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# BIOFILM FORMING POTENTIAL OF *STREPTOCOCCUS SUIS*: FOCUSING ON LUXS/AI-2-MEDIATED QUORUM SENSING SYSTEM

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***ABSTRACT***

Because of its virulence and ability to survive, the incidence of infection caused by *Streptococcus suis* (*S. suis*), an emerging zoonotic pathogen, is expected to increase significantly. A biofilm-forming process, which is a cornerstone of chronic infection, influences the survival rate of *S. suis*. The mechanism helps bacteria to live longer in host tissues, form colonies, escape immune clearance, and share genetic information. At this moment, the most studied regulatory mechanism of *S. suis* biofilm formation is Quorum Sensing (QS), mainly on LuxS/AI-2-mediated QS system, in which AI-2 is the most closely related molecule to biofilm formation. In this system, LuxS acts as the key player in the process. The understanding of biofilm formation in S. suis, especially the LuxS/AI-2-mediated QS system, is a valuable contribution to future therapeutic research frameworks.

***Keywords****: Streptococcus suis, biofilm, Quorum Sensing, LuxS, Auto Inducer-2*

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## INTRODUCTION

*Streptococcus suis* (*S. suis*) is a gram-positive facultative anaerobic bacteria which is well-known as a commensal microorganism at the respiratory system of the swine. On the other hand, in human, this bacteria is a potentially serious threat to induce fatal complications such as meningitis, septic shock, endocarditis, and peritonitis after the onset of infection (1, 2). The majority of *S. suis* infection was found in areas with pig-farming and pork industries. From 2002 until 2013, nearly more than 1.500 cases of *S. suis* infections was reported globally, most of them came from Asian countries (3). South East Asia was considered to be a potentially threatened area with its high amount of pork consumption and swine-related industries. A research conducted in Sanglah Hospital, Bali, from 2014 until 2017 has shown that there were 44 cases of *S. suis* meningitis confirmed by Polymerase Chain Reaction (PCR) of the patients' cerebrospinal fluid sample (4). The incidence of *S. suis* infections in human is predicted to elevate significantly due to unavailability of vaccines as the specific protection and the ability of its virulence factors to induce catastrophic pathological mechanisms (5).

The most prevalent strain of *S. suis* which has isolated in human is serotype 2 (86,5%), followed by serotype 14 (2,3%), and 1 (0,6%). *Streptococcus suis* serotype 2 (SS2) has considered to be the most aggressive subtype among all serotypes (6). The exacerbation of inflammatory pathway is a hallmark of SS2 infection in both human and swine. Biofilm formation by *S. suis* can cause a chronic infection that is especially difficult to treat when the pigs' immunity decreases (7). Biofilm forming ability has known to be a foundation of chronic infection (8) and the most effective mechanism to hinder from host's immunity. By aggregating into a biofilm, microorganisms may escape the dangerous host environment. The activity makes it possible for bacteria to live longer and form colonies in host tissues, as well as prevent immune clearance and share genetic information (9). To date, studies related with the

mechanism of *S. suis* biofilm formation are mainly focused Quorum Sensing (QS), mainly on LuxS/AI-2-mediated QS system. The knowledge about biofilm formation in *S. suis*, particularly on LuxS/AI-2-mediated QS system, is a worthwhile contribution in constructing research frameworks in future therapeutic strategies (8).

**The General Concepts of Bacterial Biofilm and its Formation**

Biofilm is an architecturally complex communities in which microorganisms are bound to a substratum and trapped in a matrix of polymetric substances that provide them with a high degree of tolerance and resistance (10). Biofilms have a number of effects on humans, the majority of which are harmful (11). In general, all biofilms contain an extracellular matrix that holds cells in a pack. This matrix is frequently made up of a polysaccharide biopolymer as well as proteins and Deoxyribonucleic Acid (DNA) (12). Matrix exopolysaccharide has a wide variety of properties depending on growth conditions, medium, and substrates. Biofilms have adhesive proteins in their extracellular matrix. For example, Biofilm-associated proteins (Bap) are present in the matrix of *Streptococcus aureus* (13). Extracellular DNA, in addition to exopolysaccharides and proteins, provides structural integrity to the biofilm, which was previously believed to be the product of cell lysis and subsequent genomic DNA release. The biofilms are stabilized by the eDNA (14).

There are five stages of bacterial biofilm formation in general: initial/reversible attachment, irreversible attachment, formation of micro-colonies, maturation, and cellular detachment or dispersion. The bacteria make contact with the surface through the cell pole and are transiently bound to the substratum during the reversible attachment stage. There was a reorientation to the longitudinal cell axis, cell cluster growth, nonmotile, and activation during irreversible attachment. During maturation, layered cells form clusters, and at the end of the process, the maximum cell cluster formation was observed, reaching a thickness of 100 m. The majority of cells were also displaced from the substratum at this point. Dispersion, the final stage of biofilm formation, revealed changes in cell cluster composition, the formation of pores and channels, and dispersion (15). Depending on environmental conditions and unique strain characteristics, different bacteria use different mechanisms to form biofilms (16).

Several studies have shown that *S. suis* can form biofilm in vitro using various biological models. A number of factors influence the formation of biofilm in *S. suis*, including the OCT protein, the signaling molecule autoinducer-2 (AI-2), and the collagen-binding 40 (cbp40) (8). There are four categories of bacterial biofilm's regulatory mechanism called Extracytoplasmic Function (ECF) signaling pathway, Two-Component System (TCS), intracellular second messenger cyclic diguanylate (c-di-GMP), and bacterial Quorum Sensing (QS) system (17-20). The most studied regulatory mechanism of *S. suis* biofilm formation at the moment is QS, which is primarily based on the LuxS/AI-2-mediated QS framework, with AI-2 being the most closely related molecule to biofilm formation. In S. suis, the *luxS* gene controls biofilm formation (10). According to one study, inhibiting the expression of *luxS* in *Riemerella anatipestifer* can reduce AI-2 production, which has a direct impact on biofilm formation (21).

**The LuxS/AI-2-mediated Quorum Sensing System**

In *S. suis*, biofilm formation is largely influenced by bacterial intercellular communication through QS, which is involved in a number of physiological processes including extracellular protein synthesis, biofilm maturation, and virulent factor gene expression. QS is a cell-to-cell communication system that controls the expression of genes in bacteria to promote organized adaptation of various genes (22). When bacteria are faced with a difficult situation or climate, the QS system sends information signals between cells to increase the number of bacteria, biofilm formation, and EPS production, allowing bacteria to

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better adapt to their surroundings (8). QS systems can be divided into some classes. They are LuxR-LuxI systems of Gram-negative bacteria, the auto-inducing peptide of Gram-positive bacteria, and the LuxS/AI-2 system found in both Gram-positive and Gram-negative bacteria (10).

The general mechanism of QS is to ensure that a bacteria's capacity to generate and release signaling molecules known as autoinducers (AIs) is not impaired (23, 24). The QS-controlled mechanism, which is a molecular signal network regulated by the *luxS* gene encoding the S-ribosylhomocysteinase (LuxS) enzyme found in virulent SS2, allows *Streptococcus suis* to form biofilms. LuxS has been implicated in enhancing Auto Inducer-2 (AI-2) biosynthesis, adhesions, biofilm formation, cell metabolism, and resistance to host immune responses and antimicrobial therapy in a number of studies (22, 25, 26).

The gene that encodes LuxS; the *luxS* gene, is highly conserved in bacteria. High identity and similarity of *luxS* genes were found in *Streptococcus mutans, Streptococcus pyogenes, Streptococcus pneumoniae, Lactococcus lactis, Clostridium perfringens, Neisseria meningitidis, Escherichia coli,* and *Haemophilus influenza* (27). LuxS is involved not only in the development of the AI-2 signaling molecule, but also in central bacterial metabolism and is a component of the activated methyl cycle (28). LuxS is specifically involved in the conversion of S-adenosine homocysteine to S-adenosylmethionine (SAMe).

SAMe is a typical biomolecule that primarily functions as a methyl donor. The loss of SAMe feature and inhibition of AI-2 synthesis occurs when luxS is mutated or deleted. LuxS is a critical component of the LuxS/AI-2-mediated QS system. AI-2 is a byproduct of bacterial methyl metabolism that helps activated methyl cycles regulate their metabolism (29). The methyl group is extracted from SAMe and then converted into S-Adenocylhomocysteine (SAH), which is a toxic metabolite, in the LuxS/AI-2 system. SAH is then converted to adenine and S-Ribose Homocysteine (SRH) by a 5'-methylthioadenosine/S-adenosyl homocysteine nucleosidase (Pfs). Following that, SRH is converted to 4,5-dihydroxy-2,3-pentanedione (DPD) and homocysteine acid (HCY) by LuxS (30). LuxS then catalyzes the cleavage of SRH's thioether linkage, resulting in HCY and DPD. Self-cyclization creates DPD, which is then transformed to AI-2 (Figure 1). As bacterial density grows, AI-2-mediated QS is activated (31).

When a small amount of AI-2 was applied to the growth medium, the ability of *S. suis* to form biofilm was greatly increased, while high concentrations of AI-2 inhibited the ability to form biofilm. At 24 hours, adding 2 µM AI-2 to the mix significantly improved biofilm formation, but had no effect at 48 hours (32). The capacity of *S. suis* to form biofilm is improved by overexpression of AI-2 and the incubation period, according to these studies (26).

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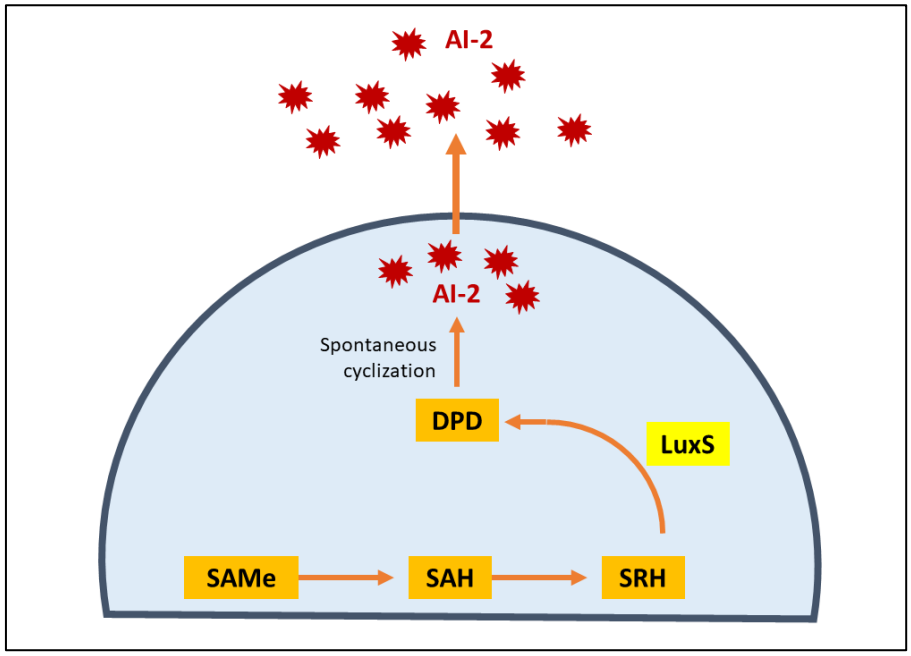


**Figure 1. AI-2 Synthesis Pathway in *S. suis*. SAMe, S-adenosylmethionine; SAH, thioglucoside homocysteine; SRH, thioglycoside-type homocysteine; DPD, 4,5-dihydroxy-2,3-pentanedione; AI-2, Auto Inducer-2.**

## CONCLUSION

The rising number of *S. suis* infections, coupled with the newly discovered importance of the LuxS/AI-2 system in *S. suis* cell-to-cell contact and virulence, necessitates the production of antibacterial strategies that target the LuxS/AI-2-mediated QS system. In order to regulate bacteria by inhibiting pathogen signaling, a better understanding of bacterial regulation and AI-2 uptake regulation, as well as the identification of genes involved in the *S. suis* QS system, is needed.

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