MALDI-TOF MS typing of bacteria

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Matrix-assisted laser desorption ionization–time of flight mass spectrometry (MALDI-TOF MS) is routinely used for bacterial identification. It would be highly beneficial to also be able to use the technology as a fast way to detect clinically relevant clones of bacterial species. However, studies to this aim have often had limited success. The methods used for data acquisition, processing and data interpretation are highly diverse amongst studies on MALDI-TOF MS sub-species typing. In addition to this, feasibility may depend on the bacterial species and strains investigated, making it difficult to determine what methods may or may not work. There is a lot of variation amongst the methods used in published studies. However, the following approaches were shared by multiple research groups when aiming to MALDI-TOF MS type bacterial clones: Multiple spectra of the same isolate were often combined before further analysis for strain distinction. Furthermore, many groups used a protein extraction step to increase resolution in their MALDI-TOF MS results, and peaks at a high mass range were often excluded for data interpretation. Three groups have found ways to determine feasibility of MALDI-TOF MS typing for their set of strains at an early stage of their project.

Keywords: MALDI-TOF MS typing ; MALDI-TOF MS ; bacterial sub-species typing ; strain typing ; bacterial strain identification

1. Background

Genotyping methods often have good discriminatory power to track bacterial strains but tend to be costly and time consuming. Whole genome sequencing (WGS), in particular, is a very powerful tool for the investigation of phylogenetic relationships and is becoming the gold standard for this purpose. However, in addition to the costs that are still high for data acquisition, considerable bioinformatics expertise is required for data analysis of WGS [1][2].

MALDI-TOF MS is routinely used in clinical laboratories for identification of bacterial species. However, the typing of bacterial clones using MALDI-TOF MS has not been established yet, and studies to this aim have shown different levels of success [3]. MALDI-TOF MS results may fluctuate due to variation in bacterial growth conditions, sample preparation or matrix used. Bacterial strain typing may be more susceptible to such fluctuations than bacterial identification due to differences being smaller between bacterial strains than between bacterial species [4].

There is a lack of standardisation amongst published studies, where MALDI-TOF MS data have been interpreted by varying bioinformatics tools, and in some cases manually. Additionally, the feasibility of MALDI-TOF MS typing may depend on the bacterial species or strains investigated, making it difficult to determine what methods make MALDI-TOF MS typing work [5][6].

2. Literature findings on MALDI-TOF MS typing:

Published studies on MALDI-TOF MS bacterial sub-species typing display a high variability of acquisition, processing and genotyping reference methods and report different levels of success. Feasibility of MALDI-TOF MS strain typing may depend on the lineages investigated [6], making it very challenging to determine what methods may make MALDI-TOF MS typing of bacterial strains work. However, the following approaches were shared by several research groups: Multiple spectra of each isolate recorded either during the same run or different runs were often combined before analysing mass spectral differences for strain distinction [7][8][9][10][11][12]. In general, any mass peaks chosen for strain discrimination were below 10,000 m/z [12] or no higher than 15,000 m/z [10][11]. There was one exception where mass peaks up to 19,293 Daltons were used for strain discrimination, and measures were taken to improve the quality of higher mass peaks. These included protein extraction, the removal of mass molecules below 3000 Daltons, the enabling of the low mass ion gate at 3950 Daltons, the optimisation of pulsed extraction at 20,000 Daltons and the use of a sinapinic acid-based matrix [13].

Ideally, the same MALDI-TOF MS data used for bacterial identification can also be used for isolate typing, allowing for on-site monitoring of outbreak strains [1]. However, to increase resolution, an extraction step has often been performed for MALDI-TOF MS typing of bacterial isolates [11][12][13]. In order to increase mass accuracy, internal calibration may be performed in addition to external calibration, along with the application of a low mass tolerance [11][12][13].

As successful MALDI-TOF strain typing may depend on the lineages investigated [6], it would be beneficial to be able to assess feasibility before embarking on big lab projects. Ribosomal proteins account for about 50% of the peaks detected in MALDI-TOF MS spectra. The use of WGS data for in silico calculation of ribosomal masses may help determine the feasibility of MALDI-TOF MS typing of bacterial isolates at an early stage. Polymorphic ribosomal proteins may be used for strain distinction, and the conserved ones may be used for internal calibration to increase mass accuracy [13]. The use of previously recorded spectra of isolates circulating in a certain setting may also help the early determination of the MALDI-TOF MS typeability of a particular bacterial strain [11].

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