Bacterial coaggregation: an integral process in the development of multi-species biofilms

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Coaggregation is a process by which genetically distinct bacteria become attached to one another via specific molecules. Cumulative evidence suggests that such adhesion influences the development of complex multi-species biofilms. Once thought to occur exclusively between dental plaque bacteria, there are increasing reports of coaggregation between bacteria from other biofilm communities in several diverse habitats. A general role for coaggregation in the formation of multi-species biofilms is discussed.

Coaggregation was first recognized between bacteria isolated from human dental plaque and is defined as the process of adhesion between genetically distinct bacterial partners. Several papers published in the 1970s [1–3] demonstrated that coaggregation was a common phenomenon between a broad range of genera from dental plaque. These early investigators showed that coaggregation between pairs of bacteria was highly specific and was typically mediated by a protein 'adhesin' on one cell type and a complementary saccharide 'receptor' on the other. Since the beginning of the 1980s, >120 peer-reviewed research papers have described coaggregation between dental plaque bacteria* and it is now known that >1000 oral bacterial strains coaggregate (reviewed by Kolenbrander [4,5]).

Coaggregation has now been observed amongst bacteria isolated from biofilms in the mammalian gut, the human urogenital tract and potable-water-supply systems, indicating that the adhesion of genetically distinct strains could be a widespread phenomenon [6]. With the current expansion of coaggregation studies to organisms from environments other than the oral cavity it is becoming increasingly clear that coaggregation could play an important role in the development of several different multi-species biofilms [4,6,7]. It is the intention of this short review to describe the process of coaggregation and discuss its role in the development of multi-species biofilms.

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Coaggregation and the development of multi-species biofilms

Microorganisms are found in a wide range of diverse ecosystems as highly structured, multi-species communities termed biofilms [8]. These are functional consortia of cells that often possess a combined metabolic activity that is greater than that of the component species [9,10]. Biofilms are most commonly found attached to a solid substratum in a moist or liquid environment from which they can derive nutrients [11]. The physiology and metabolism of multi-species biofilm communities are immensely complex and there are many excellent reviews that address aspects of biofilm biology that will not be covered here [11–13].

One current model is that the development of biofilms on surfaces proceeds as a succession of adhesion and multiplication events. The first organisms to attach are the primary (early) colonizers and primary colonization is mediated through specific or non-specific physico-chemical interactions with components of an adsorbed, organic conditioning film [14–16] (Fig. 1a). If conditions are suitable, the primary colonizers can then multiply on the substratum to form microcolonies (Fig. 1b). As environmental conditions change within the young biofilm and the substratum becomes covered by bacteria, secondary (late) colonizers are then able to attach to the primary colonizers (Fig. 1c) and the biofilm begins to develop into a multi-species community (Fig. 1d). Coaggregation interactions are believed to contribute to the development of biofilms by two routes (Fig. 1c). The first route is by single cells in suspension specifically recognizing and adhering to genetically distinct cells in the developing biofilm. The second route is by the prior coaggregation in suspension of secondary colonizers followed by the subsequent adhesion of this coaggregate to the developing biofilm. In both cases, bacterial cells in suspension (planktonic cells) specifically adhere to cells in the biofilm in a process known as coadhesion [17,18]. Coadhered cells can then become part of the biofilm community.

At least within the oral cavity both types of coaggregation events, illustrated in Fig. 1, are thought to be crucial for an ordered succession of bacterial adhesion events that

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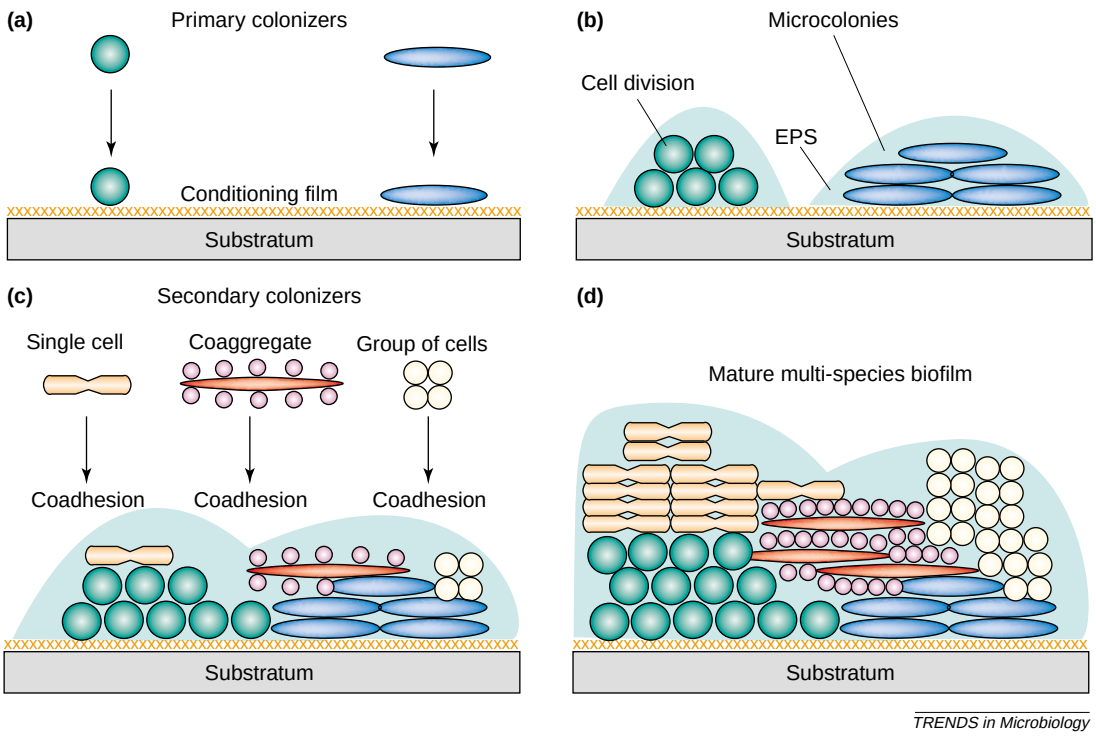

Fig. 1. Diagram illustrating the possible roles of coaggregation in the development of multi-species biofilms. (a) Primary colonization of a substratum covered in a 'conditioning film' composed of polysaccharides and proteins; (b) cell growth, division and production of extracellular polysaccharide (EPS) leading to the development of microcolonies; (c) coadhesion of single cells, coaggregated cells and groups of identical cells into the young multi-species biofilm; and (d) maturation and the formation of clonal mosaics within the multi-species biofilm.

result in the mutually beneficial pairing of bacterial species within dental plaque [14]. Multi-species biofilms eventually develop (Fig. 1d), containing specific sub-sets of species derived from the planktonic phase. An important early physiological event that can occur during the development of a biofilm, and which might be important in the adhesion of secondary colonizers, is the increased production of extracellular polymeric substances (EPS). These polymers envelop the attached cells within the biofilm, strengthen adhesion between cells within the biofilm and can also act as receptors for coaggregation interactions. Thus, as a consequence of coaggregation, coadhesion and cell division as well as the adhesive nature of EPS, clusters of genetically identical bacteria are generated within multi-species biofilms [19,20] (Fig. 1) and these are called clonal mosaics [12] (Fig. 1d). However, although it is accepted that EPS acts as an 'intercellular cement' during biofilm formation [21,22], its exact role in mediating or enhancing coaggregation between bacteria in the liquid phase (i.e. planktonic bacteria) and biofilm bacteria has yet to be determined.

Coaggregation between bacteria in human dental plaque

There are estimated to be >500 taxa of oral bacteria and ~60% of these have been cultured [23,24]. Dental plaque has been the most extensively researched biofilm ecosystem and coaggregation interactions have been detected between all of the bacteria isolated from the dental plaque community [4]. Dental plaque was studied long before the term 'biofilm' came into general use and there is a wealth of information on the ecology, physiology and taxonomy of plaque bacteria. A sequence of colonization, integrating coaggregation properties and the frequency of species in a succession of primary and secondary colonizers, has been proposed [7]. The first bacteria to colonize the pellicle on the tooth surface are streptococci and Gram-positive rods such as *Actinomyces naeslundii*. Within the first 4 h of plaque formation, *Streptococcus mitis*, *Streptococcus sanguinis* (previously *Streptococcus sanguis*) and *Streptococcus oralis* represent 60–90% of the cultivable streptococci [25]. Other bacteria are also recovered from early dental plaque and microscopy shows that early plaque is composed predominantly of cocci and short rods. After 24 h however, the surface of mature plaque contains many more morphological types of bacteria, which coaggregate to form intricate structures such as 'corn cobs' [26] (Fig. 2a).

Several matrix diagrams showing the temporal nature of the many different coaggregation pairings found between the predominant coaggregating dental plaque bacteria have been constructed [4,7,27], and a simplified diagram is presented here (Fig. 3). The partnerships between dental plaque bacteria are highly specific and primary colonizers can coaggregate with each other but not usually with secondary colonizers. However, the major periodontal pathogen *Porphyromonas gingivalis*, a secondary colonizer, can coaggregate with the primary colonizer *Streptococcus gordonii* [28] (Fig. 3). Also, secondary colonizers coaggregate with *Fusobacterium nucleatum* but not usually with each other. *F. nucleatum* is therefore proposed to be a bridge organism because it can

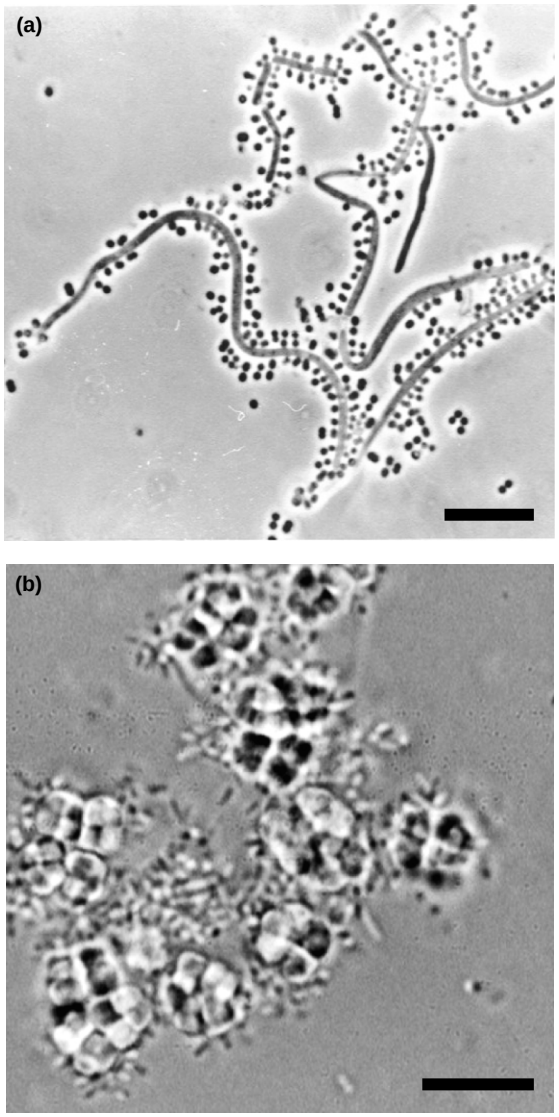

Fig. 2. (a) Phase-contrast light micrograph showing coaggregation interactions between the oral bacteria *Streptococcus cristatus* CR311 (cocci) and *Corynebacterium matruchotii* (filamentous rods), which produces a structure that resembles a corn cob. Scale bar = 10 μm. (b) Light micrograph showing coaggregation between the freshwater bacteria *Blastomonas natatoria* 2.1 (small rods) and *Micrococus luteus* 2.13 (cocci in tetrads). Scale bar = 10 μm.

coaggregate with both primary and secondary colonizers [7]. In the absence of *F. nucleatum* many other secondary colonizers cannot become part of the dental plaque community [29]. Additionally, anaerobic secondary colonizers cannot survive in the planktonic state unless coaggregated to *F. nucleatum* [29,30]. Thus, the multiplicity of its coaggregation interactions and its role as a bridging organism could make *F. nucleatum* an essential organism in the development of dental plaque. The correlation between the coaggregation ability of plaque bacteria and the temporal sequence of bacterial integration into dental plaque very strongly implicates coaggregation as a process closely linked with plaque development.

Coaggregation between bacteria in other environments

Although reports of coaggregation between oral bacteria are the most numerous it is clear that coaggregation also occurs between bacteria from several diverse ecosystems. Recently there have been reports that coaggregation occurs between different species of freshwater bacteria isolated from a biofilm formed from drinking water [31–34]. Many of the bacterial genera that were isolated are common to freshwater ecosystems [34] and both intergeneric and intrageneric coaggregation were detected amongst these strains. A light micrograph of intergeneric coaggregation *in vitro* between two freshwater strains is shown in Fig. 2b. All the strains coaggregated with at least one other strain from the biofilm and some were capable of many coaggregation interactions. Coaggregation between these freshwater strains is mediated by protein–saccharide interactions [33] and could be blocked by the addition of simple sugars (Fig. 4). Thus, the mechanism mediating adhesion between coaggregating strains is very similar for both oral and freshwater biofilm bacteria. One of the freshwater strains, *Blastomonas natatoria* 2.1, coaggregated specifically with all 18 other bacteria isolated from the original freshwater community. *B. natatoria* 2.1 might have a role as a bridging organism in the development of the freshwater biofilm community mediating adhesion between primary and secondary colonizers [34], a role analogous to that of *F. nucleatum* in dental plaque. However, the colonization sequence of organisms in the development of freshwater biofilms has not yet been investigated.

Although there are many similarities in the mechanism of coaggregation of freshwater bacteria and of dental plaque bacteria there is also a difference in the expression of coaggregation between bacteria from the two ecosystems. Coaggregation between the freshwater strains was optimally expressed only during the stationary phase of growth in batch culture and exponential cells were not able to coaggregate [32,33]. Coaggregating pairs acquired and lost their coaggregation ability at various times in stationary phase. This 'on and off' switching could indicate some form of environmental control of the expression of coaggregation adhesins and/or receptors through starvation and stress. This is in contrast to dental plaque bacteria, which do not appear to exhibit such switching 'on and off' of the coaggregation phenotype. This is presumably because loss of adhesion via coaggregation would ultimately result in oral bacteria being swallowed owing to the shear forces in the mouth [35]. There is therefore a very strong selective pressure for coaggregation interactions to occur between dental plaque bacteria.

Coaggregation has also been reported amongst organisms isolated from ecosystems other than the oral cavity and drinking water. Several papers have described coaggregation between lactobacilli and *Escherichia coli* strains isolated from the human urogenital tract [36,37], the intestinal tract of humans [38] and pigs [39], and between related lactobacilli from the crops of chickens [40]. There is so far no evidence for a growth phase influence on the phenotypic expression of coaggregation between *Lactobacillus* species and *E. coli* strains. The properties of these coaggregation interactions are not well understood and it

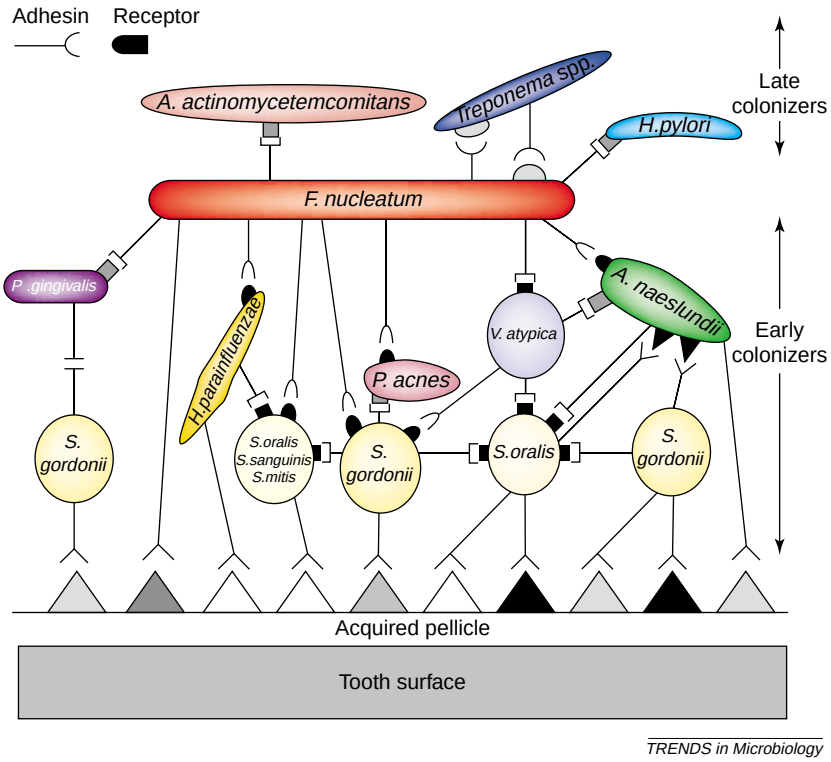

Fig. 3. Diagrammatic representation of the proposed temporal nature of human oral bacterial accretion on the tooth surface. The species represented here are *Streptococcus gordonii, Streptococcus mitis, Streptococcus oralis, Streptococccus sanguinis, Haemophilus parainfluenzae, Propionibacterium acnes, Veillonella atypica, Actinomyces naeslundii, Fusobacterium nucleatum, Actinobacillus actinomycetemcomitans, Helicobacter pylori, Treponema* spp. and *Porphyromonas gingivalis*. The complementary sets of adhesin-receptor symbols (an example is shown at the top) represent different specific coaggregation interactions or adhesion to the acquired pellicle. Coaggregation between *P. gingivalis* and *S. gordonii* is mediated by protein adhesins expressed on the surface of both cell types. Identical symbols are not intended to indicate identical molecules, but they are related functionally. The rectangular symbols represent lactose-sensitive coaggregations, other symbols represent lactose-insensitive coaggregations.

is not known whether coaggregation in these environments is restricted to these few organisms or whether a larger population of biofilm bacteria that can coaggregate remains undetected in these ecosystems.

The cell-surface components mediating coaggregation

Coaggregation adhesins

Many coaggregation adhesins have been identified on the cell surfaces of dental plaque bacteria [4,27] and the majority of the coaggregation adhesins identified so far have been found on species of *Streptococcus*, *Actinomyces* and *Fusobacterium*. The identity and specificity of some of these adhesins is summarized here.

The coaggregation adhesins expressed by the dental plaque primary colonizer *S. gordonii* DL1 have been extensively studied and this strain carries five distinct proteins involved in coaggregation interactions. In some cases, the location of the coaggregation adhesins on the cell surface has been identified. The unnamed 100-kDa protein adhesin might use lipoteichoic acid as 'scaffolding' for its presentation to its partner organism [41] and it is responsible for intrageneric, galactoside-inhibitable coaggregation with other streptococcal species. The 203-kDa Hs protein adhesin is a lectin-like protein associated with fibrillar structures and mediates adhesion to components of saliva, polymorphonuclear leukocytes and coaggregation with species of oral bacteria [42,43]. A 259-kDa protein also found on *S. gordonii* DL1, CshA, is a fibrillar protein anchored into the cell wall that can be seen by negative staining in the transmission electron microscope as peritrichous, 70-nm-long surface fibrils [44,45]. These thin, sparse, peritrichous fibrils contain several repeating amino-acid sequences and it has been suggested that the coaggregation adhesin responsible for coaggregation with *A. naeslundii* is at the distal end of the fibrillar molecule [44]. Two other coaggregation adhesins, also expressed by *S. gordonii* DL1, mediate coaggregation to *A. naeslundii* and the periodontal pathogen *P. gingivalis* and these adhesins have been designated SspA and SspB [46–48]. SspA and SspB (antigen I/II polypeptides) are multifunctional and, as well as mediating coaggregation, also mediate adhesion to salivary glycoproteins, fibronectin and collagen [46,49]. The expression of these two adhesins is enhanced when *S. gordonii* DL1 is exposed to human saliva [50]. The structural locations of SspA and SspB are unknown. The *S. gordonii* DL1 coaggregation proteins are multifunctional and can often interact with host oral surfaces and host proteins. These other properties have not been mentioned in detail and readers are referred to relevant reviews to cover other functional properties of these coaggregation proteins [5,51].

Coaggregation adhesins have also been identified on oral *Actinomyces* species and in complex with peritrichous fimbriae covering the surface of *Actinomyces* cells. For

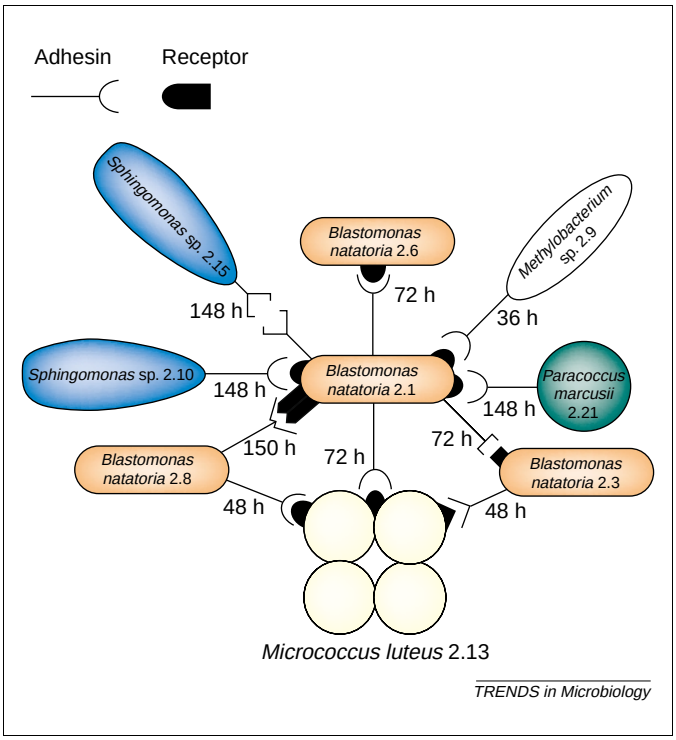

Fig. 4. Diagrammatic representation of intergeneric and intraspecies (interstrain) coaggregations between freshwater bacteria. Cells are not drawn to scale. *Micrococcus luteus* 2.13 grows as tetrads of cocci, *Blastomonas natatoria* 2.1, 2.3, 2.6 and 2.8 are symmetrical small rods, *Paracoccus marcusii* 2.21 is a coccus, *Sphingomonas* sp. 2.10 and 2.15 are club-shaped cells and *Methylobacterium* sp. 2.9 is a rod. Interactions are shown as complementary sets of stem-shaped symbols (protein adhesins) and block-shaped symbols (polysaccharide receptors). Coaggregation between *Sphingomonas* sp. 2.15 and *B. natatoria* 2.1 is mediated by protein adhesins expressed on the surface of both cell types. Identical symbols are not intended to indicate identical molecules, but they are related functionally. Semicircular symbols represent galactosamine-sensitive coaggregations, triangular symbols represent galactose-sensitive coaggregations, square symbols represent lactose-sensitive coaggregations and the 'w' shaped-symbol represents galactose- and galactosamine-sensitive coaggregations. The numbers show the length of time (hours) of growth in batch culture for maximum expression of coaggregation to occur for each pair.

example, the type 2 fimbriae of *A. naeslundii* carry and present a coaggregation adhesin for oral streptococci and the adhesins for attachment to epithelial cells and polymorphonuclear leukocytes [52]. Klier *et al.* [53] showed that *Actinomyces* serovar WVA963 probably coaggregated through a 95-kDa putative adhesin that required type 2 fimbriae to function. It was suggested that the putative adhesin is a minor subunit of the type 2 fimbriae and is most likely to be presented at the fimbrial tip. For oral streptococci, oral actinomyces and other plaque bacteria, many of the coaggregation adhesins are carried on fimbriae or fibrils, that is, surface structures projecting away from the cell wall [54]. The distancing of coaggregation adhesins away from the cell surface on fibrils and fimbriae will help partner organisms make effective contact with each other. The thin fibrils and fimbriae act to penetrate the electrostatic barrier that operates between cells in close contact (10–20 nm apart) [55], thus acting as probes to locate the appropriate receptor on the partner organism successfully.

F. nucleatum has already been mentioned as being an essential coaggregation partner for many oral bacteria and *F. nucleatum* PK1594 possesses at least three distinct multifunctional adhesin molecules, which together mediate coaggregation with seven different genera from plaque and mediate adhesion to some host surfaces [7,56]. This presence of multiple adhesins is entirely consistent with the proposed role for *F. nucleatum* as a bridging organism in plaque development.

This brief summary of some of the coaggregation adhesins on dental plaque bacteria indicates that they have evolved in parallel, as they are all different in composition from each other. It also shows that many of the adhesins are not located on the cell wall but are found associated with external appendages thus enabling cells to make more effective contact with prospective partners. It also reveals how dental plaque organisms can express more than one coaggregation adhesin simultaneously on the cell surface; this will also optimize the chances of a cell finding a suitable partner in the competition for survival in the high-shear oral environment. All these factors are consistent with the suggestion that these adhesins contribute to the build up of a multi-species plaque community.

At this time, only one coaggregation adhesin has been identified from a coaggregating biofilm bacterium not native to the oral cavity. A protein of ~70 kDa was isolated from the cell surface of the coaggregating freshwater biofilm bacterium *B. natatoria* 2.1 that has high sequence homology with a TonB-dependent receptor expressed by *Caulobacter crescentus* [A.H. Rickard *et al.* (2002) Identification of a 70-kDa coaggregation adhesin expressed by the freshwater biofilm bacterium *Blastomonas natatoria* 2.1. ASM 102nd General Meeting, 19–23 May, Salt Lake City, UT, USA; Abstract I-91]. TonB-dependent receptors are expressed by most Gram-negative bacteria and they mediate high-affinity iron acquisition [57]. Evidence is now emerging that some of these TonB-dependent receptors facilitate adhesion to surfaces [58]. It is therefore possible that in *B. natatoria* 2.1, the 70-kDa protein is a TonB-dependent receptor protein that is also a coaggregation adhesin.

Coaggregation receptors

In comparison to the coaggregation adhesin molecules, little is known about the composition and location of the receptors. Where research has been done it appears that diversity among the receptor molecules is limited. Studies on streptococcal receptors have investigated the oral streptococci including *S. gordonii*, *S. mitis*, *S. oralis* and *S. sanguinis*. The coaggregations were lactose inhibitable and each strain had one major receptor polysaccharide in the cell wall, despite the fact that these organisms could coaggregate intergenerically with a wide range of partner organisms [7]. The streptococcal receptor polysaccharides have been structurally characterized and are composed of distinct phosphodiester-linked hexa- or heptasaccharide repeating units [59,60]. The receptors contain one of only two motifs (GalNacβ1 → 3Gal and Galβ1 → 3GalNAc) [60] that have been found in each of the six structural types of receptor polysaccharides isolated from 22 strains of streptococci [60] and from all of the streptococci that exhibit galactoside-inhibitible coaggregation. This apparent lack of diversity among receptor saccharides might

indicate that the specificity of coaggregation results predominantly from the high diversity of unique adhesin molecules on the partner organisms.

Ecological significance of bacterial coaggregation

In this review it has been demonstrated that coaggregation is a common phenomenon in a variety of multi-species biofilm communities and its ecological significance must be assessed. When considering the benefits coaggregation confers on bacterial partnerships, it is probable that the strength and specificity of the interactions will be subject to natural selection. Given that most bacteria exist in environments with fluctuating conditions (e.g. shear forces, nutrient availability or physiological conditions), the bacteria within coaggregated communities will survive and proliferate under conditions that reduce the prevalence of single non-coaggregated cells.

Experimental evidence is now available indicating that shear forces can select for coaggregation ability within a multi-species biofilm community. In a water tank with a localized shear force directed across the biofilm surface and the body of water almost static, coaggregating strains occurred in a much higher frequency in the freshwater multi-species biofilm than in the surrounding bulk liquid (planktonic suspension) [A.H. Rickard *et al.* (2002) Coaggregation and biofilm formation in a freshwater ecosystem. ASM 102nd General Meeting, 19–23 May, Salt Lake City, UT, USA; Abstract N-43]. This is consistent with the hypothesis that biofilms under high shear force are subjected to a selection pressure that favours coaggregation partnerships. In fast-flowing rivers or streams, non-coaggregating organisms would be washed away from their optimum ecological niche.

Coaggregating cells on a substratum can possess a combined metabolic advantage over single cells. Palmer *et al.* [61] have recently shown, using an *in vitro* model of dental plaque, that the coaggregating partnership of *S. oralis* and *A. naeslundii* formed a nutritionally beneficial, mutualistic relationship that allowed each to grow where neither grew alone. Such mutualism has also been reported in dental plaque model systems containing many species of oral bacteria utilizing mucin as the major carbon, nitrogen and energy source [62]. Mucin catabolism involves the synergistic action of several species with overlapping patterns of enzyme activity. Bradshaw *et al.* [62] showed that such metabolic cooperation results in the liberation of additional nutrients, and this might help to maintain the characteristic diversity of biofilm communities found in many natural habitats. Clearly, in such relationships a close proximity of the participating organisms, brought about by coaggregation, would maximize the efficiency of the consortium. As a functional consortium, the whole community could therefore be considered to be the evolving entity selected for on the basis of its combined functional efficiency, as has been suggested by Caldwell [63].

For the reasons outlined here, specific coaggregation processes are likely to have an important ecological role as an integral process in the development and maintenance of mixed-species biofilm communities. The evidence available so far strongly suggests that this is true for dental plaque and probably for freshwater biofilm communities. We do not yet have a full enough understanding of how widespread coaggregation is among other multi-species ecosystems, but it could turn out to be a very widespread and truly ancient phenomenon.

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References

1 Gibbons, R.J. and Nygaard, M. (1970) Inter-bacterial aggregation of plaque bacteria. *Arch. Oral Biol.* 15, 1397–1400
2 McIntire, F.C. *et al.* (1978) Mechanism of coaggregation between *Actinomyces viscosus* T14V and *Streptococcus sanguis* 34. *Infect. Immun.* 21, 978–988
3 Cisar, J.O. *et al.* (1979) Specificity of coaggregation reactions between human oral streptococci and strains of *Actinomyces viscosus* or *Actinomyces naeslundii*. *Infect. Immun.* 24, 742–752
4 Kolenbrander, P.E. (2000) Oral microbial communities: biofilms, interactions, and genetic systems. *Annu. Rev. Microbiol.* 54, 413–437
5 Kolenbrander, P.E. *et al.* (2002) Communication among oral bacteria. *Microbiol. Mol. Biol. Rev.* 66, 486–505
6 Handley, P.S. *et al.* (2001) Coaggregation – is it a universal phenomenon? In *Biofilm Community Interactions: Chance or Necessity?* (Gilbert, P. *et al.*, eds), pp. 1–10, Bioline Press
7 Kolenbrander, P.E. *et al.* (1999) Potential role of functionally similar coaggregation mediators in bacterial succession. *Dental Plaque Revisited: Oral Biofilms in Health and Disease* (Newman, H.N., Wilson, M., *et al.* eds), pp. 171–186, Bioline Press
8 Stoodley, P. *et al.* (2002) Biofilms as complex differentiated communities. *Annu. Rev. Microbiol.* 56, 187–209
9 Moller, S. *et al.* (1998) *In situ* gene expression in mixed-culture biofilms: evidence of metabolic interactions between community members. *Appl. Environ. Microbiol.* 64, 721–732
10 Gilbert, P. *et al.* (1997) Biofilm susceptibility to antimicrobials. *Adv. Dent. Res.* 11, 160–167
11 Wimpenny, J.W.T. (2000) An overview of biofilms as functional communities. In *SGM Symposium Volume 59: Community Structure and Cooperation in Biofilms* (Allison, D.G. *et al.*, eds), pp. 1–24, Cambridge University Press
12 Gilbert, P. *et al.* (2002) The physiology and collective recalcitrance of microbial biofilm communities. *Adv. Microb. Physiol.* 46, 202–256
13 Marsh, P.D. and Bowden, G.H.W. (2000) Microbial community interactions in biofilms. In *SGM Symposium Volume 59: Community Structure and Cooperation in Biofilms* (Allison, D.G. *et al.*, eds), pp. 167–198, Cambridge University Press
14 Marsh, P.D. and Bradshaw, D.J. (1995) Dental plaque as a biofilm. *J. Ind. Microbiol.* 15, 169–175
15 Dang, H. and Lovell, C.R. (2000) Bacterial primary colonization and early succession on surfaces in marine waters as determined by amplified rRNA gene restriction analysis and sequence analysis of 16S rRNA genes. *Appl. Environ. Microbiol.* 66, 467–475
16 van Loosdrecht, M.C. *et al.* (1990) Influence of interfaces on microbial activity. *Microbiol. Rev.* 54, 75–87
17 Busscher, H.J. *et al.* (1995) Initial microbial adhesion is a determinant for the strength of biofilm adhesion. *FEMS Microbiol. Lett.* 128, 229–234
18 Bos, A. *et al.* (1994) Quantitative method to study co-adhesion of microorganisms in a parallel plate-flow chamber – basic principles of the analysis. *J. Microbiol. Methods* 20, 289–305
19 Molin, S. *et al.* (2000) Microbial communities: aggregates of individuals or coordinated systems. In *SGM Symposium Volume 59: Community Structure and Cooperation in Biofilms* (Allison, D.G. *et al.*, eds), pp. 199–214, Cambridge University Press
20 Moller, S. *et al.* (1996) Activity and three-dimensional distribution of toluene-degrading *Pseudomonas putida* in a multispecies biofilm assessed by quantitative *in situ* hybridization and scanning confocal laser microscopy. *Appl. Environ. Microbiol.* 62, 4632–4640
21 Sutherland, I.W. (2001) The biofilm matrix: an immobilized but dynamic microbial environment. *Trends Microbiol.* 9, 222–227
22 Flemming, H.C. and Wingender, J. (2001) Relevance of microbial

extracellular polymeric substances (EPSs) – Part I: structural and ecological aspects. *Water Sci. Technol.* 43, 1–8
23 Paster, B.J. *et al.* (2001) Bacterial diversity in human subgingival plaque. *J. Bacteriol.* 183, 3770–3783
24 Kroes, I. *et al.* (1999) Bacterial diversity within the human subgingival crevice. *Proc. Natl. Acad. Sci. U. S. A.* 96, 14547–14552
25 Nyvad, B. and Kilian, M. (1990) Comparison of the initial streptococcal microflora on dental enamel in caries-active and in caries-inactive individuals. *Caries Res.* 24, 267–272
26 Listgarten, M.A. *et al.* (1973) Ultrastructure of the attachment device between coccal and filamentous microorganisms in "corn cob" formations of dental plaque. *Arch. Oral Biol.* 18, 651–656
27 Kolenbrander, P.E. *et al.* (2000) Coaggregation and coadhesion in oral biofilms. In *SGM Symposium Volume 59: Community Structure and Cooperation in Biofilms* (Allison, D.G. *et al.*, eds), pp. 65–85, Cambridge University Press
28 Lamont, R.J. *et al.* (2002) Role of the *Streptococcus gordonii* SspB protein in the development of *Porphyromonas gingivalis* biofilms on streptococcal substrates. *Microbiology* 148, 1627–1636
29 Bradshaw, D.J. *et al.* (1998) Role of *Fusobacterium nucleatum* and coaggregation in anaerobe survival in planktonic and biofilm oral microbial communities during aeration. *Infect. Immun.* 66, 4729–4732
30 Diaz, P.I. *et al.* (2002) *Fusobacterium nucleatum* supports the growth of *Porphyromonas gingivalis* in oxygenated and carbon-dioxide-depleted environments. *Microbiology* 148, 467–472
31 Buswell, C.M. *et al.* (1997) Coaggregation amongst aquatic biofilm bacteria. *J. Appl. Microbiol.* 83, 477–484
32 Rickard, A.H. *et al.* (1999) Coaggregation amongst aquatic and oral bacteria is mediated by lectin-saccharide interactions. In *Biofilms: The Good, The Bad and The Ugly* (Wimpenny, J. *et al.*, eds), pp. 343–354, Bioline Press
33 Rickard, A.H. *et al.* (2000) Coaggregation between aquatic bacteria is mediated by specific-growth-phase-dependent lectin-saccharide interactions. *Appl. Environ. Microbiol.* 66, 431–434
34 Rickard, A.H. *et al.* (2002) Phylogenetic relationships and coaggregation ability of freshwater biofilm bacteria. *Appl. Environ. Microbiol.* 68, 3644–3650
35 Oliveby, A. *et al.* (1989) Influence of flow rate, pH and plasma fluoride concentrations on fluoride concentration in human parotid saliva. *Arch. Oral Biol.* 34, 191–194
36 Reid, G. *et al.* (1988) Lactobacillus inhibitor production against *Escherichia coli* and coaggregation ability with uropathogens. *Can. J. Microbiol.* 34, 344–351
37 Kmet, V. and Lucchini, F. (1997) Aggregation-promoting factor in human vaginal *Lactobacillus* strains. *FEMS Immunol. Med. Microbiol.* 19, 111–114
38 Drago, L. *et al.* (1997) Inhibition of *in vitro* growth of enteropathogens by new *Lactobacillus* isolates of human intestinal origin. *FEMS Microbiol. Lett.* 153, 455–463
39 Kmet, V. *et al.* (1995) Aggregation-promoting factor in pig intestinal *Lactobacillus* strains. *Lett. Appl. Microbiol.* 21, 351–353
40 Vandevoorde, L. *et al.* (1992) Prevalence of coaggregation reactions among chicken lactobacilli. *J. Appl. Bacteriol.* 72, 214–219
41 Clemans, D.L. *et al.* (1999) Insertional inactivation of genes responsible for the D-alanylation of lipoteichoic acid in *Streptococcus gordonii* DL1 (Challis) affects intrageneric coaggregations. *Infect. Immun.* 67, 2464–2474
42 Takahashi, Y. *et al.* (2002) Identification and characterization of *hsa*, the gene encoding the sialic acid-binding adhesin of *Streptococcus gordonii* DL1. *Infect. Immun.* 70, 1209–1218
43 Takahashi, Y. *et al.* (1997) A specific cell surface antigen of *Streptococcus gordonii* is associated with bacterial hemagglutination and adhesion to α2-3-linked sialic acid-containing receptors. *Infect. Immun.* 65, 5042–5051
44 McNab, R. *et al.* (1999) Cell wall-anchored CshA polypeptide (259 kilodaltons) in *Streptococcus gordonii* forms surface fibrils that confer hydrophobic and adhesive properties. *J. Bacteriol.* 181, 3087–3095
45 McNab, R. *et al.* (1996) Cell surface polypeptide CshA mediates binding of *Streptococcus gordonii* to other oral bacteria and to immobilized fibronectin. *Infect. Immun.* 64, 4204–4210
46 Demuth, D.R. *et al.* (1996) Tandem genes encode cell-surface polypeptides SspA and SspB which mediate adhesion of the oral bacterium *Streptococcus gordonii* to human and bacterial receptors. *Mol. Microbiol.* 20, 403–413
47 Egland, P.G. *et al.* (2001) Identification of independent *Streptococcus gordonii* SspA and SspB functions in coaggregation with *Actinomyces naeslundii*. *Infect. Immun.* 69, 7512–7516
48 Love, R.M. *et al.* (2000) Coinvasion of dentinal tubules by *Porphyromonas gingivalis* and *Streptococcus gordonii* depends upon binding specificity of streptococcal antigen I/II adhesin. *Infect. Immun.* 68, 1359–1365
49 Demuth, D.R. and Irvine, D.C. (2002) Structural and functional variation within the alanine-rich repetitive domain of streptococcal antigen I/II. *Infect. Immun.* 70, 6389–6398
50 Du, L.D. and Kolenbrander, P.E. (2000) Identification of saliva-regulated genes of *Streptococcus gordonii* DL1 by differential display using random arbitrarily primed PCR. *Infect. Immun.* 68, 4834–4837
51 Jenkinson, H.F. and Lamont, R.J. (1997) Streptococcal adhesion and colonisation. *Crit. Rev. Oral Biol. Med.* 8, 175–200
52 Sandberg, A.L. *et al.* (1995) Putative glycoprotein and glycolipid polymorphonuclear leukocyte receptors for the *Actinomyces naeslundii* WVU45 fimbrial lectin. *Infect. Immun.* 63, 2625–2631
53 Klier, C.M. *et al.* (1998) *Actinomyces serovar* WVA963 coaggregation-defective mutant strain PK2407 secretes lactose-sensitive adhesin that binds to coaggregation partner *Streptococcus oralis* 34. *Oral Microbiol. Immunol.* 13, 337–340
54 Handley, P.S. *et al.* (1999) Adhesive surface structures on oral bacteria. *Dental Plaque Revisited: Oral Biofilms in Health and Disease* (Newman, H.N., Wilson, M., *et al.* eds), pp. 145–170, BioLine Press
55 Busscher, H.J. *et al.* (1992) On the relative importance of specific and non-specific approaches to oral microbial adhesion. *FEMS Microbiol. Rev.* 8, 199–209
56 Shaniztki, B. *et al.* (1998) Characterization of a novel N-acetylneuraminic acid-specific *Fusobacterium nucleatum* PK1594 adhesin. *Oral Microbiol. Immunol.* 13, 47–50
57 Moeck, G.S. and Coulton, J.W. (1998) TonB-dependent iron acquisition: mechanisms of siderophore-mediated active transport. *Mol. Microbiol.* 28, 675–681
58 Tarr, P.I. *et al.* (2000) Iha: a novel *Escherichia coli* O157:H7 adherence-conferring molecule encoded on a recently acquired chromosomal island of conserved structure. *Infect. Immun.* 68, 1400–1407
59 Whittaker, C.J. *et al.* (1996) Mechanisms of adhesion by oral bacteria. *Annu. Rev. Microbiol.* 50, 513–552
60 Cisar, J.O. *et al.* (1997) Structural and antigenic types of cell wall polysaccharides from viridans group streptococci with receptors for oral actinomyces and streptococcal lectins. *Infect. Immun.* 65, 5035–5041
61 Palmer, R.J. *et al.* (2001) Mutualism versus independence: strategies of mixed-species oral biofilms *in vitro* using saliva as the sole nutrient source. *Infect. Immun.* 69, 5794–5804
62 Bradshaw, D.J. *et al.* (1994) Metabolic cooperation in oral microbial communities during growth on mucin. *Microbiology* 140, 3407–3412
63 Caldwell, D.E. (2002) The calculative nature of microbial biofilms and bioaggregates. *Int. Microbiol.* 5, 107–116