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Research Report

The pH changes of artificial saliva after interaction with oral micropathogen

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ABSTRACT

Backgorund: *Saliva contains several protein elements, exocrine proteins and antibodies, such as lactoferrin, sIgA, peroxidase, albumin, polypeptides, and oligopeptides that contribute to the defense of oral mucosa and dental pellicle to prevent infection caused by oral micropathogen, such as Candida albicans, Streptococcus mutans and Aggregatibacter actinomycetemcomitans (A. actinomycetemcomitans). Those micropathogens have a role to change salivary pH as an indicator of oral disease activities.* ***Purpose:*** *This study was aimed to analyze the changes of artificial saliva pH after interaction with S. mutans, C. albicans, and A. Actinomycetemcpmitans.* ***Methods:*** *The materials used in this study consist of S. mutans (ATCC 31987), C. albicans (ATCC 10231), A. actinomycetemcomitans (ATTC 702 358), and artificial saliva. To examine the pH changes of artificial saliva, those three microbiotas were cultured and incubated for 24 hours.* ***Results:*** *The results showed that the interactions of S. mutans, C. albicans, and A. actinomycetemcomitans in the artificial saliva can change the salivary on neutral. There were no significant difference with the control treatment salivary pH 4, 5, 6, 8, and 9 ($p>0.05$). Similarly, there was also no significant difference when those three microorganism interacted each other in the artificial saliva ($p<0.05$).* ***Conclusion:*** *It can be concluded that the biological activity of S. mutans, C. albicans, and A. actinomycetemcomitans in artificial saliva can change the salivary pH into neutral. It indicates that those microbiotas mutually supported and cooperated in influencing the biological cycle of the oral cavity with salivary pH as an indicator.*

Key words: *Salivary pH, Candida albicans, Streptococcus mutans, Aggregatibacter actinomycetemcomitans*

ABSTRAK

Latar belakang: *Saliva merupakan cairan eksokrin yang mengandung unsur protein dan antibodi seperti sIgA laktoferin peroksidase, albumin, polipeptida dan oligopeptida yang berperan pada pertahanan mukosa rongga mulut dan gigi guna mencegah infeksi oral mikropatogen seperti C. albicans, S. mutans, dan A. actinomycetemcpmitans. Patogenesis ketiga oral mikropatogen tersebut diawali dengan mempengaruhi perubahan pH saliva sebagai langkah invasi dan infeksi pada mukosa oral dan pelikel gigi.* ***Tujuan:*** *Penelitian ini bertujuan untuk untuk mengetahui perubahan pH saliva buatan setelah diinteraksikan dengan S. mutans, C. albicans, dan A. Actinomycetemcpmitans.* ***Metode:*** *Materi penelitian ini berupa Streptococcus mutans strain ATCC 31987, Candida albicans strain ATCC 10231, Aggregatibacter actinomycetemcomitans strain ATTC 702358, dan saliva buatan. Untuk mengetahui perubahan pH saliva, maka ketiga mikrobiota tersebut dikultur dan untuk menguji perubahan pH saliva dilakukan uji interaksi ketiga mikroorganisme tersebut dalam saliva buatan selama 24 jam dengan pengaturan pH saliva sebagai indikator hasil penelitian.* ***Hasil:*** *Hasil penelitian menunjukkan interaksi S. mutans, C. albicans, dan A. actinomycetemcomitans dalam saliva buatan mampu mereduksi perubahan pH saliva mengarah ke pH netral dengan kontrol perlakuan pH saliva 4, 5, 6, 8, dan pH 9 secara statistik tidak tidak menunjukkan perbedaan bermakna ($p>0,05$), begitu juga ketika dilakukan interakasi diantara masing-masing mikroorganisme tersebut dalam saliva buatan menunjukkan adanya perbedaan bermakna ($p<0,05$).* ***Kesimpulan:*** *Dapat disimpulkan bahwa aktivitas biologi S.*

mutans, C. albicans, dan A. actinomycetemcomitans dalam saliva buatan mampu merubah pH Saliva sekaligus mempertahankan pH netral. Hal ini menggambarkan bahwa mikrobiota tersebut saling mendukung dan bekerjasama dalam mempengaruhi siklus biologi rongga mulut dengan pH saliva sebagai indikator.

Kata kunci: *pH Saliva, Candida albicans, Streptococcus mutans, Aggregatibacter actinomycetemcomitans*

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INTRODUCTION

As pathogens in oral cavity, *Streptococcus mutans* (*S. mutans*) and *Aggregatibacter actinomycetemcomitans* (*A. actinomycetemcomitans*) are considered as the cause of dental caries and periodontitis.[1,2] Although infection caused by them is different, but it is known that both of these bacteria support each other in pathogenic process, especially in biofilm formation of dental caries infection and periodontitis.[3] These two micropathogens also play an important role on the activity of moleculer fungus, *Candida albicans* (*C. albicans*), as an agent of oral candidiasis infection. In alkaline condition, those two bacteria can facilitate *C. albicans* to form biofilms on oral mucosa,[4,5] on the other hand, in acidic condition, *C. albicans* is precisely able to adapt to changes in the acidic environment of the oral cavity, that is affected by salivary pH.[6] It means that the salivary pH can determine the pathogenicity of those micropathogens.

Saliva is exocrine fluid containing water, approximately about 99%. Other supporting elements consist of an organic component involving sodium, calcium, potassium, magnesium, bicarbonate, chloride, rodanida and thiocynate (CNS), phosphate, potassium, and nitrate; meanwhile, inorganic components consist of amylase, peroxidase, maltase, albumin protein, kretinin, mucin, vitamin C, amino acids, lysozyme, lactic acid, and hormones, such as testosterone and salivary cortisol; besides, there are also sIgA antibodies, lactoferrin, polypeptides and oligopeptides that contribute to oral mucosal defense and dental pellicle.[7, 8]

The degree of acidity (pH) of saliva is affected by diet, stimulation of salivary secretion, and activity of oral microorganisms. Diet with high carbohydrate can either lead to a decrease in salivary pH, accelerate the demineralization of tooth enamel, or produce acid through glycolysis process that can lower the salivary pH into the critical one (5.5 to 5.2).[9] In contrast, the alkaline properties of saliva can either neutralize the acidity of the mouth, reduce tooth decay, prevent the formation of plaque and calculus, or reduce the risk of periodontitis. Furthermore, calcium contained in saliva may play a role in tooth enamel remineralization.[10] The changes of the biological properties of the saliva that are likely to affect oral biological abnormalities, such as xerostomia are caused by the imbalance of salivary pH regulation.[11] In addition, the critical pH of saliva is also worsened by carbohydrate fermentation activities triggered by a number of pathogenic oral microorganisms, such as *C. albicans, S. mutans*, and *A. actinomycetemcomitans*.[12]

The changes of the salivary pH in oral soft tissues are often associated with dental caries, periodontitis, and oral candidiasis. The changes of the pH of acidic and alkaline saliva can trigger saliva viscosity and facilitate the fermentation of carbohydrates and salivary proteins that can cause the imbalance of the growth of those three micropathogens[11] since *S. mutans* can survive only at the critical pH of 4.5-5.0,[1] *C. albicans* can grow only at the pH of 4.5-6.5,[13] while *A. Actinomycetemcomitans* can only grow better at the pH of 7-8.5.[2]

In addition to being considered as pathogens in the oral cavity infections, those three microorganisms also contribute to endocarditis infection.[14] Antibacterial medicines can either accelerate the growth of fungi or provide the threat of chronic candidiasis oral infections. Thus, the pH of the oral cavity must be adjusted with salivary pH as an indicator and supporter of biological activity in oral cavity preventing the virulence factors of those three micropathogens from biofilm formation, colonization, invasion and infection of the host.

Therefore, saliva specifically becomes the controler of pathogenic microorganism activities in oral cavity through salivary pH regulation in order to maintain the pH balance of the oral cavity. As a result, this study was aimed to analyze the changes of salivary pH (acidic and alkaline) after interaction with *C. albicans, S. mutans,* and *A. actinomycetemcomitans* as well as after those interaction with each other. Finally, the results of this study are expected to be able to prevent invasion and infection caused by oral micropathogens, mainly on dental caries, periodontitis, and oral candidiasis through salivary pH control in order to regulate oral biological and ecological cycles.

MATERIALS AND METHODS

This study is an in-vitro laboratory experiment conducted in the Laboratory of Microbiology and Immunology, Faculty of Veterinary, Syiah Kuala University in Banda Aceh. Materials used in this study were a laboratory strain of *S. mutans*, ATCC 31 987, and a laboratory strain of *C. albicans*, ATCC 10231, obtained from the Laboratory of Microbiology, Faculty of Veterinary, Syiah Kuala University, as well as a laboratory strain of *A. Actinomycetemcomitans,* ATTC 702 358,

Table 1. The changes of the pH of the artificial saliva after interacted with *S. mutans, C. albicans,* and *A. actinomycetemcomitans*

Types of microorganism in the artificial saliva	The changes of the salivary pH				
	pH 4	pH 5	pH 6	pH 8	pH 9
S. mutans	5.79	5.25	6.38	7.38	7.53
C. albicans	4.71	5.59	6.42	7.16	7.29
A. actinomycetemcomitans	5.84	6.75	7.91	8.49	8.43

Table 2. The changes of the pH of the artificial saliva after *S. mutans, C. albicans,* and *A. actinomycetemcomitans* interacted each other

Types of microorganism in the artificial saliva	The changes of the salivary pH				
	pH 4	pH 5	pH 6	pH 8	pH 9
S. mutans and *C. albicans*	6.76	6.93	7.07	8.18	8.19
S. mutans and *A. actinomycetemcomitans*	6.47	6.53	7.04	7.95	7.82
A. actinomycetemcomitans and *C. albicans*	7.32	7.23	7.25	7.27	7.34

obtained from the Laboratory of Oral Biology, Faculty of Dentistry, Universitas Indonesia. Those three pathogenic microorganisms were used as biological control or balance of acid and alkaline environment changes. Other materials used as artificial saliva were 0.702 g NaCl, 0.221 g KCN, 1.495 g NaHCO3, 1.153 g KCl, 1.100 g H_2NCONH_2, 0.213 g Na2HPO_4, and 0.204 g KH_2PO_4 obtained from the Laboratory of Biochemistry, Faculty of Medicine, Universitas Indonesia. The artificial saliva was used as a reference for the interactions of those micropathogens, especially in acidic and alkaline environments. The various components of these materials were then analyzed with various forms of analysis approaches.

The laboratory strains of *A. actinomicetemcomitans* and *S. mutans* were cultured by using *treak late*. The laboratory strain of *S. mutans* was cultured on Muller Hilton agar (MHA) media, while A. actinomicetemcomitans using nutrient agar (NA) media. Each of those two bacteria was then put into anerobic jar and incubated at 37^0 C for 48 hours. For confirmatory test, those bacteria were stained. Afterwards, they were cultured in TSB liquid medium for 48 hours in order to become samples for analysis.

On the other hand, the laboratory strain of *C. albicans* was taken about 1 Ose, and then cultured on selective Sabouraud dextrose agar (SDA) media using T scratching technique. Next, it was incubated in an incubator for 24-48 hours at 37^0 C. Colonies of *C. albicans* grown on SDA media were suspended by taking a colony of *C. albicans* from the SDA media, which was then put into peptone solution. The level of turbidity of *C. albicans* in the peptone solution was compared with Mc Farland solution (1.5 x 108 CFU/ml).

S. mutans and *A. actinomicetemcomitans,* that have been cultured from TSB liquid medium, and *C. albicans,* that has been cultured in peptone, were respectively centrifuged at 2000 rpm for 5 min. Their residue was then collected and added with 15 ml PBS. Afterwards, 15 ml PBS was also added to the bacteria and *C. albicans* was added with 15 ml peptone solution. They were then vortexed and resuspended. Next, each of these microorganisms was taken and put into 2 ml of the artificial saliva prepared with pH of 4, 5, 6, 8, and 9. Afterwards, they were incubated for 72 hours, and every 24 hours the changes of the salivary pH were measured by using a pH meter, and the artificial saliva with normal pH was used as control.

Finally, data obtained from the interaction between *S. mutans*, *C. albicans* and *A. actinomycetemcpmitans* in the artificial saliva were statistically analyzed with a normality test, Kolmogorov-Smirnov test, followed with repeated measures and ANOVA test using SPSS software.

RESULTS

The results of this study were then reported in two forms of analysis, the analysis of the changes of the salivary pH after interacted with oral micropathogens (Table 1) and the analysis of the changes of the pH of the artificial saliva after interacted with oral micropathogen (Table 2). The pH of the saliva that has been interacted with *S. mutans, C. albicans*, and *A. actinomycetemcomitans* was measured 3 times during 24 hours. Each of the pH values presented in the table was the average value derived from the examintaions conducted three times.

DISCUSSION

Many researches on *S. mutans* which cause dental caries, *A. actinomycetemcomitans* which cause periodontitis, and *C. albicans* which cause oral candidiasis, have showed that they can cause oral health problems, one of which is related to saliva pH imbalance.[15] This is due to the salivary pH that has an important role to regulate both metabolic activities of normal flora microbiotas and biological balance of oral cavity. Thus, the changes of the saliva pH can cause the

normal flora microorganisms of the oral cavity evolved into a pathogen accelerating invasion, inflammation and infection of the host.[16]

The changes of the pH of the artificial saliva after interacted with *S. mutans, C. albicans*, and *A. actinomycetemcomitans* for 24 hours (Table 1) indicate that those three microorganisms have the properties of adaptation to its pH growth. Besides, it is also known that the saliva could also serve as a biological control for the activities of the oral microbiotas contained in it although in general the changes were not significantly different ($p>0.05$) from the control pH, so it is suspected that there was adjustment phase for the properties of each of these microorganisms. This nature will change as there is a change in the ecology and biology of the oral cavity caused by an imbalance triggered by various factors, such as disorders of hormonal system and oral cavity pH.[17] Temperature factors, chemical factors, and psychological factors can also become the main determinant of the pathogenic properties of oral microbiota, such as *S. mutans, C. albicans*, and *A. actinomycetemcomitans*.[18] This shows that the virulence properties of the three oral microbiotas will become active and pathogenic when influenced by those factors.[19]

It is known that the interactions between *S. mutans* and *A. actinomycetemcomitans,* and between *C. albicans* and *A. actinomycetemcomitans* as shown in table 2 were significant ($p<0.05$). It means that those oral micropathogens could lower the control pH 8 and 9 to neutral one (7.2 to 7.9). It is related with the bioactive components contained in the artificial saliva, that is possible to affect the changes of the pH caused by bacteria and fungi, while in the normal condition, sIgA and lactoferrin proteins have a role to inhibit the biological activity of the oral microbiota associated with the changes of the salivary pH.[17] Interaction of *S. mutans* and *C. albicans* in the artificial saliva can actually make the pH more alkaline, specifically the control pH 4, 5, and 6. It can be assumed that the microorganisms, in addition to making the pH stable in an acidic environment, is also able to adapt to an alkaline environment, called the instability of acid.[19]

S. mutans and *C. albicans* are two microorganisms that always express virulence properties when the instability of acid occurs in the oral cavity, and their growth is still stable despite the critical acidic pH (3 to 4.5).[1] The instability of acid can trigger and stimulate the virulence properties of the microorganisms to become more pathogenic, as well as can affect the biological properties of saliva.[20] *S. mutans* not only have both aciduric and acidogenic properties by producing a dextran, which play a role in the formation of biofilm on the tooth surface before causing colonization, invasion and infection of the tooth enamel and chronic caries.[21] Meanwhile, *C. albicans* can live in various acidic and alkaline pH as well as in neutral pH although at 4.5-6.5 pH. The activity and expression of manoprotein of *C. albicans* are affected by changes in temperature and pH that can inhibit protein synthesis and reduce manan activity (cell wall protein) that affect the growth of *C. albicans*.[22]

As bacteria which are stable in alkaline environments, *A. actinomycetemcomitans* and *C. albicans* were able to make the pH of the artificial saliva neutral or alkaline (Table 2). This is because *A. actinomycetemcomitans* has strong adhesion properties and can colonize well in saliva, so the condition of the salivary pH can be controlled by the bacteria in order to balance the pH and the growth of *C. albicans*.[23,24]

In molecular level, *S. mutans* (39-864) molecules, played a role in attachment to the salivary protein, so the localization of acid products occured with a high concentration on the surface of tooth enamel. This acid will lower the pH of the oral cavity, so it can cause demineralization of the enamel.[14] In cellular level, two proteins of the cell wall were hydrolyzed, such as fructose hydrolyzed by *fructosyltransferase* and dextran hydrolyzed by *glucosyltransferase* (GTF). This process aims to maintain the instability of the critical pH of GTF and *glucan binding protein* (GBP) as trigger protein to produce lactic acid either by S. mutans or by other bacteria that mediates colonization on tooth enamel, so glucose- dextran can lower saliva properties either as a protector or as antibacteria on tooth surface.[25]

Interactions among microbiotas in the oral cavity were either facilitated by components of salivary proteins or caused by interactions of molecule bondings, such as interactions between proteins, hydrophobicity bonding on cell surface, electrostatic bonding, and biofilm matrix protein molecule bonding that interacts with either *C. albicans, S. mutans* or *A. actinomycetemcomitans*. The bonding and interaction among those protein molecules then can form coaggregasion and cause adhesion among microorganisms.[15] One function of the molecular interaction among the microbiotas is to maintain the pH balance of oral cavity, including salivary pH, as biology and ecology control of the oral cavity.

The acidity of the saliva is also dependent on hydrogen ions contained in the solution. At alkaline pH, *C. albicans* unable to adapt because the increasing of cell wall polysaccharide reactiviting and components of salivary proteins, thereby affecting cell wall resistance and instability of the absorption of hydrogen ions caused by cytoplasma that can interfere energy supply as a results of cell lysis. [24]

The research of *oral candidiasis* in denture users indicates that the interaction of *C. albicans*, *Streptococcus Sp,* and *Actinomyces Sp* in the saliva may decrease the population of *C. albicans*. In addition, this condition occurs as a result of the activity of lipopolysaccharide molecules produced by bacteria that are able to inhibit the formation of hyphae of *C. albicans*, so this condition then can facilitate the penetration of protein molecules of the bacterial surface into the cells of *C. albicans*.[12, 26]

Therefore, this research showed that there was a close relation between the influence of oral activity of microphatogen and the changes of the pH of the artificial saliva since the results of this research provide information

that in normal conditions, *C. albicans, S. mutans*, and *A. Actinomycetemcomitans* can consistently maintain pH of the oral cavity in order to maintain their stable life as normal floras, but they will change into pathogens when the salivary pH changes. This research also provides information that the salivary pH will change when there is biological imbalance of oral cavity, thus, it is necessary to balance the salivary pH as an attempt to control the growth of oral pathogenic microbiotas and to support ecological and biological activities in oral cavity. In other words, it can also be considered as an attempt to maintain the integrity of pathogenic bacteria as normal flora microorganisms, so it can prevent infection and oral diseases, such as dental caries, periodontitis and oral candidiasis.

It can be concluded that biological activities of *S. mutans*, *C. albicans*, and *A. actinomycetemcomitans* in artificial saliva can change salivary pH as well as maintain its neutral pH. This condition indicates that the microbiotas can support and co-operate each other in influencing the biological cycle of the oral cavity with saliva pH as an indicator.

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