Adhesion and biofilm formation by non-typeable Haemophilus influenzae isolated from patients with Pneumonia, Chronic Obstructive Pulmonary Disease and healthy carriers

Carmen Puig (1), A Domenech (1), P Mayer (2), C Ardanuy (1), J Liñares (1), S Marti (1)
(1) Hospital Universitari Bellvitge. IDIBELL. CIBERes, Spain
(2) Biofilm Control, Saint-Beauzire, France

Objective: Non-typeable Haemophilus influenzae (NTHi) are frequently identified as the etiologic agent of recurrent infections in children and adults. Our work aimed to study and compare biofilm formation in isolates from patients with Chronic Obstructive Pulmonary Disease (COPD), Community Acquired Pneumonia (CAP) and healthy children. Methodology: NTHi strains were isolated from oropharyngeal samples of healthy children (n=120), sputum samples of COPD (n=62) and CAP (n=96) patients. Biofilm formation was performed in a static biofilm assay with crystal violet staining. Initial bacterial adhesion to a solid surface was assessed with the Biofilm Ring Test methodology (Biofilm Control, France) with readings after 2 and 4 hours of incubation at 37°C. Results: NTHi adhesion to a solid surface in the initial 2 hours of growth was very low (5-9%) for all the tested groups. After four hours growth, the rate of NTHi adhesion was variable among the three groups of isolates: CAP (36%), COPD (61%) and healthy children (56%). Isolates from CAP patients presented a significantly lower adhesion than isolates from COPD patients (p=0.0021) and healthy children (p=0.0028). Overall biofilm formation on a solid surface (24h static growth) ranged between 67% (CAP) and 80% (COPD and healthy carriers); biofilm formation was also significantly lower in NTHi isolated from CAP patients (p=0.049 and p=0.046). Conclusion: NTHi isolates from CAP patients showed a delayed ability for adhesion to solid surfaces and a reduced biofilm formation when compared to isolates from COPD patients and healthy children.

Lysis buffer properties: influence on S. epidermidis biofilm proteome analysis

Virginia Carvalhais (0), N Cerca (1), R Vitorino (2)
(1) IBB - Institute For Biotechnology and Bioengineering, Centre of Biological Engineering, University of Minho, Braga, Portugal
(2) QOPNA, Mass Spectrometry Center, Department of Chemistry, University of Aveiro, Portugal

Besides being part of human commensal flora, S. epidermidis has the ability to colonize and form biofilms in artificial implants. Due to the particular characteristics of biofilms, conventional methods used to disrupt and lyses biofilms from Gram positive bacteria may include association between mechanical, enzymatic and chemical methods. Nevertheless, proteomic characterization is highly dependent of the extraction procedure. In order to characterize proteome from S. epidermidis biofilms grown in glucose excess, we used mechanical lysis (glass beads) associated with two distinct lysis solution with different charge characteristic detergents, namely, SDS (an ionic detergent) or CHAPS (a zwitterionic detergent). Protein extracted was separated by SDS-PAGE and identified by LC-MS/MS. SDS lysis buffer combined with glass-beads showed the highest number of identified proteins (332 proteins). With zwitterionic detergent extraction, most the identified proteins presented a lower GRAVY value (grand average of hydropathy) and a protein molecular weight under 30 KDa. In overall, this work evidence that SDS lysis buffer is the optimal protocol to proteome analysis of S. epidermidis biofilms. This work was funded by Fundação para a Ciência e a Tecnologia (FCT) and COMPETE grants PTDC/BIA-MIC/113450/2009, FCOMP-01-0124-FEDER-014309. Thanks are due to Fundação para a Ciência e a Tecnologia, European Union, QREN, FEDER and COMPETE for funding the QOPNA research unit (project PEst-C/UI0062/2011) and the Portuguese National Mass Spectrometry Network (RNEM), also supported by funds from FCT. VC was funded by FCT fellowship SFRH/BD/78235/2011.