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Caries Ecology Revisited: Microbial Dynamics and the Caries Process

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Key Words

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Abstract

In this essay we propose an extension of the caries ecological hypothesis to explain the relation between dynamic changes in the phenotypic/genotypic properties of plaque bacteria and the demineralization/remineralization balance of the caries process. Dental plaque represents a microbial ecosystem in which non-mutans bacteria (mainly non-mutans streptococci and *Actinomyces*) are the key microorganisms responsible for maintaining dynamic stability on the tooth surface (dynamic stability stage). Microbial acid adaptation and subsequent acid selection of 'low-pH' non-mutans bacteria play a critical role for destabilizing the homeostasis of the plaque by facilitating a shift of the demineralization/remineralization balance from 'net mineral gain' to 'net mineral loss' (acidogenic stage). Once the acidic environment has been established, mutans streptococci and other aciduric bacteria may increase and promote lesion development by sustaining an environment characterized by 'net mineral loss' (aciduric stage). Hence, high proportions of mutans streptococci and/or other aciduric bacteria may be considered biomarkers of sites of particularly rapid caries progression. This cascade of events may change the surface texture of caries lesions from smooth to rough (enamel) or hard to soft (dentin). These clinical surface features can be reversed at any stage of lesion development provided that the acidogenic/aciduric properties of the biofilm are resolved. From an ecological point of view it is therefore not only important to describe which bacteria are involved in caries, but also to know what the bacteria are doing.

The dental biofilm supports a 'micro-ecosystem' of bacteria that exhibit a variety of physiological characteristics. In particular, the production of acid resulting from sugar metabolism by these bacteria and the subsequent decrease in environmental pH is responsible for demineralization of the tooth surface and formation of dental caries [for review, see Marsh and Nyvad, 2008].

Much research has suggested that mutans streptococci (MS) are the major pathogens of human dental caries. This is because, first, MS are frequently isolated from cavitated caries lesions; second, MS induce caries formation in animals when fed a sucrose-rich diet; third, MS are highly acidogenic and aciduric [Hamada and Slade, 1980; Loesche, 1986], and fourth, MS are able to produce water-insoluble glucan, which promotes bacterial adhesion to the tooth surface and to other bacteria [Hamada and Slade, 1980]. A systematic literature review by Tanzer et al. [2001] confirms a central role of the MS in the initiation of dental caries on enamel and root surfaces.

However, some recent studies indicate that the relationship between MS and caries is not absolute: high proportions of MS may persist on tooth surfaces without le-

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sion development, and caries can develop in the absence of these species [Nyvad, 1993; Bowden, 1997; Aas et al., 2008]. Under such circumstances, it is suggested that acidogenic and aciduric bacteria other than MS, including 'low-pH' non-MS and *Actinomyces* [van Houte et al., 1994, 1996; Sansone et al., 1993] are responsible for the initiation of caries. Recent molecular analyses have strengthened this concept by showing that the microflora associated with white spot lesions is more diverse than hitherto appreciated and that novel phylotypes and species including *A. gernesceriae, A. naeslundii,* and *A. israelii* as well as a broad range of non-MS and *Veillonela* spp. may also play a role [Becker et al., 2002; Aas et al., 2008]. Since all the bacteria that have been associated with caries belong to the normal microflora of the oral cavity, dental caries has been described an endogenous infection [Fejerskov and Nyvad, 2003]. Endogenous infections may occur when members of the resident flora obtain a selective advantage over other species whereby the homeostatic balance of the biofilm is disturbed [Marsh and Martin, 1999]. Therefore, an ecological hypothesis is attractive [Marsh, 1994].

Concurrently with these changes in the interpretation of the microbial etiology of caries, novel concepts have evolved around the caries process itself. Thus, there is a growing awareness that caries lesions can be managed by non-operative interventions [Fejerskov, 1997; Fejerskov et al., 2008]. Moreover, it has been demonstrated that the effect of such interventions is reflected in the clinical appearance and activity of the lesions [Nyvad and Fejerskov, 1997; Nyvad et al., 2003, 2005; Thylstrup et al., 1994].

So far, these clinical and microbiological advances have not been integrated into a comprehensive concept that may broaden our understanding of caries. Given these circumstances, the aim of this paper is to revisit the 'ecological plaque hypothesis' pioneered by Carlsson [1986] and Marsh [1994, 2003] and to clarify the relationship between the ecological succession of bacteria in dental plaque and the caries process.

Recent Concepts of the Caries Process

Dental caries has been described as a chronic disease that progresses slowly in most individuals. The disease is seldom self-limiting and, in the absence of treatment, caries progresses until the tooth is destroyed. The localized destruction of the hard tissues, often referred to as the lesion, is the sign or symptom of the disease [Fejerskov et al., 2008]. Lesion progression is often depicted on a linear scale ranging from initial loss of mineral at the ultrastructural level to total destruction of the tooth. In reality, however, caries lesion development is a highly dynamic series of processes with alternating periods of progression and arrest/regression [Backer-Dirks, 1966; Nyvad et al., 2003]. Lesion progression may be arrested at any stage of lesion development, even at the stage of frank cavitation [Lo et al., 1998], provided the local environmental conditions, e.g. biofilm control and topical fluoride exposure, are favorable [Nyvad and Fejerskov, 1997]. Hence, the clinical stages of caries represent nothing but historical signs of past caries experience. What may be perceived clinically as an 'incipient' or 'early' lesion may in reality turn out to be an 'aged' established lesion that has been present in the oral cavity for months or years. Likewise, carious cavities may have experienced major differences in their history in the oral cavity.

Changes in the progression rate of caries are associated with alterations of the surface features of the lesions, active non-cavitated enamel lesions being dull and rough and inactive non-cavitated enamel lesions being shiny and smooth [for review, see Thylstrup et al., 1994]. These clinical distinctions have been shown to provide a reliable and valid classification of caries lesion activity [Nyvad et al., 1999, 2003]. Furthermore, such classification has offered novel information about caries lesion transition patterns [Baelum et al., 2003; Lima et al., 2008] and served as a useful basis of selecting high-risk patients [Hausen et al., 2007] in randomized clinical trials. Therefore, when trying to understand the clinical dynamics of caries, assessment of the surface texture of lesions may be a more sensitive parameter than merely assessing the stage of severity of a lesion as revealed by the presence or absence of a cavity.

From a biochemical point of view, the caries process is much more complex. Metabolic processes are constantly taking place in the dental plaque as a result of microbial activity, and this is reflected by continuous, rapid fluctuations in plaque pH, both when the plaque is starved and fed [Newman et al., 1979]. Hence, any clinically sound or carious tooth surface that is covered by an undisturbed plaque may experience minute mineral losses and mineral gains depending on the metabolic status of the microflora. The key point is that only when the cumulative result of the de- and remineralization processes produces a net mineral loss over time may a caries lesion develop or progress [Manji et al., 1991]. Such situations are likely to occur when there is a drift of pH in the biofilm, e.g. as a consequence of increased carbohydrate availability or reduced salivary clearance. By contrast,

when the integrated de- and remineralization processes result in a net mineral gain over time, this may lead to deposition of minerals in the tooth surface and arrest of lesion development. This explains why the caries process has been regarded as a ubiquitous and natural phenomenon [Manji et al., 1991]. Because of the constantly metabolically active biofilm, these processes cannot be prevented, but they can be controlled to the extent that caries does not appear clinically [Fejerskov, 1997; Kidd and Fejerskov, 2004]. This new microdynamic concept of caries suggests that an updated explanation of the microbial ecology of caries must take into consideration that the caries activity may change over time in response to pH drifts in the biofilm.

Microbial Characteristics and the Caries Process

Distribution of MS and Non-Mutans Bacteria in Supragingival Dental Biofilm at Clinically Healthy Sites and in Carious Lesions

In situ studies have shown that the initial colonizers of newly cleaned tooth surfaces constitute a highly selected part of the oral microflora, mainly *S. sanguinis*, *S. oralis* and *S. mitis* 1 [Nyvad and Kilian, 1987]. Together, these three streptococcal species may account for 95% of the streptococci and 56% of the total initial microflora [Li et al., 2004; Nyvad and Kilian, 1987]. Surprisingly, MS comprise only 2% or less of the initial streptococcal population, irrespective of the caries activity of the individual [Nyvad and Kilian, 1990a]. These observations imply that the vast majority of the early colonizers on teeth belong to the 'mitis group'. These bacteria as well as other viridans group streptococci, except for the MS, are often referred to as the non-MS, which are genetically distinguished from the MS that belong to the 'mutans group' [Kawamura et al., 1995]. As the microflora ages it shifts from *Streptococcus*-dominant to *Actinomyces*-dominant [Syed and Loesche, 1978; van Palenstein Helderman, 1981]. The predominant species in mature smooth surface plaque belong to *Actinomyces* and *Streptococcus*, most of which are non-MS [Ximénez-Fyvie et al., 2000]. MS are found in very low numbers [Bowden et al., 1975].

The proportion of MS in plaque covering white spot lesions in enamel is often higher than at clinically healthy sites, although still rather low, ranging between 0.001 and 10% [van Houte et al., 1991b]. Meanwhile, non-MS and *Actinomyces* still remain major bacterial groups in enamel lesions. In fact, it has been shown that in the absence of MS and lactobacilli, the initial dissolution of enamel can be induced by members of the early microflora, exclusively [Boyar et al., 1989].

In cavitated lesions in dentine, including rampant caries, MS constitute about 30% of the total flora [Boue et al., 1987; Loesche et al., 1984; Milnes and Bowden, 1985], indicating that these species are associated with progressive stages of caries. By contrast, MS are encountered less frequently at the advancing front of dentin caries where lactobacilli, prevotellae and *Bifidobacterium* are more prevalent [Aas et al., 2008; Becker et al., 2002; Chhour et al., 2005; Edwardsson, 1974; Munson et al., 2004].

Non-MS as Generalists and MS as Specialists: How Non-MS Can Become Dominant in Supragingival Plaque

Most non-MS have adhesins [Gibbons, 1989; Kolenbrander, 2000] which adhere to proteins and sugar chains of acquired pellicles coating the tooth surface. This seems to be one of the reasons for the dominance of non-MS at the initial stage of plaque formation. In addition, most oral streptococci produce extracellular polysaccharides such as glucans and fructans [Banas and Vickerman, 2003; Whiley and Beighton, 1998]. Polysaccharides can fill the gaps between bacteria and form the matrix of plaque, and accelerate plaque formation.

On the other hand, MS do not attach efficiently to the acquired pellicle [Nyvad and Kilian, 1990b], although they have adhesins such as the antigen I/II. Instead, these bacteria have been emphasized to produce water-insoluble glucans, which are adhesive and capable of accelerating bacterial accumulation. However, it should be noted that glucans only act as additional factors in plaque formation, and that not only MS but also non-MS can produce glucans [Banas and Vickerman, 2003; Vocca-Smith et al., 2000].

Both non-MS and MS metabolize various sugars and produce acids. When sugar is supplied in excess, streptococci can store the extra sugars as intracellular polysaccharides (IPS) [Hamilton, 1976; Takahashi et al., 1991; van Houte et al., 1970], and they can utilize the IPS as an energy source to produce acids when sugar is limited as occurs between meals. The final pH values of non-MS when grown with sugars are heterogeneous, ranging from 4 to 5.2, whereas those of MS are more homogeneous, being around 4 [Hardie, 1986]. In general, on the basis of final pH values, MS are more acidogenic and aciduric than non-MS. It should be realized, however, that the final pH values of non-MS can be much lower than pH 5.5 [Hardie, 1986], the 'critical' pH for the demineralization of enamel.

Non-MS have a variety of extracellular glycosidases [Whiley and Beighton, 1998] that can liberate sugars and amino-sugars from glycoproteins such as the mucin contained in saliva. Furthermore, all non-MS grow on amino-sugars [Byers et al., 1996; Whiley and Beighton, 1998]. This is an advantage for non-MS in the oral cavity, where salivary glycoproteins are always available.

In addition, most non-MS can utilize arginine or arginine-containing peptides available in saliva through the arginine deiminase system, which degrades the arginine molecule to ammonia and carbon dioxide with production of ATP. Overall, this metabolic pathway produces alkali and neutralizes the intracellular and the environmental pH [Burne and Marquis, 2000]. Arginine deiminase system is helpful for non-MS not only to utilize arginine as an energy source but also to survive under the acidic conditions in the oral cavity. However, most MS do not have these metabolic features.

In summary, non-MS have diverse physiological activities, suggesting that they are generalists, versatile enough to adapt to various conditions in supragingival biofilm, and this could be the reason why they are the dominant streptococci in supragingival biofilm. On the other hand, MS are aciduric specialists in sugar metabolism and acid production, which make them less competitive in clinically sound supragingival environments.

Acidogenicity and Acidurance of Non-MS: Key Factors in the Caries Process

It is clear that an ability both to produce acid (acidogenicity) and to tolerate a low-pH environment (aciduranc) is a crucial feature for microorganisms responsible for caries. Sansone et al. [1993] compared the microbial composition of dental plaque at clinically healthy sites and white spot lesions and found that non-MS were dominant at both sites while MS were present at low and similar levels at both sites. However, the ability of plaque to reduce pH in vitro was significantly greater at white spot lesions (pH 4.13) than at clinically healthy sites (pH 4.29). These results suggest that MS are neither a unique causative agent for white spot lesions, nor a main determinant of the acidogenicity of plaque.

In order to evaluate the acidogenicity of the non-MS, Sansone et al. [1993] further grew these bacteria in liquid culture media supplemented with 1% glucose and measured the final pH of the culture media. In agreement with Svensäter et al. [2003], they found that non-MS are heterogeneous for acidogenicity: some strains lowered the culture pH to below 4.4, a pH comparable to that produced by MS, whereas for other strains the pH-lowering capacity was less pronounced. In addition, the proportion of acidogenic non-MS was higher at white spot lesions than at clinically healthy sites. The acidogenic non-MS, identified as *S. gordonii*, *S. oralis*, *S. mitis* and *S. anginosus*, were subsequently designated as 'low-pH' non-MS [van Houte, 1994], and it was suggested that the pH-lowering capacity of plaque may be related to the proportion of 'low-pH' non-MS [van Houte et al., 1991a, 1991b]. Later observations by van Ruyven et al. [2000] have supported this notion.

The question still remains: Which of the non-MS are to be considered 'low-pH' non-MS? Alam et al. [2000] obtained two groups of *S. oralis* – one comprised the total *S. oralis* population in dental plaque, whereas the other comprised aciduric strains that were able to grow at pH 5.2. They then differentiated these strains into 15 genotypes on the basis of genetic similarity. The distributions of genotypes were different between the total bacterial group and the aciduric group; only some genotypes of *S. oralis* seemed to be aciduric and to form an aciduric subpopulation. These results are in line with another study showing that strains of non-MS differ distinctly by their rate of acid production at decreasing pH; in particular some strains within *S. mitis* 1, *S. oralis* and *S. gordonii* are capable of producing acids as rapidly as many *S. mutans* strains at pH 5.0 and 5.5 [de Soet et al., 2000]. Collectively, it is suggested that the group of 'low-pH' non-MS comprise a mosaic of acidogenic subpopulations of each species of non-MS.

Involvement of Actinomyces

Most of our knowledge about the role of *Actinomyces* in caries stems from studies of root surface caries. However, there is no evidence that *Actinomyces* spp. have a specific role in root caries. In fact, a review of the literature has concluded that the basic patterns of microbial colonization are identical on enamel and root surfaces, structurally as well as microbiologically [Nyvad, 1993].

As with enamel caries, MS comprise only a small proportion of the microflora of root surface caries lesions. van Houte et al. [1996] reported that non-MS and *Actinomyces* spp. were dominant in dental plaque covering root surface caries and that the isolated *Actinomyces* strains were heterogenous with respect to acidogenicity: strains isolated from root surface caries were more acidogenic than those from clinically healthy root surfaces. Meanwhile, Brailsford et al. [2001] observed that, in subjects with root surface caries, aciduric bacteria able to grow at pH 4.8 comprised 21.6% of the total microflora in root surface caries lesions (lactobacilli and *Actinomyces* were

dominant), whereas aciduric bacteria comprised 10.7% in clinically sound root surfaces (*Actinomyces* dominant). However, in subjects without root surface caries, aciduric bacteria comprised only 1.4% of total microflora in clinically sound root surfaces. These findings indicate an association between acidogenic/aciduric *Actinomyces*, i.e. 'low-pH' *Actinomyces* and root surface caries.

Actinomyces are as versatile to adapt to the dental biofilm environment as are the non-MS; they have adhesin-mediated adhesion to tooth surfaces, produce acids from various sugars, and synthesize intracellular and extracellular polysaccharides. In addition, *Actinomyces* have a unique glycolytic system [Takahashi et al., 1995] in which they utilize high-energy polyphosphate and pyrophosphate compounds for synthesis of hexokinase and phosphofructokinase, respectively, acting as phosphoryl donors instead of ATP. This means that *Actinomyces* are able to exploit a surplus ATP to synthesize polyphosphate as an energy reservoir, and salvage energy from pyrophosphate, a high-energy-phosphoryl-bond-containing byproduct from the metabolism of polymers such as nucleic acids and glycogens. In addition, *Actinomyces* are often ureolytic [Kleinberg, 2002; Yaling et al., 2006] and can utilize lactate as a carbon source for growth [Takahashi et al., 1996]. These diverse physiological characteristics of *Actinomyces* seem to be advantageous to survive and dominate in supragingival plaque [Takahashi and Yamada, 1999b].

Acid Adaptation and Acid Selection: Adaptive Changes in Acidurance and Acidogenicity and the Consequent Selection of 'Low-pH' Non-MS

Non-MS are not only genotypically heterogenous, but they are also able to change their physiological characteristics adaptively. Takahashi and Yamada [1999a] have shown that when these bacteria were exposed to an acidic environment, they increased their acidogenicity. These bacteria were grown first at pH 7.0 and afterwards at pH 5.5 for a short time: 30, 60 and 90 min. The bacteria were then harvested, washed and incubated with glucose, and the final pH values were measured as a marker of acidogenicity. Their acidogenicity or final pH values varied (pH 4.04–4.33), but after incubation at pH 5.5 for 60 min, all the bacteria increased their acidogenicity (pH 3.93–4.12).

Non-MS were also able to increase their acidurance adaptively [Takahashi and Yamada, 1999a]. Bacteria initially grown at pH 7.0 were killed by acid stress in a strain-dependent manner following exposure to pH 4.0 for 60 min (survival rate: 0.0009–71%), but after pre-acidification at pH 5.5 for 60 min, all the bacteria increased their acidurance (survival rate: 0.4–81%).

The biochemical mechanisms underlying the acid adaptation are considered to involve the following mechanism [Quivey et al., 2000]: (1) an increase in proton impermeability of the cell membrane; (2) induction of proton-translocating ATPase (H^+-ATPase) activity that expels proton from cells; (3) induction of the arginine deiminase system that produces alkali from arginine or arginine-containing peptides, and (4) induction of stress proteins that protect enzymes and nucleic acids from acid denaturation. In non-MS, the increase in activities of H^+-ATPase and arginine deiminase and expression of stress proteins (homologues of heat shock protein, Hsp60 and Hsp70) were observed following incubation at pH 5.5 [Takahashi and Yamada, 1999a].

In the oral cavity, acidification of the biofilm due to frequent sugar intake or poor salivary secretion can be a driving force to enhance the acidogenicity and aciduranceof the non-MS, resulting in establishment of a more acidic environment. Even if acid adaptation occurs, non-MS are still so heterogeneous with respect to acidurance that the population of more aciduric strains, i.e. 'low-pH' non-MS will increase selectively in this environment. This will cause a shift in the composition and acidogenic potential of the biofilm, which, provided the demineralization/remineralization balance is disturbed over an extended period of time, leads to dental caries. Similar microbial acid adaptation and acid selection processes may occur in *Actinomyces*.

Competition between Non-MS and MS

Transient Acidification. Although 'low-pH' non-MS can adaptively increase their acidurance and acidogenicity, and take over the position in supragingival plaque, MS are more competitive under severely acidic conditions. Following a rapid exposure to pH 4.0 for 60 min as often observed in dental plaque after a sugar exposure, *S. sanguinis* ATCC 10556, a strain of 'low-pH' non-MS, was able to survive. However, this bacterium temporarily lost the ability to grow, along with the inactivation of glycolytic enzymes, and did not start growing again until 90 min after the pH had returned to neutral [Takahashi et al., 1997]. By contrast, the growth of *S. mutans* NCTC 10449 at pH 4.0 was not influenced at all. In view of this observation, it is expected that the population of non-MS decreases gradually during frequent acidification, whereas the proportion of MS would increase.

Prolonged Acidification. Experiments using in vitro cultures of mixtures of oral bacterial species have clearly

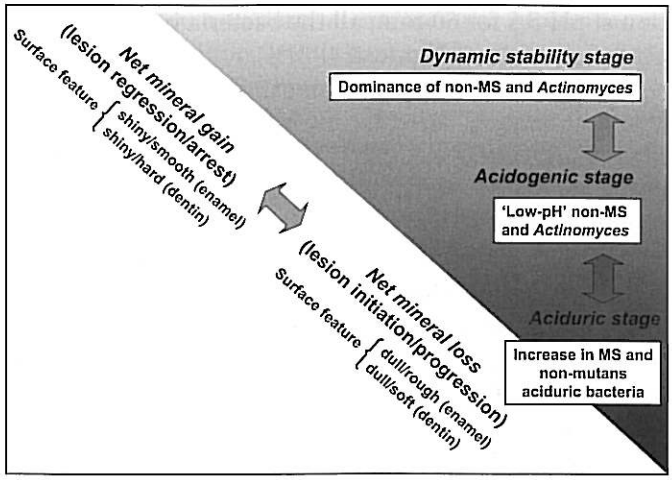

Fig. 1. An extended caries ecological hypothesis explaining the relationship between acidogenic and aciduric shifts in the composition of the dental biofilm and changes in the mineral balance of the dental hard tissues. Note that the cascade of ecological events in the biofilm is reversible and is reflected in the surface features of the dental hard tissues at any stage of lesion formation. MS = Mutans streptococci. For detailed explanation, see text.

shown that prolonged acidification is the driving force behind the emergence of MS in dental plaque. Bowden and Hamilton [1987] demonstrated that *S. sanguinis* (formerly *S. sanguis*) was dominant when the pH was kept at 7.0–6.0 in a mixed continuous culture, whereas when pH was shifted to 5.5, *S. mutans* overcame *S. sanguinis*, although *S. sanguinis* survived in the culture at pH 4.5. A similar phenomenon was observed by Bradshaw and Marsh [1998]. They established a continuous culture with 9 oral bacterial species and demonstrated that non-MS *(S. oralis* and *S. gordonii)* were dominant when the pH was kept at pH 7.0 during daily pulses of glucose for 10 days, whereas when the pH was allowed to fall to a preset value of 5.0, MS as well as lactobacilli became dominant; non-MS were excluded from the consortium when pH was allowed to fall without control (final pH = 3.83). Similarly, Takahashi et al. [1997] showed that a strain of 'low-pH' non-MS (*S. sanguinis* ATCC 10556) was not able to grow at pH $\leq$ 4.2 in a complex liquid medium under anaerobic conditions, while *S. mutans* was still able to grow at pH 4.2. Given these observations, it is suggested that prolonged acidic conditions around pH 5.5 may cause the emergence of MS in the microbial flora and that more severe acidic conditions around pH 4 may exclude the non-MS. In the oral cavity, prolonged acidic conditions (pH $\leq$ 5.5) can occur in carious cavities [Dirksen et al., 1962; Hojo et al., 1994], where clearance of acids is hampered. This may be the reason why MS and particularly lactobacilli are frequently isolated from established carious cavities.

An Extended Caries Ecological Hypothesis

In the light of the foregoing we suggest an extended caries ecological hypothesis that explains the relationship between the composition of the dental plaque and the caries process (fig. 1). In this hypothesis, dental plaque is a dynamic microbial ecosystem in which non-mutans bacteria such as non-MS and *Actinomyces* are the key players for maintaining dynamic stability. These bacteria can produce acids from sugary foods and the resulting acids can demineralize the enamel. However, the temporary decreases in pH are easily returned to neutral level by homeostatic mechanisms in the plaque [Marsh and Martin, 1999]. This is a natural pH cycle, which occurs numerous times daily in supragingival plaque (dynamic stability stage).

However, when sugar is supplied frequently or salivary secretion is too scarce to neutralize the acids produced, the pH decreases in the plaque may enhance the acidogenicity and acidurance of the non-mutans bacteria adaptively. Under such conditions the population of the 'low-pH' non-MS and *Actinomyces* then increases via acid selection, leading to a microbial shift to a more acidogenic microflora. These changes in the phenotype and genotype of the microflora may shift the demineralization/remineralization balance from 'net mineral gain' to 'net mineral loss' and initiate lesion development (acidogenic stage). At this stage, lesion development could also be arrested with de-adaptation of the microflora, provided that the mineral balance is restored to a 'net mineral gain' by reduced environmental acidification.

If prolonged acidic environments prevail, lesion development ('net mineral loss') is likely to progress. In these environments, more aciduric bacteria such as MS and lactobacilli may replace the 'low-pH' non-mutans bacteria and further accelerate the caries process (aciduric stage). However, even at this highly aciduric stage, the mineral balance and composition of the microflora could possibly be reversed by modification of the acidic environment, e.g. as a result of sugar restriction [de Stoppelaar et al., 1970].

In this scenario, the microbial acid adaptation and the subsequent acid selection of 'low-pH' non-mutans bacteria play a crucial role in destabilizing the homeostasis of

the biofilm and facilitating lesion development. Moreover, once the acidic environment has been established, the proportion of aciduric bacteria such as MS and lactobacilli may increase and act as promoters of lesion progression by sustaining an environment characterized by 'net mineral loss'. Hence, high proportions of MS and/or other aciduric bacteria may be considered biomarkers of sites that undergo particularly rapid caries development [Bowden et al., 1976; Chhour et al., 2005; Macpherson et al., 1990; Nyvad and Kilian, 1990b]. We suggest that this cascade of events is associated with changes in the surface texture of the dental hard tissues from smooth to rough (enamel) or from hard to soft (dentin) [Nyvad et al., 1999, 2003].

Two decades ago, Carlsson [1986] presented a caries microbiological hypothesis by which he speculated that ecological changes in the oral flora were determined by competition for nutrients. Carlsson proposed that at low levels of sugars, the oral microflora would be dominated by bacteria with a high affinity for sugars (the 'gleaners'), whereas at consistently higher concentrations of sugars, bacteria with lower affinity for sugars, but with high growth rates, would be favored (the 'exploiters'). Under the latter condition the metabolic end products established acidic environments favoring an outgrowth of aciduric bacteria, the so-called 'pH-strategists' [Carlsson, 1986]. This concept was further developed as the ecological plaque hypothesis by Marsh [1994, 2003], who focused on the dynamic and reversible processes of de- and remineralization in the plaque by linking between sugar supply, pH change and microflora shift. We suggest that the ecological concept of caries should be extended and strengthened by including clinical manifestations of caries lesion processes, and by detailing the microbial acid adaptation and acid selection processes.

Clinical and Scientific Perspectives

The extended caries ecological hypothesis supports the 'mixed-bacteria ecological approach' proposed by Kleinberg [2002] that the proportion of acid- and base-producing bacteria is the core of caries activity. Clearly, the extended hypothesis undermines the view that dental caries is a classical infectious disease, and therefore that prevention and control of this condition by elimination of a specific group of microorganisms, such as the MS, through vaccination, gene therapy or antimicrobial treatment, is unwise. Rather environmental control of the microflora should be achieved by stimulating the non-mutans bacteria such as non-MS and *Actinomyces* by avoiding acidification of the dental biofilm.

Practical solutions to this strategy may include mechanical plaque control, reduction/substitution of the intake of sugary foods and/or application of pH-neutralizing techniques such as saliva stimulation. Even if the effect of such interventions on the composition of the microflora is sparsely documented, it has been shown that dietary modification may facilitate such changes. Hence, de Stoppelaar et al. [1970] observed a clear reduction in the proportion of MS at carious and filled sites at the expense of *S. sanguis* following a 3-week period of sucrose restriction. These changes were reversed when individuals resumed a normal diet containing sucrose. Conventional culture studies of young dental plaque in caries-inactive individuals have failed to reveal a consistent microbial response pattern to sucrose-regulated diets [Staat et al., 1975; Scheie et al., 1984]. In these studies, sucrose-related modulation of the microflora was found to depend on prior oral colonization by mutans streptococci, and these species were not entirely eliminated on a low-sucrose diet. It is interesting to speculate that differences in the propagation of MS might reflect differences in acid tolerance between clones of these species [Welin-Neilands and Svensäter, 2007]. Future studies describing the site-specific microbial shifts in response to sucrose should therefore focus on both the MS and the non-mutans bacteria, e.g. by applying molecular identification methods.

An important consequence of the extended hypothesis is that knowledge about the acidogenic and aciduric properties of bacteria, i.e. the phenotypic characteristics, and their regulatory mechanism may be a more relevant parameter than knowledge about their taxonomy. The phenotypes of most bacteria have already been well described in textbooks such as the Bergey's Manual of Systematic Bacteriology [Holt, 1984]. Nevertheless, such descriptions are not particularly helpful to explain the in vivo behaviors since bacterial phenotypic characteristics may change depending on the local environmental conditions. Therefore, from an ecological point of view it is not only important to describe which bacteria are involved in caries but also to know what the bacteria are doing [Takahashi, 2005].

Recently, van Ruyven et al. [2000] have detected non-mutans aciduric bacteria other than non-MS and *Actinomyces* from dental biofilms covering white spot lesions. They found that these bacteria consisted of various species including lactobacilli and *Bifidobacterium*. Interestingly, the samples differed with respect to dominance of

particular bacterial species, suggesting that any bacterial species can participate in the development of caries as long as they are aciduric and dominant [Bowden, 1984]. In this essay we have focused on the non-MS and the *Actinomyces* as the major non-mutans aciduric bacteria because detailed studies have been conducted for these bacteria. However, it would not be surprising if other non-mutans aciduric bacteria were found to be associated with dental caries. As stated above, it is not the genotype per se, but the phenotype in a certain environment, i.e. the acidogenic and aciduric potential of the bacteria, that is conducive to a microbial shift leading to caries.

According to the extended hypothesis, there is a firm association between the de- and remineralization balance of caries lesions and the overall composition of the microflora. In the in situ study of Nyvad and Kilian [1990b], root surface caries lesions experiencing the highest mineral loss, as assessed by quantitative microradiography, were dominated by uniform *Actinomyces* spp., or a combination of MS and *Lactobacillus* spp., whereas lesions experiencing a smaller mineral loss were associated with a more diverse microbiota including non-MS, MS, *Actinomyces*, lactobacilli, *Bifidobacterium* as well as lactate-metabolizing species (*Veillonella* spp.). Such differences in the pattern of the microflora in response to different lesion progression rates not only lend support to the suggested acidogenic and aciduric stages of bacterial succession in caries, but also conform with the concept that microbial diversity may exert a protective effect on the dynamic stability of the biofilm community, recently referred to as the 'insurance hypothesis' [Yachi and Loreau, 1999; Boles et al., 2004]. Therefore, in the future, if we truly wish to advance the ecological understanding of caries, it is important to describe the total microbiota of caries lesions by studying lesions with a known age and history in the oral cavity or, alternatively, employ clinical caries diagnostic methods that reflect the activity state of lesions [Nyvad et al., 1999, 2003].

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