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CHARACTERIZATION OF PROTEIN-PROTEIN INTERACTION NETWORK IN *Staphylococcus aureus* BIOFILM

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ABSTRACT

Staphylococcus aureus is a Gram-positive bacterium inhabiting soft tissues such as the epidermis and the nasal cavity. In chronic diseases, including osteomyelitis, endocarditis and wound infections, biofilm formation in *S. aureus* causes an increase in antibiotic resistance. To date, the information about the protein interaction network in *S. aureus* biofilm is still limited. The present work was performed to characterize *S. aureus* proteins and their interaction networks using *in silico* approach, and to identify the proteins expressed in *S. aureus* biofilm using tandem mass spectrometry. The preliminary characterization of protein interaction network was performed using the STRING database. Following that, *S. aureus* biofilm was developed in 6-well microplate and harvested at 6 h, 12 h, 18 h, and 24 h. Expression of *S. aureus* proteins in all biofilm stages was determined using a combination of one-dimensional SDS-PAGE and HPLC-ESI-MS/MS. Results demonstrated that a total of 430 nodes and 958 functional interactions were produced in the network. The clustering coefficient values was predicted to be 0.411. There were 421 (96%) proteins predicted as hub proteins as they showed more than 10 functional interactions with other proteins in the network. A total of 147 biological processes, 46 molecular functions, 17 cellular components and 15 biological pathways were found to be significantly ($P<0.05$) enriched in the protein interaction network of *S. aureus*. Meanwhile, six SDS-PAGE gel bands (80 kDa, 60 kDa, 58 kDa, 47 kDa, 35 kDa, 32 kDa.) were consistently expressed in 24 h *S. aureus* biofilm. Identified *S. aureus* proteins from these protein bands included L-lactate dehydrogenase (quinone), malate:quinone oxidoreductase, carbamoyl-phosphate synthase large chain, chaperone protein DnaK, serine hydroxymethyltransferase, enolase, protein translation elongation factor G, citrate synthase, isocitrate dehydrogenase, S-adenosylmethionine synthase, phosphoglycerate kinase, pyruvate kinase, L-lactate dehydrogenase, catabolite control protein A, GTPase era, elongation factor Tu, pyruvate dehydrogenase E1 component subunit alpha, and histidine--tRNA ligase which were also identified *in silico*. There were 6 novelty proteins found in *S. aureus* biofilm formation which Malate quinone oxidoreductase, pyruvate dehydrogenase, histidine--tRNA ligase, GTPase Era, S-adenosylmethionine synthase and serine hydroxymethyltransferase. In conclusion, the biofilm formation by *S. aureus* may involve complex protein interaction network. The findings from the present study may be useful for identification of potential drug targets to control *S. aureus* infections.

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CHAPTER ONE

INTRODUCTION

1.1 Background of Study

Bacteria predominately exist in nature as complex microbial species attached to surfaces and held together by a network of extracellular polymeric substances (EPS). These microbial communities are often referred to as biofilms, where members of the community are subject to microbial competition, interaction, and coordination. Physiological coordination and spatial organization are considered to be used by biofilm bacteria to enhance both their metabolic efficiency and their tolerance to fluctuations in the local climate. Bacteria grow biofilms not only on underwater materials like normal freshwater environments but also on living tissues, water pipes, oral surfaces, implants and indwelling medical devices (Chew & Yang, 2016).

Staphylococcus aureus is a gram-positive bacterium inhabiting soft tissues such as the epidermis and the nasal cavity. In chronic diseases, including osteomyelitis, endocarditis and wound infections, biofilm formation in *S. aureus* causes an increase in antibiotic resistance. Infections of biofilms are clinically important because biofilm bacteria exhibit recalcitrant antimicrobial compounds and persistence against sustained host defences (Jenul & Horswill, 2019). A complex, multi-factorial process is the creation of a bacterial biofilm and can be divided into three stages that involve particular molecular factors: attachment, accumulation/maturation, and detachment/dispersal. A variety of macromolecules, including exopolysaccharides, proteins, extracellular eDNA and other polymers, are released by bacteria living in biofilms. *S. aureus* is considered to be one of the most common causes of nosocomial bacteremia, inflammation of the bloodstream and pneumonia in the hospital. *S. aureus* surface protein C and G (SasC and SasG), clumping factor B (ClfB), serine aspartate repeat protein (SdrC), biofilm-associated protein (Bap) and fibronectin/fibrinogen-binding protein (FnBPA and FnBPB) are independently involved in the development of biofilm matrix (Speziale *et al*., 2014).

Information of protein interaction networks is essential for defining and narrowing protein structure and protein function. Protein interaction network (PIN) in