The Assessment of Metabolic Changes and Stress Response of *Streptococcus Mutans* Growth in Saliva by Fourier Transform Infra-Red

Dharli Syafriza[1]*, Ayudia Rifki[1], Vera Yulina[1], Basri A. Gani[2]

1. Department of Pediatric Dentistry, Dentistry Faculty, Universitas Syiah Kuala, Darussalam, Banda Aceh, Aceh Indonesia.
2. Department of Oral Biology, Dentistry Faculty, Universitas Syiah Kuala, Darussalam, Banda Aceh, Aceh, Indonesia.

Abstract

To examine the stress response and metabolic changes in S. mutans cells following contact with saliva that has been linked to growth and ionic characteristics.

The saliva and S. mutans isolate ATCC 25175 were employed in this work. A spectrophotometer was used to measure growth. The ionic changes in saliva were determined using an ionic meter. Meanwhile, FTIR was used to examine S. mutans' stress response and metabolic alterations (Fourier Transform Infra-Red).

At the 24 hours, salivary growth response was 12.5% against S. mutans (100%), 25% saliva (82%), saliva 6.25%, and CHX (97%). At the incubation time of 48 and 72 hours, 12.5% and 6.25% of saliva had a better growth response to S. mutans (81-87%) than other saliva concentrations. Salivary concentrations of 12.5% and 6.25% can reduce the stress response of S. mutans by 54% and 64%, respectively) from the stress response to the tolerance of S. mutans (71%). Saliva 6.25% affected the cellular metabolism of S. mutans (15%) compared to saliva concentrations of 50 (16%), 25 (17%), and 12.5 (18%). S. mutans cell affinity metabolism, which is influenced by saliva and environmental factors, causes acid challenge activity to reach 245%, metabolic changes (102%), amino acid and nucleotide (88%), protein (73%), nucleic acid (69%), carbohydrates (17%), and lipids (6%).

Saliva and its ionic properties can reduce and control the growth of S. mutans which is characterized by a decrease in the stress response of S. mutans , an increase in the growth response of saliva and cellular metabolism, and the metabolic affinity of S. mutans cells.

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Introduction

Streptococcus mutans (S. mutans) was the primary infectious agent responsible for dental caries infection, and it was imperative in the pathogenesis of early childhood caries (ECC). Due to its acidogenic and aciduric qualities, *S. mutans* is challenging to eradicate even after antibiotic treatment[1]. Additionally, the ability of *S. mutant* to develop quorum sensing and a rise in the spread of biofilms on the dental pellicle to support higher growth influenced the increase in *S. mutants* in caries infection[2].

Corresponding author:
Dharli Syafriza,
Department of Pediatric Dentistry, Dentistry Faculty,
Universitas Syiah Kuala, Darussalam, Banda Aceh, Aceh
Indonesia.
E-mail: dharli.usk@gmail.com

As a commensal, *S. mutans* acts as a biological control against the development of other pathogens in the oral cavity. However, changes in the oral environment cause these bacteria to be pathogenic and increase their communication activity with other bacteria to form quorum sensing as an initiator of biofilm formation, adhesion, and invasion on the surface of the dental pellicle, causing dental caries[3]. Saliva can act as a barrier to the oral cavity because it contains several essential proteins to control the development of oral pathogens like proline-rich proteins, histatins, mucins, and sIgA. Saliva can act as a barrier to the oral cavity because it contains many essential proteins to control the development of oral pathogens[4].

The effect of the interaction of salivary proteins with bacterial protein-binding ligands can suppress or control their development, including *S. mutans*, if the growth is not balanced. The exchange of salivary proteins can increase the

stress response by bacterial cells. This increase in stress tends to cause changes in cell metabolism for nutrient intake and disrupt communication between oral pathogens[5]. Ray (1999) reported that the 65-kDa fimbrial protein of *S. mutans* was recognized by salivary amylase[6]. In contrast, the sIgA was able to bind to the glucan binding protein of *S. mutans* plays a role in biofilm formation[7].

Numerous approaches for reading stress response activity or changes in bacterial cell metabolism have been introduced, including cellular and molecular methods. However, this procedure is costly and necessitates complex results reading. Helm and Naumann pioneered Fourier Transform Infrared (FTIR) to detect biofilm cell components in 1995[8]. FTIR is a beneficial instrument for detecting cellular components, including the bacterial life cycle, because physicochemical considerations play a critical role in microbial cell communication and signaling[9]. Additionally, because it is based on variations in transmission associated with each molecule's absorption intensity, FTIR can detect signals of bacteria's stress response or metabolic changes (cm-1)[10].

Attenuated Total Reflectance (ATR) mode spectroscopy can be used to test bacterial stress response analyses. This methodology utilizes more rapid samples and requires little or no sample preprocessing. Additionally, ATR accessories cut analysis costs and give all infrared sampling techniques the highest data quality and reproducibility [11, 12]. The results of the sample vibration spectrum measurements are utilized to calculate the signal ratios of protein peaks and compound functional groups [13]. Chemicals and functional groups classified according to their absorption class are the determinants of the type of compounds that exhibit the severity of the *S. mutans* bacteria's stress response when exposed to saliva.

The ability to enhance the stress response or alter the metabolism of *S. mutans* cells may serve as a proxy for various salivary defense proteins in regulating *S. mutans* development throughout the pathogenesis of dental caries infection. This study evaluated the stress response, *S. mutans* metabolism, and affinity metabolism following adaptation in saliva, all connected with bacterial growth and saliva's ionic characteristics during transformation to *S. mutans*. The results of this study will be used to determine the degree of stress response and metabolism of *S. mutans* cells in a minimal intervention program designed to eradicate ECC and control the population as oral commensals. Additionally, we evaluated the efficacy of FTIR technology for microbiological analysis.

Materials and methods

The study was conducted in vitro with saliva, and *S. mutans* isolate ATCC 25175 was collected from the Oral Biology laboratory at Syiah Kuala University, Darussalam, Banda Aceh. Saliva was used to characterize *S. mutans'* stress response, cellular metabolism, and metabolic affinity.

Saliva preparation and culture of *Streptococcus mutans*

The saliva was dissolved in 1% phenylmethylsulfonyl fluoride (Sigma Aldrich, Darmstadt, Germany). Additionally, saliva was concentrated to 50%, 25%, 12.5%, and 6.25%. Saliva was used to determine its effect on the metabolic activity of *S. mutans* cells and the growth and ionic activity of saliva when infected with *S. mutans* . The *S. mutans* ATCC 25175 bacteria were isolated from a 50% glycerol stock and re-cultured on Tryptic soy broth (TSB) media (Merck KGaA, Darmstadt, Germany). Then Mc. Farlan 0.5 was substituted ($1.5x10^8$).

Assay of *Streptococcus mutans* Growth

The growth of *S. mutans* was determined using spectrophotometry. Saliva was prepared at concentrations of 50%, 25%, 12.5%, and 6.25%, using 0.2 percent chlorhexidine (CHX) as a positive control. Each well in the 96-well plate was put 50 L of TSB medium and incubated for 15 min. Then thrice rinsed with PBS pH 7.0. Additionally, it was placed into a well containing 25 l of *S. mutans* in medium and incubated for 15 min at 27 ^{0}C, followed by the addition of saliva at a preset concentration into a well containing 100 l. (1:4). The cells were then incubated for 24 h, 48 h, and 72 h in an aerobic environment. *S. mutans* growth was quantified using spectrophotometry-Elisa Reader (Bio-Rad, USA) with an optical density (OD) of 620 nm. The optical density of 0.08-0.1 nm is identical to that of Mc. Farland. 0.5 (1.5x108) or 300 CFU [14].

Extraction of *Streptococcus mutans* whole-cell

The *S. mutans* equivalent to Mc Farlan 0.5 (300 CFU/mL) were introduced at various doses to saliva. Additionally, adaptation was performed for 15 min at room temperature at a speed of 200 xg. The whole-cell extraction procedure for *S. mutans* was modified somewhat from He (2009)[15]. Bacterial homogenization using a saliva check based on turbidity changes. Additionally, these mixes were cultured in anaerobic conditions for 48 h. Centrifugation for 5 min at 7000 xg was used to initiate the entire cell extraction procedure of *S. mutans* (separation of *S. mutans* cells in saliva). After collecting the pellet (*S. mutans* cells), it was vortexed for 30 sec, and 0.1 M HCL (200 mL in 1 mL) was added. After another 30 sec of vortexing, it was incubated at 4 °C for 15 min. After centrifuging at 7000 xg for 15 min and collecting the residue, 500 mL PBS was added and vortexed for 30 sec before centrifuging at 500 xg for 10 min. The residue is then washed with 70% ethanol (1:3) and centrifuged at 7000 xg for 15 min. Additionally, the precipitate was collected entirely as an extract (surface protein and inner protein). Following that, a 2 mL solution of sample buffer was added. The FTIR equipment was then used to evaluate the stress response of *S. mutans* in the presence of saliva.

FTIR Assay for assessment of cell metabolism of *Streptococcus mutans*

The FTIR analysis of *S. mutans'* stress response under the impact of saliva was performed using functional groups contained in the sample. The FTIR-ATR technique generates transmittance spectra with a wavenumber of 4000 cm^{-1} - 400 cm^{-1}. The first stage involves placing a sample of *S. mutans* on the surface of a clear IR prism with a refractive index that is always greater than the sample (the refractive index of bacterial cells is estimated at 1.39). The radiation beam is directed at the prism walls for the prism-sample interface at an angle greater than the barrier. Under these conditions, complete reflection occurs on the internal prism's side. The reflected light emerges through the walls of the second prism, which records the light's intensity and absorption spectrum[16]. The ATR-FTIR (cm-1) model was utilized to determine the stress response of *S. mutans* in the presence of saliva.

The assessment of bacterial stress response is based on the FTIR analysis described in Zarnowiec's paper (2015). Wl (windows I) – lipids (3,000-2,800 cm-1), W2- (Windows 2) – proteins (1,700-1,500 cm-1), W3 (Windows 3) – nucleic acids (1,500-1200 cm-1), W4 (Windows 4) – carbs (1200-900 cm-1), and W5 (Windows 5) – carbohydrates (1200-900 cm-1) (900-700)[17]. Additionally, the metabolic affinity of *S. mutans* cells for acid challenges (600-500 cm-1, Window 6) and metabolic alterations was determined (600-500, window 7).

Statistical Analysis

Kruskal Wallis analysis was used to determine the growth rate of *S. mutans* and the ionic activity of saliva, with a significance level of $p<0.05$ and $r=1$, indicating a strong connection between variables. Meanwhile, variations in *S. mutans* cell metabolism were studied descriptively using the percentage value of each cell's metabolic activity, including stress response, cellular metabolism, and affinity metabolism.

Results

The findings of this study included data on *S. mutans* growth under the influence of saliva. Spectrophotometry was used to investigate the growth data of *S. mutans* in response to changes in the incubation period. The ionic value produced by saliva during the interaction with *S. mutans* further validated the growth value of *S. mutans*. These two values were found to correlate with the intensity of the stress response and alterations in the metabolism of *S. mutans* based on changes in the functional groups of lipids, proteins, nucleic acids, carbohydrates, and fingerprint areas.

Saliva's capacity to control the growth of *S. mutans* is illustrated in Figure 1. After 24, 48, and 72 hours of incubation, *S. mutans* overgrew with an average OD of 0.171 nm (minimum 0.002 and maximum 0.406). According to the Mc Farland Standard, it is 300 CFU or Mc. Farland 0.5. (1.5x108). Optical Density 0.05 nm (<150 CFU/mL), 0,08-0,1 nm (Mc Farlan 0,5; <300 CFU), OD 0,11-0,29 nm (Mc Farland 1; 300-600 CFU); OD 0,3-0,49 nm (Mc Farland 2; 600-1200 CFU). These scales were adopted by Mc Farland Standard for in vitro use only, Catalogue No. TM50-TM60, Scott Sutton, Measurement of Microbial Cells by Optical Density, 2011) [14].

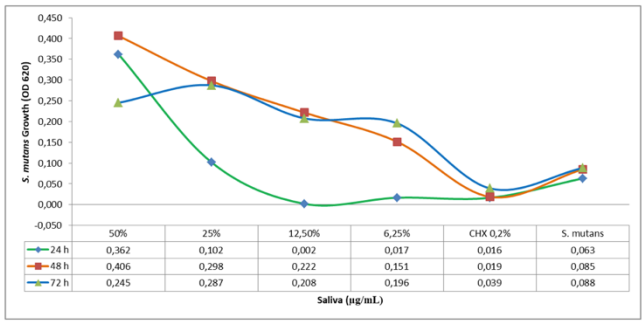

Figure 1. Depicts the growth of *S. mutans*. Saliva at various concentrations could control the growth of *S. mutans* , particularly after 24 hours of incubation, starting at 25, 12.5, and 6.25 percents. The data was collected three times in a row.

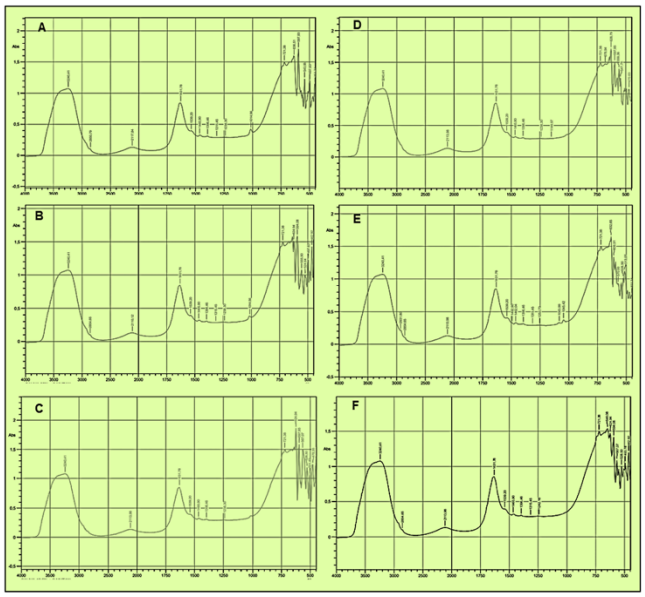

Figure 2. FTIR graph pattern of *S. mutans* cell activity after being influenced by saliva at various concentrations. Frequency range 4000-500 cm-1. (A) *S. mutans* (saliva 50%), (B) *S. mutans* (25% saliva), (C) *S. mutans* (12.5% saliva), (D) *S. mutans* (saliva 6.25%), (E) *S. mutans* (CHX 0.2%), and (F) *S. mutans* ATCC 25175. Y-axis (absorbance value) and X-axis (Wavenumbers; cm-1).

The investigation results in Figure 1 are consistent with Table 1, where a salivary concentration of 12.5%represented only 0.4% of *S. mutans* in the category of 100% salivary growth response after 24 h of incubation. While a saliva concentration of 25% induced an 82% growth response, *S. mutans* grew at an 18% rate. Then comes the 6.25% concentration and CHX. At 48 and 72 hours of incubation, salivary concentrations of 12.5% and 6.25 percent grew *S. mutans* better than other saliva concentrations, but increased CHX grew *S. mutans* (98.42% and 96.33%). According to Kruskal Wallis analysis, there was no significant difference in the development of *S. mutans* in saliva concentrations depending on incubation period ($p>0.05$; 0.273) or concentration ($p>0.05$; 0.065). Both have a positive correlation with *S. mutans* development at varied saliva concentrations.

Figure 2 shown the representative IR spectrum showing peak assignments from 4,000-450 cm-1, where v = stretching vibrations, σ = bending vibrations. The presented spectrum is an attenuated total reflection-type-spectrum (ATR-FTIR) from *S. mutans* ATCC 25175. According to the peak profile of FTRI, Figure E (*S. mutans* in CHX 0.2 percent) has changed peaks, indicating an increase in *S. mutans'* stress response (Table 2). According to Table 2, *S. mutans* exposed to CHX (chlorhexidine) exhibit a strong stress response in comparison to the effect of saliva content. Meanwhile, *S. mutans* has a 71% tolerance for environmental stress (test material). Saliva at any concentration can attenuate the stress response of *S. mutans* to less than 71%. It is indicated that saliva can control the stress response to environmental changes caused by cell metabolism. The formula for calculating the stress response is as follows: stress response=100% - (percent area+percent intensity).

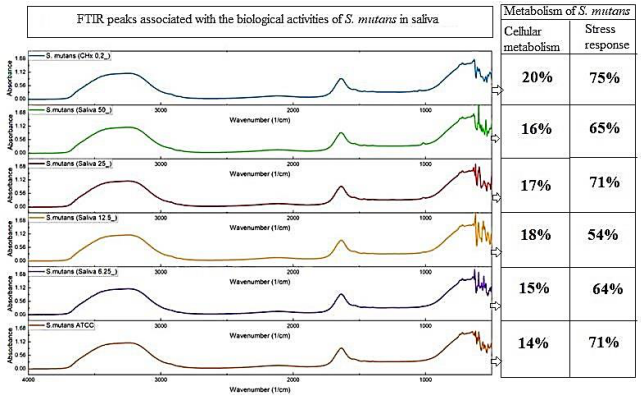

Figure 3. FTIR was performed of the biological activities of *S. mutans* in saliva (4000-450 Wavenumbers; cm-1). FTIR data can be described as the metabolism of *S. mutans* during interaction with saliva and CHX, which is indicated by changes in cellular metabolism with the value of cellular metabolism of *S. mutans* as a control change.

The value of cellular metabolism is calculated in Figure 3 using the total absolute size of the molecular absorbance intensity of *S. mutans'* activity product with each test material at a wavenumber of 4000-450. (cm^{-1}). CHX, used as a positive control, possesses antimicrobial characteristics. This antibacterial potential is demonstrated by the significant stress response (75%) induced by 20% of *S. mutans* cellular metabolic activity. Meanwhile, saliva, in addition to lowering *S. mutans'* stress response, tolerates its cellular metabolic activity (below the CHX value for antibacterial activity), allowing *S. mutans* to continue functioning as a commensal in the oral cavity and saliva acting as a barrier to *S. mutans'* growth balance. In principle, it has been demonstrated using FTIR data that *S. mutans* has a stress response tolerance limit of up to 71%, with alterations in cellular metabolism reaching 14%. The remainder may pose a hazard to the growth of *S. mutans* .

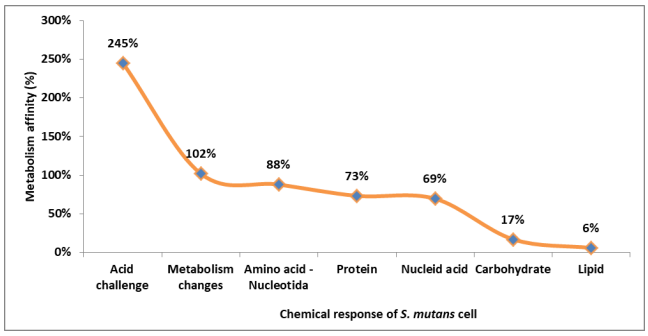

Figure 4. Cell affinity metabolism of *S. mutans* under the influence of saliva. The acid challenge gave the highest contribution to *S. mutans* cells to increase acid tolerance and cell metabolism. Data were taken from the total of each analysis window based on the absorption value (cm-1) of each wavelength as an indicator of the chemical response of *S. mutans* cells.

According to Figure 4, the first thing *S. mutans* did after being exposed to saliva was the acid challenge-response, which resulted in alterations in cell metabolism, including amino acid and nucleotide activity, particularly RNA activity and DNA activity in protein synthesis. Carbohydrates and lipids are used to synthesize nucleic acids.

Table 3 illustrates the affinity metabolism of *S. mutans* upon exposure to saliva. The wavelength of the absorbed molecule intensity was determined due to the biological activity between saliva and *S. mutans* (value in cm-1 units). The assessment of affinity metabolism is based on the wavelength range 4000-450, which is separated into seven windows. Window 1, lipids (3000-2800), window 2, protein (1700-1500), window 3, nucleic acid (500-1200), window 4, carbs (1200-900), window 5, fingerprint region/amino acid (900-700), window 6, fingerprint region (acid challenges) (600-500), and window 7, metabolism changes (500-450). In general, the acid challenge affinity dominates the metabolic relationship of *S. mutans* cells (245%) with changes in cell metabolism (102%) and the activity of amino acids and nucleotides (88%) as well as nucleic acids (69%) and proteins (73%) followed by with carbide fermentation (17%) and fatty acid synthesis only (6%).

Discussion

The FTIR technique was used to determine the cellular activity of *S. mutans* , which was corroborated by the development of *S. mutans* in saliva. FTIR spectroscopy can effectively detect biospecific interactions of the type "target molecule-recognition molecule." This experiment evaluated the viability of FTIR spectroscopy supplementing analytical methods used in biology, specifically microbiology. The stress response, cellular metabolism, and affinity metabolism of *S. mutans* cells were all examined during interaction with saliva (activity of lipids, proteins, nucleic acids, carbohydrates, amino acids and nucleotides, acid challenges, and metabolic changes).

The findings of this study indicate that saliva has a role in regulating the metabolism of *S. mutans* cells, as evidenced by a decrease in *S. mutans* growth in saliva, which is a characteristic in response to *S. mutans*. Additionally, this study discovered that saliva could act on the cellular level by preventing *S. mutans* from exhibiting an excessive stress response by enhancing cellular metabolic activity. Additionally, saliva maintains the metabolic affinity of *S. mutans* cells throughout the environmental adaptation[18].

As shown in Figure 1 and Table 1, the study's results indicate that saliva had a favorable effect on *S. mutans* growth, with an average decrease in the development of *S. mutans* (100 -36 percent) at various saliva concentrations. It is suggested that in addition to acting as a barrier for oral mucosal defense, it

also contributes to the oral biological balance by inhibiting the growth of *S. mutans* as an oral commensal. Additionally, the results indicated that the growth limit of *S. mutans* remained within the usual range (300 CFU/mL) but rose at 48 and 72 hours of incubation. This rise can be attributed to a decline in protecting several active components of saliva (salivary defense proteins) between 48 and 72 hours[19].

Saliva has been identified as a medium for *S. mutans* to convert and cling to tooth surfaces. The *S. mutans* utilize several sugars in the tooth pellicle produced from saliva to form quorum sensing and biofilm mass[20]. In addition, the decreased growth of *S. mutans* during the 24-hour phase by salivary activity correlated with the content of several active proteins, such as proline-rich protein and salivary sIgA, which controlled the excessive growth of *S. mutans* [21]. However, other salivary components such as salivary peroxidase and catalase may also be possible[22]. Additionally, saliva can act as a growth inhibitor for *S. mutans* by reducing the bacteria's ability to synthesize glucan from sucrose, preventing colonization of the tooth pellicle and interfering with the bacteria's acidogenic and aciduric capabilities[23]. Thus, the saliva utilized in this study can indirectly keep the growth of *S. mutans* within the limits of the oral cavity's natural flora.

Figure 2 depicts a representative IR spectrum from *S. mutans* ATCC 25175, with peak assignments ranging from 4,000 to 450 cm-1. The FTRI peak profile reveals a change in peaks as an indicator of the metabolic response of *S. mutans* cells. Table 3 also showed that saliva at all concentrations could reduce *S. mutans'* stress response to less than 71%. As a result, saliva is thought to regulate *S. mutans'* stress response to environmental changes related to cell metabolism (Figure 3). Saliva can tolerate *S. mutans'* cellular metabolic activity to perform its function as an oral cavity commensal, and saliva acts as a barrier to balance *S. mutants* growth.

According to Baker (2017), carbohydrate fermentation influences *S. mutans* stress tolerance by producing acid accumulates in the biofilm. Bacterial stress is a change in conditions that causes bacteria to deviate from their ideal growth rate [24]. In line with the cellular metabolic activity of *S. mutans* cells, the stress response facilitated by saliva against *S. mutans* provides growth restriction to prevent stress frequency by limiting acid formation to increase tolerance to the acid produced. This action represents a continuum of stress tolerance in *S. mutans* during adaptation to saliva growth.

Figure 4 shows that after being influenced by saliva, the acid challenge is the essential metabolic affinity for *S. mutans* . This response suggests that S. mutans produce an acidic pH that functions and protects and influences the environment's adaptation response as lactic acid bacteria. Furthermore, *S. mutans'* acid challenge breaks down carbohydrates into simple sugars, which are required as a source of nutrition and cell energy. Streptococcus mutans is widely regarded as the primary causative agent of dental caries; the bacterium's ability to produce acid from fermented sugars (acidogenicity) is a significant virulence factor in the etiology of dental caries development [25]. The *S. mutans'* acidogenic and aciduric properties have been identified as one of the virulence factors contributing to the pathogenicity of dental caries infection[26]. Matsui (2010) reported that *S. mutans* perform stress tolerance responses by increasing organic acids to increase the formation of quorum sensing to survive. Furthermore, *S. mutans'* stress tolerance is associated with sensitivity to acid challenges[27]. Changes in the phospholipid cardiolipin's fatty acid composition have also been identified as essential to acid tolerance[28].

Streptococcus mutans has been described as an acid-producing bacterium that can survive in highly acidic environments (4-5)[29]. This phenomenon is demonstrated when saliva and antibacterial agents interact. The FTIR data (Table 4) correlated with the cell metabolism theory and principle of *S. mutans* as a biofilm-forming bacterium. The data in Table 4 are described in detail for the metabolic affinity of *S. mutans* cells during saliva adaptation. During the adaptation phase with saliva, lipid affinity (Window 1) had the same value between *S. mutans* under the influence of 50%, 25%, and *S. mutans* (control) which was 1%. Meanwhile, CHX caused 3 percent damage to semi-layer lipids on the surface of *S. mutans* cells. Streptococcus mutans produced a 14 percent active (virulent) protein response; however, all saliva concentrations tested in this study were able to reduce the activity of *S. mutans'* functional (virulent) protein below 14 percent;

even CHX was able to reduce up to 4 percent of the total 14 percent virulent protein of *S. mutans* (window 2).

Saliva can regulate the nucleic acid of *S. mutans* cell (window 3). It has to prevent *S. mutans* from growing, transmitting, and storing genetic information. Thus, it is possible to maintain intermediary metabolism and information exchange for energy and other biomolecular components and be involved in the oxidation and reduction of cell activities[30]. Data from Windows 1 and 2 demonstrated that protein synthesis and nucleic acid activity are consistent with the environment's response (14% protein synthesis and 14% nucleic acid/DNA activity).

The *S. mutans* do not respond to carbohydrate synthesis activity, as demonstrated in window 4. Saliva is thought to inhibit the activity of glucokinase, fucosyltransferase, and sucrose, hence avoiding the creation of acid (acid challenge). Salivary concentrations of 50%, 25%, and 6.25% elicited responses of between 3% and 4%, while CHX elicited a response of 6%. *S. mutans ,* as a lactic acid bacterium (LAB), generate energy primarily by glycolysis. This organism is unique in its ability to digest various carbohydrates. All carbohydrates produced by *S. mutans* biological activity have developed many pathways for sucrose catabolization to create acid, which aids in biofilm deposition via cellular adhesion to tooth surfaces[31].

The observations in window 3 are consistent with those in window 5, where saliva can lower the transcription and translation of amino acids and nucleotides in protein synthesis activity to less than 17%, on average, between 16% and 13%. In comparison, CHX reached 12% (in this investigation, *S. mutans* (17%) demonstrated amino acid and nucleotide activity in response to the environment without being given antibacterial. Peng (2016) showed that *S. mutans* possess the nucleotides pGpp and c-di-AMP, engaged in inter-bacterial competence signaling and modulating biofilm formation and stress response[32]. Increased c-di-AMP levels stimulate the production of biofilms by interaction with the VicRK-gtfB network, which activates transcriptional gtfB[33].

The results from window 4 are consistent with those from window 6, indicating that saliva can control *S. mutans'* acid challenges. The *S. mutans* were able to diminish carbohydrate fermentation as a source of nutrition during adaptation to saliva, with the average acid challenge activity of *S. mutans* being 40%. At 50% saliva, acid challenges activity reduced to 30%, while at 25% saliva, it decreased to 34%. Meanwhile, 12.5% and 6.25 percent of saliva concentrations included CHX, which did not influence *S. mutans'* acid challenges. The acid challenges response of *S. mutans* is required for communication between oral bacteria to build biofilms that infiltrate the tooth surface during the etiology of caries. According to Syafriza (2020), the production of *S. mutans* biofilms is always regulated by environmental factors such as pH and temperature. In general, saliva has an excellent reaction to changes in pH following contact with *S. mutans*[19].

Window 7 demonstrates that metabolic alterations in *S. mutans* metabolism decreased due to 6.25 percent saliva, similar to CHX (affinity 12%). Meanwhile, saliva concentrations of 50%, 25%, and 12.5% increased the metabolism of *S. mutans* cells. This increase in metabolism can be attributed to *S. mutants* acid challenge-response during adaptation to environmental changes generated by saliva. Numerous salivary proteins, including proline-rich protein, lysozyme, lactoferrin, lactoperoxidase, immunoglobulins, agglutinins, and mucins, regulate oral microbial metabolism to maintain oral biological equilibrium[34]. The acid challenge-response of *S. mutans* cells responds to changes in cell metabolism to keep cells alive in response to various environmental effects, most notably antibacterial drugs[35].

Saliva has an effect on the development of *S. mutans* via affinity metabolism. Saliva stimulates *S. mutans'* acid challenge activity, followed by metabolic changes in response to the action of amino acids and proteins required for cell adaptation to the environment. Cells synthesize proteins on a molecular level, assisted by nucleotides and nucleic acids. The *S. mutans* cells produce carbohydrates and fatty acids to adapt to acidic conditions encountered during interaction with saliva.

Conclusions

Saliva inhibits and controls *S. mutans* growth during interactions lasting 24 hours, 48 hours, and 72 hours. Saliva can help alleviate stress responses of *S. mutans* by enhancing cellular metabolism and regulating changes in

lipid metabolism, glucose metabolism, protein metabolism, amino acid metabolism, DNA (nucleotides and nucleic acids), and acid challenges. The acid challenges the response of *S. mutans* cells in the metabolic pathway with the highest propensity for responding to saliva's biological activity.

Declaration of Interest

We declare no conflict of interest.

S. mutans in saliva (%)	N	24 h				48 h				72 h			
		OD	S.Dev	Freq	Growth Response	OD	S.Dev	Freq	Growth Response	OD	S.Dev	Freq	Growth Response
50%	3	0,362	0,272	64%	36%	0,406	0,180	34%	65,59%	0,245	0,136	23%	76,93%
25%	3	0,102	0,024	18%	82%	0,298	0,019	25%	74,80%	0,287	0,070	27%	72,98%
12,5%	3	0,002	0,006	0,4%	100%	0,222	0,068	19%	81,23%	0,208	0,017	20%	80,47%
6,25%	3	0,017	0,027	3%	97%	0,151	0,033	13%	87,19%	0,196	0,036	18%	81,60%
CHX 0,2%	3	0,016	0,007	3%	97%	0,019	0,009	2%	98,42%	0,039	0,001	4%	96,33%

Table 1. Distribution and growth frequency of *S. mutans* under the influence of saliva.

S. mutans	Freq Range (cm-1)	Bands	Intensity			Area			Stress Respon	Comments
			Value	Corr	Freq	Value	Corr	Freq		
S.mutans (saliva 50%)	3240.41-451.34	16	12,56	0,166	16%	722	6,46	19%	**65%**	Decrease
S.mutans (saliva 25%)	3240,41-462,92	17	12,39	0,197	16%	489	9,84	13%	**71%**	Standard
S.mutans (saliva 12.5%)	3240,41-470,63	15	14,56	0,297	19%	1.028	10,25	27%	**54%**	Decrease
S.mutans (saliva 6.25%)	3240,41-470,63	15	13,59	0,156	17%	721	6,26	19%	**64%**	Decrease
S.mutans (CHX 0.2%)	3240,41-451,34	20	11,84	0,103	15%	381	6,59	10%	**75%**	Increase
S.mutans	3240,41-462,92	17	12,95	0,143	17%	463	5,96	12%	**71%**	Standard

Table 2. Intensity and area of FTIR spectrum for identified the stress response activities of *S. mutans* that influenced by functional saliva (Frequency range 4000-400 cm-1).

Areal	Absorption (cm-1)	Types	Group	Compound Class	The affinity of cell metabolism of *S. mutans* (%)					
					S. mutans (50% Saliva)	*S. mutans* (25% Saliva)	*S. mutans* (12.5% Saliva)	*S. mutans* (6.25% Saliva)	*S. mutans* (0.2% CHX)	*S. mutans* (medium)
Window 1	3.000-2.800	Lipid	N-H stretching	Amine salt	0.1 (1%)	0.1 (1%)	-	-	0.32 (3%)	0.1 (1%)
Window 2	1.700-1.500	Protein	C=O stretching	Amine II	1.24 (13%	1.24 (13%)	1.25 (11%)	1.25 (14%)	1.26 (10%)	1.25 (14%)
Window 3	1.500-1200	Nucleic acid	C-H bending	Amine III	1.33 (13%)	1.21 (11%)	0.93 (8%)	0.92 (10%	1.56 (13%)	1.21 (14%)
Window 4	1200-900	Carbohydrate	C-O stretching	Amine IV	0.42 (4%)	0.39 (4%)	-	0.30 (3%)	0.71 (6%)	-
Window 5	900-700	Finger print regions/amino acid and nucleotides	C-H bending	1,2,4-trisubstituted	1,5 (15%)	1.50 (14%)	1.50 (13%)	1.49 (16%)	1.50 (12%)	1.5 (17%)
Window 6	600-500	Finger print regions/ Acid Challenges	C-I stretching	Halo compound	2.94 (30%)	3.66 (34%)	5.32 (46%)	4.14 (45%)	6.05 (50%)	3.5 (40%)
Window 7	500-400	Metabolism changes	None	None	2.35 (24%)	2.65 (25%)	2.50 (22%)	1.08 (12%)	1.08 (12%)	1.2 (14%)

Table 3. The metabolic affinity of *S. mutans* under the influence of saliva by cluster window (1-7) FTIR.

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