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Quantitative proteomic analysis of sub-MIC erythromycin inhibiting biofilm formation of *S. suis* in vitro

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ABSTRACT

Streptococcus suis (*S. suis*) is a swine pathogen and also a zoonotic agent. Biofilms of *S. suis* may cause persistent infections by the host immune system and antibiotics. Sub-minimal inhibitory concentration (sub-MIC) of erythromycin can inhibit biofilm formation in bacteria. Here, we performed comparative proteomic analyses of cells at two different conditions: sub-MIC erythromycin treated and nontreated cells. Using iTRAQ strategy, we found some novel proteins that involved in biofilm formation. 79 differentially expressed proteins were identified in sub-MIC erythromycin inhibiting planktonic cell when the protein had both a fold-change of more that a ratio >1.2 or <0.8 (p-value <0.05). Several cell surface proteins (such as Primosomal protein N′, L-fucose isomerase, and ABC superfamily ATP binding cassette transporter, membrane protein), as well as those involved in Quorum-sensing, were found to be implicated in biofilm formation. Overall, our results indicated that cell surface proteins played an important role in biofilm formation. Quorum-sensing played a crucial role leading to biofilm formation. ABC superfamily ATP binding cassette transporter, membrane protein and comD might act as channels for erythromycin uptake in Quorum-sensing system. Thus, our data analyzed rough regulatory pathways of biofilm formation that might potentially be exploited to deal with biofilm infections of *S. suis*. This article is part of a Special Issue entitled: Microbial Proteomics.

Biological significance
In this study, we identified many proteins involved in cell transport, biological regulation and signal transduction, stress responses and other metabolic processes that were not previously known to be associated with biofilm formation of *S. suis* and target spot of erythromycin. Therefore, our manuscript represents the most comprehensive analysis of protein profiles of biofilm formation of *S. suis* inhibited by sub-MIC erythromycin and provides new proteomic information about biofilm formation.

Abbreviations: *S. suis*, *Streptococcus suis*; QS, Quorum sensing; MICs, the minimum inhibitory concentrations; SEM, scanning electron microscopy; SCX, Strong Cation Exchange; CBD, Calgary Biofilm Device; TCP assay, crystal violet.

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1. Introduction

Streptococcus suis (*S. suis*) is one of the most important swine pathogens worldwide causing meningitis, arthritis, septicaemia, bronchopneumonia, and other pathologies. Furthermore, *S. suis* is an important zoonotic agent [1]. Two human outbreaks in China in 1998 and 2005 were associated with increased severeness of clinical symptoms, a high rate of mortality, and streptococcal toxic shock-like syndrome [2,3]. Thirty five serotypes of *S. suis* (types 1 to 34 and type 1/2) have been described, but type 2 is considered to be the most pathogenic for both human and swine [4]. It is reported that current studies indicate that *S. suis* maybe achieve through persistent infections in vivo by forming biofilms [5] and hence *S. suis* infections might be difficult to treat. Biofilms play a key role in the pathogenesis and persistence of several bacterial infections [6].

Throughout the biological world, bacteria thrive predominantly in surface-attached, matrix-enclosed, multicellular communities or biofilms, as opposed to isolated planktonic cells. Costerton et al. [7] proposed a basic definition of biofilm as "a structured community of bacterial cells enclosed in a self-produced polymeric matrix and adherent to an inert or living surface." The matrix components can be exopolysaccharides, proteins, nucleic acids, or other substances (referred to as extrapolymeric substances, or (EPS) that are believed to provide the cells with an array of advantages as compared to planktonic cells [7–10]. It is important, especially in the clinical context, where it is estimated that about 60% of all microbial infections involve bacterial biofilms [11].

Some studies have demonstrated that *S. suis* has the ability to form biofilms [12,13]. It is an important requirement for chronic colonization of human tissues and persistence in implanted medical devices. In addition, these structures are associated with multiple drug resistance [14,15]. Considerable investigation is required to gain a better understanding of biofilm formation. Bacterial biofilm formation involves several complex molecular mechanisms in which some proteins are thought to play a major role during important events such as cell adhesion, maturation, signaling and others [16–19]. Recent results indicate that biofilm cells have an active, although altered cell metabolism [20,21].

Biofilm initiation is mainly characterized by adhesion of cells to biotic or abiotic surfaces. Cell surface attachment is mediated by proteins [22]. Within the biofilm, the bacteria use cell-to-cell communication systems to pool their activities and act in a multicellular organized manner. One such activity is to launch their arsenal of virulence factors at the strategically right moment, and hence coordinate the progressive attack on the host. This process is termed quorum sensing (QS), whereby bacteria produce diffusible chemical signals (autoinducers) that interact with specific receptors on itself and on neighboring cells, which in turn regulate the expression of specific target genes [23].

QS may also function to control the population size in a biofilm. Biofilm formation has been described as a developmental cycle (Fig. 1), and QS may serve as the checkpoint for reinitiating the cycle by promoting dispersion or dissolution of a subpopulation of cells. In this case, dispersing cells might escape the nutritional stress that accompanies or follows

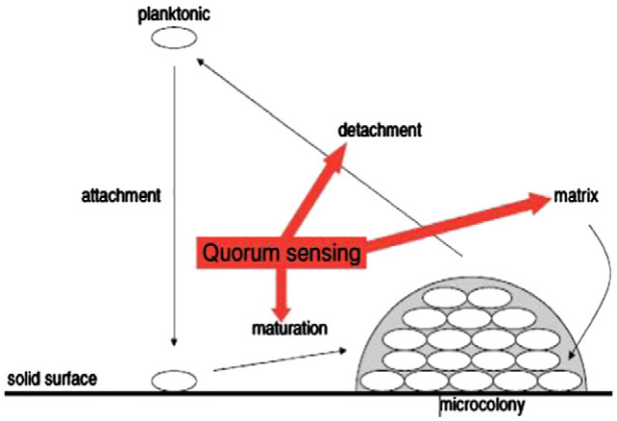

Fig. 1 – Developmental biology of biofilms. Unattached free-swimming bacteria (planktonic) initially attach to a solid surface, eventually maturing into structured aggregates called microcolonies. Biofilms are composed of these microcolonies, often encased in extracellular polymeric substances known as the matrix. Some biofilm bacteria detach from microcolonies and become planktonic, presumably to colonize a new surface. QS is believed to be involved in regulating different steps of biofilm development, depending on the organisms and growth conditions [24].

inducing concentrations of QS signal. For nonmotile species, QS might serve to regulate population density in a biofilm using a different mechanism. Some Gram-positive bacteria initiate autolysis in response to reaching a quorum [24].

Bacterial biofilms play relevant role in persistent infections, which are rarely eradicated with antimicrobial therapy [7]. Macrolides inhibit the transcription of several of these genes [25,26]. It has also proved to present potential effects on inhibition of bacterial biofilm with reduction of bacterial virulence factor when used in sub-inhibitory concentrations [27]. Erythromycin is produced by a strain of *Saccharopolyspora erythraea* (formerly *Streptomyces erythraeus*) and belongs to the macrolide group of antibiotics. It is reported that sub-MIC of erythromycin modified bacterial surface properties and enhanced not only biofilm formation but also cell-surface hydrophobicity. Erythromycin-induced biofilm formation may contribute to the inconsistent success of antimicrobial therapy for *C. diphtheriae* infections [28]. Naoko Takahashi reported that erythromycin reduced biofilm development of *Actinobacillus actinomycetemcomitans* in the early phase, and it enhanced biofilm development of *A. actinomycetemcomitans* in the mature phase [29].

Previous studies have investigated different immunogenic components of planktonically grown *S. suis* proteins e.g., secreted or cell wall associated proteins using immunoproteomic assays [30–33]. But the results of the proteomic research of sub-MIC erythromycin didn't find it to inhibit biofilm formation of *S. suis* in vitro. In this study, we identified several proteins in sub-MIC erythromycin inhibiting biofilm formation of *S. suis* by using iTRAQ technology. It is known about proteins targeted of erythromycin, antimicrobial agents against pre-formed biofilms of *S. suis* in vitro. The goal of the present study will lay a theory foundation for therapy of *S. suis* biofilm infection.