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Comparative Proteomic Profiling of Planktonic and Biofilms from Staphylococcus aureus Using Tandem Mass Tag-Based Mass Spectrometry

Authors : Arifur Rahman, Ardeshir Amirkhani, Honghua Hu, Mark Molloy, Karen Vickery

Abstract : Introduction and Objectives: Staphylococcus aureus and coagulase-negative staphylococci comprises approximately 65% of infections associated with medical devices and are well known for their biofilm formatting ability. Biofilm-related infections are extremely difficult to eradicate owing to their high tolerance to antibiotics and host immune defences. Currently, there is no efficient method for early biofilm detection. A better understanding to enable detection of biofilm specific proteins in vitro and in vivo can be achieved by studying planktonic and different growth phases of biofilms using a proteome analysis approach. Our goal was to construct a reference map of planktonic and biofilm associated proteins of S. aureus. Methods: S. aureus reference strain (ATCC 25923) was used to grow 24 hours planktonic, 3-day wet biofilm (3DWB), and 12-day wet biofilm (12DWB). Bacteria were grown in tryptic soy broth (TSB) liquid medium. Planktonic growth was used late logarithmic bacteria, and the Centres for Disease Control (CDC) biofilm reactor was used to grow 3 days, and 12-day hydrated biofilms, respectively. Samples were subjected to reduction, alkylation and digestion steps prior to Multiplex labelling using Tandem Mass Tag (TMT) 10-plex reagent (Thermo Fisher Scientific). The labelled samples were pooled and fractionated by high pH RP-HPLC which followed by loading of the fractions on a nanoflow UPLC system (Eksigent UPLC system, AB SCIEX). Mass spectrometry (MS) data were collected on an Orbitrap Elite (Thermo Fisher Scientific) Mass Spectrometer. Protein identification and relative quantitation of protein levels were performed using Proteome Discoverer (version 1.3, Thermo Fisher Scientific). After the extraction of protein ratios with Proteome Discoverer, additional processing, and statistical analysis was done using the TMTPrePro R package. Results and Discussion: The present study showed that a considerable proteomic difference exists among planktonic and biofilms from S. aureus. We identified 1636 total extracellular secreted proteins, of which 350 and 137 proteins of 3DWB and 12DWB showed significant abundance variation from planktonic preparation, respectively. Of these, simultaneous up-regulation in between 3DWB and 12DWB proteins such as extracellular matrix-binding protein ebh, enolase, transketolase, triosephosphate isomerase, chaperonin, peptidase, pyruvate kinase, hydrolase, aminotransferase, ribosomal protein, acetyl-CoA acetyltransferase, DNA gyrase subunit A, glycine glycyltransferase and others we found in this biofilm producer. On the contrary, simultaneous down-regulation in between 3DWB and 12DWB proteins such as alpha and delta-hemolysin, lipoteichoic acid synthase, enterotoxin I, serine protease, lipase, clumping factor B, regulatory protein Spx, phosphoglucomutase, and others also we found in this biofilm producer. In addition, we also identified a big percentage of hypothetical proteins including unique proteins. Therefore, a comprehensive knowledge of planktonic and biofilm associated proteins identified by S. aureus will provide a basis for future studies on the development of vaccines and diagnostic biomarkers. Conclusions: In this study, we constructed an initial reference map of planktonic and various growth phase of biofilm associated proteins which might be helpful to diagnose biofilm associated infections.

Keywords : bacterial biofilms, CDC bioreactor, S. aureus, mass spectrometry, TMT
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