INTRODUCTION

In recent years MALDI-TOF Mass Spectrometry has risen to the forefront of diagnostic microbiology largely because its comprehensive database which enabled its resolution to reach, and often surpass, the current gold standard, 16S rRNA for microbial identification. Furthermore, its simplicity, speed of analysis and low cost has impelled MALDI-MS to gain universal acceptance in clinical laboratories. New developments in data analysis are allowing some species to be subtyped but for other pathogens the limits of resolution have been reached and new approaches are required.

Bacterial-typing techniques, such as SDS-PAGE profiles generated by gel electrophoresis have been used for decades as a basis for electrophoretic typing of closely related microorganisms and shows excellent confluence with DNA-based methods. SoCo-PAGE in combination with electrospray tandem MS (designated GeLC-MS/MS) is frequently used for proteomic analysis. However, its use as a platform for microbial proteotyping has not been systematically investigated. Here, we subjected members of the family Enterobacteriaceae including taxonomically indistinguishable species such as E. coli and Shigella spp. to such analyses to explore the potential of this approach.

The results show that by using the optimised database and proteome profiling MS-generated data, all taxa could be confidently delineated to the species and subspecies levels. Furthermore, in addition to comparative mass spectral analysis, data could be obtained on the biological properties of a strain. Thus, we could confidently identify E. coli, characterise strain-specific virulence factors and differentiate Shiga toxin negative from positive strains. This proof of concept study demonstrates that GeLC-MS/MS, which combines the traditional SDS-PAGE platform with LC-MS/MS, has the potential to simultaneously combine strain identification with key pathogenic properties of an isolate and significantly extends the clinical applications of mass spectrometry in microbiology.

METHODS

A panel of 33 strains, comprising 70 enteric bacterial strains (Table 1) were cultured, lysed prior to protein identification by LC-MS/MS using a Thermo LTQ Orbitrap. The MS/MS data were then searched against a series databases using Mascot (Matrix Science, UK) for bottom-up protein identification (Figure 1).

Marker Database Generation

In order to generate marker database, a series of standalone NCBI BlastP search against databases which increased in size: i) genus specific protein sequences; ii) Enterobacteriaceae family and iii) NCBI rRNA.

Analysis of an Outbreak E. coli 014 Strains

A selection of pathogenic E. coli strains including three isolates from the 2011 German outbreak (H112160280, H112160540 and H112160541), and four strains representative for the two characterised pathovars, EAEC (E93518), EHEC (EDL933 and H13002) and a Non-toxic, shigatoxin negative EHEC strain (NCTC12900), were analysed using the bottom proteomics method described in this study.

DISCUSSION

Investigation of the total proteome for each of the seven strains revealed a varied list of proteins, covered a wide range of functional categories, including virulence factors.

The outbreak strains expressed both EAEC and EHEC features as well as features specific to outbreak strains, including antibiotic resistance, heavy metal resistance and toxins; proteins unique to each pathovar were also identified, indicating that the approach has the potential to delineate pathogenic E. coli into subgroups;

Shiga-like toxins were identified from Shiga toxin positive isolates (H112160280, H112160540, H112160541, EDL933 and H13002), which could be used to differentiate Shiga toxin positive strains from Shiga toxin negative strains (E93518 and NCTC12900) (Figure 3).

Table 1. The 70 strains Enterobacteriaceae used in this study.

Table 3.

REFERENCES

