**ED2 (02/10/2018):** Interações polimicrobianas em biofilmes orais.

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1. Quais alterações ocorrem na ecologia do biofilme dental quando ocorre a introdução frequente de sacarose na dieta do hospedeiro?

Commensals have a significant advantage over cariogenic pathogens when the host’s diet is not rich in fermentable carbohydrates, particularly sucrose.

Many commensal bacteria associated with oral health can adhere more avidly to saliva-coated tooth surfaces, can grow substantially better than S. mutans and many other aciduric species, and have multiple mechanisms to interfere with their establishment and growth.

However, the ‘microbial battlefield’ can change dramatically if sugar is supplied frequently, promoting EPS matrix synthesis, acid production, and the creation of localized acidic microenvironments, where S. mutans can work synergistically with other aciduric species and cause ecological changes to shape the biofilm community, structure, and metabolism conducive to caries development (Figure 3).

2. Explique as interações de competição e antagonismo que ocorrem entre bactérias comensais e *S. mutans* em biofilmes.

The inverse association between the abundance of commensals with mutans streptococci has been well documented, indicating that these organisms can control the growth of cariogenic bacteria.

1. The simplest mechanism involves the production of basic (alkaline) compounds that maintain pH values near neutrality, allowing commensals to outcompete S. mutans and acidtolerant organisms that are able to grow and dominate at lower pH conditions.

The two prevailing pathways for alkali production are urea metabolism by bacterial ureases, mainly S. salivarius, certain Actinomyces species, and a few oral haemophili [38]. **Urea** hydrolysis yields ammonia and CO2. While the CO2 can provide some modest buffer capacity, ammonia can rapidly equilibrate with a proton to yield NH4+, raising the pH and providing the bacteria with a source of nitrogen.

**Arginine** can be metabolized by numerous oral bacteria via various pathways. A dominant route for arginine catabolism by oral commensals is the arginine deiminase system (ADS), which metabolizes internalized arginine yielding ornithine, ammonia, CO2, and ATP.

**Urea and arginine** metabolism not only results in alkalinization of oral biofilms, preventing demineralization and promoting remineralization, it also provides bioenergetic advantages to the organisms that harbor ureases and the ADS. This can provide ecological advantages to commensals and deter the growth of caries pathogens, promoting the development of healthy oral biofilms.

b) In addition to adhesion and alkali generation, many commensals have more ‘active measures’ to interfere with the growth and biological processes of caries pathogens, and S. mutans in particular. The **production of H2O2** via pyruvate oxidase and other enzymes, and the **secretion of bacteriocins** and other antimicrobial compounds by commensals, are important ‘chemical weapons’ that are inhibitory to the growth of S. mutans [39]. Interestingly, S. mutans can ‘counter-attack’ by releasing mutacins (lantibiotic and non-lantibiotic peptide antibiotics), which are effective against a variety of commensals. However, certain commensal streptococci (e.g., S. gordonii) have the ability to interfere with intercellular signaling systems required for the production of mutacin [40]. In particular, a recent clinical isolate, designated as Streptococcus A12, produces a protease (similar to S. gordonii challisin) that breaks down competence-stimulating peptide (CSP), which activates mutacin production by S. mutans via a twocomponent signal transduction systems [41]. Alkali-generating or bacteriocin/toxin-producing bacteria within biofilms can influence interspecies interactions, local pH, and microenvironment dynamics [42].

c) The development of dental caries is also intertwined intimately with stress tolerance by caries pathogens. In addition to the aforementioned competitive interactions with commensals, coping with a large influx of carbohydrates in the diet by rapidly shifting metabolism can induce a variety of stress pathways. Perhaps, though, **the most significant stress under cariogenic conditions is acid**. The in situ measurements of plaque pH following a carbohydrate challenge reveal that the pH can drop within 2–3 min to values as extreme as 4.0 and below [43]. This is a true ‘acid shock’ that would be intolerable to most health-associated commensals as they cease growth below 6 or 5.5, and are rapidly killed at pH 4.0 [44,45]. By contrast, S. mutans, lactobacilli and other aciduric bacteria can cope with such suddenly dropped pH by using various adaptive strategies. A primary determinant of acid tolerance in S. mutans is the **proton-extruding F1F0-ATPase pump,** which is highly active, has a much lower optimal pH than the same enzyme in commensal streptococci, and is produced in greater quantity when the organisms are challenged with low pH [45]. However, acid tolerance is a complex trait with many factors contributing to constitutional and adaptive acid resistance, including restructuring the bacterial membrane structure–composition. These mechanisms have been reviewed elsewhere [43,46].

3. Explique as interações de cooperação e sinergismo que ocorrem em biofilmes cariogênicos.

If the environmental acidic stress persists, other acidogenic and aciduric bacteria such as nonmutans streptococci, actinomyces, lactobacilli, bifidobacteria, and Scardovia species are detected, which can synergize to enhance acidification of the biofilm milieu as detailed in excellent in-depth review articles [8,11,47]. Cariogenic biofilms are acidic, hypoxic, but rich in carbohydrates, which creates an ideal microenvironment for opportunistic organisms like lactobacilli to grow and accelerate caries progression.

a) Concomitantly, EPS production by S. mutans Gtfs is induced under acidic pH. Lactobacillus casei, frequently isolated from the cariogenic plaque with S. mutans, is known for its high capacity to produce and tolerate acid, although it has poor ability to colonize teeth. The **presence of S. mutans and sugar exposure promotes colonization by certain lactobacilli [48] through Gtf binding and a glucan-mediated adhesion mechanism**, increasing accumulation of both organisms within biofilms.

b) Further, some Lactobacillus reuteri strains possess two different enzymes: 4,6-a-glucanotransferase that uses starches to synthesize a-glucan-type polysaccharides, and a typical glucansucrase that synthesizes a-glucans from sucrose [49]. In addition, the presence of amylase bound on bacterial (e.g., S. gordonii, Streptococcus parasanguinis) and tooth surfaces produce a variety of starch hydrolysates in situ that can be metabolized into acids and incorporated into glucans synthesized by Gtfs forming hybrid polymers [50–52]. **Additional EPS produced by other oral bacteria may also contribute to biofilm matrix assembly, even if the proportion of S. mutans is declining as the biofilm matures and the caries lesion worsens**. Thus, we speculate that co-colonization with a diverse group of EPS-producing or EPS-modifying organisms can expand the substrate repertoire that allows oral bacteria to build complex EPS matrix with properties that can enhance caries development that glucans alone cannot achieve.

c) The creation of acidic microenvironments benefits not only acid producers and strongly acidtolerant species [53] **but also those organisms that use lactate as a carbon source**, whereas other species that are acid sensitive, or that cannot metabolize the acids present in their surroundings, may perish. Veillonella, an obligate anaerobic Gram-negative bacterium, is considered a bridge organism in the oral biofilm [54]. Veillonella does not utilize carbohydrates as a source of energy; instead, it utilizes lactate. Such nutritional preference renders this organism dependent on streptococci that produce copious amounts of lactic acid. In fact, Veillonella often coaggregates with oral streptococci and, not surprisingly, it is often identified as a signature organism in caries-active subjects [55]. It has been long perceived that the presence of this bacterium in plaque neutralizes the acidic pH of the oral biofilm, thereby preventing enamel demineralization. However, evidence from microbiome studies demonstrates that both Veillonella and S. mutans are highly associated with caries lesions [56]. Acetate produced from Veillonella lactate catabolism can be damaging to enamel, while Veillonella promotes S. mutans growth despite the presence of antagonistic S. gordonii in vitro [57].

Conversely, some bacteria found in cariogenic biofilms do not fit the classical profile of being acidogenic–aciduric, such as Prevotella and Atopobium [58]. Whether they are just bystanders or play an active role in caries pathogenesis remains to be elucidated.

d) Intriguingly, results from several clinical studies reveal that the fungus C. albicans is frequently detected in higher numbers in plaque-biofilms from toddlers with ECC, as reviewed in [19]. In the mouth, C. albicans is known to form mixed microbial communities on soft-tissue and prosthetic surfaces, causing mucosal infections. However, C. albicans can coadhere with S. mutans and colonize tooth surfaces in the presence of sucrose [59,60]. Specifically, this cross-kingdom interaction appears to be largely mediated by S. mutans-derived Gtfs that bind avidly onto the Candida surface and produce large amounts of glucans on the fungal surface, boosting the ability of both microbes to form biofilm together while increasing the amount of EPS-matrix. Once together within biofilms, these organisms can cooperate by providing substrates/metabolites and growth-stimulating factors [61,62], while enhancing Gtfs production [60,62,63]. Using a rodent model, a synergistic enhancement of biofilm virulence was observed when S. mutans was coinfected with C. albicans and exposed to a sucrose-rich diet, leading to rampant caries on teeth similar to those found clinically in ECC [60]. Further investigation into how other metabolic pathways contribute to the symbiotic interactions and matrix production may offer additional insights into the disease process.

Dental caries is a highly dynamic pathological process where the host’s diet fuels the assembly of virulent biofilms by promoting EPS matrix assembly and polymicrobial interactions that cause profound changes in the local environment. EPS-producing pathogens such as S. mutans appear to battle with commensal organisms and, when conditions are conducive, build-up a ‘habitat’ to work synergistically with other aciduric species to acidify the biofilm milieu and cause demineralization of the tooth surface. Yet, it is indeed difficult to fully assess the absolute contribution of many species to the caries process because of the spatial/chemical heterogeneity, the continually changing microenvironments, and the fact that organisms could be beneficial or detrimental depending on the conditions and degree to which a lesion had progressed (e.g., proteolytic bacteria could accelerate the caries process in advanced lesions where dentin is exposed). Nevertheless, this evolving view of ecological battles and polymicrobial synergies within a heterogeneous yet structured environment has direct implications in understanding the pathogenic mechanisms of dental caries and in developing effective therapeutics (Box 3).