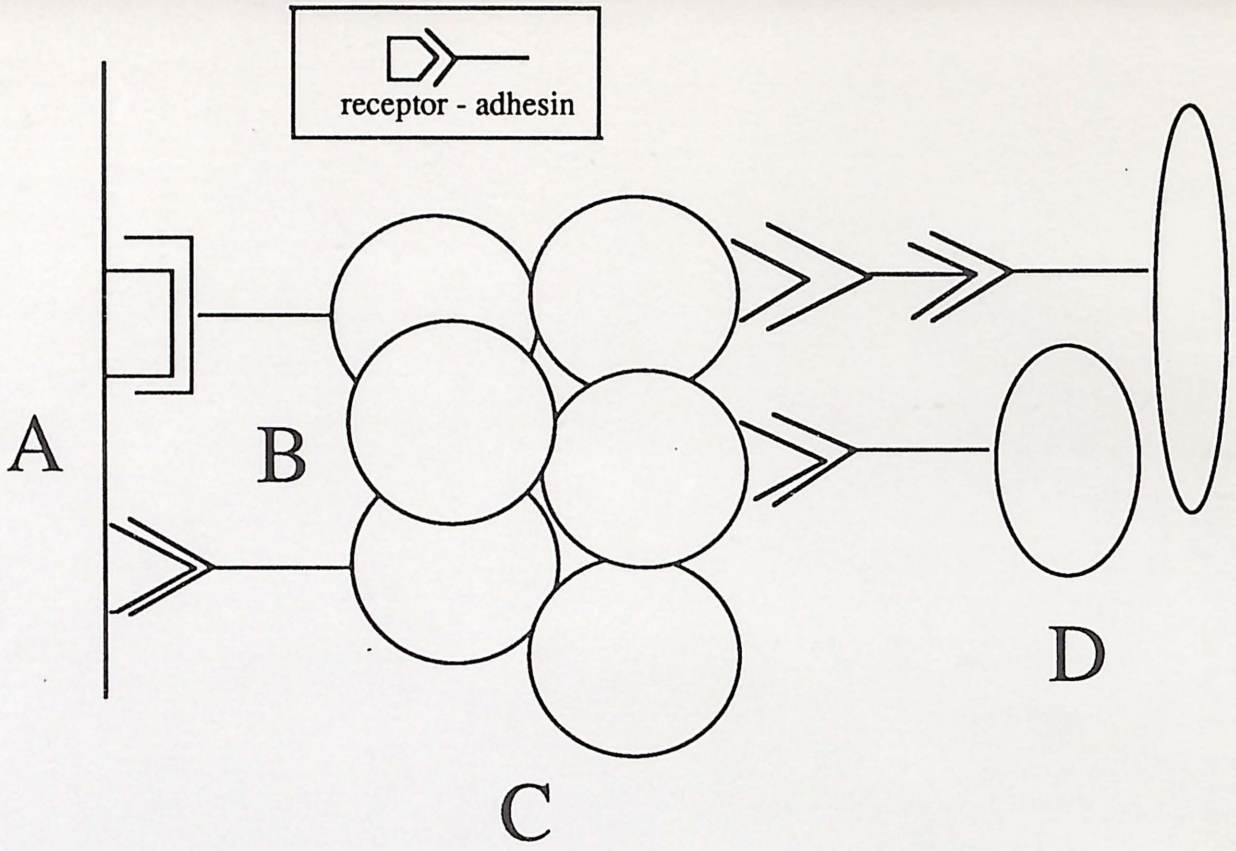

FIGURE 1. Stages of plaque formation. A - salivary pellicle; B - initial attachment of bacteria; C - accumulation of bacteria by bacterial growth; and D - accumulation of bacteria by interbacterial adherence. The complementary sets of adhesin-receptor symbols (an example is shown in the box at the top) represent the various kinds of coaggregations as well as interactions with molecules in the salivary pellicle.

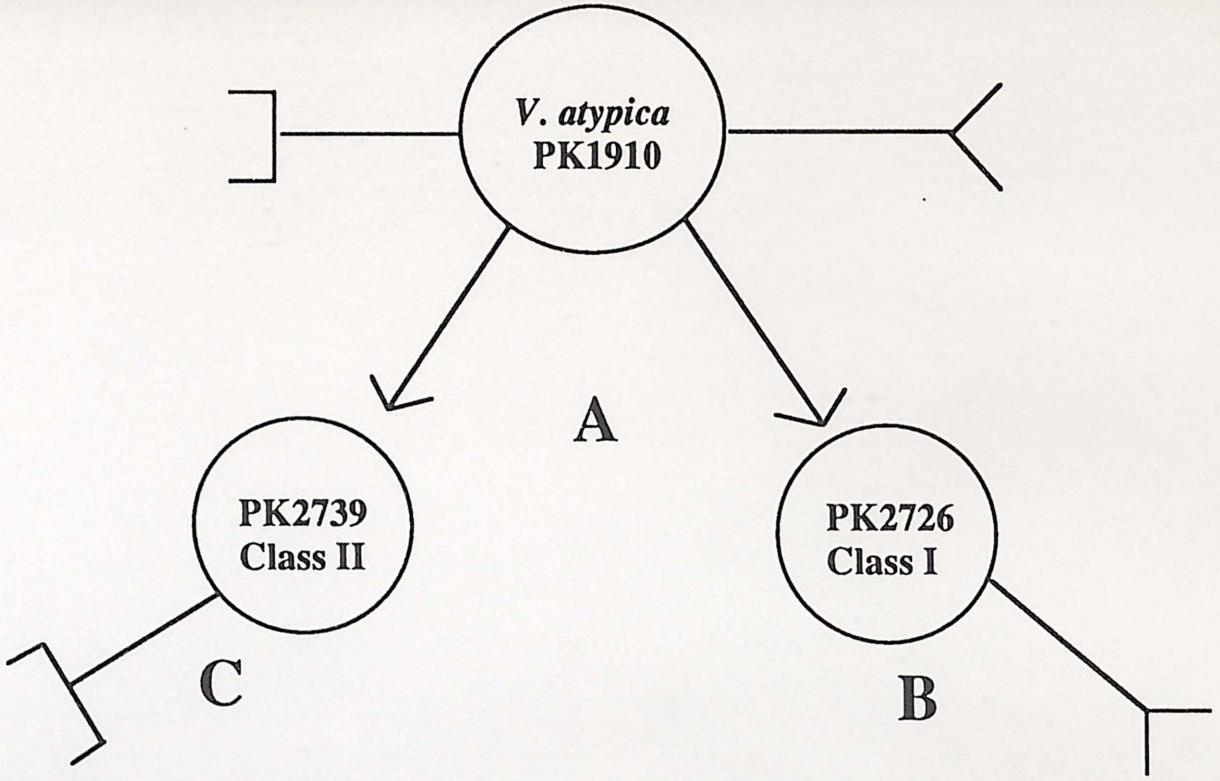

FIGURE 2. Representation of *V. atypica* PK1910 and its coaggregation-defective mutants. A - *V. atypica* PK1910 with both adhesins; B - *V. atypica* PK2726, Class I mutant, which does not participate in lactose- inhibitable interactions with *streptococci* and does not have the 45 kDa adhesin; C - *V. atypica* PK2739, Class II mutant, which does not participate in lactose-resistant interactions with *streptococci* and does have the 45 kDa adhesin. Class III mutants (not represented here) lack both kinds of interactions.

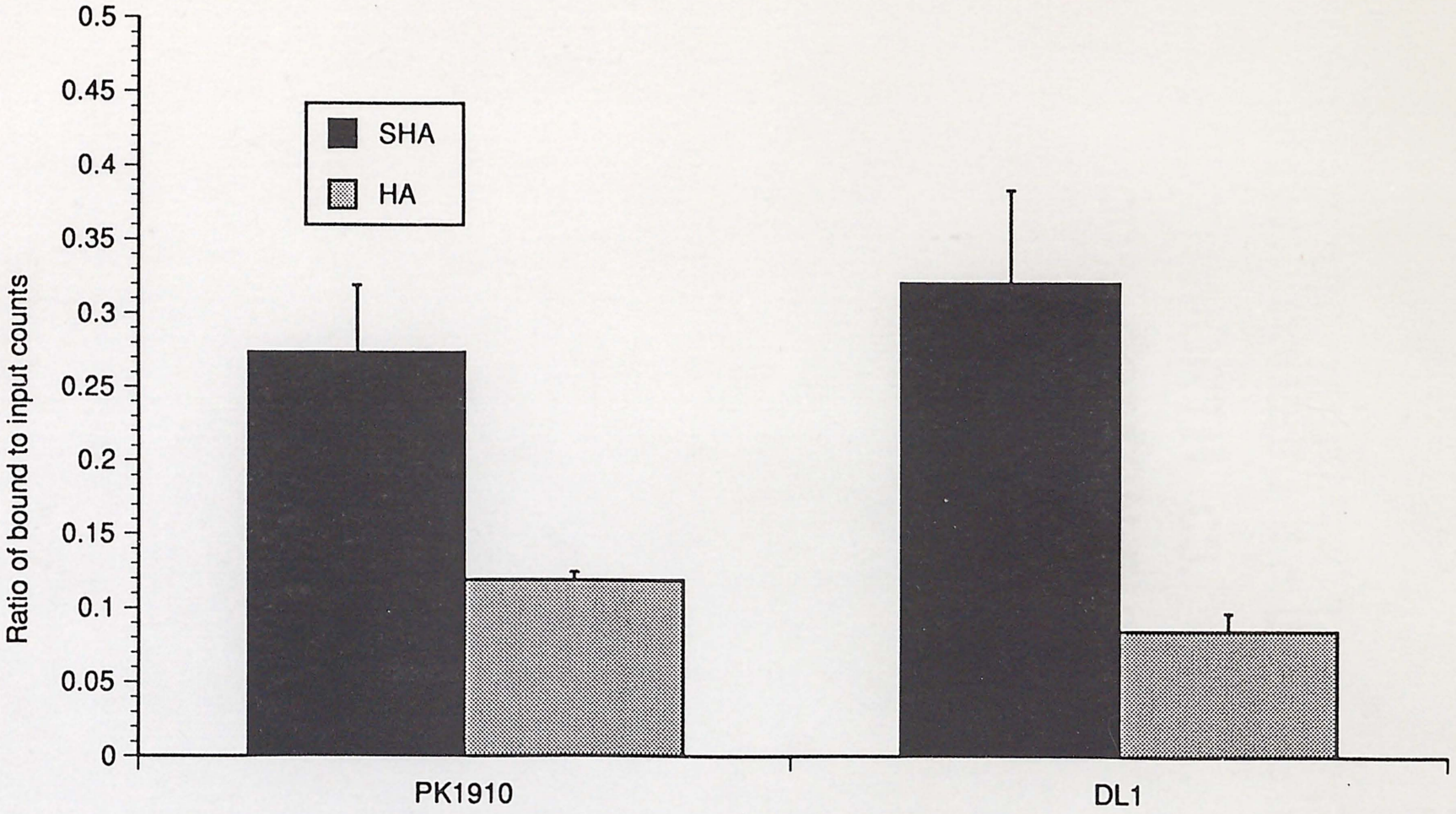

FIGURE 3. Mean ratio ± SEM (standard error of the mean) of bound to input counts for binding of *V. atypica* PK1910 and *S. gordonii* DL1 to SHA and HA.

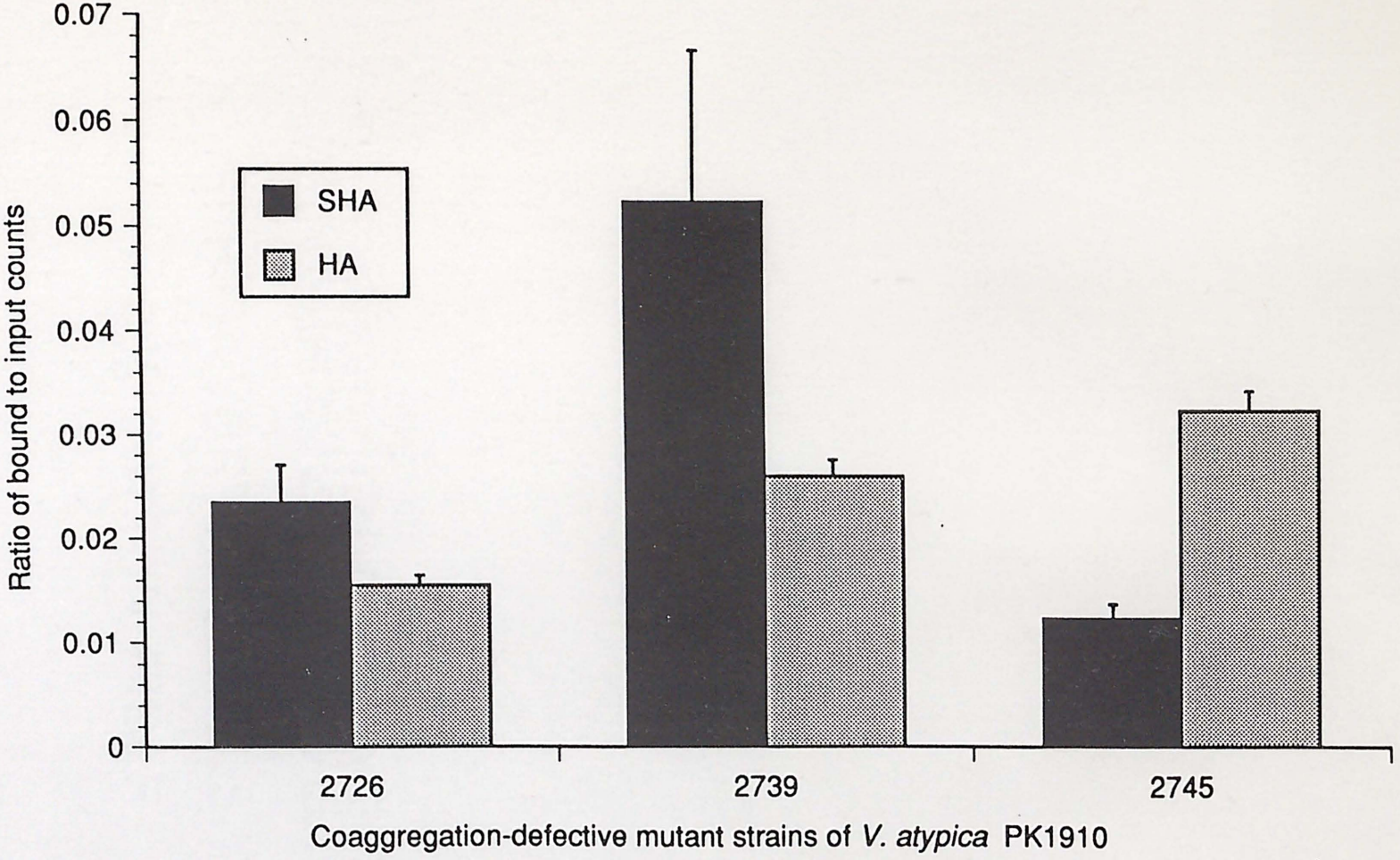

FIGURE 4. Mean ratio ± SEM (standard error of the mean) of bound to input counts for the binding of the coaggregation-defective mutant strains of *V. atypica* PK1910 to SHA and HA.

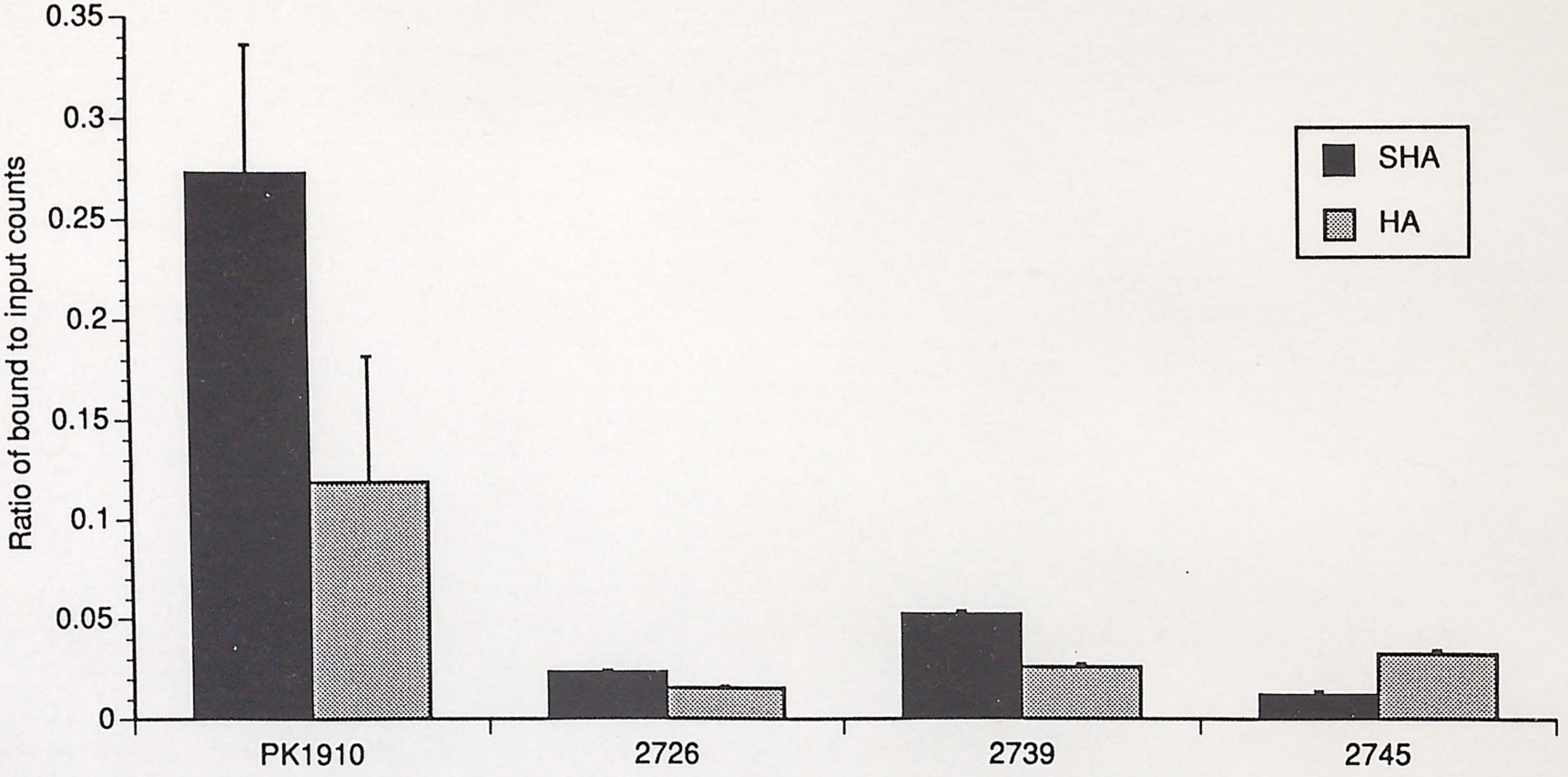

FIGURE 5. Comparison of the mean ratio ± SEM (standard error of the mean) of bound to input counts for the binding of *V. atypica* PK1910 and its coaggregation-defective mutant strains to SHA and HA.

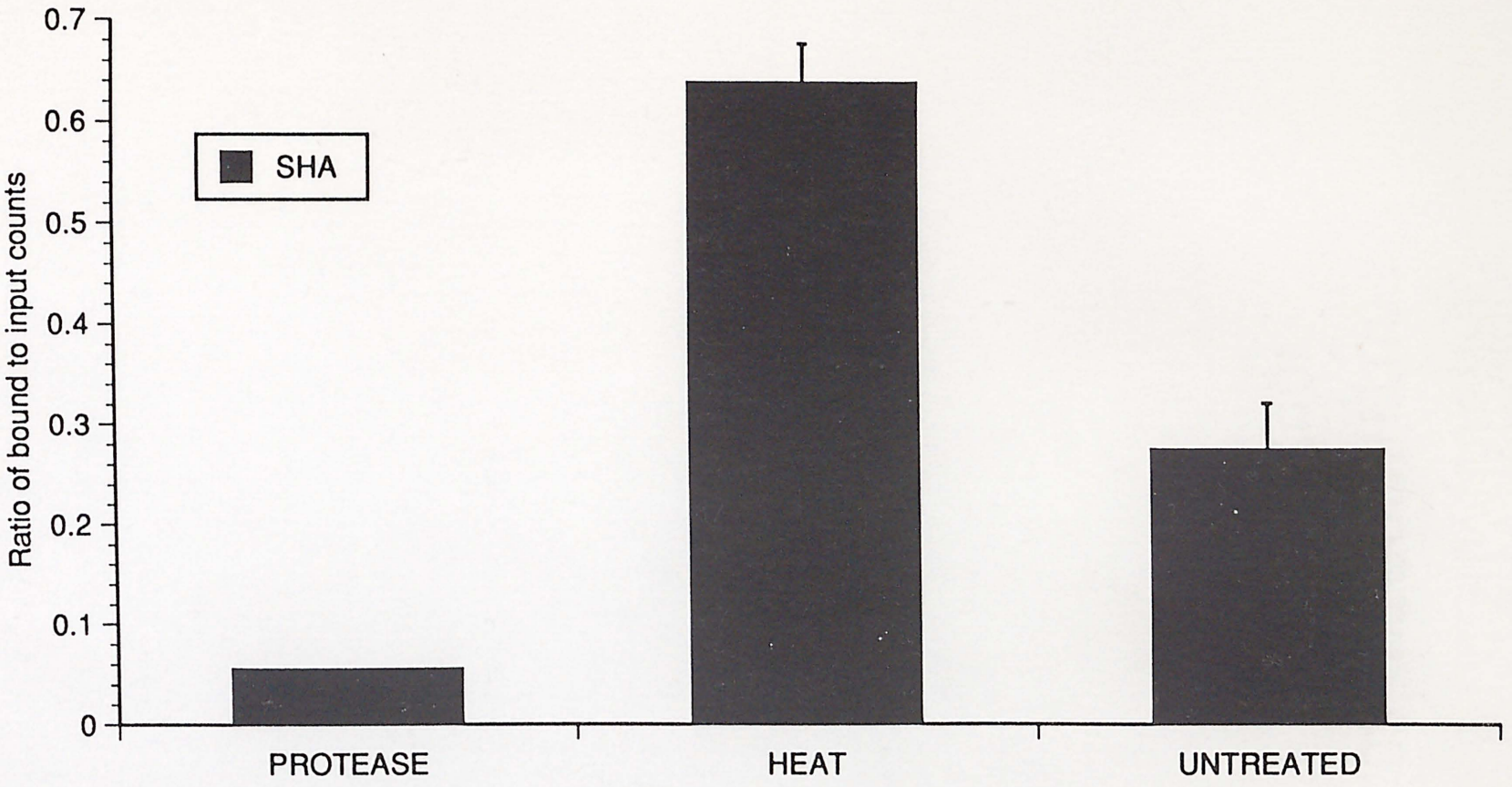

FIGURE 6. The mean ratio ± SEM (standard error of the mean) of bound to input counts for the binding of protease-treated, heat-treated and untreated *V. atypica* PK1910 to SHA.

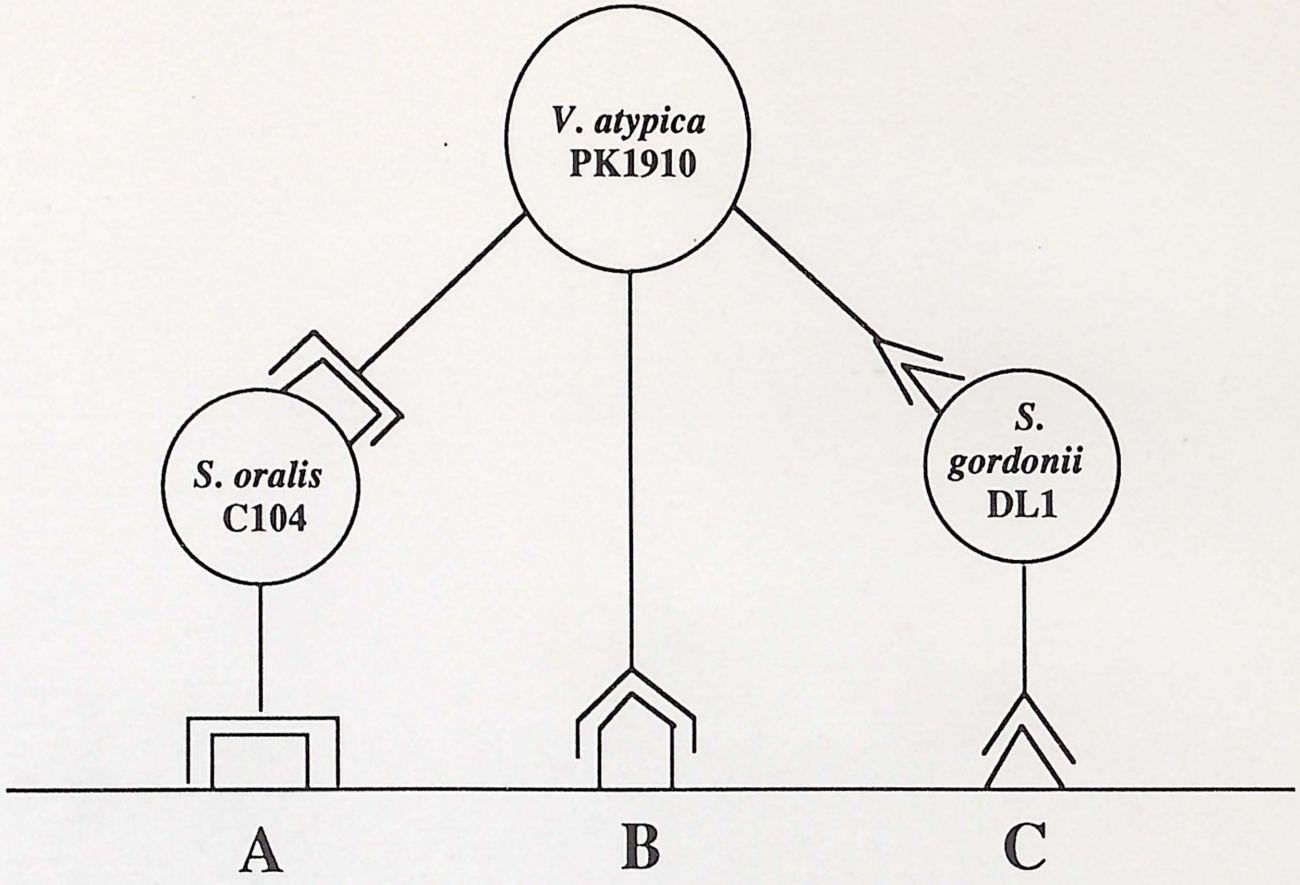

FIGURE 7. Model of three distinct mechanisms by which *V. atypica* PK1910 accumulates in dental plaque. A and C - The two types of coaggregation with *streptococci*; and B - binding directly to salivary pellicle.

TABLE I

Strains of *Streptococcal* species and *Veillonella atypica* used

Strain	Properties or origin	Streptococcal coaggregation group	Reference
Streptococcal species:			
S. gordonii			
DL1	Reference strain	1	25
PK488	Reference strain	6	27
S. oralis			
H1	Reference strain	2	25
34	Reference strain	3	25
J22	Reference strain	4	25
C104	Reference strain	3	25
Streptococcus SM			
PK509	Reference strain	5	28
V. atypica strains			
PK1910	Wild type; Reference strain		12
PK2726	PK2148 (Class I COG^- mutant)		23
PK2739	PK2148 (Class II COG^- mutant)		21
PK2745	PK2726 (Class III COG^- mutant)		23

TABLE II

The average ratio of bound to input counts for binding of *V. atypica* PK1910 to SHA and HA of five different experiments including the mean and standard error of the mean of these five experiments

Experiment No.	SHA	HA
1	0.151	0.141
2	0.356	0.111
3	0.366	0.116
4	0.317	0.108
5	0.176	0.119
Mean	0.273	0.119
S.E. Mean	0.046	0.006

TABLE III

The average ratio of bound to input counts for binding of *S. gordonii* DL1to SHA and HA of five different experiments including the mean and standard error of the mean of these five experiments

Experiment No.	SHA	HA
1	0.224	0.119
2	0.308	0.050
3	0.372	0.085
4	0.532	0.102
5	0.170	0.068
Mean	0.321	0.085
S.E. Mean	0.063	0.012

TABLE IV

The average ratio of bound to input counts for binding of *V. atypica* PK2726 to SHA and HA of five different experiments including the mean and standard error of the mean of these five experiments

Experiment No.	SHA	HA
1	0.037	0.012
2	0.023	0.017
3	0.021	0.018
4	0.021	0.016
5	0.015	0.015
Mean	0.023	0.015
S.E. Mean	0.004	0.001

TABLE V

The average ratio of bound to input counts for binding of *V. atypica* PK2739 to SHA and HA of five different experiments including the mean and standard error of the mean of these five experiments

Experiment No.	SHA	HA
1	0.026	0.021
2	0.015	0.027
3	0.092	0.031
4	0.073	0.026
5	0.055	0.025
Mean	0.052	0.026
S.E. Mean	0.014	0.002

TABLE VI

The average ratio of bound to input counts for binding of *V. atypica* PK2745 to SHA and HA of five different experiments including the mean and standard error of the mean of these five experiments

Experiment No.	SHA	HA
1	0.009	0.032
2	0.016	0.028
3	0.015	0.039
4	0.011	0.030
5	0.011	0.033
Mean	0.012	0.032
S.E. Mean	0.001	0.004

TABLE VII

Statistical significance ($p < \text{or} > 0.05$) of the binding ability to SHA and HA of the bacterial strains studied

Bacterial Species and Strains	P value
V. atypica PK1910	0.02
S. gordonii DL1	0.01
V. atypica PK2726	0.07
V. atypica PK2739	0.06
V. atypica PK2745	0.0001

TABLE VIII

Statistical significance ($p < \text{or} > 0.05$) of the binding ability to SHA of the bacterial strains studied as compared to each other

Bacterial Species and Strains	P value
V. atypica PK1910 and *S. gordonii* DL1	0.280
V. atypica PK1910 and *V. atypica* PK2726	0.003
V. atypica PK1910 and *V. atypica* PK2739	0.005
V. atypica PK1910 and *V. atypica* PK2745	0.002
V. atypica PK2726 and *V. atypica* PK2739	0.062
V. atypica PK2726 and *V. atypica* PK2745	0.016
V. atypica PK2739 and *V. atypica* PK2745	0.025
Protease treated and untreated *V. atypica* PK1910	0.028
Heat treated and untreated *V. atypica* PK1910	0.009

DISCUSSION

As seen in the results, the wildtype *V. atypica* PK1910 binds well to SHA. This ability to adhere to SHA is not shared by its coaggregation-defective mutant strains of *V. atypica* PK2726, *V. atypica* PK2739 and *V. atypica* PK2745. Interestingly, in previous studies, it has been stated that *Veillonella* spp. adhere poorly to hard and soft tissues.[78] This study indicated that *V. atypica* PK1910 binds as well to the salivary pellicle as some early colonizers such as *S. gordonii* DL1. It is proposed that *V. atypica* PK1910 has the ability to bind directly to salivary pellicle by means of a distinct adhesin or molecule not yet identified. The presence of a distinct adhesin on *V. atypica* PK1910 that mediates binding to SHA may also mediate the binding to other bacteria.

The coaggregation of *V. atypica* PK1910 to other streptococcal strains has been well established. Two distinct types of *veillonella-streptococcal* coaggregations have been described.[81] In 1990 Hughes and associates[113] isolated three classes of coaggregation-defective mutants of *Veillonella* that did not bind to certain streptococcal strains, presumably due to the absence of an adhesin(s). The first class, Class I mutants, failed to participate in lactose-inhibitable coaggregations with these streptococcal cells. The second class, Class II mutants, failed to coaggregate by the lactose-resistant interactions. The third class, Class III mutants, failed to coaggregate with any of these oral

streptococci. Utilizing these mutants, Hughes *et al.*[58] identified a 45 kDa protein that appeared to mediate the lactose-inhibitable interactions of *V. atypica* PK1910 and these oral streptococci. The protein appears to be absent in Class I and Class III mutants but present in the wild type and Class II mutants.[61]

It was a premise of this study that the 45 kDa adhesin in addition to mediating binding to certain streptococci might regulate adherence to other oral surfaces, in particular, the salivary pellicle. If so, binding to the SHA should have been demonstrated by cells expressing the 45 kDa protein, *V. atypica* PK1910 and the Class II mutant, *V. atypica* PK2726. Conversely, those cells that do not express this protein would be expected to show reduced binding to SHA. The results of this study however, suggest that all three classes of coaggregation mutants showed poor binding to SHA.

V. atypica PK1910 and *S. gordonii* DL1 bound well to SHA, much more so than to HA. In contrast, the coaggregation-defective mutant strains, *V. atypica* PK2726, *V. atypica* PK2739, and *V. atypica* PK2745 did not adhere significantly to either SHA or HA. Therefore, the 45 kDa protein expressed in both *V. atypica* PK1910 and the Class II mutant (*V. atypica* PK2739) would not appear to mediate adherence to the salivary pellicle. Another possibility might be that the 45 kDa protein on *V. atypica* PK2739 is stereochemically inhibited from binding to SHA but not oral streptococci.

Subtle differences in binding to SHA were detected between the three classes of mutants. Two of the coaggregation-

defective mutant strains of *V. atypica* PK1910 (*V. aytpica* PK2726 and *V. atypica* PK2739) showed a significantly ($p < 0.05$) greater adherence to SHA than *V. atypica* PK2745. Both *V. atypica* PK2726, and *V. atypica* PK2739 each have a distinct adhesin not present on the other. Neither of these adhesins are present on *V. atypica* PK2745. The data from this study reflect that the actual ratio of bound to input counts for binding to SHA ranged from 0.009 to 0.09. In comparison to the binding of *V. atypica* PK1910 or *S. gordonii* DL1 to SHA, the binding of *V. atypica* PK2726, *V. atypica* PK2739 and *V. atypica* PK2745 to SHA appears dramatically reduced. This suggests that coaggregation properties may at least partially influence binding to SHA.

The *V. atypica* PK1910 used for this study was routinely examined by suspension with *S. gordonii* DL1, *S. gordonii* PK488, *S. oralis* H1, *S. oralis* 34, *S. oralis* J22, *S. oralis* C104, and *S. SM* PK509 in coaggregation buffer to insure the validity of coaggregation properties. Once this was established, the SHA and HA adherence tests were conducted. It is interesting to note that one culture of *V. atypica* PK1910 did not exhibit positive coaggregation properties and likewise showed poor binding to SHA. Thus, the reproducible decreased binding of all three classes of coaggregation mutants suggests that a distinct mechanism for binding of *V. atypica* PK1910 to SHA may exist.

To further test this hypothesis, this study examined the influence of protease or heat treatments on SHA binding by *V. atypica* PK1910. In previous studies, heating or protease

treatment inactivated the lactose-inhibitable coaggregations with streptococci. In contrast, while heat treatment inactivated the lactose-resistant interactions, protease treatment had no effect on these interactions.[113] The results from the protease tests showed that protease inhibited binding by *V. atypica* PK1910 to SHA.

In contrast, heat treatment of the *V. atypica* PK1910 did not inhibit its adherence to SHA. In fact, these results suggest that heat treatment augments the binding ability of *V. atypica* PK1910 to the salivary pellicle. This would appear to contradict results of previous studies investigating the effects of heat treatment on the coaggregation of *V. atypica* PK1910 with streptococci. Hughes *et al.* in 1990[113] demonstrated that heat treatment inactivated both lactose-inhibitable and lactose-resistant coaggregation interactions of *V. atypica* PK1910 with certain streptococci. The heat treatment in this study may enhance binding to SHA by causing a stereochemical change in the *V. atypica* PK1910 causing increased adhesin-receptor recognition.

Bacteria can have multiple adhesins that recognize distinct and diffferent receptors. This adhesin-receptor interaction leads to adherence and coaggregation to other bacteria and the salivary pellicle. These data suggest that there is yet another distinct adhesin present on *V. atypica* PK1910 that allows it to adhere to the salivary pellicle. This unique adhesin has not been previously isolated and would appear to be protease inactivated and not only heat resistant but enhanced by heat treatment.

This novel molecule that acts as a specific adhesin targeting receptor sites in the salivary pellicle as seen in this study's experiments with the SHA would appear to be expressed on *V. atypica* PK1910 only. The coaggregation-defective mutant strains of *V. atypica* PK1910 (*V. atypica* PK2726, *V. atypica* PK2739, and *V. atypica* PK2745) exhibited reduced binding to SHA, therefore indicating an absence of this adhesin on their cell surface. It would then appear that, in addition to altered coaggregation properties, these cells are altered in other properties.

Perhaps the mutation by which the coaggregation-defective mutant strains of *V. atypica* PK1910 (*V. atypica* PK2726, *V. atypica* PK2739, and *V. atypica* PK2745) have each lost their ability to bind to certain streptococcal strains may explain how these strains lost the ability to bind to SHA. Mutations in genetic loci that encode regulation of cell surface molecules can have such multiple effects.[116] This unique adhesin may be present on a coaggregation-defective mutant not yet isolated.

Based on these observations, it is proposed that there is a distinct third adhesin or molecule present on *V. atypica* PK1910 that is not present on its coaggregation-defective mutant strains, *V. atypica* PK2726, *V. atypica* PK2739, and *V. atypica* PK2745 (Fig. 7). This adhesin allows for the binding of *V. atypica* PK1910 to the salivary pellicle and may also mediate binding to other streptococcal strains. Two of this adhesin's unique

properties include inactivation by protease treatment and resistance, possibly even enhancement, by heat treatment.

SUMMARY AND CONCLUSIONS

Dental caries is a prevalent disease of the 20th century. Bacteria in dental plaque play a role in caries formation. Dental plaque is composed of a complex and dynamic microbial community. *Veillonella* is a prominent bacterial species found in dental plaque. It has been found in large numbers in both caries-active and caries-free sites.

In this study, it was hypothesized that *Veillonella* binds directly to the salivary pellicle. It was further proposed that this binding may be mediated by a 45 kilodalton adhesin previously identified by Hughes and associates.[58] By utilizing *V. atypica* PK1910 and its three classes of coaggregation-defective mutants, the relationship between *Veillonella* and the salivary pellicle was studied. The results attained lead to the following conclusions:

1. *V. atypica* PK1910 binds to the salivary pellicle. Its binding ability to SHA was comparable to the binding of *S. gordonii* DL1 to SHA.
2. All three classes of mutants show greatly reduced binding to SHA. Thus, the 45 kilodalton adhesin previously identified by Hughes and associates[58] does not appear to mediate this binding of *V. atypica* PK1910 to the salivary pellicle.The coaggregation-defective mutant of *V. atypica* PK1910 which also expresses this molecule (*V. atypica* PK2739) exhibited greatly reduced binding to

SHA in comparison to the binding of *V. atypica* PK1910 to SHA.

3. The binding of *V. atypica* PK1910 to the salivary pellicle may involve a distinct mechanism possibly mediated by a unique molecule that appears to be present only on the wild type (*V. atypica* PK1910 and not on its three classes of coaggregation-defective mutants. The binding is heat stable and protease sensitive.

V. atypica PK1910 is a prominent bacterial species found in dental plaque. Previous studies suggested it colonizes dental plaque by adhering to other plaque bacteria. These studies suggest that it can also recognize receptors in the salivary pellicle. Understanding the mechanisms by which bacteria adhere to the tooth surface and the salivary pellicle during plaque formation can aid in the development of agents to prevent dental disease.

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ABSTRACT

BINDING OF ORAL *VEILLONELLA* SPP. TO SALIVA-COATED HYDROXYAPATITE

by

Sonya L. Wu

Indiana University School of Dentistry
Indianapolis, Indiana

Veillonella spp. are found in high numbers in the mouth in dental plaque and on the mucosa. Veillonellae utilize lactic acid for their metabolic needs. A symbiotic relationship between Veillonellae and other oral bacteria, including a nutritional relationship with some streptococci, has been demonstrated both *in vitro* and *in vivo*. Thus, Veillonellae may protect the host from dental caries. Adherence is the initial step in bacterial colonization of oral surfaces. Recent evidence suggests that certain oral bacteria express molecules (adhesins) on their cell surface, which recognize receptors on other oral bacteria and/or in salivary pellicle. It has been previously demonstrated that *Veillonella* spp. bind avidly to *Streptococcus*. spp. found in subgingival plaque. The present study investigated the ability of *V. atypica* PK1910 to bind to saliva-coated hydroxyapatite (SHA), a model for adherence to the salivary pellicle. The results show that there was statistically significant enhanced binding of

Veillonella atypica PK1910 to saliva-coated hydroxyapatite beads. ($p < 0.05$) Three classes of coaggregation-defective mutants of V. atypica PK1910 were tested for their ability to bind to SHA. Interestingly, they did not demonstrate any enhanced binding to saliva-coated hydroxyapatite beads. Heating of PK1910 did not effect binding to SHA. In contrast, protease treatment of the veillonella cell surface inactivated binding. Therefore, it appears that *V. atypica* PK1910, in addition to binding to oral *Streptoccoccus* spp. in dental plaque, may also colonize the tooth surface by binding directly to the salivary pellicle. It appears that a distinct heat stable protein may mediate this binding to SHA.

CURRICULUM VITAE

SONYA LEE WU

April 23, 1966	Born in Columbus, Ohio
December 29, 1990	Married to Richard I. Ng Cheng Hin
May 1991	D.M.D., Washington University School of Dental Medicine, St. Louis, Missouri
July 1991-June 1993	M.S.D. Program, Pediatric Dentistry Indiana University School of Dentistry, Indianapolis, Indiana

Professional Organizations

American Dental Association
American Academy of Pediatric Dentistry
Omicron Kappa Upsilon, Gamma Chapter
Delta Sigma Delta, Upsilon Chapter