Identification of Staphylococcus aureus isolates by shotgun spectral matching

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Staphylococcus aureus is a Gram-positive bacterium that can be pathogenic when expressing specific toxins. S. aureus is a common and notorious cause of skin infections, respiratory disease (pneumonia), sepsis and food poisoning. Due to the production of altered penicillin binding proteins by the mecA-gene, S. aureus has resistance to the most active antibiotics such as flucloxacillin or methicillin.[1] Therefore S. aureus infections are a threat to both individual and public health. Rapid characterization of virulence and resistance factors may aid in providing clues for treatment of individual patients as well as prevent nosocomial spread.

In the last two decades, mass spectrometry (MS) based proteomics have matured into a technology that is suitable for large scale protein analysis, also in a clinical setting. Current strategies in clinical microbiology focus on the detection of antimicrobial peptides or their immunological counterparts in clinical samples, as well as pursue comprehensive characterization of the proteome of pathogens.[2-5] Most MS-based proteomics approaches for pathogen identification follow a pattern recognition or profiling strategy, or rely on the measurement of specific peptides or proteins. Recently, an alternative MS-based method on spectral matching using spectral libraries in a bottom-up proteomics approach was demonstrated on food species authentication.[6] In the current study we will attempt to show the applicability of this method to pathogen identification of S. aureus isolates, a task that has proven to be challenging with the routinely used pattern recognition platform.

The experimental setup is a simple, common MS-based bottom-up proteomics workflow including protein extraction, digestion, and data acquisition on an ion-trap mass spectrometer using LC-MS/MS.(figure 1). A 45-minute, linear gradient from 4 to 35% acetonitrile in 0.05% formic acid was used.

The bottom-up proteomics approach was applied to 12 different Staphylococcus aureus isolates with known toxin genes. Isolates are derived from a single colony that is presumed to arise from a single bacterium. The toxins produced by these are only a small part of the total amount of proteins present, so differences between the isolates are expected to be small. Other samples of the same type of S. aureus isolates were searched against other reference libraries for sample authentication based on the number of spectra matching the different spectral libraries. It should be stressed that this method does not require any genomic sequence information and uses all acquired data. Preliminary data indicates the majority of the tested S. aureus samples were correctly identified down to the strain level.

A phylogenetic tree was also generated from these spectra using a previously published algorithm for molecular phylogenetics, illustrating how shotgun proteomics can be used for a taxonomical comparison.

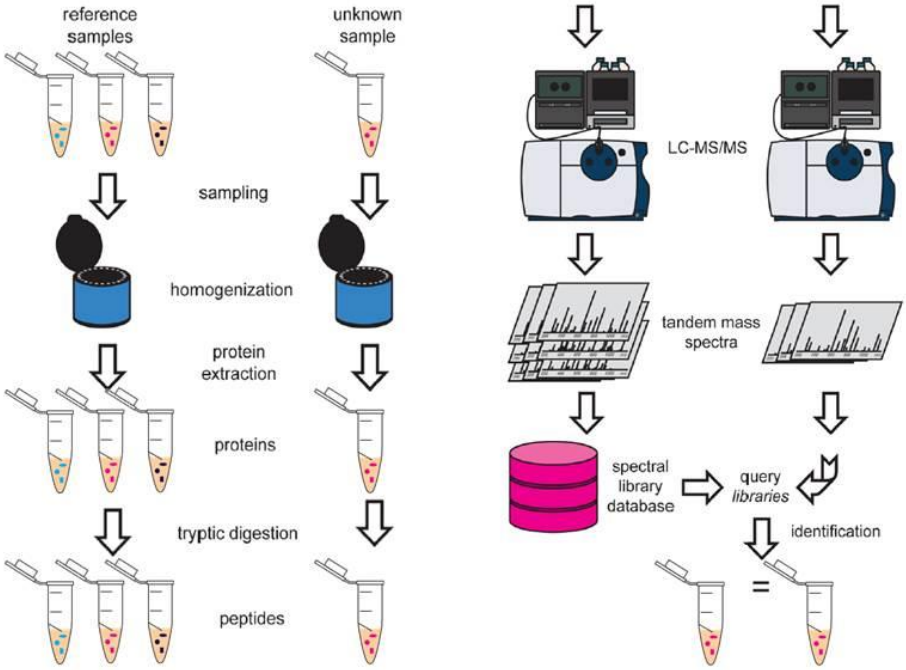

Figure 1 : A reference database was first constructed based on 12 Staphylococcus aureus spectral libraries using a common proteomics methodology including protein extraction, digestion and measurement by liquid chromatography-tandem mass spectrometry. Unknown samples are prepared following the same workflow and the tandem mass spectra searched against all references in the database.

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