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Chapter

Virulence Factors of *Streptococcus mutans* Related to Dental Caries

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Abstract

Streptococcus mutans (*S. mutans*) has important virulence factors related to the etiology and pathogenesis of dental caries. Through mechanism of adhesion to a solid surface, *S. mutans* is able to colonize the oral cavity and form dental biofilm, which is composed of a matrix of exopolysaccharides that affect the physical and biochemical structure of the biofilm. The additional properties that allow *S. mutans* to colonize the oral cavity include the generation of acid (acidogenicity), the interaction with other bacterial species colonizing this ecosystem and the ability to survive in an acidic environment. In addition, these microorganisms can tolerate the acidic environment (aciduricity) due, at least in part, to the ATPases located in the plasma membrane responsible for the extrusion of the cytoplasmic proton. Changes in environmental pH can modify the fatty acid and proteins composition of the plasma membrane of *S. mutans*, inducing the alteration of its permeability. The different dental surfaces or biofilms can affect the lipid composition of the bacterial membrane by altering the virulence factors of *S. mutans*, such as acid survival and ATPase activity.

Keywords: *Streptococcus mutans*, virulence factors, biofilms, membrane fatty acid, ATPases

1. Introduction

In the oral cavity, on the teeth surface, organic and inorganic substances coming from the saliva, the gingivocrevicular fluid and microorganisms accumulate. These deposits are called integuments acquired from the enamel and are related to the appearance of the most prevalent diseases of the oral cavity, such as dental caries and periodontal disease. Therefore, it is necessary to understand the chemical composition and odontopathogenic power of these acquired integuments, particularly the role played by *S. mutans* in the oral cavity, since this microorganism is considered one of the most common agents in the development of dental caries in humans [1]. One of the characteristics that contributes to the pathogenicity of this microorganism is its ability to metabolize fermentable sugars (sucrose) and consequently the production of various organic acids, which lower the pH of the oral medium. Over time, the low pH of the medium produces the demineralization of the dental element, hence the development of dental caries. *S. mutans* has diverse virulence factors such as acidogenesis, acid survival and proton ATPase activity (related to the lipid and protein components of its membrane), among others. This enables it to survive in low pH environments and provides it survival advantages over other common bacteria in the oral ecosystem [2].

2. Acquired integuments from enamel

The acquired structures of enamel include following structures:

- Acquired enamel pellicle (AEP)
- Dental biofilm (DB)
- Dental calculations or dental tartar

In this chapter, the acquired enamel pellicle and dental biofilm will be specifically explained.

3. Acquired enamel pellicle

Saliva is almost never in direct contact with the surface of the tooth, since there is a separation between them provided by a heterogeneous coating: the AEP.

It is an acellular organic 0.1–10 μm thick pellicle, totally free of microorganisms. The AEP forms on the surfaces of the teeth by selective adsorption of proteins, peptides and other molecules present in saliva and gingivocrevicular fluid, as well as others from bacteria and desquamated epithelial cells [3, 4].

3.1 Chemical composition of the acquired enamel pellicle

AEP has a very complex and heterogeneous chemical composition. The chemical composition of this integument plays an important role in the formation of dental biofilm, since some of its biomolecules act as receptors that enable the adherence of oral microorganisms.

The following proteins constitute the AEP: proline-rich acidic proteins (PRPs), statins, high molecular weight mucins, cystatins, histatins, amylase, lactoferrin, lactoperoxidase and secretory immunoglobulin A (IgAs). Also to a lesser extent, serum albumin, carbonic anhydrase, IgG, IgM, various complement fractions and enzymes such as glycosyltransferases of microbial origin. The existence of peptides, a product of the partial proteolysis of salivary proteins, has also been demonstrated.

Regarding the carbohydrates that compose it, there are mainly glucose, galactose, hexuronic acid and amino sugars such as glucosamine and galactosamine. Other glucides, such as sialic acid, are present in the AEP. Carbohydrates are involved in the process of colonization of the pellicle, because many of the adhesins found on the surface of microorganisms bind to carbohydrates located in the AEP.

Other representative biomolecules in this integument are lipids such as glycolipids, acylglycerols, cholesterol, phospholipids and free fatty acids.

The hydrophobic character of the lipids averts the demineralization of the enamel by preventing the diffusion of the acids originated by the metabolism of the microorganisms present in the biofilm, together with its capacity to modulate the colonization of microorganisms of the AEP.

On the other hand, the chemical composition of the AEP varies depending on the type of surface on which it was formed (different faces and regions of the tooth, restoration materials, prosthesis or orthodontic appliances). For example, the chemical composition of the one formed on the root cement differs considerably from the one formed on the enamel, due to the special chemical and structural characteristics of both tissues and the higher proportion of proteins provided by the gingivocrevicular fluid on the cement.

The AEP is formed within minutes and modified later by enzymes, which is coming from the saliva, bacteria, desquamated epithelial cells and polymorphonuclear leukocytes (transported by the gingivocrevicular fluid).

In this way, various components adsorbed at first to the enamel are rapidly degraded, which is the reason why they cannot be found in the mature integument. The most susceptible to enzymatic degradation proteins are those rich in proline, statins and histatins, whereas cystatins, amylase and other proline-rich proteins are more resistant [3, 4].

3.2 Acquired enamel pellicle training process

The formation of this integument is given by the combination of ionic, hydrophobic, hydrogen bridge and van der Waals attractions that are established between the buccal surfaces (dental enamel, cement, mucous membranes, etc.) and the organic components suspended in the saliva.

The enamel or adamantine substance is made up of 96% mineral salts of hydroxyapatite (HA) crystals. The negative charge (phosphate groups) of the HA crystals attracts the ionic calcium present in saliva (Ca++). The amino acids with anionic side chains, which form part of the salivary proteins, establish electrostatic bonds with calcium. The amino acids with cationic side chains interact directly with the phosphate groups of the HA through ionic bonds and in this way the proteins are adsorbed to it.

The formation of AEP occurs in two stages. In the initial stage, the protein cover increases three times its thickness, mainly proline-rich proteins are added forming globular structures (from 20 to 300 nm arranged in the form of clusters), which are afterwards fused, forming long units that cover the whole enamel. In the second stage, by action of proteolytic enzymes the molecular conformation of the pellicle is altered and the globular structure gets lost. The first phase is quantitatively the most important, while the second one, which corresponds to the process of maturation of the integument, has functional importance in relation to the bacterial colonization [3, 4].

3.3 Acquired enamel pellicle functions

AEP has different functions, among which are:

- Protective barrier of enamel from acidic substances from the diet or formed during bacterial metabolism. Therefore, it prevents demineralization and acid erosion.

- Promotion of the enamel remineralization process. Constituent proteins of the acquired film such as staterins and proline-rich proteins favor the stabilization of calcium and phosphate ions under supersaturation conditions. These participate in the maturation of the enamel after the eruption of the teeth (posteruptive maturation).

- Reduction of tooth friction forces developed during chewing.

- Prevention of the drying of the surfaces it covers, due to the presence of mucoproteins that are capable of retaining water.

The AEP also provides sites for the adhesion of oral microorganisms, giving rise to the formation of the dental biofilm. In addition, lactoferrin (another protein present in the film) fixes iron, essential for bacterial metabolism. As counterpart,

has been observed that certain proteins and enzymes present in the AEP affect the development of the biofilm bacteria. The presence of lysozyme in the pellicle destabilizes the bacterial wall producing cell lysis and lactoperoxidase forms compounds that inhibit the metabolism of glucose in bacteria [3, 4].

4. Dental biofilm

The DB is a dense bacterial mass, constituted by different types of microorganisms organized in a coccoid, filamentous or bacillary form, embedded in an intermicrobial matrix that accumulates on the structures of the tooth. It is metabolically active, organized and potentially pathogenic. Inside the DB a continuous reorganization and bacterial succession take place. Therefore, this organized bacterial complex survives the challenges of a constantly changing environment.

It is of utmost importance to understand the dental biofilm as an ecosystem, where a complex of bacteria and their products are embedded in an abiotic extracellular material of bacterial, salivary and dietary origin.

70% of the DB consists of microorganisms. The remaining 30% is made up of organic and inorganic components. Water represents 80% (it allows the dissolution of minerals and nutrients within the environment), proteins 40%, carbohydrates (CH) between 13 and 18%. Glucose is the principal CH, which can be found in two forms: extra and intracellular polysaccharides. The proteins of the biofilm come from bacteria, gingivocrevicular fluid and saliva. The main proteins are amylase, lysozyme, albumin and immunoglobulins IgA and IgG.

The architecture of the dentobacterial deposits is an important factor both for the regulation of microbial physiology and for the ecology of the place. The microbial behavior depends on the thickness of the deposit, its density, its ratio of bacterial cells/organic matrix and the presence of channels in this structure [5].

4.1 Factors that influence the development of dental biofilm

In the development of DB, various factors are involved:

- Anatomy, position and structure of the teeth: bacteria colonize different types of dental surfaces because they have fimbriae, exopolysaccharides and hydrophobic. All these properties are implicated in its capacity to adhere to the surface.
- Bacterial metabolism: aerobic or anaerobic.
- Presence and quantity of bacterial nutrients.
- Composition of saliva and gingivocrevicular fluid: pH, ions, temperature and fluidity favor the production of biofilm on the tooth surface.
- Host diet: fermentable carbohydrates (cariogenic), consistency, frequency and speed of sweep of the oral cavity.
- Oral hygiene maintenance.

4.2 Structure of dental biofilm

The three-dimensional character of the bacteria is reflected in the structure of the DB. It may be: (a) immature: the one that takes place after a few hours of formation or (b) mature: the one that takes place several days after its appearance, which is considered

metabolically active and organized. The accumulation of bacteria on the surface of the tooth is a result of the balance between adhesion, growth and removal of bacteria. It reaches a saturation point where it is no longer possible to increase its volume. Inside the DB structure there is reorganization and bacterial succession continuously [5].

4.3 Supragingival dental biofilm

The supragingival dental biofilm is located from the gingival margin to the dental crown. Gram-positive microorganisms predominate, mainly *Streptococcus* spp., among them the most abundant are *S. sanguis, S. sobrinus* and especially *S. mutans*. *Actinomyces* sp., such as *A. viscosus, A. naeslundii* and *A. israelii* as well as other varieties. *Among the Gram-negative bacteria anaerobic such as Fusobacterium, Prevotella and Porphyromonas predominate* [6].

4.4 Dental biofilm formation process

The process of forming the dental biofilm is characterized by a series of ordered stages:

1. Bacterial transport to the acquired film: almost simultaneously with the formation of the acquired film, microorganisms proceeding from the salivary flow, tongue, desquamated cells of the mucosa (bacteria adhered to aforesaid cells) and other microorganisms with inherent mobility capacity found in the oral environment start to adhere to the dental surface. Between zero and 4 hours after correct oral hygiene, few bacteria are observed on the surface of the teeth.

2. Primary colonization: later interactions between bacterial proteins (adhesins) and specific receptors of the acquired film allow the irreversible adhesion of the first colonizing microorganisms. Gram-positive and Gram-negative bacteria predominate, including coccoid and filamentous forms. Diverse ionic and electrostatic interactions, covalent bonds as well as other molecular interactions are established. Probably one of the most important adhesive mechanisms is mediated by glucans (extracellular polysaccharides synthesized by bacteria). This allows bacteria not only to adhere to the surface but also to add to each other.

Adherent microcolonies are originated by a mucosal layer surrounding several cells. At this stage the DB is still very thin; *Streptococcus sanguis* is probably the first colonizer. The metabolism is aerobic and the microbial nutrition comes from the salivary glycoproteins and sugars in the diet. Other primary colonizers are *Streptococcus mitis, Streptococcus oralis, Actinomyces naeslundii*, and to a lesser extent other bacteria, most of them aerobic and facultative anaerobes.

3. Coadhesion phenomenon (secondary and tertiary colonization): it takes place between the primary and late colonizers and is characterized by an active multiplication of bacteria, aggregation (taxonomically related bacteria) and coaggregation (bacteria that have little to do from the taxonomic point of view). Interactions are mediated between adhesins, lectins, and specific receptors, which increases the complexity of the microbial mass. Diverse phenomena occur, leading to qualitative changes in the ecosystem, such as competition for nutrients, production of H_2O_2, release of bacteriocins, oxygen consumption and others. The extracellular polymers constitute the matrix that surrounds the bacteria, which guarantees their firm adhesion. Among bacteria there are numerous water channels that allow the diffusion of nutrients and elimination of metabolic waste.

4. Detachment of bacteria: the attached bacteria can be released in response to changes that occur in the environment (e.g. pH, concentration of specific ions, etc.). The production of inhibitory substances against other bacteria can also influence this phenomenon. These changes lead also to a competition for nutrients and the creation of unfavorable conditions for the growth of certain bacterial species [7].

In the already formed DB, channels or open spaces that go from the outside to the surface of the enamel are observed, which allow the penetration and distribution of molecules in its interior. The bacterial metabolism guarantees the right environment for the growth of bacteria (pH and adequate amount of oxygen). Some of them split polymers into smaller units, others are able to reduce sulfates, and others obtain energy from simple metabolic products.

The surface of the tooth is an indispensable natural habitat for *S. mutans*, since this organism cannot be detected in the mouth until the eruption of the teeth and disappears shortly after the loss of the teeth. *S. mutans* is able to form DB through a series of mechanisms such as the expression of the SpaP surface adhesin and the ability to synthesize insoluble extracellular polysaccharides that improve its accumulation in the tooth [8].

Within biofilms, bacteria have developed their own signals or communication systems through small molecules which allows them to survive in unfavorable and densely populated environments. In addition, this signal system favors the expression of genes associated with the caries or with the acid tolerance of the environment in which they develop. The transfer of genetic information, explaining the antibiotic resistance among native microbiota species, has also been described. Bacteria regulate these physiological processes through a mechanism called quorum sensing (QS), defined as a bacterial mechanism of intercellular communication to control the expression of genes in response to population density. Due to a particular environmental stimulus, bacterial cells can communicate and behave collectively to provide significant benefits in host colonization, defense against competitors and adaptation to different physical variants [8].

The QS system consists generally of three components: (a) a signal peptide and (b) a double regulatory system (DRS), which in turn has a (c) histidine kinase (HK) sensor attached to the membrane and an intracellular response regulator (RR). *S. mutans* is a bacterium that has evolved into a "lifestyle" biofilm in order to survive and persist in its natural environment. The Quorum detection system, essential for genetic competence in *S. mutans* (cell–cell signaling system), involves at least five genetic products encoded by cslAB (comAB) and comCDE [9].

The genes comC, comD, and comE are adjacent within the chromosome and constitute a peptide signaling system (PSS) including a generation pathway and a response pathway, respectively. The comC and comDE genes encode, respectively, a peptide precursor of the PSS, the sensor protein (HK) and an intracellular response regulator (RR) [9].

The other two genes, cslA and cslB, are located in a separate region of the chromosome and encode a CSP-specific secretion apparatus consisting of an ATP-binding cassette transporter (ComA) and an accessory protein to ComA (ComB), which presumably participate in the processing and export of the CSP. ComE is responsible for the regulatory response of the QS system producing activities dependent on cell density (bacteriocins) (**Figure 1**).

This quorum sensing system works optimally when cells live actively in the growing dental biofilm, suggesting that cell–cell signaling is the system that could play a role in the formation of *S. mutans* biofilms [9, 10].

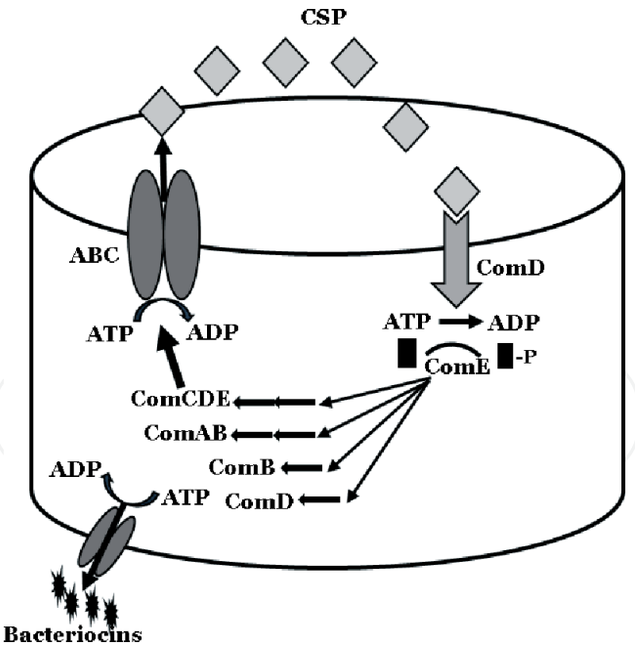

Figure 1.
Scheme of the quorum detection system ComCDE and its genes regulated in S. mutans. The ComCDE encodes a signal peptide precursor, which is exported through a specific ABC transporter and generates a competing stimulating peptide (CSP). The ComCDE encodes a component transduction system that specifically detects and responds to CSP. When it reaches a critical concentration CSP interacts with the ComD receptor protein and activates its ComE response regulator. The phosphorylated ComE, in turn, activates the genes downstream, which triggers the signaling cascade for the production of activities dependent on cell density (bacteriocins).

4.5 Cariogenicity of dental biofilm

The DB of the oral surfaces establishes a dynamic balance with the host's defenses, compatible with the maintenance of the integrity of the tissues. Oral diseases occur when the composition and metabolic activities of dental biofilm communities are disturbed by the pH, oxygen tension and nutrients of the environment. These ecological changes in the DB result in an increase in the proportion of pathogenic microorganisms, which have more virulent structural and enzymatic determinants than those of microorganisms related to healthy conditions. Caries is a disease associated with the supragingival dental biofilm. It is produced by the action of acidogenic bacteria, which degrade fermentable carbohydrates of the diet and produce acids as result of their metabolism [11].

The cariogenicity of the dental biofilm increases with the retention of bacterial nutrients and the greater permanence of organic acids in it. The structure of the dental biofilm allows these acids to be longer in contact with the tooth surface, causing the demineralization process [12].

S. mutans has important properties that enable it to predominate in the dental biofilm and induce the development of caries. These properties are known as virulence factors and provide the microorganism with survival advantages over other bacteria common to the oral ecosystem [13].

Among these cariogenicity factors are:

Acidogenic: production of acid (mainly lactic acid) through the fermentation of refined sugars such as sucrose and glucose. This causes the environmental pH to drop to values of 5.5 or 4.5, pH called critical, which initiate the demineralization of tooth enamel.

Aciduric: factor that refers to the capacity of the microorganism to continue lowering the pH in acidic conditions.

Acidophilic: ability to grow and survive in acid pH. Not all bacteria resist these conditions. This is a fundamental domain element in the dental biofilm.

Synthesis of intracellular polysaccharides: these are homopolysaccharides and have a major role as nutritional reserve.

Synthesis of extracellular polysaccharides: permit the synthesis of a mucous layer constituted by polysaccharides, provided a high availability of sugars in the medium, such as water, soluble glycans (dextrans), hydroinsoluble glycans (mutans) and water-soluble fructans (levans).

Post-short pH effect: despite rapid decreases in ambient pH, a rapid physiological recovery of the microorganism occurs. This mechanism prevents the entry of new molecules of sucrose by activating pyruvate kinase or generating alkaline products resulting from protein catabolism.

Synthesis of cell adhesion proteins: antigenic proteins found in the wall of *S. mutans* and initiate adhesion to the tooth surface. They may have different functions depending on the region of the protein in question: aggregation (hydrophobic amino terminal region), and adhesion (amino terminal region rich in alanine). On the other hand, they can also bind to collagen in the dentinal tubules, an important property in the development of root caries. This suggests that these molecules possess several receptors, which interact with the secretory component of IgAs, albumin, agglutinins and salivary glycoproteins.

Glucan receptor proteins: these are extracellular products of bacteria which associate or bind glucans in the presence of sucrose and therefore are involved in the formation processes of the dental biofilm. All glucan binding proteins show affinity for glycans rich in α-1.6 glycosidic bonds.

Mutacin production: also called bacteriocins, are substances of peptide nature with antimicrobial activity. *S. mutans* through these molecules can eliminate other bacterial species from the dental biofilm, giving this microorganism an ecological advantage for colonization.

Each of these properties acts in coordination to alter the ecology of the dental biofilm by increasing the proportions of *S. mutans* over other acidogenic and acid-resistant species in the environment [13].

In addition, the ability of *S. mutans* to use sucrose to promote its adhesion and accumulation in the dental biofilm is very significant. This provides the microorganism a pathogenic potential if the physiology of the host and the general ecology of the oral microbiota allow it.

4.6 Biochemistry of the dental biofilm

Bacteria possess the ability to metabolize a large amount of organic and inorganic compounds. Said metabolism has as its purpose the supply of carbon and energy necessary for its growth and reproduction.

In the absence of oxygen, *S. mutans* metabolizes glucose giving as final product lactic acid. Depending on the metabolic conditions, it can also synthesize glycogen for energy reserves and, in the event of a sucrose deficit, degrade it to obtain energy.

The fermentable carbohydrates of the diet are the main source of energy for most of the bacteria present in the dental biofilm. The association of the consumption of sugars with dental caries depends on the bioavailability and the structural characteristics of the DB [5].

4.7 Extracellular cleavage of sucrose

Sucrose is a low molecular weight disaccharide, soluble in water, easy to diffuse through the biofilm and converted into organic acids by bacteria such as *S. mutans* and *Lactobacillus* sp.

For their splitting, these bacteria produce and secrete a family of enzymes called glucosyltransferases (Gtfs), which hydrolyze sucrose into glucose and fructose. These enzymes take the glucose molecule and bind it to a pre-existing glucose chain. In this way, the chain lengthens and gives rise to the extracellular polysaccharides (glucans).

S. mutans produces at least three genetically different Gtfs, each of which synthesizes a glucan structurally different from sucrose. GtfB synthesizes mainly insoluble glucan rich in α-1.3 glycosidic bonds. GtfC produces a mixture of solubles (with mostly α-1.6 bonds) and insoluble glycans, and GtfD predominantly forms soluble glucans. Each one plays a different role in the formation of the dental biofilm [5].

GtfC adsorbed to the enamel within the biofilm, while GtfB binds avidly to the bacteria, promotes cell clustering and improves the cohesion of the biofilm. GtfD, on the other hand, forms a soluble polysaccharide, easily metabolizable and acts as a primer for GtfB.

When the enzymes involved in the sucrose break are the fructosyltransferases (Ftfs), the final product are extracellular polysaccharides of the levans type: water solubles fructans, glycosidic bond type: α-2.6 and α-1.2 (**Figure 2**).

Soluble glucans and fructans have a nutritional function in the absence of exogenous carbohydrate intake. This is because they are easily degradable by enzymes such as glucanases and fructanases. On the other side, insoluble glucans are difficult degraded by bacteria and have greater adherent properties, intervening in the so-called glucan-mediated unions; important for the formation of the dental biofilm in which the Gtfs themselves and host receptors also participate [5].

The organic acids resulting from the bacterial metabolism of fermentable carbohydrates are, in addition to lactic, acetic, butyric and carboxylic. Lactic acid produces the most notorious changes; since the greater extracellular concentration of it, the more accentuated is the pH drop of the dental biofilm, up to the critical level of dissolution of hydroxyapatite (inorganic enamel component) which is around 5.5 (**Figure 3**).

4.8 Acid survival of *Streptococcus mutans* in the dental biofilm

The microorganisms involved in the caries must be able not only to grow, but must possess the ability to survive in acidic environments.

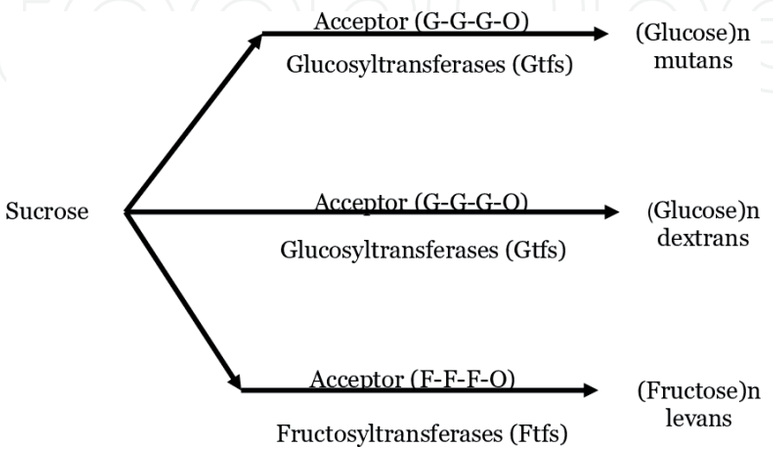

Figure 2.
Enzymatic cleavage of sucrose. Glucosyltransferases (Gtfs) hydrolyze sucrose into glucose and fructose. These enzymes take the glucose molecule and bind it to a pre-existing glucose chain [acceptor (GGGO)]. The chain lengthens and gives rise to extracellular polysaccharides (mutans and dextrans). Fructosyltransferases (Ftfs) hydrolyze sucrose, take the fructose molecule and bind it to a pre-existing fructose chain [acceptor (FFFO)] and the final product is the levans polysaccharides.

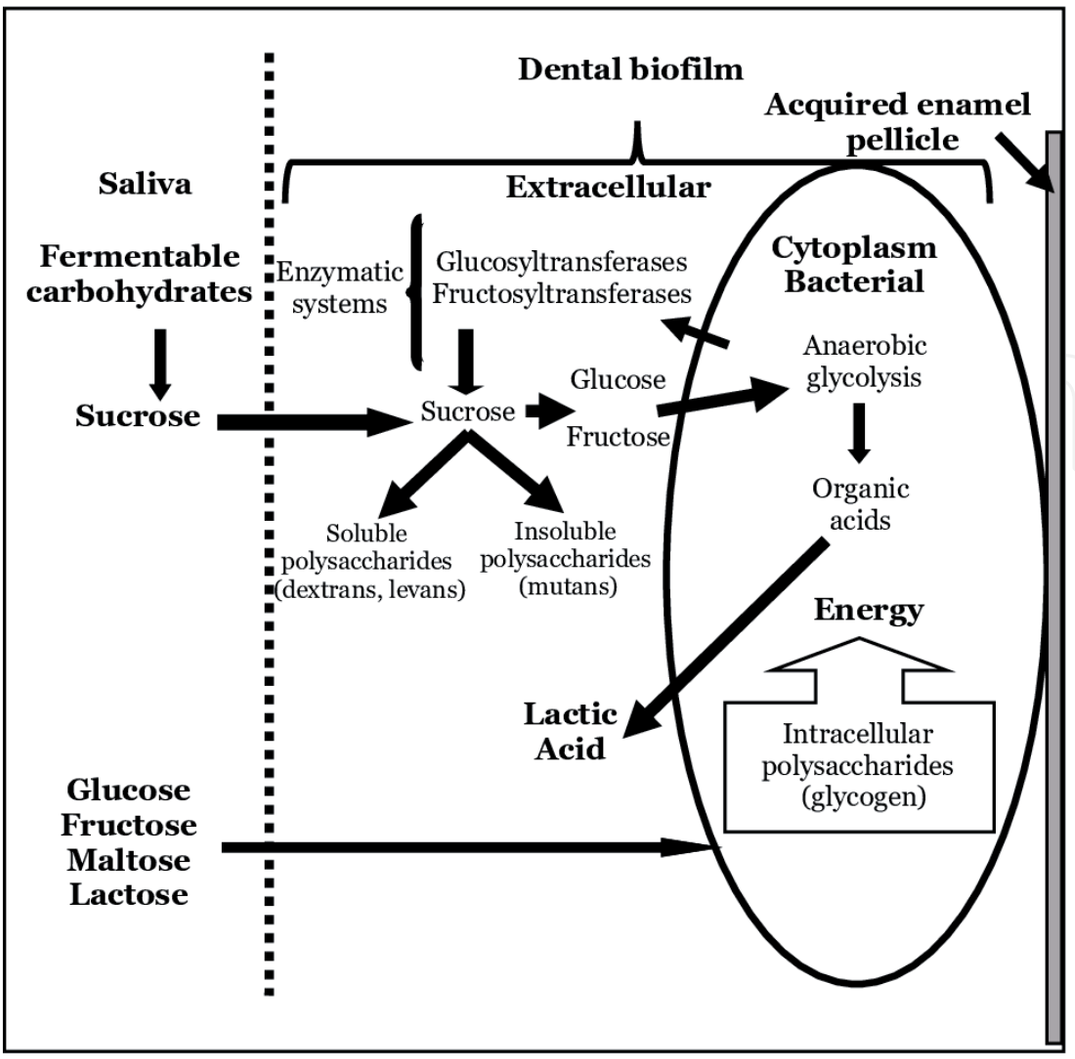

Figure 3.
Schematic representation of bacterial metabolic activity in the dental biofilm. Metabolism of various carbohydrates (including glucose and fructose) by bacterial biofilm. Production and secretion of a significant amount of lactic acid, which can cause demineralization of teeth structure that can finally result in the development of decay.

S. mutans and *S. sobrinus* have a higher average acid production rate compared to other oral streptococci such as *S. mitis, S. oralis, S. gordonii, S. sanguis, S. intermedius, S. anginosus, S. constellatus and S. vestibularis* [14].

There is a different acidification capacity of the *S. mutans* medium as it comes from different carious dental surfaces: either a carious smooth tooth surfaces (CSTS) or a carious occlusal tooth surfaces (COTS).

The strains developed in CSTS possess greater cariogenic capacity, probably induced by the different ecosystem developed in the smooth surface decayed, showing the particular importance of it [15].

The acidogenic power (virulence factor) of *S. mutans* can lead to ecological changes in the microbiota of the dental biofilm, which includes an increase in the proportion of acid-producing *S. mutans* to the environment and the decrease in the microbiota sensitive to said acidity (*Streptococcus mitis*, *oralis* and *sanguis*).

Accompanying its acidogenic power, *S. mutans* possesses the characteristic of acid tolerance. It retains its ability to survive even at pH levels that are inhibitory for some bacterial species (pH 4.4), this being a distinctive feature of this species [14].

In CSTS, *S. mutans* is more acidogenic and has greater resistance and survival to the acidity of the medium, therefore these microorganisms developed in CSTS have greater cariogenic capacity compared with those developed in COTS [15].

One of the strategies for increasing the acid survival of *S. mutans* in CSTS is to change the fatty acid profile of its membrane from saturated and short chains to monounsaturated and long chains at pH 5. These changes induce an increase in the interrelation of the acidic chains in the lipid bilayer, which allows correlate it with the increase of its stability (greater rigidity) [15].

5. Activity of the F-ATPase and P-ATPase of *Streptococcus mutans* membrane

F-ATPase and P-ATPase are present in the membrane of *S. mutans* to maintain intracellular homeostasis. These constitutive enzymes represent ATPases with proton pump function. The induction of proton pumps in acidic environments and the consequent expulsion of protons from the cell to the exterior helps to maintain a high cytoplasmic pH in comparison to its environment. The activity of these enzymes is very important for the acid tolerance of microorganisms such as *S. mutans*, in such a way that the optimum pH of the F-ATPases are directly related to the capacity of the microorganism to survive in acidic conditions (pH 5) [15].

In addition to the F-ATPases, there is a 100-kDa membrane protein called P-ATPase that can maintain a cytosolic pH close to neutrality during the growth of the microorganism. This is a different protein from F-ATPase, due to its sensitivity to orthovanadate and lansoprazole [16].

In the intracellular metabolism of *S. mutans*, protons from the anaerobic glycolytic pathway acidify the cytoplasm, but glycolytic enzymes as well as other cellular functions, are sensitive to inhibition by intracellular acidity. Therefore, the function of the F- and P-ATPase is to translocate of protons to the outside and to maintain a pH gradient across the cytoplasmic membrane compatible with life.

The increase of the activity of the F-ATPase and the membrane P-ATPase of microorganisms developed in acid environment indicates that the enzymatic activity is one of the main mechanisms of the acid tolerance for oral streptococci [16].

S. sobrinus has a higher acid resistance than *S. mutans*; the differences in the mechanisms of acid tolerance between the two microorganisms are due to the fact that *S. sobrinus* is genetically tolerant acid [15].

As mentioned above, in the plasma membrane there are integral or transmembrane proteins (F-ATPase and P-ATPase). The interactions between this type of membrane proteins and the surrounding lipid environment are important to determine its structure and function.

The integral proteins of the lipid bilayer require that the hydrophobic transmembrane region of the protein matches the hydrophobic region of the lipids, in order to avoid an unfavorable energetic contact in said regions. When there are no coincidences in the hydrophobic lipid-protein zones, misalignments occur, leading to a phase separation or segregation of the lipid components with the proteins, creating domains enriched in one of the two components.

Furthermore, the length of the acidic chains of the bilayer lipids affects the state of protein aggregation or hydrophobic lipid-protein mismatch [15].

When the length of the acidic chain is greater than the hydrophobic extension of the protein, it tends to aggregate forming dimers, separating from the lipid and decreasing the hydrophobic interaction between the two. On the other hand, when the length of the chain is smaller than the hydrophobic zone of the protein, monomeric aggregates of the protein are produced with lipids trapped inside them, establishing interactions with the protein. With lengths of intermediate chains, coincidences with the hydrophobic zones of the protein take place and this adopts a monomeric form, leaving the totality of its hydrophobic surface in total coincidence with that of the lipids [15].

The non-coincidence in the hydrophobic zone leads to the separation of the lipids in relation to the protein components, and to the formation of domains with a predominance of lipids or proteins.

The greater hydrophobic contact of the lipid with the protein occurs in the CSTS and this favors the greater activity of the ATPase. On the other side, the lower hydrophobic lipid/protein contact in the COTS produces a mismatch between the lipid and protein component, which negatively affects the enzymatic functionality (total ATPase).

Therefore, a correlation between the hydrophobic regions of the protein and that of the surrounding lipids is necessary for the optimal functionality thereof.

In addition, the hydrophobic thickness of the bilayer must match the hydrophobic thickness of the protein embedded in the bilayer because of the high-energy costs that occur when the acyl chains of the fatty acids or the hydrophobic amino acids contact with water. The lack of coincidence between the hydrophobic thicknesses of the lipid bilayer and the protein leads to the distortion of the lipid bilayer, or of the protein, or both, to minimize the mismatch [15].

The acyl chains neighboring a membrane protein adjust its length to match the hydrophobic thickness of the protein. Indeed, when the hydrophobic thickness of the bilayer is less than that of the protein, the lipid chains neighboring the protein are "stretched" to provide a thicker bilayer, creating a positive curvature (exocytosis). Conversely, when the hydrophobic thickness of the bilayer is greater than that of the protein, the lipid chains are "compressed" to provide a thinner bilayer, creating a negative curvature (endocytosis). The relatively small changes in the binding of lipids with proteins are due to changes in the lengths of the acyl chains.

In conclusion, the acyl chains are "stretched" or "compressed" to provide, as much as possible, a complete hydrophobic match with the protein zone. This leads to changes in the spontaneous curvature of the lipid bilayer coupled with possible conformational changes or distortion of the membrane protein to provide the strongest interactions. Both the lipid and the protein modified to favor the best interaction, with the result of an optimal activity. The function of the protein is dependent on the structure of the lipid that surrounds it.

The different organization of the microbial membrane according to the dental surface where *S. mutans* are developed are exemplified in **Figures 4** and **5**, which show how lipids and proteins interact and how this interaction can affect the enzymatic activity of ATPase (virulence factor).

In CSTS, the increase of long-chain unsaturated fatty acids improves the interrelation of these with the hydrophobic sectors of the ATPase protein, which contributes to the greater enzymatic activity or greater expulsion of H^+ to the outside (**Figure 4**), so in CSTS, the virulence factor of *S. mutans* is increased. On the other hand, when chains of membrane fatty acids are shorter and more saturated as in COTS, spaces or "pockets" can occur between the lipid and the protein, influencing the behavior of ATPase. In this case, the contact zones between the hydrophobic portion of the protein and that of the fatty acids did not coincide completely (**Figure 5**). Consequently, of this mismatch the enzymatic activity is diminished and there is not sufficient expulsion of H^+ to the exterior (decreased virulence factor).

The change in the membrane fatty acid profile of *S. mutans* and the changes in total ATPase activity are simultaneous processes.

The greater enzymatic activity in CSTS would relate to changes in the organization of the membrane, induced by changes in lipid composition, which favor the best interaction between the hydrophobic segments of both components lipid and protein [15].

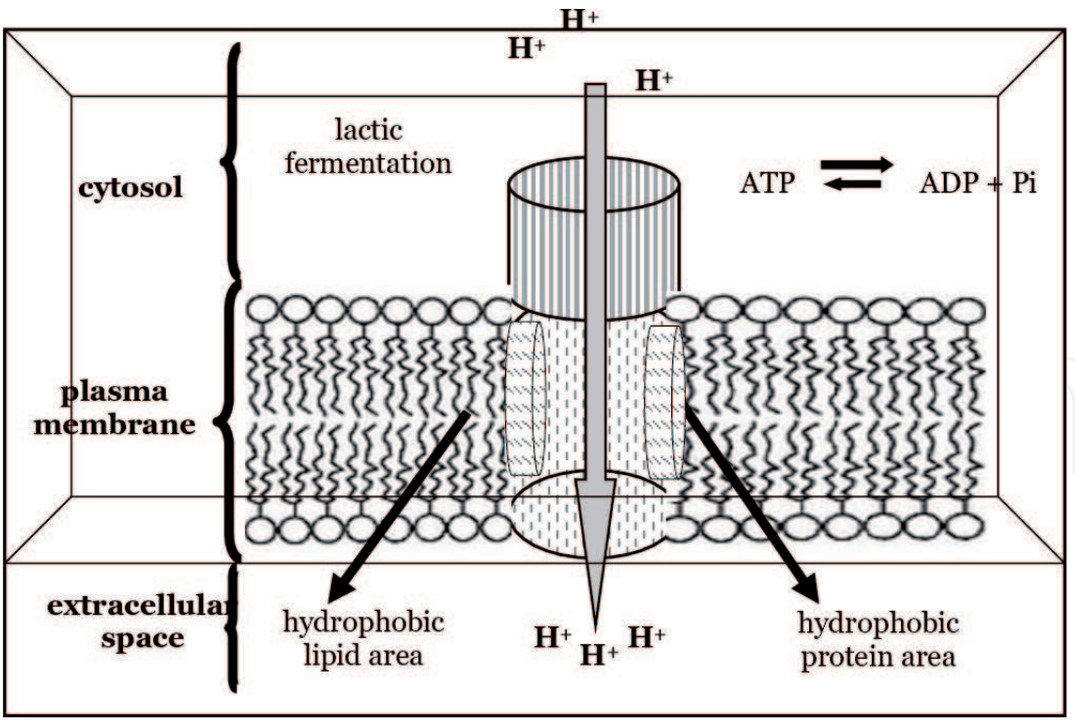

Figure 4.
Lipid-protein interaction in CSTS. The hydrophobic region of the acyl chains coincides with the entire hydrophobic region of the protein. In this case, the enzyme functionality (higher ATPase activity) is favored by better protein-lipid hydrophobic matching, preventing distortion of both parties.

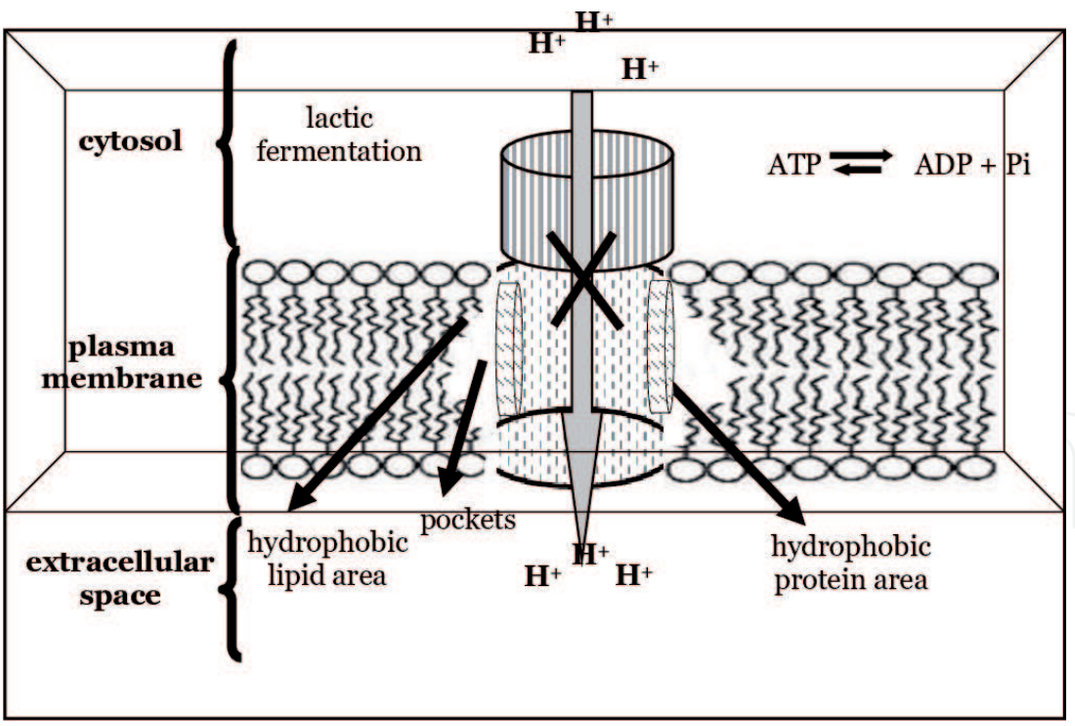

Figure 5.
Lipid-protein interaction in COTS. The hydrophobic region of the acyl chains does not fully coincide with the hydrophobic region of the protein, creating "pockets" due to a mismatch between the lipid and protein components, affecting adversely the enzyme functionality.

6. Conclusions

The main virulence factors of *S. mutans* are its ability to utilize sucrose to promote adhesion and accumulation in dental biofilms, its acidogenicity and its

tolerance to acids. The acid survival of *S. mutans* depends both on the pH of the medium and on the composition of fatty acids and proteins plasma membrane (F-ATPase and P-ATPase).

As with most host–microbe interactions, these attributes only provide the organism with pathogen potential. The physiology of the host and the overall oral flora ecology may or may not suppress this potential.

The advance in the knowledge of how complex and heterogeneous can be the disease of the caries, according to the surface or the biofilms where it develops, can be useful to design new strategies of therapy in the treatment of this disease.

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Conflict of interest

We declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research reported.

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