

ANALYSIS ON THE INHIBITION OF *AGGREGATIBACTER ACTINOMYCETEMCOMITANS* BY *STREPTOCOCCUS SALIVARIUS* ISOLATED FROM SALIVA AND TONGUE DORSUM OF ADULTS

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ABSTRACT

Objective: To analyze the inhibitory potential of *S. salivarius* isolated from saliva and the dorsum of the tongue, and the protein it produces, in inhibiting the growth of *Aggregatibacter actinomycetemcomitans*.

Methods: Examine the inhibition zone of *A. actinomycetemcomitans* formed by the treatment of *S. salivarius* isolated from saliva and the tongue dorsum, along with the protein produced, in 10 participants using a deferred antagonism test with well-diffused gelatin.

Results: The inhibitory zone of *S. salivarius* isolated from saliva and the tongue against *A. actinomycetemcomitans* is insignificantly different ($p \geq 0.05$). There is no inhibition of the growth of *A. actinomycetemcomitans* shown by whole-cell and spent medium proteins of *S. salivarius*.

Conclusion: *S. salivarius* isolated from both saliva and the tongue dorsum is able to inhibit the growth of *A. actinomycetemcomitans*, but not the protein they produce.

Keywords: Streptococcus salivarius, Aggregatibacter actinomycetemcomitans, Lantibiotic, Probiotic.

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INTRODUCTION

Probiotics, according to the World Health Organization, are live microorganisms which when administered in adequate amounts are capable of providing health benefits for the recipient [1]. In recent years, research related to the use of oral probiotics for the prevention of dental caries and plaque biofilm formation, as well as for the treatment of halitosis and pharyngitis, has been successfully performed [1]. The aim of probiotic use in the oral cavity is to help prevent these diseases. Probiotics work by inhibiting the adhesion of pathogens in host tissues, stimulating and modulating the immune system of the mucosa, modulating apoptosis in cell proliferation, and improving the integrity of the barrier. Probiotics also kill or inhibit the growth of pathogens through the production of antimicrobial compounds such as bacteriocins or other products that are antagonistic against pathogens [2]. Unlike antibiotics, bacteriocins kill the bacteria within a relatively narrow spectrum [3]. Several studies have been conducted on the various multidrug-resistant pathogens that require natural antibiotic alternatives to restrict the use of antibiotic additives. One such alternative is the lantibiotic produced by *Streptococcus salivarius*.

S. salivarius is an essential element of biofilms in healthy individuals that colonize the oral mucosa, the dorsum of the tongue, and the pharyngeal mucosa [4]. *S. salivarius* is found as a pioneer colony in the human oral cavity from an early age. Its presence in the oral cavity can be identified within 2 days after birth and will settle as the predominant bacteria [1]. The number of *S. salivarius* in healthy adults can be as much as 2% from the isolated buccal mucosa, 17% from the tongue, and 30% from the pharynx. In the saliva samples of an adult population, *S. salivarius*' levels range from 10^6 to 10^7 colony-forming units (CFU)/ml [1]. *S. salivarius* has strains that are able to produce oral probiotics by releasing lantibiotics in large amounts in saliva, which in turn are able to eliminate harmful bacteria [4]. A lantibiotic is an antimicrobial peptide or small protein produced by the bacteria, which is able to kill or inhibit the growth of microorganisms. The lantibiotic produced by *S. salivarius* plays an important role in stabilizing the oral microbiota, preventing the

excessive growth of pathogens, and preventing infections such as periodontitis.

In Indonesia, as in other developing countries, periodontal disease is an oral health problem for all age groups. Periodontal disease in Indonesia has the second highest prevalence after caries, which has an incident rate of 60% [5]. This high prevalence should encourage dentists to take a more proactive role with patients in preventing periodontitis in the coming years. Periodontitis is an infection of the supporting structures of the teeth caused by inflammation due to the formation of plaque and calculus from bacteria. This is followed by periodontal pocket formation which then results in progressive damage to the periodontal ligament and alveolar bone structure. The disease is stimulated by 90% of facultative anaerobic bacteria and 75% of which are Gram-negative bacteria. Some of the main causes of periodontal disease include inflammation of the periodontal tissues, attachment loss in the alveolar bone structure, and the accumulation of bacteria such as *Aggregatibacter actinomycetemcomitans*, which triggers the formation of microbial plaque.

A. actinomycetemcomitans is part of the normal flora in healthy individuals and is the predominant pathogen associated with localized aggressive periodontitis. *A. actinomycetemcomitans* has a variety of virulence factors, including the leukotoxin gene, which plays a role in the decline of the immune response and degradation of gingival epithelial attachment on periodontal tissues. Treatments given to patients with periodontitis may include conventional mechanical treatments such as scaling, root planing, and surgical intervention. However, patients with periodontitis caused by the bacterium *A. actinomycetemcomitans* often require not only conventional mechanical treatment but also antibiotic therapy. This is because the bacteria are able to invade the entire soft tissue and quickly colonize the periodontal pocket after doing mechanical therapy without antibiotics. Therefore, probiotic agents are expected to play a role in stopping or reducing the bacterial growth of *A. actinomycetemcomitans* in the oral cavity. Although the effects of *S. salivarius* probiotics are widely known, there has not been any research until now that proved the inhibitory potency of the lantibiotic released

from *S. salivarius* against the growth of *A. Aggregatibacter*. Thus, this study is expected to show the presence/absence of antimicrobial activity of the lantibiotic produced by *S. salivarius* from the isolated saliva and tongue dorsum of healthy adult participants that affects the growth of *A. actinomycetemcomitans*.

METHODS

The procedure began with the selection and preparation of 10 participants. They were chosen in accordance with the inclusion criteria and given an explanation of the research to be conducted. They were each asked to sign an informed consent form if they understood and agreed to become a research participant. The participants were asked not to eat for 3 hrs before sampling and to rinse with water to remove food debris. We then took samples of stimulated saliva and swabbed the dorsum of the tongue for each participant. Stimulated saliva sampling was conducted in the morning in the form of whole saliva up to 10 ml. Participants were instructed to chew Parafilm M®, and their saliva was collected in a sterile vial. Dorsum of the tongue swab sampling was also done in the morning using a Cytobrush from the circumvallate papillae to the tip of the tongue. Swabs collected on the Cytobrush were placed in a vial containing a solution of phosphate-buffered saline (PBS). The samples of saliva and tongue dorsum swabs that had been obtained were stored in the refrigerator (freezer) at 4°C.

Next, we conducted the identification and confirmation of the *S. salivarius* colonies. Samples of saliva and tongue dorsum swabs were cultured on the selective medium mitis *Salivarius agar* (MSA) to isolate colonies of the *S. salivarius* strain. Afterward, colonies morphologically considered *S. salivarius* were confirmed by polymerase chain reaction (PCR), with *S. salivarius* ATCC 13419 as the positive control. PCR was performed in 30 cycles with a denaturation temperature of 94°C for 30 seconds, an annealing temperature of 55°C for 30 seconds, and an elongation time of 30 seconds at a temperature of 65°C, using primers SalAUS (5'-GTAGAAAATATTTACTACATACT) and SalADS (5'GTAAAGTA

TTCGTAATAACTGATG). PCR results were then input running on electrophoresis for 30 minutes (100 V) using a 1% agarose. They were stained with GelRed™, and then observed in the Gel Doc system. A DNA band of 118 base pairs indicated that the colonies identified on the medium MSA were *S. salivarius*. Next, the identification of the protein produced by *S. salivarius* through sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) started with the clinical culturing of *S. salivarius* colonies and ATCC 13419 in brain-heart infusion (BHI) broth for 18 hrs. Then, the pellets and spent medium were separated by centrifugation, and the pellets resuspended with a cell lysate buffer solution. These proteins were then analyzed using SDS-PAGE (150 V, 80 mA, 60 minutes) and stained with Coomassie blue solution to see whether the *S. salivarius* proteins had the same molecular mass (kDa). Samples with the same protein profile were then calculated its concentration with Bradford test to be used in the well-diffused gelatin methods.

Subsequently, the *S. salivarius* inhibitory potency test was performed against *A. actinomycetemcomitans*. Clinical *S. salivarius* and *S. salivarius* ATCC 13419 were diluted to 4 concentrations (CFU/ml), then inoculated in a 1-cm line in the middle of the BHI gelatin medium and incubated for 24 hrs. Afterward, *S. salivarius* that grew on the surface was removed with the edge of the glass slide. The surface was sterilized with chloroform for 30 minutes then left for 15 minutes to remove the remnants of chloroform. We took one colony from *A. actinomycetemcomitans* and placed it in an Eppendorf tube which contained soft gelatin, in vortex, and removed it using a cotton bud. The cotton bud was then rubbed in a line perpendicular to the former *S. salivarius* swabs that had been cleaned. Each procedure was performed twice in duplicate. After that, colony was incubated for 24 hrs. Then, the growth inhibition zone of *A. actinomycetemcomitans* was measured by calculating the distance between the edges of the sweep of *S. salivarius* with a bacterial colonies growing test.

After the inter-bacterial inhibitory potency test, we tested the potential of the inhibitory protein *S. salivarius* against *A. actinomycetemcomitans*. *A. actinomycetemcomitans* was taken as many as 2 ose, put in an Eppendorf tube containing PBS, and in vortex until homogeneous. Then, 100 ml of BHI gelatin was sterilized and cooled to 55°C. The *A. actinomycetemcomitans* which had been dissolved in PBS was inoculated into the BHI gelatin that was still molten, put on an orbital shaker for 30 seconds, and poured into a Petri dish. When the gelatin had hardened, we made four wells of gelatin, each with a diameter of 4 mm. The protein with four different concentrations (pg/ml) was inserted into each well and incubated for 24 hrs. We then observed and measured the distance between the edge of the well and the growth of the bacterial colonies. To process and analyze the data, we began with the Shapiro-Wilk data normality test. We then calculated the value of the average zone of inhibition and the protein produced by *S. salivarius* from both isolated sources to the growth of *A. actinomycetemcomitans*. We compared the results using the one-way ANOVA test and followed that with a *post-hoc* test. We compared the inhibitory potential of *S. salivarius* isolated from the saliva with the *S. salivarius* isolated from the tongue and its protein against *A. actinomycetemcomitans* using a paired t-test. Then, we used the Pearson's test to examine the correlation between the increase in concentration of *S. salivarius* and the protein with the inhibitory zone widening of *A. actinomycetemcomitans*.

RESULTS

The identification was done by comparing the morphology of *S. salivarius* from the culture of clinical samples with *S. salivarius* ATCC 13419 that was grown on the MSA medium. Twelve samples from six participants were tested using a PCR test to ensure that the colony was a colony of *S. salivarius*. Based on the PCR test results, we confirmed that all 12 colonies derived from the six participants were colonies of *S. salivarius*. Thus, it can be said that the results were not significant ($p \geq 0.05$) based on the identification of *S. salivarius* colonies on the mitis *Salivarius* gelatin medium and confirmed by PCR. Only 6 out of 10 participants showed evidence of *S. salivarius* in the oral cavity, as shown in Table 1.

Table 2 shows the results of the inhibitory potential of clinical *S. salivarius* isolated from both sources and *S. salivarius* ATCC 13419 against *A. actinomycetemcomitans* Y4 evaluated using a deferred antagonism test. However, of the four participants tested, only one participant's results could be interpreted. Consequently, for this part of the study, we only used two samples isolated from the saliva and tongue of a single participant.

The test results of both types of protein inhibitory potency against *A. actinomycetemcomitans* can be seen in Tables 3 and 4. The amount of *A. actinomycetemcomitans* mixed in the gelatin medium in this method was 1.2×10^{12} CFU/ml.

Table 3 shows that there is no inhibition zone of *A. actinomycetemcomitans* that is generated by the whole-cell protein concentrations of *S. salivarius* isolated from either the saliva or the tongue. This is indicated by the colony of *A. actinomycetemcomitans* in the area around the well. The same can be seen in the test results of the protein inhibitory potential of the *S. salivarius* spent medium from both isolated sources against *A. actinomycetemcomitans*. Table 4 shows the absence of barriers that may occur from spent medium proteins. In addition, there were no obstacles against *A. actinomycetemcomitans* from the protein produced by *S. salivarius* ATCC 13419.

Table 1: Identification results of *S. salivarius* in clinical samples

Isolated source	<i>S. salivarius</i> colonies	
	Positive (%)	Negative (%)
Saliva	6 (60)	4 (40)
Dorsum of the tongue	6 (60)	4 (40)

S. salivarius: *Streptococcus salivarius*

Table 2: The result of the inhibitory potential of *S. salivarius* isolated from saliva and the dorsum of the tongue against *A. actinomycetemcomitans*

Inhibitory potential	Culture results			
<i>S. salivarius</i> ATCC 13419 concentration (CFU/ml)	1.7×10 ⁷	1.7×10 ⁸	1.7×10 ⁹	1.7×10 ¹⁰
Inhibitory zone	0 mm	0 mm	0 mm	0 mm
Clinical <i>S. salivarius</i> strain concentration (saliva) (CFU/ml)	4.4×10 ¹⁰	4.4×10 ⁹	4.4×10 ⁸	4.4×10 ⁷
Average of inhibitory zone	3 mm	3.5 mm	6.5 mm	9 mm
Clinical <i>S. salivarius</i> strain concentration (tongue dorsum) (CFU/ml)	3.7×10 ¹⁰	3.7×10 ⁹	3.7×10 ⁸	3.7×10 ⁷
Average of inhibitory zone	3.25 mm	6.75 mm	6.5 mm	7.75 mm

S. salivarius: *Streptococcus salivarius*, *A. actinomycetemcomitans*: *Aggregatibacter actinomycetemcomitans*, CFU: Colony-forming units

Table 3: The results of the inhibitory potency test of every whole-cell protein concentration of clinical *S. salivarius* and ATCC against *A. actinomycetemcomitans*

Inhibitory potential	Culture results based on protein concentration			
Whole-cell <i>S. salivarius</i> ATCC 13419 protein concentration (µg/ml)	1.46×10 ⁻²	1.46×10 ⁻¹	1.46	14.6
Inhibitory zone	0 mm	0 mm	0 mm	0 mm
Whole-cell <i>S. salivarius</i> clinical strain protein concentration (saliva) (µg/ml)	2.06×10 ⁻²	2.06×10 ⁻¹	2.06	20.6
Inhibitory zone	0 mm	0 mm	0 mm	0 mm
Whole-cell <i>S. salivarius</i> clinical strain protein concentration (tongue dorsum) (µg/ml)	2.03×10 ⁻²	2.03×10 ⁻¹	2.03	20.3
Inhibitory zone	0 mm	0 mm	0 mm	0 mm

S. salivarius: *Streptococcus salivarius*, *A. actinomycetemcomitans*: *Aggregatibacter actinomycetemcomitans*

Table 4: The results of the inhibitory potency test of every protein concentration of spent medium clinical *S. salivarius* and ATCC 13419 against *A. actinomycetemcomitans*

Inhibitory potential	Culture results based on protein concentration			
Spent medium <i>S. salivarius</i> ATCC 13419 protein concentration (µg/ml)	1.81×10 ⁻²	1.81×10 ⁻¹	1.81	18.1
Inhibitory zone	0 mm	0 mm	0 mm	0 mm
Spent medium <i>S. salivarius</i> clinical strain protein concentration (saliva) (µg/ml)	5.33×10 ⁻²	5.33×10 ⁻¹	5.33	53.3
Inhibitory zone	0 mm	0 mm	0 mm	0 mm
Spent medium <i>S. salivarius</i> clinical strain protein concentration (tongue dorsum) (µg/ml)	2.79×10 ⁻²	2.79×10 ⁻¹	2.79	27.9
Inhibitory zone	0 mm	0 mm	0 mm	0 mm

S. salivarius: *Streptococcus salivarius*, *A. actinomycetemcomitans*: *Aggregatibacter actinomycetemcomitans*

DISCUSSION

This study aimed to analyze the effectiveness of bacteria isolated from two sources: Saliva and the dorsum of the tongue, and the protein produced by *S. salivarius*, in inhibiting the growth of bacteria *A. actinomycetemcomitans*. *S. salivarius* colonies were identified by culture samples on the selective medium MSA and confirmed by PCR. We obtained data from 20 samples of isolated saliva and dorsal tongue swabs from 10 healthy adult participants and found that only 60% of the samples were positive for *S. salivarius* in the oral cavity ($p \geq 0.05$). This result differs from that of Wescombe *et al.*, who found that *S. salivarius* is the pioneer colony in the oral cavity and will settle as the predominant bacteria on all human life [6].

The absence of *S. salivarius* in 40% of participants was also caused by the participants' dietary habits. In a previous study, it was demonstrated that the intake of sucrose is directly proportional to the number of *Streptococcus salivarius* on the tongue and saliva, which plays a role in the regulation of sucrose metabolism of this species [7,8]. One of the extracellular enzymes that are secreted by *S. salivarius*, fructosyltransferases, catalyzes the synthesis of sucrose into levan fructan with structures that are known to have a significant probiotic effect. In addition to dietary factors, enzymes in the saliva that have antibacterial properties such as lysozyme, lactoperoxidase, and amylase also affect the growth of *S. salivarius* in the oral cavity [9].

After identifying the colonies by PCR, we identified specific proteins produced by the colonies using SDS-PAGE. With this procedure, the whole-cell protein profiles (proteins that are inside the cell membrane) can be better identified than with the protein profile of the spent medium. This is consistent with research conducted by Barbour and

Philip (2014) which stated that 60-70% of bacteriocins discovered were peptides associated with producer cells of the cell wall, whereas 30-40% of the inhibitory peptide was secreted into the extracellular liquid medium. In addition, this study revealed that the production of lantibiotics by *S. salivarius* is regulated by a system of quorum sensing or interactions between cells, which occurs when the bacteria secrete peptide molecules that aid in bacterial communication. During the growth of bacteria, these peptides accumulate in the environment, and then, on the threshold of a certain concentration, trigger the production of lantibiotics in high concentrations. It can be concluded that the undetected proteins in the test results may have been caused by not achieving the threshold concentration through quorum sensing so that the production of lantibiotics was not triggered. The inhibitory potential of *S. salivarius* and the protein it produced against *A. actinomycetemcomitans* is distinguished based on the isolated source, the concentration of *S. salivarius* bacteria, and protein concentration. All culture procedures, bacteria inhibitory potency tests, and protein tests were done from the time of incubation for 24 hrs, with the goal of reaching the stationary growth phase of *S. salivarius*. In this phase, it produced secondary metabolites of *S. salivarius*, including the lantibiotic, and obtained stability of the inhibitory activity of the lantibiotic [9,10].

In contrast to the Gram-positive bacteria, the Gram-negative bacteria have a cell wrapper structure consisting of three layers (trilayer): The outer membrane, the middle layer which is the cell wall, and the plasma membrane. According to Stevens *et al.*, antibiotics have a bactericidal effect against Gram-positive bacteria by inducing the formation of porous bacterial cytoplasmic membranes and inhibiting the biosynthesis of the cell wall by binding with the molecular precursor cell walls and lipid II,

which is located in the peptidoglycan [11]. Gram-negative bacteria such as *A. actinomycetemcomitans* are generally insensitive to lantibiotics, because the outer membrane of the Gram-negative bacteria which serves as a permeability barrier prevents the entry of macromolecules such as lantibiotic cells [9]. However, the vulnerability of cells to lantibiotics will increase when the outer membrane of the bacteria has been damaged or weakened by a permeabilizer.

A permeabilizer is an external agent capable of causing the loss of integrity of the outer membrane of the Gram-negative bacterial cell. Based on the research of Alakomi *et al.* (2000), the lactic acid produced by *S. salivarius* as a result of the fermentation of glucose is proven to work as a permeabilizer. Another study also stated that *S. salivarius* was able to ferment 93.6% of glucose in the medium and is the largest producer of lactic acid in the viridans group [12]. Therefore, the high concentration of lactic acid produced by *S. salivarius* may increase the sensitization of lantibiotics against *A. actinomycetemcomitans*. Alakomi *et al.* also stated that lactic acid as a permeabilizer is not bactericidal or bacteriostatic against Gram-negative bacteria. However, by increasing the permeability of the outer membrane, other compounds such as lantibiotics are able to penetrate and enhance the antimicrobial potency of these components against Gram-negative bacteria [10].

The interaction between the *S. salivarius* bacteria of isolated saliva and the dorsum of the tongue are one of the participants with *A. actinomycetemcomitans* bacteria which was tested with the deferred antagonism test. The test indicated that there was no significant difference between the inhibition zone of *A. actinomycetemcomitans* produced by *S. salivarius* isolated from saliva and the inhibition zone produced by the isolated samples from the tongue ($p \geq 0.05$). In the *S. salivarius* isolated saliva, the inhibition zone of *A. actinomycetemcomitans* was produced at a concentration of 10^8 CFU/ml greater than the inhibition zone produced at a concentration of 10^7 CFU/ml, but not statistically significant. A significant difference was shown by the inhibition zone produced at a concentration of 10^9 - 10^8 CFU/ml, and 10^{10} with 10^9 CFU/ml ($p \leq 0.05$). Meanwhile, judging from the results of the inhibition zone of *A. actinomycetemcomitans* that was produced by *S. salivarius* isolated from the tongue, the only significant difference was found between the concentrations of *S. salivarius* 10^7 CFU/ml with 10^8 CFU/ml ($p \leq 0.05$).

In addition, the isolated saliva showed an increase in the concentration of *S. salivarius* followed by an increase in the inhibition zone. Based on the results of the Pearson's correlation analysis, the concentration of *S. salivarius* from the isolated saliva showed a very strong positive correlation ($p \geq 0.05$; $r = 0.887$) with the resulting inhibition zone against *A. actinomycetemcomitans*. The same positive correlation was also demonstrated by *S. salivarius* isolated from the tongue, but only with a relatively strong correlation ($p \geq 0.05$; $r = 0.685$). This proves that the inhibition zone of *A. actinomycetemcomitans* is affected by the concentration of *S. salivarius*. Based on the results of the paired t-test comparing the inhibition zone produced by *S. salivarius* from both isolated sources with the inhibition zone produced by *S. salivarius* ATCC as a positive control, the inhibitory potential of *S. salivarius* from both the saliva and the dorsum of the tongue (clinical strain) against *A. actinomycetemcomitans* was significantly better than *S. salivarius* ATCC (laboratory strains) ($p \leq 0.05$). This is consistent with the research of a previous study, which stated that the results of the SDS-PAGE mutant strain or laboratory strain were weaker than the clinical strain or wild-type [13]. Weerkamp and McBride also stated that the wild-type strain has adhesive properties that allow attachment to the buccal surface, teeth, and dorsum of the tongue, as well as aggregate with other bacteria, whereas *S. salivarius* ATCC laboratory strains do not adhere to the epithelial cells [14].

Furthermore, to analyze the antimicrobial activity exhibited by *S. salivarius* against *A. actinomycetemcomitans* in the deferred antagonism test, we tested the interaction of a protein produced by *S. salivarius* using well-diffused gelatin. In contrast to the results of

the interaction between bacteria in the previous method, the protein interaction of *S. salivarius* with *A. actinomycetemcomitans* did not show the formation of barriers, either by whole-cell protein and spent medium, or from isolated saliva and tongue samples. The test results were similar with the *S. salivarius* ATCC protein. In accordance with the previous explanation of the bactericidal mechanism of lantibiotics against Gram-negative bacteria, the lantibiotic produced by *S. salivarius* showed antimicrobial action after the integrity of the outer membrane of bacteria was disturbed. Due to the relatively large size of the lantibiotic molecule (1800-4600 Da), it could not penetrate the outer membrane of Gram-negative bacteria [15]. In this case, it needed a permeabilizer such as the lactic acid produced by *S. salivarius* to penetrate the outer membrane [12]. In this method, exposure of whole-cell proteins and the spent medium was only done to test the bacteria, so the glucose fermentation process did not occur. Therefore, the lactic acid that was supposed to act as an agent permeabilizer to potentially disintegrate the outer membrane of *A. actinomycetemcomitans* was not formed, and the lantibiotic was not able to penetrate the cytoplasmic membrane of bacteria. This resulted in *A. actinomycetemcomitans* becoming insensitive to the lantibiotic [9,10].

Furthermore, the previous study stated that a greater number of lantibiotics would be produced by *S. salivarius* if they were grown in a solid medium, whereas lantibiotics produced by *S. salivarius* cultured in a liquid medium would show a very weak expression of inhibitory activity [16]. According to their study, a liquid medium can be used if the auto-induction method is used to obtain bacterial proteins. The protein interactions test of *S. salivarius* against *A. actinomycetemcomitans* was derived from cultures of *S. salivarius* in a broth medium (liquid medium). Therefore, the protein produced by both the clinical *S. salivarius* and *S. salivarius* ATCC had a very weak antimicrobial action. In this study, we did not do a protein purification procedure or autoinduction to get a specific *S. salivarius* lantibiotic, due to available time and materials. In addition, the working mechanism of the lantibiotic produced by *S. salivarius* isolated from saliva and the dorsum of the tongue against *A. actinomycetemcomitans* cannot be explained further. Therefore, additional research is required to study the interaction of specific lantibiotic clinical strains of *S. salivarius* from isolated saliva and the dorsum of the tongue against *A. actinomycetemcomitans*.

CONCLUSION

We concluded that *S. salivarius* has an inhibitory potency against *A. actinomycetemcomitans*, but there is no difference in the potential inhibitory effect between *S. salivarius* isolated from saliva or the dorsum of the tongue. In addition, there are no barriers to the growth of *A. actinomycetemcomitans* by the protein produced by *S. salivarius* isolated from saliva and the tongue.

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