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REVIEW

Role of acyl-homoserine lactone quorum-sensing system in oral biofilm formation: A review

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Abstract

Background: The function of Acyl homoserine Lactone (AHL) as a communication system in oral biofilm formation by oral microorganisms is essential. A complete understanding of the role of AHL in oral microflora leads to new strategies for controlling biofilms and promoting oral health. The relationship between AHL and oral biofilm formation is not fully understood. Closing this knowledge gap leads to a better understanding of how oral AHL influences the microbiota and enables the development of more effective oral healthcare strategies**.** **Objective:** To summarise the connection between AHL and oral biofilm formation, including the mechanisms involved and the impact of Quorum sensing (QS) on oral and dental health. **Methods**: The authors performed a comprehensive literature review utilising Google Scholar and PubMed to investigate the association between the AHL and the development of biofilms by oral microflora. **Result:** The research included a comprehensive overview of the existing knowledge regarding the interplay between AHL signalling and the formation of oral biofilms. **Conclusion**: The AHL quorum sensing system plays a fundamental function in developing and organising oral biofilms, which contribute to oral and dental diseases such as gum disease and dental caries.

Introduction

Normal microflora is generally harmless, and it often causes biofilms to grow in the oral cavity. It was observed that bacterial communication is not only necessary for the survival of the microorganisms but also a major factor in their ability to develop and live in biofilms, causing oral diseases. The fight against oral bacteria biofilms has been ongoing for a long time, even before the current understanding of biofilms (Niazy, 2021).

Dental plaque is a very intricate biofilm created by the build-up and interaction of microorganisms that reside in the oral cavity, which adhere to the surfaces of the teeth and are planted in a matrix of extracellular polymers (Fuqua & Greenberg, 2002). Indeed, an imbalance in the population of resident microorganisms caused by changes in the local environment is responsible for the two primary diseases caused by oral bacteria: periodontal diseases and tooth decay (Muras *et al.*, 2020).

Gram-negative bacteria such as *P. gingivalis* have been identified as producers of AHL, a QS signal, which is believed to affect the development of oral biofilms. The high frequency of this bacterium found in dental plaque and saliva samples shows that AHL, by up to 60%, underscores the importance of this signalling system in oral microbiology (Muras *et al.*, 2020; Basavaraju *et al.*, 2016; Grandclément *et al.*, 2016).

Quorum sensing (QS) is a procedure by which bacteria react to extracellular molecules known as autoinducers (AIs) produced when the population grows. AHL is better understood and characterised among several QS signalling system molecules (Perry & Cvitkovitch, 2011). QS, facilitated by AHL, plays a significant role in oral biofilm formation, such as dental plaque formation. However, despite evidence of AHLs in saliva, sputum,

and dental plaque samples, we still do not fully understand how the signalling relationships and processes in dental biofilms affect commensal microbiota and dysbiosis (Marsh, 2006). The limited understanding of the interspecies interactions and dynamics within the oral microbiome is a gap in the research on the role of the AHL in biofilm formation. Further investigation is needed into the effect of AHL on these interactions and the effect on microbiome stability and composition, which will contribute to discovering novel therapeutic methods to limit and control biofilm formation.

Novel approaches are necessary to control oral biofilms. Developing chemical agents to control oral biofilms is crucial as current removal methods rely solely on mechanical means with defects such as not reaching a deep point. With the increasing problem of antibiotic resistance, alternative methods of treating infections are necessary. On the other hand, using antibiotics creates selective pressure on bacteria. It disrupts their essential functions, which has led to the proposal of Quorum quenching (QQ) as an attractive option for antimicrobials. These alternatives to antibiotics help address the rise of antibiotic resistance and provide modern strategies for treating bacterial infections (Asahi *et al.*, 2010; Bhardwaj *et al.*, 2013).

This review aimed to explore the potential influence of the role of AHL molecules on biofilm formation and oral diseases. The focus was primarily on elucidating whether AHL assists in initiating and progressing oral biofilm formation, either through direct or indirect mechanisms. To address these topics, this review intended to improve our understanding of the role of AHL in oral microbiota.

Mechanism of action for AHL

In the oral cavity, the main QS are AHL molecules, which are essential for regulating a variety of bacterial cellular processes, such as virulence gene expression, growth inhibition, antibiotic synthesis, biofilm formation, antibiotic resistance, and bioluminescence (Li & Nair, 2012) as seen in Table I.

Table I: Overview of the most significant oral biofilm microorganisms producing AHL

No	Bacterium	Inducer	Important notes	Source
1	*P. gingivalis*	AHL	Demonstrated that introducing specific types of AHL affects disease-related characteristics, such as the activity of proteases and the production of lactic acid, in laboratory models of oral biofilm.	(Parga *et al.*, 2023)
2	*A. actinomycetemcomitans*	AHL	Gram-negative bacteria communicate using AHL as signal molecules. *A. actinomycetemcomitans* primarily use AI-2 as a signalling molecule.	(Abdullah *et al.*, 2021)
3	*Pseudomonas aeruginosa*	AHL	*P. aeruginosa* has three quorum-sensing signalling systems related to AHL. LuxI-type synthases make them and LuxR-type receptors are responsible for their detection and produce the biofilm.	(Galloway *et al*., 2011)
4	*A. hydrophila*	AHL	*A. hydrophila* produces AHL molecules, allowing communication and coordination through the AhyI/AhyR QS response to population density system changes. This results in the regulation of virulence gene expression and host colonisation.	(Chen *et al*., 2010)
5	*Agrobacterium tume-faciens*	AHL and AHL lactonase s	The AHL and AHL lactonase from the bacterium *Agrobacterium tumefaciens* disrupt virulence factor production regulated by QS, and its active highlights specific roles in disrupting tyrosine and aspartate.	(Liu *et al*., 2007)
6	*Burkholderia pseudomallei*	AHL	The formation of biofilms by *Burkholderia pseudomallei* in the oral cavity is regulated through QS by AHL, which contributes to the persistence of the bacterium. Biofilm-defective mutants showed biofilm production, and the amount of AHL in biofilm cells was higher than in planktonic cells.	(Sawasdidoln *et al*., 2010)
7	*Fusobacterium nucleatum*	AHL, AI-2	*Fusobacterium nucleatum*, AHL, is critical in oral cavity infections and cancer progression. According to studies, AHL-facilitated QS causes endodontic and periodontal infections and OSCC. AHL causes cancer by inhibiting immune responses.	(Sankar *et al.*, 2023)

The signalling function of AHL is contingent upon the configuration of the carbon chain. The AHL structure exhibits functional diversity by incorporating oxo- or hydroxyl groups within these chains (Nagi *et al*., 2023). Studies have revealed that the concentration of AHL is essential for maintaining bacterial balance and that

bacteria-produced AHL promotes the colonisation of pathogens in periodontium in oral biofilms (Grandclément *et al*., 2016).

Furthermore, AHL stimulates the rise of commensal bacteria at low concentrations, whereas, at high concentrations, it prevents the growth of harmful bacteria and hinders the formation of diseases that transform multispecies microbial populations from favourable to pathogenic conditions. AHL, therefore, functions as a modulator (Grandclément *et al*., 2016; Kolenbrander *et al.*, 2010).

AHL molecules are generated by dedicated synthases while accumulating both inside and outside of the bacterial cells. AHL molecule concentration rises as the bacterial community does, achieving a concentration level known as "*quorum*." At this point, the molecules are recognised by specific receptors called "*R-proteins,"* such as *Lux*R and *Las*R, and target genes' transcription is induced. The AHL molecules vary in length and chemical composition, with each species producing specific signal molecules for intraspecific communication. The diverse AHL signal molecules all contribute a common homoserine lactone ring and are synthesised by dedicated, non-promiscuous AHL synthases Figure 1 (Li & Nair, 2012).

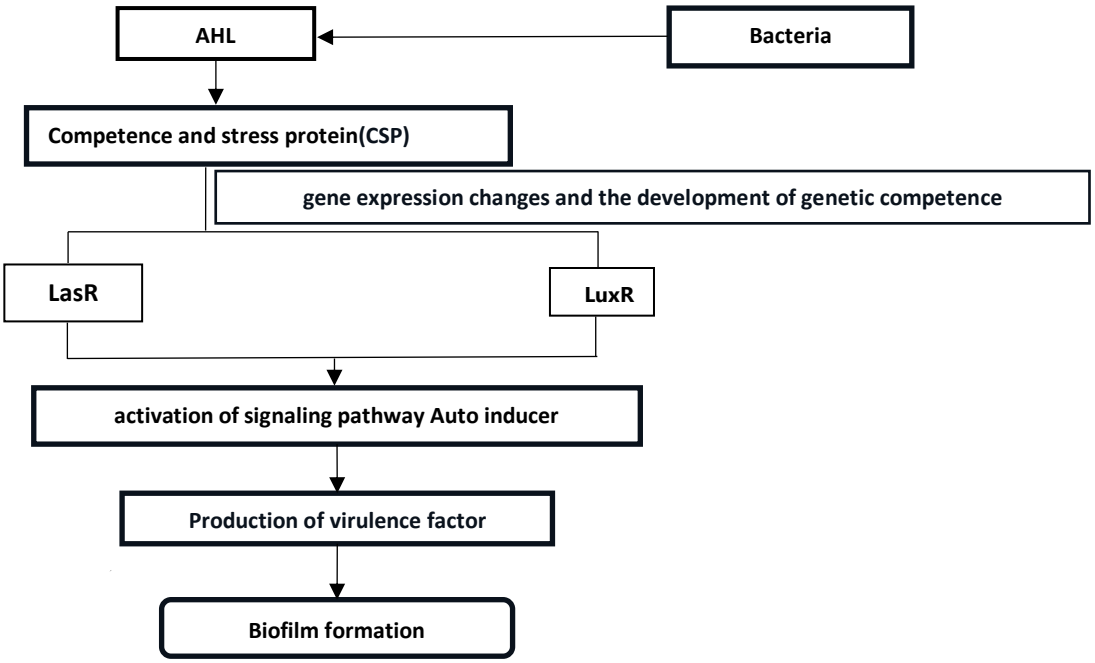

Figure 1: Schematic presentation of mechanism of action for AHL.

Biofilms and the AHL mediator of oral bacteria:

Dental plaque is a biofilm of billions of microbes that colonise healthy oral cavities and is the main cause of caries and gum disease. Communication among plaque microbes is crucial for its development, involving physical and metabolic connections, along with cell-to-cell communication via QS. Autoinducer 2 (AI-2) signals, produced by Gram-negative and Gram-positive bacteria, are a key part of multispecies oral biofilm (Muras *et al.*, 2020).

The AHL role in dental plaque development after Saliva and sputum samples have shown the presence of AHL in large amounts in many AHL-producing strains of *Klebsiella* sp., *Burkholderia* sp., *Citrobacter amalonaticus* (*Levinea amalonaticus*), *Pseudomonas putida*, *Klebsiella pneumonia*, and *P. gingivalis* were shown to contain homologues of a *Lux*R-type receptor and the AHL-synthase gene (Muras *et al.*, 2020; Chawla *et al.*, 2010; Wu *et al*., 2009).

A recent study suggests an applicable role for QS depending on AHL in dental plaque formation and dysbiosis and creates fresh possibilities for periodontal disease treatment or prevention (Muras *et al*., 2020). Cytoplasmic receptor proteins play a significant influence in the detection of AHL as signalling molecules. The dimerization of these receptor proteins occurs in response to binding with AHL, thus allowing for the detection of these signalling compounds. Additionally, these protein receptors attach to the areas of the target gene promoters and modulate gene expression through either activation or inhibition of

transcription. This mechanism of regulation serves as a crucial component in the cellular response to environmental stimuli (Huang *et al*., 2011).

Recently, it was shown that the exogenous addition of AHL influences pathological characteristics such as protease activity and lactic acid generation in (*in-vitro*) oral biofilm models (Muras *et al.*, 2020).

Advances and future directions

A major advance in AHL research is the discovery of novel AHL molecules and their diverse functions (Guo, He, & Shi, 2014). Traditional AHL were primarily found in Gram-negative bacteria, but recent studies have identified AHL molecules in Gram-positive bacteria and other microorganisms. These findings have expanded our understanding of AHL signalling systems and their potential roles in different bacterial communities and ecological niches (Kusada *et al*., 2019).

Another exciting advancement is the development of new tools and techniques for studying AHL. These include biosensors, reporter systems, and high-throughput screening methods that allow for the detection and characterisation of AHL molecules and their interactions with receptors. These tools provide valuable insights into the dynamics of AHL networks, as well as the identification of specific AHL-receptor pairs (Brooks & Alper, 2021).

Furthermore, bacteria often employ multiple signalling mechanisms, including other QS systems, two-component systems, and small molecule-mediated signalling pathways. Investigating the interplay between AHL signalling and these other systems uncovers intricate regulatory networks and sheds light on the complex behaviour of bacterial communities. Additionally, exploring the diversity and functional significance of AHL molecules across different bacterial species and ecosystems provides insights into the variations in AHL systems and their implications for oral health. Investigating the ecological and evolutionary implications of AHL, unravelling the mechanisms underlying AHL-mediated host-microbe interactions, and exploring AHL-based therapeutic strategies for oral diseases are also important directions for future research. By addressing these areas, we can advance our knowledge of AHL in the oral environment and improve oral healthcare strategies.

Studies on therapies based on AHL

The limitations of traditional treatment techniques and the link between oral microorganisms and oral and systemic diseases highlight the need for new and effective approaches to prevent plaque formation (Lee *et al*., 2021).

Antibiotic resistance is a significant obstacle in the medical field, but the development of new antibiotics is proceeding at a slow pace. An alternative solution is to focus on anti-virulence strategies, which have gained increasing attention as a complement to modern antimicrobial treatments. This strategy reduces the evolutionary pressure on antibiotic resistance, among other advantages, expanding antibacterial targets and preserving the host's native microbiome. For example, AHL reacts with its *Lux*R receptors to trigger virulence gene expression. So, the control of AHL-based QS reduces biofilms and bacterial virulence (Wang & Ma, 2014). The production of AHL plays a crucial role in bacterial communication and virulence. However, there are ways to inhibit its production to prevent biofilm formation, including inhibiting the synthesis of its precursor molecules or blocking the activity of the AHL synthases. The precursor molecules for AHL synthesis are acyl carrier protein and S-adenosyl-L-methionine (SAM), which are necessary for bacterial metabolism and survival. Therefore, inhibiting these precursors can be toxic to bacteria and affect the production of other important chemicals (Christensen *et al.*, 2013).

The quest for QS signalling inhibitors has been a main area of research-creation, particularly those based on AHL. AHL is inactivated by hydrolytic enzymes from other bacteria, and human paraoxonases have also been demonstrated to hydrolyse AHL. Despite the possibility that these enzymes are effective treatments, there are drawbacks, including severe immunogenicity for the bacterial enzymes and extensive substrate promiscuity for the mammalian enzymes (Debler *et al*., 2007). Some studies mentioned an integrated therapeutic strategy after evidence of its effectiveness both in vitro and *in vivo*. Such as antibiotics combined with biofilm-dispersing agents or QQ rather than as a stand-alone treatment by QQ (Grandclément *et al*., 2016).

Finally, given the AHL molecules' extracellular dispersion and evolutionarily conserved parts, the microbial QS systems constitute an appealing target for immunotherapy against infections caused by biofilm (Kaufmann *et al*., 2008). Future studies should explore the cross-regulation between AHL signalling and other pathways, as well as their combined effects on bacterial physiology, biofilm formation, and virulence (Wang *et al*., 2020). Future research may also focus on developing more sensitive and specific detection technologies and methods (Coccia *et al*., 2021). Accurate characterisation of AHL molecules in the oral environment leads to a deeper comprehension of their role in biofilm formation and oral health.

Conclusion

The AHL plays a fundamental role in developing and organising oral biofilms, which contribute to oral and dental diseases such as gum disease and dental caries. However, the exact mechanisms of AHL signalling and microbial interactions in oral biofilms still need to be fully understood. Further research is needed to unravel the intricate relationships between the AHL system, oral microflora, and biofilm formation. Understanding the AHL mechanism in oral microflora offers valuable insights for developing novel strategies to control oral biofilm formation and promote oral health**.**

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Abbreviations

ACP: acyl-carrier protein; Acyl-CoA: acyl-coenzyme A; AHLs: N-Acyl-L-homoserine lactones; AAS: Acyl-ACP synthase; AI: Auto inducer; AI-2: Autoinducer 2; HPLC: High-performance liquid chromatography; MTA: methylthioadenosine; OSCC: oral squamous cell cancer. QQ: Quorum quenching; QS: Quorum sensing; QSI: Quorum Sensing Inhibition; SAM: S-adenosyl-L-methionine; CSP: Competence and stress protein; TLC: thin layer chromatography.

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