Streptococcus sanguinis as an opportunistic species in human oral cavity: adherence, colonization, and invasion

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ABSTRACT

Streptococcus sanguinis (formerly *S. sanguis*) is a Gram-positive, facultative anaerobe, nonmotile , normal inhabitant of the human oral cavity, and a member of the viridans group of streptococci. Among the streptococcus, *S. sanguinis* is a primary colonizer in the human tooth surface or it is recognize as a 'pioneer' by forming dental plaque. The aim of this paper is to review the role of *Streptococcus sanguinis* in the adherence to and invasion of human tissues. *S. sanguinis* has been reported that it is associated with healthy tooth surfaces but not with caries. *S. sanguinis* tend to involved in an interspecies interactions with *Streptococcus mutans*, which is known as competition/coexistence within dental biofilm. In their colonization, this bacteria used enzyme sortase A (SrtA) to cleave LPXTG-containing proteins sequence and anchored the cell wall, while virulence factors in infective endocarditis involved housekeeping functions such as cell wall synthesis, amino acid and nucleic acid synthesis, and the ability to survive under anaerobic conditions.

Keywords : Streptococcus sanguinis; primary colonizer; adherence; colonization; invasion

ABSTRAK

Streptococcus sanguinis (nama sebelumnya S. sanguis) adalah bakteri Gram-positif, anaerob fakultatif, nonmotile, normal ditemukan pada rongga mulut manusia, dan merupakan anggota grup streptokokus viridans. Diantara anggota streptokokus, S. sanguinis dikenal sebagai bakteri yang pertama berkolonisasi pada permukaan gigi manusia atau sebagai 'pioneer' pada pembentukan plak gigi. Tujuan penulisan makalah ini adalah telaah pustaka peran Streptococcus sanguinis dari mulai pelekatan sampai invasi ke jaringan manusia. S. sanguinis telah dilaporkan berhubungan dengan permukaan gigi yang sehat tanpa karies. S. sanguinis cenderung terlibat dalam interaksi antar species dengan Streptococcus mutans, dan interaksinya dikenal sebagai kompetisi/ko-eksistensi pada biofilm gigi. Saat berkolonisasi S. sanguinis menggunakan ensim sortase A (SrtA) untuk memotong protein yang memiliki sisi pemotongan LPXTG yang menancap pada dinding sel. Pada infective endocarditis factor-faktor virulensi juga melibatkan gen-gen yang berperan pada pemeliharaan umum 'housekeeping' seperti sintesis dinding sel, sintesis asam amino dan asam nukleat, dan kemampuan untuk hidup pada kondisi anaerobic.

***Key words**: Streptococcus sanguinis; koloni pertama; pelekatan; kolonisasi; invasi*

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INTRODUCTION

Streptococcus sanguis was found by White and Niven in 1946 at isolate of endocarditis patient, then by 1980 the name has been approved to change to "sanguinis" due to conform to the rules of Latin grammar. *Streptococcus sanguinis* is gram-positive bacterium and a member of the group of oral streptococci.[1-3] According to 16S rRNA gene sequences, *S. sanguinis* comprises the mitis group along with *S. gordonii*, *S. cristatus*, *S, parasangguinis*, *S. infantis*, *S. oralis*, *S. pneumoniae*, and *S. mitis*.[2,3] Almost all species of mitis group may be isolated from the human oral cavity or nasopharynx. *S. sanguinis* is the human indigenous biota and has been recognized for a pioneer in colonization of the tooth surfaces. It will be found in any sample contaminated with dental plaque or saliva.[1,3,4]

Like most oral streptococci, *S. sanguinis* produces green area nearby its colony on blood agar. This is a characteristic linked to the ability of viridans streptococci to oxidize hemoglobin in erythrocytes by secretion of hydrogen peroxide. The taxonomy and biochemical identification reveal that *S. sanguinis* hydrolyzes arginine and esculin, ferments raffinose and inulin, and produces a glucan. One study found that 98.6% (291 of 295) of the isolates which had selected as *S. sanguinis* from MM10-sucrose medium were indeed *S. sanguinis* based upon their ability to cleave arginine and produce H_2O_2. The glucan imparts to colonies a firmness and a tenacity to sucrose agar that requires the colony to be cut out of the agar if it is to be cultured further.[1,5]

General genomic features of the *S. sanguinis* genome (SK36) is comprised of a 2,388,435-base pair circular DNA molecule, which is 7 to 24% larger than previously described streptococcal genomes. The G+C content of the genome is 43.40%, which is higher than the G+C content of any of the 21 other completed streptococcal genomes. The genome contains genes encoding 2,274 predicted proteins and accounting for more than 90% of the sequence. *S. sanguinis* can apparently use a broad range of carbohydrate sources for survival. The *S. sanguinis* genome contains the genes required for cell wall sugar, peptidoglycan, and teichoic acid biosynthesis and degradation. In the *S. sanguinis* genome were also identified genes which are expressing proteins potentially relevant to adhesion in the oral cavity or to virulence in invasive disease, and genes that are responsible for assembly of the pili.[2,4]

Several researcher have been studied separately *S. sanguinis* in terms of adherence, colonization, and to invasion. In this paper the author would provide short review of *S. sanguinis*. In particular, the review is very useful for providing a better understanding of *S. sanguinis* in adherence and colonization, competition and coexistence in dental biofilm, and invasion on endocardium

LITERATURE REVIEW

1. Adherence and colonization

Bacteria in the natural environment often grow upon surfaces. Streptococcal adherence and colonization on human tooth surface was mediated by *S. sanguinis* that is known as a pioneer colonizer. Firstly, *S. sanguinis* associate with a conditioned surface using pili, which can penetrate salivary glycoprotein coated surface. Some of the pioneers form stronger bonds with the surface molecules engaging multiple adhesins. Then, cells involve in intermicrobial signaling, produces extracellular polymeric substance (EPS) to form societies. Next, Incorporation of other microorganisms, including intergeneric coaggregation and cell-cell signaling, leads to the development of complex communities or biofilm. Finally, These communities contain specific microbial associations within metabolic networks, ensuring more efficient utilization of nutrients and reduced susceptibility to antibiotics and immune surveillance.[3,4,6]

Different streptococci vary in their propensities to form biofilm communities, but in all cases, biofilm formation depends first upon the adherence of cells to a surface. Cell division and multiplication then occur to produce a society (clonal), and the integration of other microorganisms within the society leads to the formation of a community (mixed species). Environmental conditions such as pH, temperature, oxygen availability, and organic metabolites, etc. influence the development of these communities, and signaling molecules for cell-cell communication are integral to population control.[3,4,6]

The study of *S. sanguinis* (SK3) genome showed that there are genes expressing several

proteins that is potentially relevant to adhesion in the oral cavity or to virulence in invasive disease. SSA_1099 (Stx) expressing protein which exhibits homology to RTX-type toxins in gram-negative bacteria. The HylB ATPase and HlyD "membrane fusion protein" components of an RTX toxin export system are encoded by adjacent ORFs (SSA_1100 and SSA_1101, respectively). SspC and SspD are orthologs of the SspA and SspB adhesins of *Streptococcus gordonii*.[2]

Many streptococcal surface proteins are attached to the bacterial cell wall by membrane-associated transpeptidases of the sortase family. These enzymes function by cleaving target proteins at a C terminal cell wall sorting signal, typically LPXTG, to form an acyl enzyme intermediate[3]. Lipoproteins (LP) and cell-wall anchored proteins (CWA), two classes of proteins that are surface exposed and prevalent among reported virulence factors, were predicted in *S. sanguinis*. *S. sanguinis* possess abundant numbers of surface proteins (60 LPs and 33 CWAs). The lgt and lspA genes expected for LP processing are present (SSA_1546 and SSA_1069, respectively), as are genes encoding three sortases (SSA_0022, SSA_1219, and SSA_1631) for CWA processing. Surface proteins may contribute to the ability of *S. sanguinis* to colonize the tooth and interact with a diverse group of oral bacteria and may account for its predominance as a cause of streptococcal endocarditis.[2,3,7]

Fibrils or pili are involved in streptococcal adherence and virulence. *S. sanguinis* strains possess both short fibrils and long fibrils. Gene SSA_0829 or *SrpA*, is thought expressing the structural component of long fibrils, and its orthologs are important for adhesion to platelets, saliva-coated hydroxyapatite, and salivary agglutinin. Then products of SSA_0830 to SSA_0841 exhibit homology to the proteins shown to be required for the glycosylation and export of SrpA orthologs in *S. parasanguinis* and *S. gordonii*.[2]

There is locus contains three putative pilin subunit genes encoding CWA motifs and one to three sortase genes that are required for assembly of the pili *S. sanguinis* also contains an apparent pilus locus, with SSA_1632 to SSA_1635 encoding LPXTG proteins and SSA_1631 encoding a sortase. SSA_1632 to SSA_1634 also each contain a conserved "E box" domain found in many pilin genes[2]. Interestingly, the experiment demonstrated that the pili of *S. sanguinis* bound to multiple salivary proteins, including α-amylase. Amylase, the most abundant salivary component in humans, is an enzyme which catalyzes the hydrolysis of dietary starch. Although it has been reported that pili of pathogenic streptococci bind extracellular matrix proteins such as fibronectin and collagen. the ability of pili to bind to α-amylase and other salivary proteins is the possible the unique binding specificity of *S. sanguinis*.[4]

Pili may allow the organism to adapt better to the oral environment. Although the structure of *S. sanguinis* pili remains unknown, however the western blot analysis and immunogold electron microscopic findings suggested that *S. sanguinis* pili contains three region similar to other strptococci. Firstly, PilA may be the backbone of the pili. PilC has been reported to bind to fibronectin, thus it is likely that PilC is located at the distal end of *S. sanguinis* pili. PilB was not detected in western blot analysis, however, based on present knowledge regarding the structure of *S. pneumoniae* pili, it is possible that PilB is located at the proximal end of the pili and covalently linked to peptidoglycan in the streptococcal cell wall.[2,4]

Cell wall polysaccharides (CWP) serve as important receptors for agglutination and coaggregation in oral streptococci. Three genes, encoding a periplasmic lipoprotein involved in iron transport (SSA_1129), an iron-dependent peroxidase (SSA_1130), and a high-affinity Fe^{2+} permease (SSA_1131) associated with the Tat genes in *S. sanguinis*. Two glucosyltransferases (Gtf) were found in *S. sanguinis* that are GtfB and GtfP. The SSA_0613 product is a homolog of GtfR of *Streptococcus oralis* ATCC 10557, which synthesizes water-soluble glucans with no primer dependence. The SSA_1006 product is a homolog of GtfA, an enzyme that, in the presence of inorganic phosphate, converts sucrose to fructose and glucose-1-phosphate. Furthermore, the products of several ORFs exhibit homology to *S. mutans* non-GTF glucan-binding proteins (GBP), including the products of SSA_0019, SSA_0303, and SSA_0956. Non-GTF GBPs are cell surface receptors for glucan or secreted proteins that can become cell associated when glucan coats the bacterial cells.[2]

Initial colonization of *S. sanguinis* in infants had been study from the natural history cohort. All

45 infants acquired *S. sanguinis* sometime after the emergence of their primary teeth. Twenty-five percent of the infants had acquired *S. sanguinis* by 8.0 months of age, and 75% had *S. sanguinis* by 11.4 months. The median age of the emergence of the first tooth was 7.1 months range, 3.9 to 9.5 months). The time of initial colonization by *S. sanguinis* was significantly correlated to the infant's age at first tooth emergence (Fig. 1).[1] The time of the initial detection of *S. sanguinis* in plaque preceded its detection in unstimulated saliva by an average of 4.4 months. More specifically, *S. sanguinis* was first detected in plaque of infants at a median age of 9.0 months and in saliva at a median age of 12.7 months (Table 1.).[1]

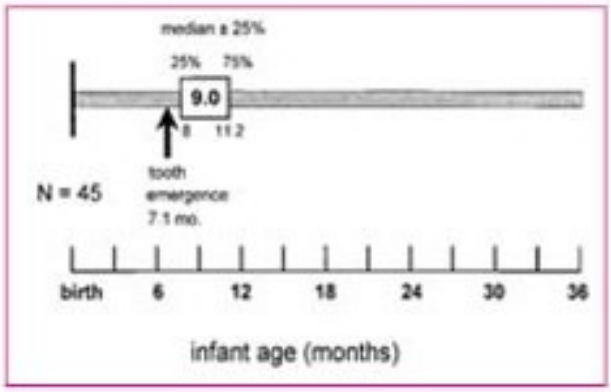

Figure 1. Time line of infectivity for Streptococcus sanguinis. Infants become colonized by S. sanguinis at a median age of 9.0 months. Colonization followed the emergence of primary teeth; The first tooth emerged at a median age of 7.1 months.

Table1. Age of infants at initial detection of S. sanguinis in either plaque or saliva samples.[1]

Sample	Age (mo)		
	Mean ± SD	Median	Range
Saliva	13.9 ± 6.0	12.2	4.0-29.5
Plaque	10.6 ± 5.2	9.1	5.2-36.5
Either	9.7 ± 3.1	9.0	3.9-20.9

Competition and coexistence in dental biofilm

S. sanguinis is one of the pioneer colonizers of the oral cavity and may initiate biofilm formation on tooth surfaces. Several putative biofilm-related genes are found in *S. sanguinis* and most other streptococci. SSA_0135 to SSA_0137 genes are clustered in an arrangement similar to that observed for their orthologs in the adc operon, which is involved in biofilm formation in *S. gordonii*. Genes of the inducible fructose phosphotransferase operon, which is also related to biofilm formation in S. gordonii, are similarly clustered in S. sanguinis (SSA_1080 to SSA_1082). The SSA_1909 product is more than 60% identical to biofilm regulatory protein A (BrpA) in *S. mutans*. BrpA codes for a predicted surface associated protein with functions not only in biofilm formation, autolysis, and cell division but also in the regulation of acid and oxidative stress tolerance in S. mutans. SSA_1853 is an ortholog of the LuxS gene in *S. oralis*, which is responsible for the catabolism of S-ribosylhomocysteine, producing autoinducer 2, a universal signal molecule mediating cell-cell and interspecies communication (quorum sensing) among bacteria, biofilm formation, and virulence.[2,6]

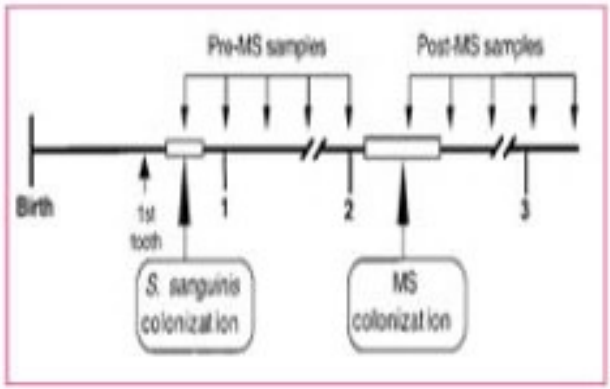

Figure 2. Study design of the natural history study: sampling periods relative to the infant time line. Bacteriological samples were taken at 3-month intervals. Pre- and post-mutans streptococci levels of S. sanguinis/ total cultivable bacteria in saliva were averaged and compared relative to the time to colonization by the mutans streptococci.[1]

S. sanguinis, like S. mutans, resides on the surfaces of teeth, produces extracellular glucans from sucrose. The glucans of *S. sanguinis* are structurally different and are not adhesive. *S. sanguinis* may compete with the mutans streptococci for colonization sites on tooth surfaces, since both groups of bacteria require the presence of teeth for colonization and may exhibit direct biochemical antagonism in situ (Fig. 2).[1] Because the cariogenic potential of *S. sanguinis* is deemed low compared to that of the

mutans streptococci, several investigators have suggested that the S. mutans/S. sanguinis ratio may serve as an indicator for caries risk, i.e., the smaller the ratio, the lesser the risk of caries [8].

In another study involved Caries Free (CF) and Severe-Early Childhood Caries (S-ECC) children, *S. sanguinis* was detected in almost all children [9,10]. All CF children had higher relative levels of *S. sanguinis* to *S. mutans* than all S-ECC children, suggesting that CF children were colonized by an absolutely high count of *S. sanguinis* over the level of *S. mutans*. Additionally, the interaction between *S. sanguinis* and *S. mutans* was significantly associated with caries outcome (Table 2) [1,9]. These findings not only support the notion that the presence of *S. mutans* alone may not be the sole indicator for increased risk for caries, but also suggest that an interactive effect of *S. mutans* and *S. sanguinis* may play an important role in children's caries experiences [9].

Table 2. Comparison of the mean levels (log value) and prevalence of oral bacteria examined between the S-ECC and CF children [9].

Microorganism	Mean $\log_{10}$ value (SD)			Prevalence, %		
	S-ECC (n = 14)	CF (n = 8)	p value[1]	S-ECC (n = 14)	CF (n = 8)	p value[2]
S. mutans[3]	6.0 (1.5)	2.2 (0.8)	0.000	100	37.5	0.002
S. sobrinus[3]	2.5 (1.3)	2.1 (1.2)	0.457	35.7	12.5	0.351
S. sanguinis[3]	6.4 (1.3)	6.6 (0.6)	0.432	92.9	100	1.000
Total oral lactobacilli	4.1 (1.5)	4.3 (1.3)	0.785	100	100	
Total oral streptococci	8.0 (0.4)	7.4 (0.4)	0.005	100	100	
Total cultivable count	8.2 (0.3)	7.9 (0.5)	0.133			

[1] Nonparametric Mann-Whitney U test. [2] Fisher's exact test. [3] Zero value was replaced with detection limit.

Invasion

Endocarditis is an infection of heart valves or the endocardium. The viridans group streptococci are the common cause of infective endocarditis, and among these bacteria, *S. sanguinis* is the most common. The oral streptococci may invade into the bloodstream through oral surgery or everyday activities including brushing and chewing. Blood-borne bacteria may colonize endocardium or cardiac valves that have been damaged by congenital conditions or degenerative processes, resulting in infective endocarditis [11,12]. Endocarditis causes substantial morbidity and mortality. Recent retrospective studies have reported endocarditis mortality rates ranging from 12 to 46%. In addition, infective endocarditis is the fourth leading cause of life-threatening infectious disease syndromes [1,6].

S. sanguinis has frequently reported as the most common isolate from the blood or heart valves of patients with endocarditis. In native-valve endocarditis, infecting bacteria adhere to "vegetations" composed of host components such as fibrin and platelets that form at sites with preexisting injuries. There is an indications that the production of glucans enhances attachment of streptococci to the endocardium, although many glucan-negative isolates [11,12].

Some streptococcal surface proteins play important roles in the adherence to and invasion of human tissues. These are usually proteins that recognize specific receptors, often sugars or oligosaccharides, expressed at various host body sites. Adhesins are protein surface that are linked directly to the cell surface or components of surface structures, e.g., pili projected away from the confines of the cell wall. The protein subunits of pili may themselves mediate adherence, or they may carry the adhesins along their lengths or at their tips. *S. sanguinis* possesses at least one adhesin that works through an fibrinogen (Fn)-independent pathway and is anchored to the cell surface along with SrtA. In addition, the loss of some proteins, catalyzed by SrtA, from the surface of *S. sanguinis* led to exposure of invasin molecules on the bacterial surface, thereby increasing the invasion activity of strain [2,3,7].

Surface proteins of Gram-positive bacteria are digested with sortase A (SrtA) at the recognized sequence, LPXTG, and become anchored to the cell

wall. Inactivation of the srtA gene in *Streptococcus gordonii* and *Streptococcus pneumoniae* has been reported to cause multiple defects in their pathogeneses. *S. sanguinis* possesses only a single sortase gene in its genome. The present findings showed that SrtA of *S. sanguinis* was catalyzed to anchor more than 6 surface proteins in the cell wall. It is also suggesting that *S. sanguinis* cells lacking SrtA activity are associated with virulence loss, which is mediated by cell surface proteins. In addition, the results of in vitro opsonization and bactericidal assays suggest that SrtA of *S. sanguinis* may link with one or more surface proteins related to antiopsonization, while it also plays an essential role in evasion from the human immune system. SrtA of *S. sanguinis* is an important molecule for infection and colonization, and may be a reasonable target to prevent bacterial infection and disease progression.[2,7]

Another findings indicate that housekeeping functions such as cell wall synthesis, amino acid and nucleic acid synthesis, and the ability of the bacteria to survive in anaerobic conditions are important virulence factors for *S. sanguinis* endocarditis. *S. sanguinis* mutant defective housekeeping genes showed reduction in virulence in rabbit model [11]. Several genes that involved in housekeeping e.g *thrB* gene encoding homoserine kinase involved in threonine -isoleucine synthesis, *purB* gene involved in purine biosynthesis, and *bacA* gene is also required for formation of the biofilm that occurs in infected "vegetation". The *bacA* gene product, undecaprenol kinase, is a membrane-bound isoprenol kinase that can phosphorylate cytoplasmic undecaprenol monophosphate, which overcomes the inhibition caused by bacitracin.[2,11]

DISCUSSION

Adhesin of *S. sanguiiss* as surface protein is anchored to the cell wall peptidoglycan through the LPxTz motif that will be digest by Sortase. The adhesin attach bacteria to the host receptor, which acts as a bridge between *S. sanguinis* and host cells. So, there is adhesion-receptor interaction between *S. sanguinis* and host cell. Then, the adherence forces will vast spectrum of proteins to the host environment. Complex arrays of molecules are present upon host surfaces, e.g., salivary pellicle and epithelia, etc., with which streptococcal cells interact. Therefore, adhesin-receptor interactions between Streptococcus and the host will be multiply mediated. Therefore, it is strongly suggested that cell wall anchoring by SrtA-dependent proteins containing the LPXTG motif is required for colonization of *S. sanguinis* and invasion as well. Given that most of the enzymes involved in these processes are not found in humans, those enzymes can be suggested for several possible new drug targets.

It has been explained that pili of oral *S. sanguinis* bind to salivary amylase, have been implicated in mediating adhesion to host cells and to extra cellular matrix. Pili is thought to facilitate the interaction with salivary α-amylase, involved in facilitating interbacterial coaggregation and this interaction promotes biofilm formation, thereby contributing to the colonization of *S. sanguinis* in the human oral cavity. Pili have also been implicated as putative virulence factors, because it have been associated with mediating adhesion to a wide variety of host epithelia including cells derived from the lungs, cervix, nasopharynx, tonsils, and intestine. As the protein subunits, pili have been shown to elicit protective immunity against the corresponding pathogen in mouse models of infection, making them potential vaccine candidates.

The initiation of oral biofilm development is caused by the adhesion of primary microorganisms, such as streptococci, to a salivary glycoprotein-coated surface. In addition to mediating cell attachment, Glucosyltranferase secreted will degrade host, and supply additional nutrients material contribute to a developing extra cellular matrix. In brief, the pioneer organisms provide a new surface and appropriate metabolic or other signals for the attachment of succeeding organisms in the biofilms.

Some salivary components have been examined for specific binding to oral bacteria, and salivary proline-rich proteins, agglutinins and α-amylase were shown to bind to oral streptococci. Regarding to α -amylase, the binding is reported to be associated with its maltose oligosaccharides-binding activity. Next, Gene cloning study have revealed that multiple surface proteins of oral streptococci contribute to bacterial adherence and binding to salivary components to form biofilms.

Furthermore, biofilm formation in saliva-coated plates was proximately 2-fold more efficient than that in noncoated plates. It is suggesting that saliva promoted the dental biofilm formation. In this regard, a number of genes relating to the biofilm formation have also been identified in oral streptococci, and they are reported to be associated with a variety of biological processes, including microbial adhesion, signal transduction and quorum sensing, carbohydrate metabolism, and exopolysaccharide biosynthesis.

Relationship between colonization by *S. sanguinis* and mutans streptococci. Colonization by *S. sanguinis* not only precedes that by mutans streptococci but, like that by mutans streptococci, is dependent on the presence of teeth. Infants with higher premutans streptococci proportions of *S. sanguinis* to total cultivable bacteria in saliva exhibited a significant delay in the time to colonization by mutans streptococci compared with infants who had lower proportions. Moreover, the time until *S. sanguinis* colonization significantly influences the time until mutans streptococci colonization, i.e., early *S. sanguinis* colonization correlates to late mutans streptococci colonization.

Endovascular infection is believed to occur when bacteria in the bloodstream adhere to damaged heart valves. Fibrin and platelets are deposited at the site of endothelial cell trauma, forming a sterile vegetation where bacteria may adhere and colonize during bacteremia. However, antibiotics cannot realistically be used to prevent such occurrences. Therefore, a vaccine would be a preferable prophylactic if one were available. It is a challenge to make a new vaccine for *S. sanguinis* based on its adhesion protein , sortase A, GtfB and GtfP, or genes that are correlate to assembly pili.

CONCLUSION

S sanguinis from a single cell/several cell in line deposited onto a surface in the human body, developed to a mixed-species community through quorum sensing and cell-cell interaction, and finally formed complex biofilm. It is naturally proved that *S sanguinis* like other oral streptococcus colonize of the tooth surfaces within communities of bacteria growing as biofilms and the integration of adhesins, receptors, signals, adaptation, and nutrition is needed in biofilm formation. In addition, it also involved cell-cell signaling, housekeeping gene that controlling nutritional adaptation, and host modulation as well.

As an opportunistic organism, The existence *S. sanguis* in oral cavity has to be aware. Ones bacteria enter the bloodstream, they can colonize endocardium or cardiac valves resulting in infective endocarditis. The better understanding in *S. Sanguis* genom has reveal some virulence factors. In terms of therapeutic or prophylactic agents to against *S. Sanguis*, some protein can be determinant to be candidates or provide epitopes for incorporation into new vaccines. For example vaccine for adhesion or sortase. We can also search compounds that can terminated regulator genes which responsible for biofilm formation, assemble for pili, or inactivated Gtf to suppress the number of *S. Sanguis* .

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