independent sets of all experiments will be performed in triplicate. The isolates were then process with both top down and bottom up proteomics, including high resolution narrow 2D electrophoresis coupled with MALDI-TOF analysis. **Results and Discussion:** Mono and 2D electrophoresis were compared from different S. aureus ATCC and isolated strains, together with the capacity to produce biofiilm, and proteins were identified. **Conclusion:** Proteomics analysis through strong and weak producers revealed differencesin the profile of strains cultured in the plactonic form in comparison to the sessile form, and in the profile of different food isolated giving new insights to conteract the biofilm formation.

Keywords: Staphylococcus aureus, food, biofim

P09.21 Immunoproteome Analysis of Bordetella Bronchiseptica by IP-nLC-MS/MS

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Introduction and Objectives: Bordetella bronchiseptica is a gram-negative pathogen and the evolutionary progenitor of Bordetella pertussis, cause of whooping cough in humans. B. bronchiseptica usually causes asymptomatic infections in humans and causes acute and chronic respiratory infection in a variety of animals. This study aimes to identify the antigenic proteins of B. bronchiseptica. The identification of new antigenic proteins may be a resource for further development of a new vaccine candidates. **Methods:** Secreted proteins, membrane-related and hydrophilic fractions of B. bronchiseptica were isolated and treated with B. bronchiseptica whole cell immune sera. Magnetic bead-based immunoprecipitation (IP) method was applied to select the antigenic ones. Then the antigenic proteins were separated by one-dimensional gel electrophoresis and identified by nLC-MS/MS. Gene ontology, cellular location, molecular function, and biological process terms were analysed by using UniProt and protein interaction networks were generated using STRING. **Results and Discussion:** 15 antigenic secreted proteins were identified, of which 11 were reviewed for the first time. By GO analysis, secreted proteins were linked to pathways such as metabolism, genetic and environmental information processing. Also, most of the proteins were located throughout the cytoplasm, outer membrane, periplasm. STRING-generated protein network showed no connectivity between secreted antigenic proteins without 50S ribosomal protein subunit L14 and L22. 41 antigenic membrane related proteins located on plasma membrane, outer membrane or membrane part were detected. Of which, 31 were reviewed for the first time in this study. Membrane-related antigenic proteins were distributed throughout biological procesees; pathogenesis, signal transduction, cell adhesion, biological regulation. 159 antigenic proteins of hydrophilic fraction were identified of which 94 were reviewed for the first time in this study. **Conclusion:** These newly identified proteins may promote a better understanding of the pathophysiology of B. bronchiseptica and also a possible development of new vaccine candidates.

Keywords: Bordetella bronchiseptica, immunoprecipitation, nLC-MS/MS, Immunoproteome

P09.22 SILAC Labelling Dynamics in Staphylococcus Aureus MSSA/MRSA Strains Isolated Form Mastitis

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Introduction and Objectives: Staphylococcus aureus, the main causative agent of nosocomial infections, has become a relevant healthcare problem because of the widespread occurrence of strains resistant to multiple antibiotics, in particular methicillin-resistant S. aureus (MRSA). Although MRSA represents a serious problem for human health, also relevant infections of animal-associated MRSA have been recently identified. Animals involved in food production, their products are therefore a potential reservoir of MRSA for humans (EFSA-Q-2009-00612), and MRSA today has been considered as a potential dangerous zoonosis. The main aim of this preliminary work is to optimize the SILAC labelling strategy in methicillin-resistant (MRSA) and methicillin-sensible (MSSA) Staphylococcus aureus (SA) isolates from mastitis for the next investigation on the host-pathogen subcellular protein dynamics during the phagocytosis of these bacteria. **Methods:** SILAC labelling has been performed on several MSSA and MRSA isolates. Different parameters were taken in account during this task (CFU, growth media composition, labelling time, strain type, genome) in order to correlate this parameters to the incorporation dynamics of the isotopic label into the MSSA/MRSA proteome. Heavy and light protein samples were mixed and separated by SDS-PAGE. Protein bands were digested and peptides analyzed by nLC-MS/MS. Spectra were processed using MASCOT 2.4.1 and MaxQuant 1.5.2.8. Protein functional analysis has been performed using InterProScan 5 **Results and Discussion:** The levels of Isotopic label incorporation and biological and technical reproducibility of SILAC quantitation that has been also correlated to the different parameters mentioned before has been reported. An optimized workflow with the evaluation of the critical key points has been summarized in this work. **Conclusion:** This experimental approach will allow quantitative insights into the bacterial response as well as the turnover of the protein expressed by MSSA and MRSA Staphylococcus aureus during the host-pathogen interaction. Work supported by MIUR under grant “Futuro in ricerca 2013” project code RBFR13PIQE_001 to A.S.

Keywords: Staphylococcus aureus, SILAC, host-pathogen interaction, MRSA/MSSA isolate

P09.23 Proteomics Investigation of Pseudomonas Fluorescens Chromogenic Strains: Insight in Blue-Mozzarella

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Introduction and Objectives: In the last years an high number of mozzarella cheese coming from Italian and German establishments caused complaints in consumers due to unusual pigmentation of dairy products Many microbiological analysis have been performed revealing an ubiquitous non-pathogenic bacteria, Pseudomonas fluorescens, as a causative agents liable for this phenomenon. Some specific strains of this microorganism own gene sequences coding for enzymes, able to produce dyes that can induce anomalous coloration to food. It has been verified

that the occurrence of blue pigmentation in mozzarella cheese samples is however not related to chemical conditions, bacterial concentration, or critical temperature level. Both ribotyping and PFGE methodologies have underlined differences in Pseudomonas fluorescens strains isolated in food coming from different geographical areas. Nevertheless the phylogenetic assessment of bacteria pointed out no correlation between the genetic profile of the microorganism and its chromogenic behavior. the aim of this study was a deeper proteomic investigation of P. fluorescens isolates in order to evaluate how these bacteria could represent contamination source. **Methods:** A shotgun proteomic approach has been performed on isolated Pseudomonas fluorescens cultures coming from samples of mozzarella cheese either showing anomalous pigmentation or not. LC-MSMS experiments have been carried on by nanoHPLC runs and auto MS^n acquisitions on a Bruker amaZon-ETD Ion Trap instrument. Protein identification has been executed either versus Pseudomonas dedicated DBs or on the open-reviewed databases. **Results and Discussion:** Obtained data highlighted several differential expressed proteins in two conditions. Among these, key proteins are, on one hand, the major cold shock protein that increases its level in the anomalous pigmentation, and, on the other, the phosphate starvation-inducible protein that decreases respect to normal condition. **Conclusion:** Proteomics analysis could allowed us to differentiate among chromogenic and non chromogenic strains at the molecular level, widening the knowledge on this bacteria and the molecular mechanism underlying this contamination

Keywords: Pseudomonas fluorescens, Blue-Mozzarella, dairy products, proteomics

P09.24 Investigating HIV-Mediated Dynamics of Cullin RING E3 Ligases by AP-MS and Proximity Biotinylation

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Introduction and Objectives: Human immunodeficiency virus (HIV) is known to hijack cellular components for its replication. A major target is the ubiquitin machinery, in particular Cullin-RING E3 ligases (CRLs). To interfere with antiviral pathways, HIV proteins, Vpu, Vpr and Vif, hijack CRLs to promote ubiquitination and degradation of host restriction factors. Here, we aim to systematically characterize HIV-mediated CRL complex dynamics and potentially identify new ligase-substrate relationships combining affinity purification mass spectrometry (AP-MS) and proximity biotinylation. **Methods:** We generated stable Jurkat T-cell lines expressing tagged CRL complex components. Purifications are performed following infection with wildtype HIV and Vif-, Vpu- or Vpr-deleted HIV mutants to link specific CRL PPIs to each viral protein. To obtain a high coverage of the CRL interactome we combine classical AP-MS for purifying native protein complexes with a novel technique for proximity-dependent biotin identification (BioID). For BioID proteins of interest are fused to the promiscuous biotin ligase BirA* that, upon addition of biotin, biotinylates vicinal proteins which are subsequently purified and quantified by MS. The covalent attachment of biotin allows for identification of transient interactors, such as ligase-substrate interactions, and captures interactions over the time of biotinylation. **Results and Discussion:** We demonstrated that AP-MS and BioID are complementary in identifying high-confidence CRL interactors. Both approaches confirmed a large number of previously reported CRL interactors, including CRL substrate receptors and regulators. Comparing HIV infected with mock-infected conditions, we detected known HIV-mediated dynamics of the CRL complexes, such as Vif-dependent recruitment of CBFB to CUL5 complex for the degradation of APOBEC3G. Furthermore, we identified novel interactors and quantified changes in PPI in the context of HIV infection that are potentially interesting for functional follow up studies. **Conclusion:** This project will provide the first systematic, quantitative CRL PPI network in the context of HIV infection and potentially reveal new ubiquitin ligase-substrate relationships relevant for HIV infection.

Keyword: HIV-host interactions

P09.25 Elucidation of the Ebola Virus VP24 Cellular Interactome

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Introduction and Objectives: Viral pathogenesis in the infected cell is a balance between antiviral responses and subversion of host-cell processes. Many viral proteins specifically interact with host-cell proteins to promote virus biology. Understanding these interactions can lead to knowledge gains about infection and provide potential targets for antiviral therapy. One such virus is Ebola, which has profound consequences for human health and causes viral haemorrhagic fever where case fatality rates can approach 90%. The Ebola virus VP24 protein plays a critical role in the evasion of the host immune response and is likely to interact with multiple cellular proteins. To map these interactions and better understand the potential functions of VP24, label-free quantitative proteomics was used to identify cellular proteins that had a high probability of forming the VP24 cellular interactome. **Methods:** VP24 was fused to GFP and over-expressed in 293T-cells. IPs were performed with a GFP-trap and label free quantitative proteomics used to discriminate background interactions. **Results and Discussion:** Several known interactions were confirmed, thus placing confidence in the technique, but new interactions were also discovered including one with ATP1A1, which is involved in osmoregulation and cell signaling. Disrupting the activity of ATP1A1 in Ebola-virus-infected cells with a small molecule inhibitor resulted in a decrease in progeny virus. **Conclusion:** Quantitative label-free proteomics was invaluable in identifying potential therapeutic targets in the viral pathogenesis of Ebola.

Keywords: label-free proteomics, Interactome,, Ebola, VP24

P09.26 High-Throughput Genetic Analysis of Salmonella-Host Interactions

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Introduction and Objectives: Salmonella directly injects effector proteins into host cells to subvert their function, ultimately leading to disease such as enteritis or enteric fever. Salmonella mutants deficient in the ability to inject effector proteins are dramatically attenuated, indicating the importance of this process, but mutants deficient in individual effectors often have little or no phenotype. To address the hypothesis that effectors may act in concert to exert their effects, we are pursuing