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(RESEARCH ARTICLE)

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The effect of hydrolase enzyme on antibiofilm activity in oral cavity microbes

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Abstract

Background : Every surface of the human body that comes into contact with the outside environment is covered with a layer of microorganisms called the human microbiome, characterized by species diversity and a high number of cells. Biofilms are surface-related, three-dimensional multicellular structures whose integrity depends on the extracellular matrix produced by their constituent bacteria. Hydrolase enzymes are a class of enzymes belonging to the anti-biofilm enzymes and are categorized as polysaccharide-degrading enzymes, proteolytic enzymes, and antiquorum sensing enzymes.

Purpose : To know the effect of hydrolase enzyme towards antibiofilm activity of oral cavity microbe.

Method : The method used is literature study by searching databases from various national and international sources.

Results : There is an anti-biofilm effect of several types of hydrolase enzymes on oral microbes such as *P. aeruginosa*, *S. aureus*, *E. faecalis*, and *E. coli*. The greatest effect was found in the enzymes PelAh & PslGh with the mechanism of action of polysaccharide-degrading enzymes and their microbial target, *P. aeruginosa*, can reduce biofilm biomass by 58-94%.

Conclusion : Of the several existing mechanisms can provide different effects depending on the microbial target.

Keywords: Antibiofilm; Hydrolases Enzyme; Oral Microbiome; Oral Cavity; Microbes

1. Introduction

Every surface of the human body that comes into contact with the external environment is covered with a layer of microorganisms called the human microbiome which is characterized by high species diversity and cell numbers. The oral cavity is one of the most diverse in terms of species of bacterial microbiomes of the human organism. Until now, many chronic infectious diseases that are fatal cause human suffering caused by bacterial biofilms.

A balanced microbial ecosystem is maintained in the oral cavity by the contribution of these microorganisms to normal development and defense. However, an imbalance in the oral microbiome from both external and internal factors can lead to undesirable changes in the microbiome of opportunistic pathogenic organisms, causing several diseases such as dental caries, inflammation of the oral mucosa, and so on. Several types of opportunistic pathogenic microbes in the oral cavity include Streptococcus, Staphylococcus, Enterococcus, Lactobacilli, Fusobacterium, Pseudomonas, Candida, and so on. Treatment of systemic diseases related to teeth is not only well-known in dentistry, but also in all medical fields [1]. Some prevention of these diseases can certainly focus on reducing or preventing the formation of biofilms and uncontrolled colonies of opportunistic pathogenic microbes.

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Biofilms are three-dimensional multicellular structures associated with surfaces whose integrity depends on the extracellular matrix produced by their constituent bacteria [2]. Biofilms are composed of a mixture of polymers such as polysaccharides, lipids, and nucleic acids produced by microorganisms supported by biotic and abiotic surfaces [3]. Favorable conditions for biofilm formation include nutrients, humidity, oxygen availability, variable pH conditions, enzymes, temperature, salinity, and redox potential that can affect the ecosystem in changing the composition of biofilm species in each place [4].

Enzymes are stereospecific proteins or natural catalysts that are able to accelerate and act as natural biocatalysts without being consumed. There are several factors that can interfere with the activity and specificity of enzymes such as temperature, pH, substrate, and the presence or absence of activators, co-factors or inhibitors. Enzymes can be classified into 6 main classes, namely oxidoreductases, transferases, hydrolases, lyases, isomerases, and ligases or synthetases [5].

Enzyme Class	Example of Enzyme	Function
Transferases	Transaldolase, lipid kinase, phosphomutase, acyl-, methyl-, glucosyl-, phosphoryl-, transferase, etc.	It allows the transfer of an atom or group of atoms from one molecule to another
Oxidoreductases	Glucose oxidase, alcohol dehydrogenase, catalase, heme oxygenase, dihydrofolate reductase, phenylalanine hydroxylase, etc.	Catalyze redox reactions and transfer oxygen or hydrogen atoms
Isomerases	Isomerase, epimerase and racemase	Catalyze rearrangement reactions in a molecule
Hydrolases	Serine protease, pectinesterase, aminopeptidase, pyrophosphatase, glycosylase, oligoribonuclease, etc.	Catalyze hydrolytic reactions
Lyases	Pyruvate decarboxylase, synthase, hydratase, aldolase, etc.	Catalyze reactions by removing an atom or group of atoms
Ligases or synthetases	Carboxylase and synthetase	It can join two molecules together with a covalent bond.

Figure 1 Enzyme Classification

Hydrolase enzymes are one class of enzymes included in antibiofilm enzymes and are categorized as polysaccharide-degrading enzymes, proteolytic enzymes, and antiquorum sensing enzymes [3]. Hydrolase enzymes are enzymes that catalyze the hydrolytic cleavage of C–C, C–O, C–N bonds, and other covalent bonds [6]. Glycoside hydrolases are a class of enzymes that target glycosidic bonds between sugars and several types of this class of enzymes have been found to be effective in previous studies in reducing and preventing biofilm formation by *P. aeruginosa* bacteria [7].

The purpose of this article is to better understand the mechanism of biofilm formation and how the hydrolase enzyme class intervenes in the formation of biofilms in several bacteria found in the oral cavity.

2. Methods

This article was written using a literature study method that searches databases from various references, such as national and international research journals, journal reviews, textbooks and data related to antibiofilms, hydrolase enzymes, and oral microbes published for a maximum of 10 years. Literature searches were conducted using the sites "Google, Google Scholar, PubMed, Science Direct, Scopus, Biomedical Journal, SpringerLink, Research Gate, Encyclopedia Pub and Repository" in addition to the keywords: hydrolase enzymes, antibiofilms and oral microbes.

3. Results

From the literature study, journals related to different types of hydrolase enzymes with antibiofilm effects on microbes were obtained. The antibiofilm effects of several types of hydrolase enzymes on oral cavity microbes are shown in Table 1.

Table 1 Results of literature study

Enzyme	Mechanism of Action	Target Oral Microbes	Effects	References
$PelA_h$ & $PslG_h$	Enzim *polysaccharide-degrading*	*P. aeruginosa*	Reduces biofilm biomass by 58-94%	Baker *et al*., 2016
Amylase	Enzim *polysaccharide-degrading*	*S. aureus & P.aeruginosa*	The highest antibiofilm activity against SW pathogens at pH 7 and 37ºC	Rasool *et al*., 2022
Lactonase (*expressed by an engineered T7 bacteriophage*)	Enzim *antiquorum sensing*	*P. aeruginosa & E. coli*	Inhibition of biofilm formation	Pei & Lamas-Samanamud, 2014
DNase	Enzim *oxidative*	*E. faecalis*	Biofilm removal before 24 hours	Schlafer *et al*., 2018
Serine protease (dihasilkan *S. epidermidis*)	Enzim proteolitik	*S. aureus*	Biofilm inhibitor activity ≥ 50%	Fredheim *et al*., 2015

4. Discussion

4.1. Biofilm Formation

Biofilm is a community of microorganisms attached to a surface and enclosed in an extracellular polymer matrix. Biofilm-forming microorganisms have been shown to acquire specific mechanisms at the initial stages, namely initial attachment to the surface, microcolony formation, development of structure and maturation of the three-dimensional community until detachment [8]. A requirement for biofilm formation is a sufficiently close distance between the bacteria and the surface. When bacteria approach the surface, there are attractive and repulsive forces that work. When the distance of bacteria to the surface is around 10-20 nm, the negative charge on the bacterial surface is repelled by the negative charge found in most surface environments. This repulsive force can be overcome by van der Waals attraction between bacterial cells and the surface and also the use of fimbriae and flagella for motility and provide mechanical attachment to the target surface [9]. Initial attachment to the surface is divided into 2, namely reversible attachment and irreversible attachment. In reversible attachment, the first attachment of bacteria is influenced by attractive or repulsive forces that vary depending on nutrient levels, pH, and temperature. Flagella and chemotaxis in this step also play an equally important role to avoid the action of hydrodynamic and repulsive forces and to select their respective surfaces. Second, irreversible attachment in the case of *P. aeruginosa* and other Pseudomonas species requires an ATP-binding cassette (ABC) encoded by the lap gene to carry out this process, on the other hand *P. aeruginosa* requires the SadB protein and the BfiSR two-component regulatory system for irreversible attachment [10].

After the initial attachment of bacteria to an inert/living tissue surface, the association becomes stable for subsequent microcolony formation. Bacteria start to multiply while emitting chemical signals that "communicate" between the bacterial cells present. When the signal intensity exceeds a certain threshold, the genetic mechanisms underlying exopolysaccharide production are activated. Thus, in this way, bacteria proliferate within the embedded exopolysaccharide matrix, leading to microcolony formation [2].

This three-dimensional structure with macrocolony morphology depends on extracellular matrix components that are self-produced when the biofilm reaches maturity. To produce this structure, extracellular polymeric substance (EPS), adhesins, proteins, amyloid formers, and exopolysaccharides are required [11]. The attachment itself can initiate the synthesis of the extracellular matrix in which sessile bacteria are embedded and is followed by the formation of water-filled channels [2].

When the biofilm is fully mature, detachment can occur. The process of biofilm detachment can be divided into three processes, namely erosion, abrasion, and peeling. Erosion occurs as a result of fluid shear forces and only affects the surface of the biofilm and removes single or small groups of cells. Abrasion, like erosion, only affects the surface of the

biofilm but this detachment process occurs due to collisions between particles. Sloughing refers to the instantaneous removal of large groups of biofilm which has a significant effect on the morphology and strength of the biofilm, which can cause complete removal of the biofilm. The rate of detachment is not only determined by the accumulation of biofilm and shear forces, but also depends on the internal structure and composition of the biofilm structure so that the release of cells, both individually and on a large scale, is influenced by a series of biotic and abiotic processes [12].

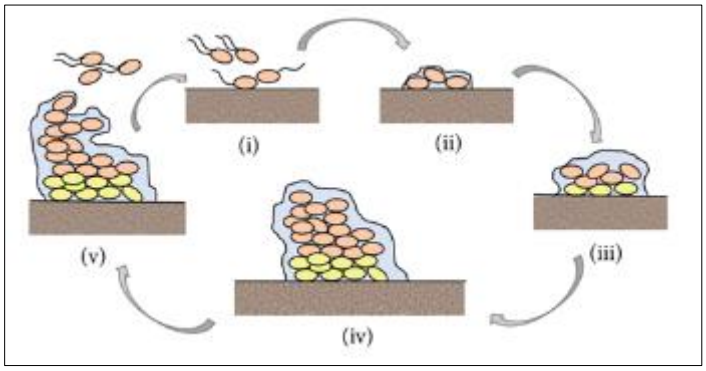

Figure 2 Stages of biofilm formation. (i) Reversible attachment of planktonic bacteria to the surface. (ii) Irreversible attachment to the surface. (iii) Formation of external matrix. (iv) Biofilm acquires three-dimensional structure. (v) Detachment of biofilm

4.2. Enzim polysaccharide-degrading

Polysaccharides are an important part of the EPS matrix that helps microbes adhere to different surfaces, provides strength and inertia to the biofilm wall, filters antimicrobials, acts as a nutrient reservoir, and facilitates the formation of a suitable microbiome. Several studies have suggested that polysaccharide-degrading enzymes can play an important role in the decomposition of biofilms produced by many pathogenic microbes and selective targeting of biofilms using glycoside hydrolase enzymes is possible because the chemical structure and nature of glycosidic bonds in some polysaccharides present in the biofilms of these microbes have been studied [13].

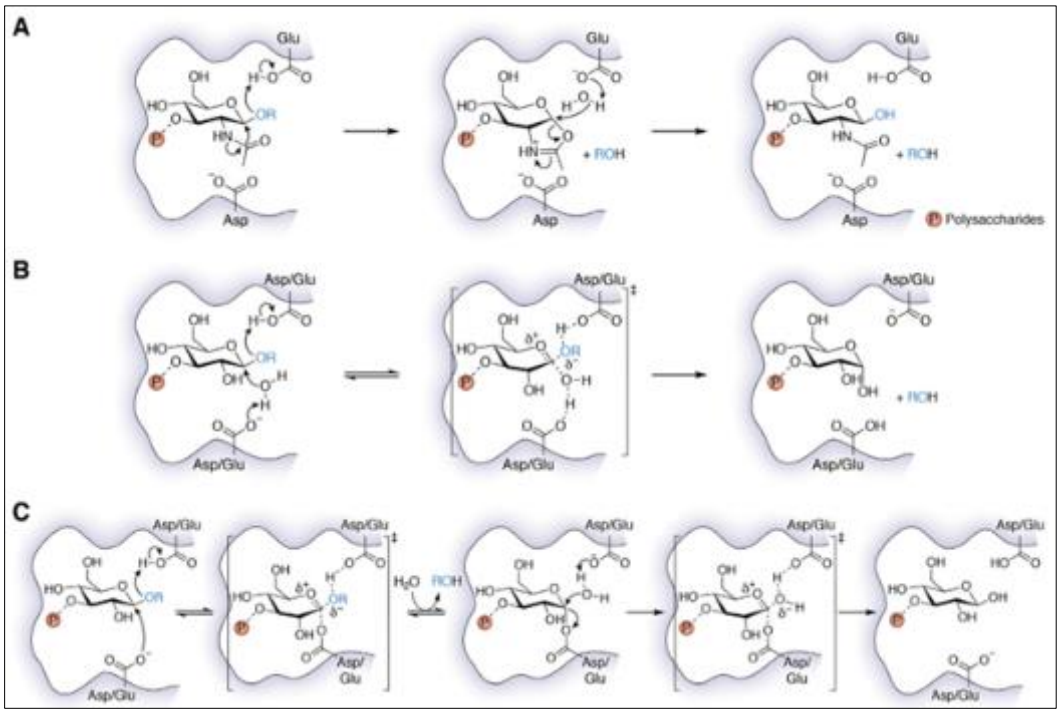

Figure 3 Possible mechanisms of cleavage of glycosidic bonds of polysaccharides in biofilms by glycoside hydrolase enzymes. A. Dispersin B mechanism involving an oxazoline intermediate during catalysis. B, mechanism of glycoside hydrolase conversion following the intended pathway. C, mechanism of glycoside hydrolase involving formation of a covalent enzyme-substrate intermediate

PelAh and PslGh enzymes belong to the polysaccharide-degrading enzyme mechanism that binds eDNA and dimatrix proteins to form a cohesive and structurally strong antibiofilm. The production of these extracellular matrix glycans provides protection not only for the cells that synthesize these molecules but also for other bacteria residing in the biofilm matrix. Pel can promote mixed species interactions with *S. aureus* and Psl can provide protection against detergent and antibiotic stress in *E. coli* and *S. aureus* [14].

Pel and Psl also play important roles at various stages of *P. aeruginosa* biofilm development and maturation. PelAh and PslGh are compatible with each other with antibiotics and neutrophils because the enzymes act as adjuvants for the innate immune system and antibiotics do not alter growth and therefore do not exert direct selective pressure on the bacteria. The rapid action of these enzymes avoids the need for bacteria to be exposed to the molecules for a long time and should reduce the risk of resistance [14].

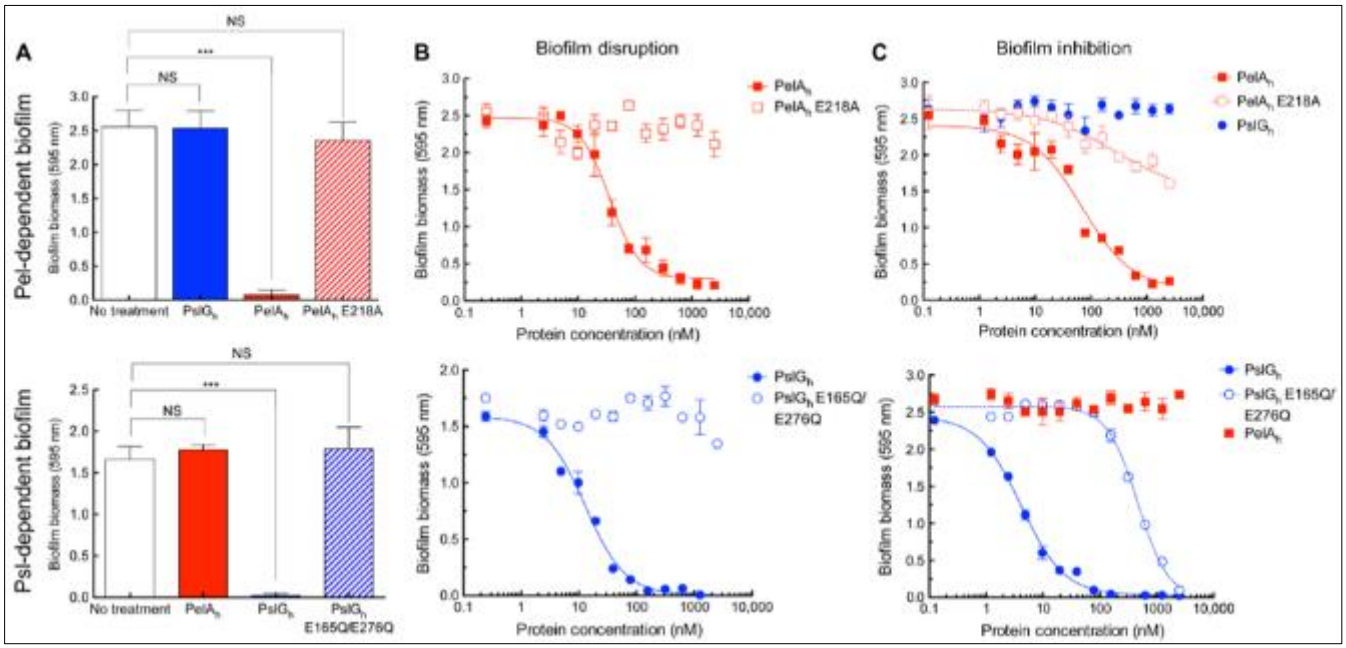

Figure 4 Glycoside hydrolases PelAh and PslGh catalyze inhibition and disruption of *P. aerungiosa* biofilms

Amylase enzyme belongs to the class of hydrolase enzymes that digest starch and has a mechanism of action as a polysaccharide-degrading enzyme. Amylase enzyme works by hydrolyzing starch and can remove organic adhesive residues from paper. Amylase enzyme shows effectiveness in good antibiofilm activity on *S. aureus* and *P. aeruginosa*. Strands of amylase-producing bacteria are also used to extract amylase enzyme to reduce the development of biofilm formation. The ability of amylase enzyme to remove and decompose biofilms makes it very suitable for the treatment of invasive bacteria and diseases. The highest antibiofilm activity of amylase enzyme was recorded at pH 7 and 37oC [15].

4.3. Enzim antiquorum sensing

Quorum sensing (QS) is a bacterial communication system that is important in regulating bacterial physiology such as motility, virulence, symbiosis, competence, conjugation, sporulation, antibiotic production, and biofilm formation [13]. Antiquorum sensing treatment is quite reliable in preventing biofilm formation by inhibiting the motility of microbial gathering to form biofilms [16]. Intervention in the bacterial QS communication system or activity causes a decrease in microbial virulence by inactivating or degrading QS signal molecules which can be done in several ways such as antibody formation, enzymatic destruction, or inhibitory agents against QS signal molecules [17].

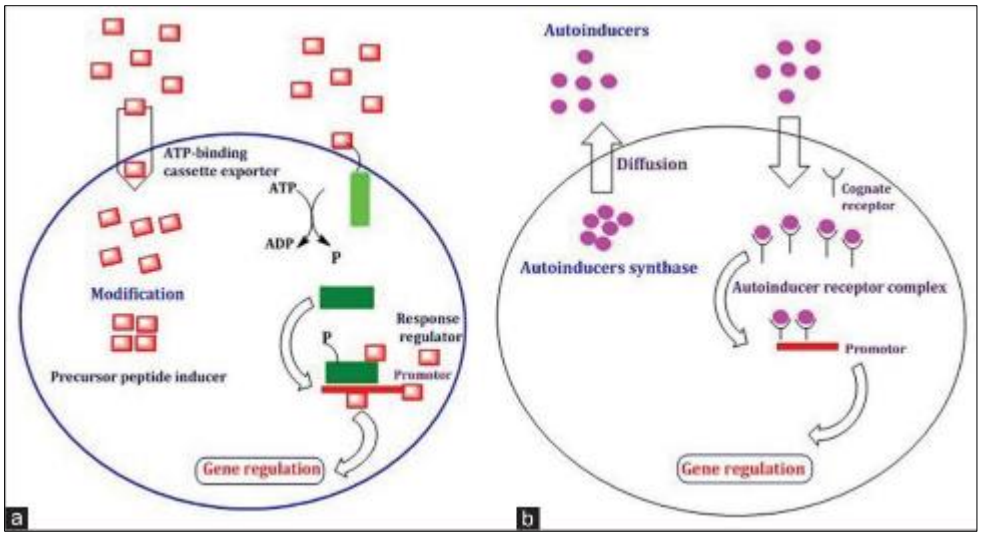

Figure 5 QS molecular signaling network graph in (a) gram-positive bacteria and (b) gram-negative bacteria

Quorum cooling has been investigated as a biofilm control approach in many clinical and industrial settings due to the important role of quorum sensing in regulating biofilm formation [18]. In the results of the study, the researchers combined phage treatment and quorum quenching into a single unit, namely phage quorum quenching. The results showed that such phage has a two-pronged effect that lyses host bacteria in the biofilm and expresses the enzyme AiiA to disrupt quorum sensing between bacteria. Consequently, compared to wild-type phage, the engineered phage T7aiiA showed increased antibiofilm effects in mixed-species biofilms consisting specifically of *P. aeruginosa* PAO1, *E. coli* TG1, and *E. coli* Bl21. The increased inhibition by T7aiiA was due to the expression of AiiA because the antibiofilm effects of T7aiiA and T7wt were similar in mixed-species biofilms [19].

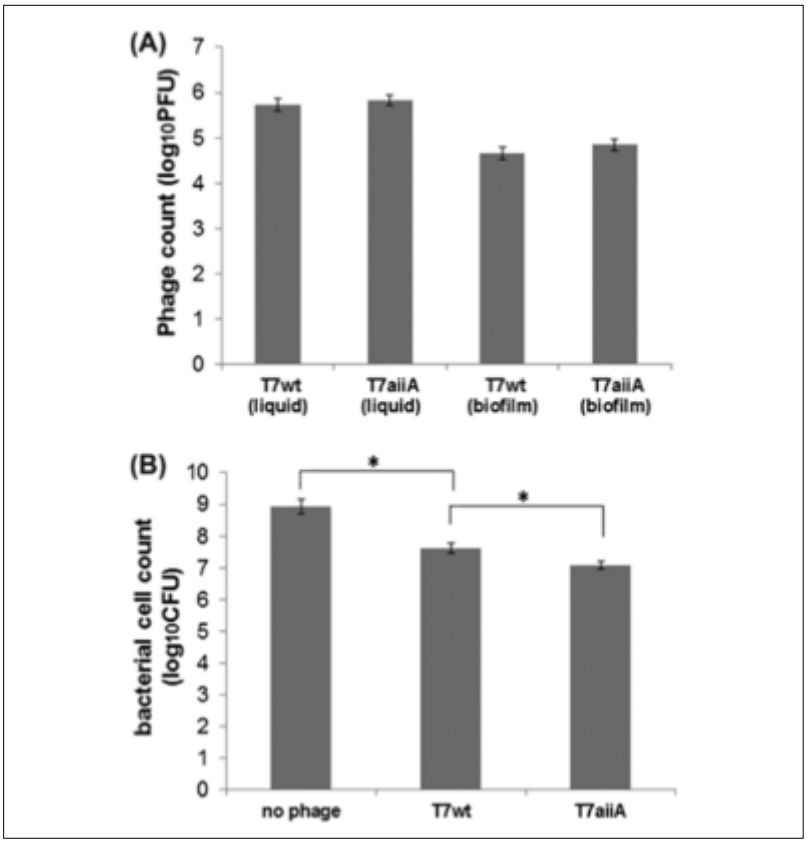

Figure 6 Effect of phage T7aiiA on cell number and phage number in mixed-species biofilms

4.4. Oxidative enzymes

Root canal treatment failure commonly associated with Enterococcus faecalis biofilm formation is dependent on the production of extracellular DNA (eDNA). DNase treatment can remove eDNA released by *E. faecalis* during bacterial attachment and initial biofilm formation. The study found that DNase treatment had little impact on *E. faecalis* biofilm attachment and stability. However, the effect of DNase treatment is still believed to be more focused on locations that are inaccessible when cleaned mechanically. The matrix-degrading effect of DNase can increase the penetration of antiseptic agents into the biofilm. The use of a mixture of DNase and NaOCl or Ca(OH)2 in chlorhexidine is not allowed because it will inactivate the enzyme due to the highly alkaline environment [20].

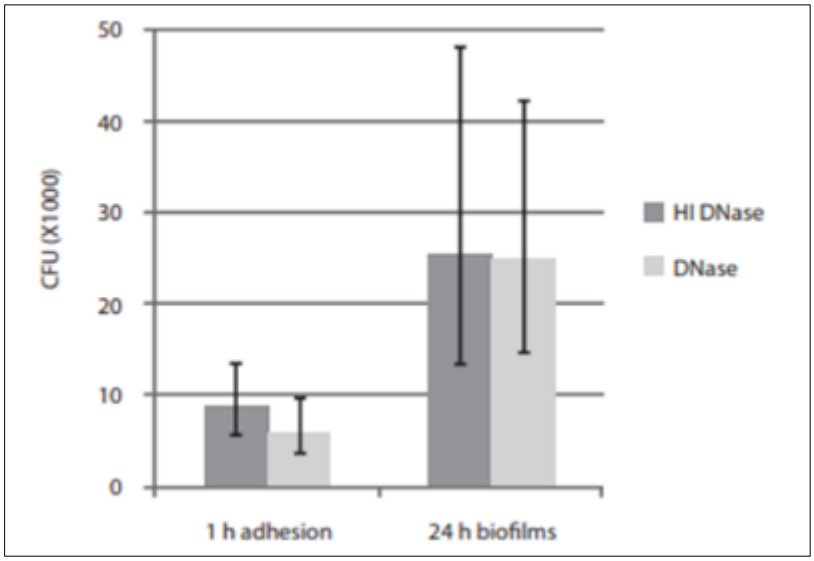

Figure 7 Effect of 1-hour treatment with heat-activated DNase (HI DNase) or DNase or after 24 hours of biofilm formation. The number of adherent cells was quantified using CFU counting

4.5. Proteolytic enzymes

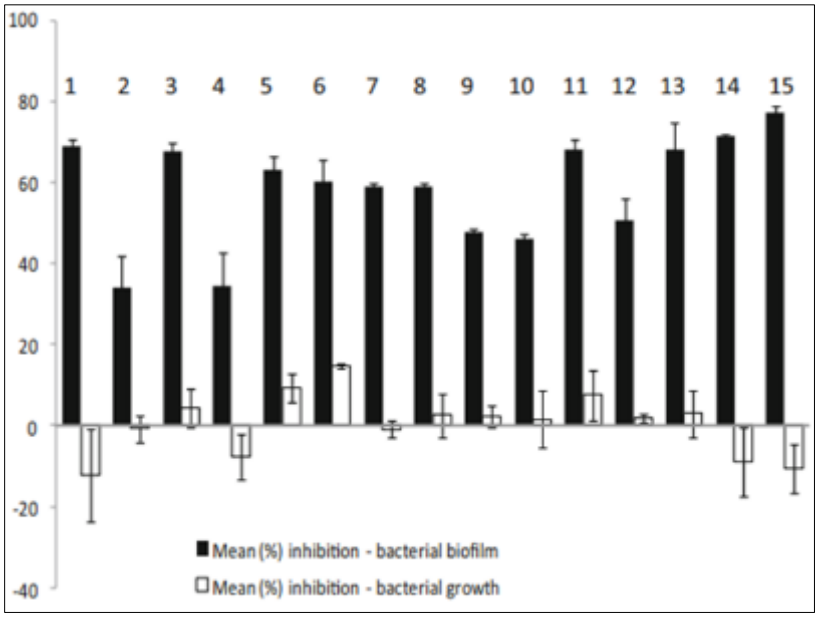

Figure 8 Inhibitory effect of *S. epidermidis* culture supernatant from strands colonizing the nose with *S. aureus*
Conclusion (Heading 1, WJS heading level 1)

Proteolytic enzymes, also known as proteases, cleave peptide bonds connecting two amino acids by a hydrolytic reaction mechanism [21]. Proteases are a class of proteolytic enzymes and can hydrolyze protein macromolecules

attached to the surfaces of food processing facilities that are difficult to access such as pipes [3]. The results of the EPS index composition and biofilm removal showed that serine protease was better at removing Bacillus biofilms than polysaccharidase, while the effect of polysaccharidase was better at eliminating P. fluorescens biofilms [16].

Serine protease enzymes produced by *S. epidermidis* have been reported to inhibit *S. aureus* biofilm formation and nasal colonization. The effects of serine proteases (Esp) may be mediated by the degradation of specific proteins important for biofilm formation and host-pathogen interactions [22].

5. Conclusion

Hydrolase enzymes are one class of enzymes included in anti-biofilm enzymes for oral bacteria such as *P. aeruginosa*, *S. aureus*, *E. coli*, and *E. faecalis* with mechanisms that are categorized as polysaccharide-degrading enzymes, proteolytic enzymes, and antiquorum sensing enzymes. From several existing mechanisms, it can provide different effects depending on the target microbe.

Compliance with ethical standards

Acknowledgments

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Disclosure of conflict of interest

The author(s) declare that they have no Conflict of Interests.

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