

D. Dare¹, H. Sutton¹, M. Lunt², H. Shah³;¹Manchester Metropolitan University, Manchester, UNITED KINGDOM, ²Waters Corporation, Manchester, UNITED KINGDOM, ³Health Protection Agency, London, UNITED KINGDOM.

D.Dare@mmu.ac.uk

Abstract

Microbial taxonomy and identification of bacteria has traditionally been based upon morphology and physical properties. The inclusion of chemotaxonomic and biochemical analysis, have extended the range of phenotypic characteristics for bacterial cells. Nucleic acid analysis, in particular 16S rDNA analysis, is also providing further information concerning the classification of bacteria. The development of these techniques has resulted in a major revision of the established classification system resulting in many nomenclature changes. Recently MALDI-TOF MS has proved a powerful tool for microbial identification, matching spectral profiles with those of a database of over 4000 spectral fingerprints. This extensive database also represents a powerful new tool for comparing strains within a species. This paper examines the diversity of spectral profiles exhibited by a number of strains from the same species and uses these to develop microbial taxonomy and identification of bacteria based upon MALDI-TOF MS.

To achieve this intact microbial cells are transferred from a culture plate to a MALDI target plate and overlaid with MALDI matrix. The co-crystallised sample is then irradiated with a N₂ laser and the resulting plume of positive ions separated using time-of-flight mass spectrometry, producing a characteristic mass spectral fingerprint pattern. This fingerprint has been used for microbial identification against a database containing representative spectra of microbial strains. Extending the database to include a number of fingerprint spectra for different strains of the same species has generally resulted in conclusive species identification where all top matches are correct to species. This implies that the fingerprint pattern may be extended to aid microbial taxonomy.

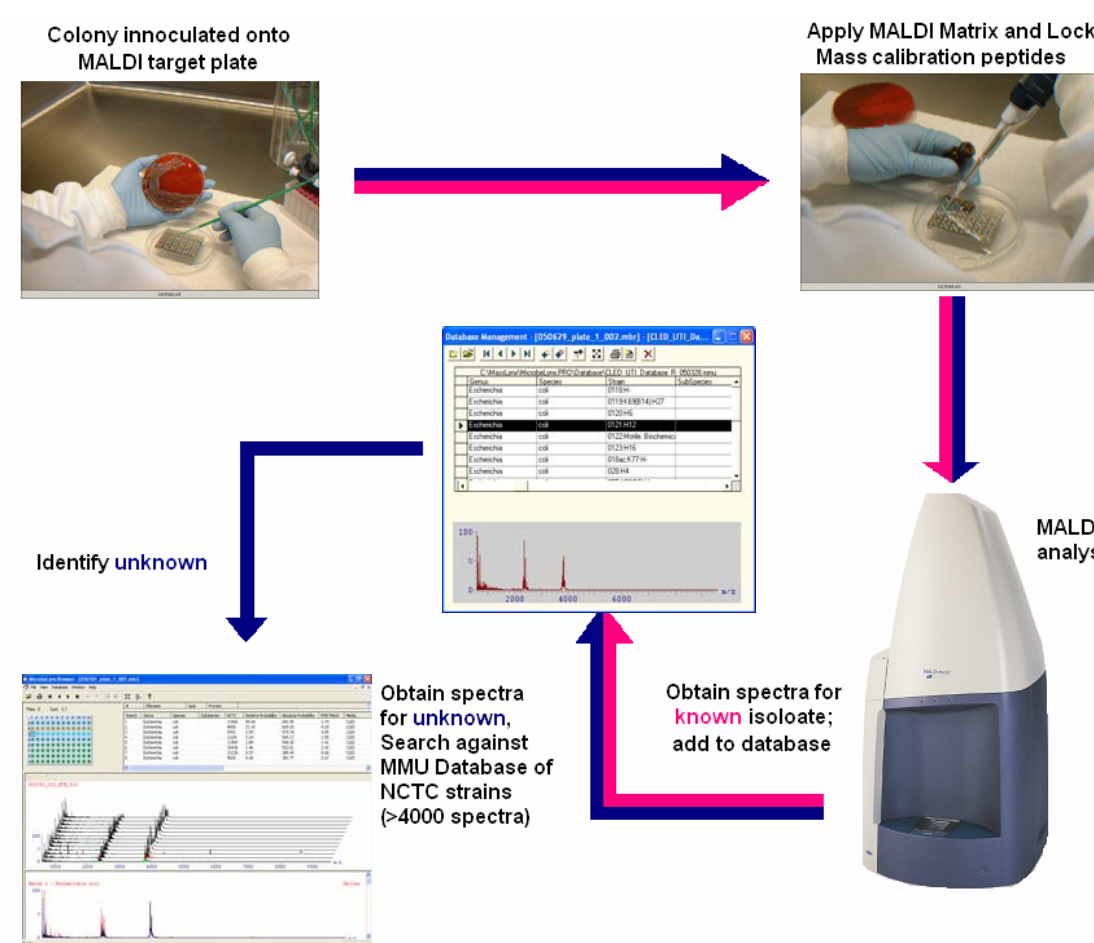
Identification of microbial species against an extended MALDI-TOF MS database has highlighted some anomalous strains. For example *Bacillus subtilis* NCTC 10452 often appears in a list of matches containing *Bacillus licheniformis*. The inference in these cases is that the taxonomical classification is incorrect.

Introduction

The identification of bacterial isolates associated with clinical infections from human, animal and terrorist sources, as well as food, drug and environmental sources, make this an important objective in many fields of microbiology, e.g. clinical and veterinary diagnosis, homeland defence, food, pharmaceutical and water industries. The goal in each case is to gain understanding of the microbial agent associated with the "problem sample" in order to define the possible outcomes and initiate strategies to counteract the effect.

The basis of all identification is a comparison of isolate characteristics with those of similar bacteria to define the taxonomical species to which the isolate belongs. Various techniques are used to achieve this including API, DNA sequencing and fatty acid profiling. In this study MALDI-TOF MS is used to aid the taxonomical classification of bacteria.

Methods



Results

A selection of NCTC strains were tested against the MMU database in a parallel study carried out by the Health Protection Agency. In a number of cases where the number of strains per species represented in the database was substantial, the top 8 matches suggested the same species identification. In some instances however the list contained an anomalous species, suggesting the taxonomical classification for the strain may need to be revised. A selection of these is discussed below:

Bacillus subtilis NCTC 10452:

- ◆ The HPA test spectrum was searched against the MMU database of >4000 spectral entries and the results presented in Figure 1.
- The first spectral match is to the **correct strain** and demonstrates the **reproducibility** of the spectra **across two different sites**, Figure 2.
- The next **seven spectral matches** are to *Bacillus licheniformis* and the **similarity of the spectral pattern** to this species suggests this strain should be **reclassified as *Bacillus licheniformis***, Figure 3.
- **16S rDNA** for NCTC 10452 supports reclassification to *Bacillus licheniformis*¹.

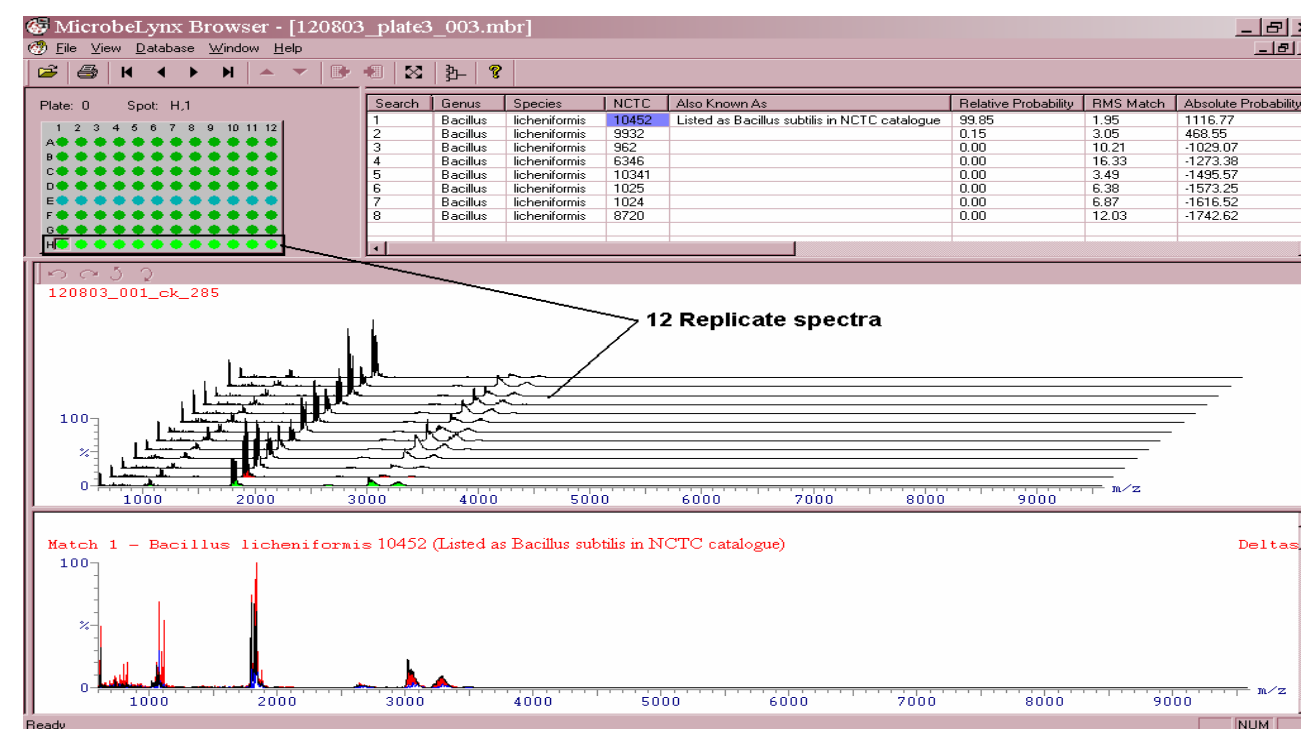


Figure 1: A browser display of the tabulated search results, the test spectrum *Bacillus subtilis* NCTC 10452 and the matching database spectrum are presented in a browser format.

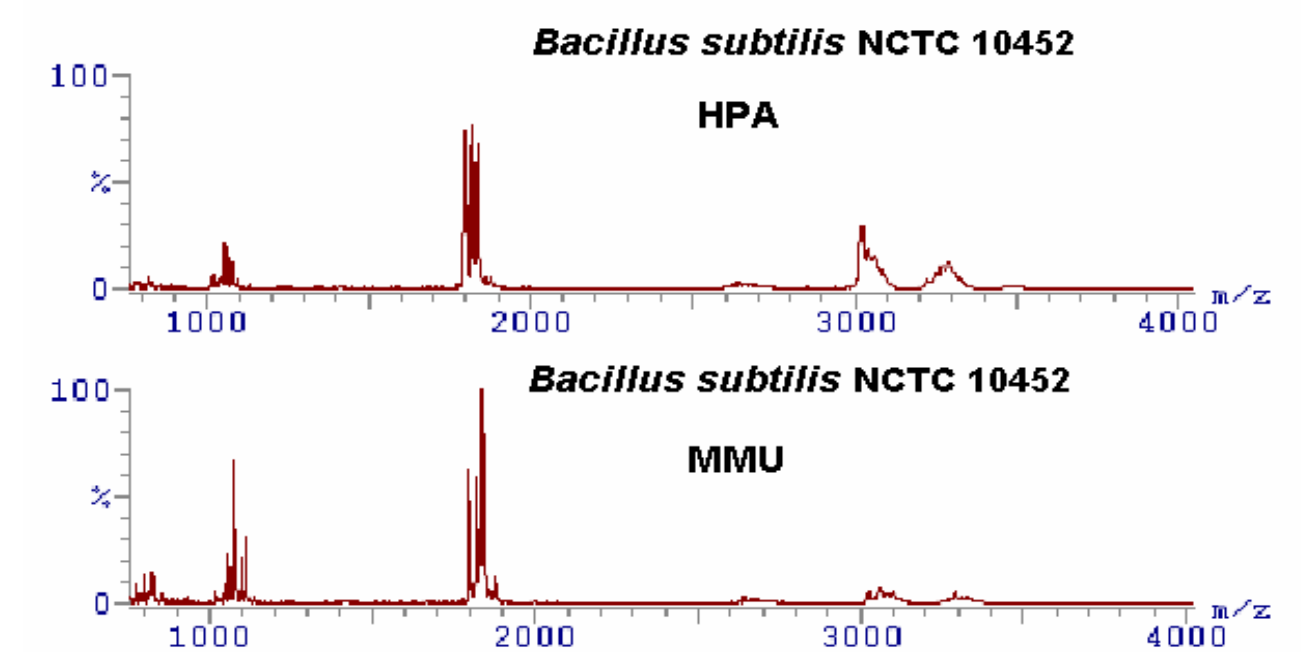


Figure 2: MALDI-TOF MS spectral fingerprints for two ampoules of *Bacillus subtilis* NCTC 10452, one ampoule cultured and analysed at HPA (top spectra) and the other cultured and analysed at MMU (bottom spectra), showing reproducibility of spectral pattern across two different sites, over m/z 500 to 4000.

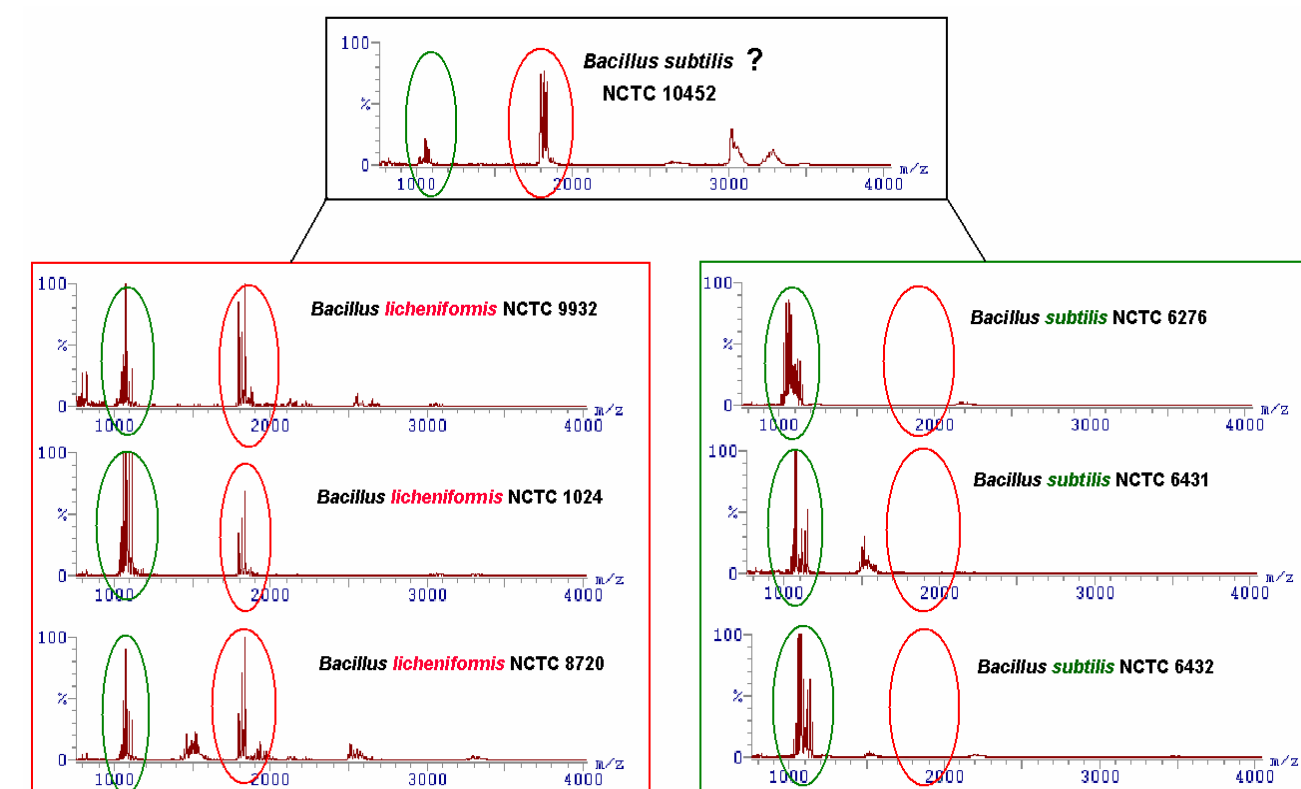


Figure 3: Comparison of MALDI-TOF MS spectral fingerprint of *Bacillus subtilis* NCTC 10452 (Top) to current database representatives of *Bacillus subtilis* (bottom right, green box) and *Bacillus licheniformis* (bottom left, red box). Evaluation of the spectra suggests that the taxonomic classification of NCTC 10452 should be reassigned to *Bacillus licheniformis*.

Serratia marcescens NCTC 10036:

- ◆ The **HPA test spectrum** for *Serratia marnorubra* NCTC 10845 was searched against the MMU database of >4000 spectral entries and the results presented in Figure 4.
- The **first match** is to a strain deposited as *Serratia marcescens* NCTC10036, although NCTC subsequently identifies this strain as *Serratia marnorubra*, (www.hpa.org.uk).
- The **second spectral match** is to the **correct strain** and demonstrates the **reproducibility** of the spectra **across two different sites**, Figure 5.
- The third and fourth significant matches are to *Serratia rubidaea*, homotypic synonym *Serratia marnorubra* (www.bacterio.cict.fr/s/serratia.html)
- The similarity of the **spectral pattern** of *Serratia marcescens* NCTC10036 with the test spectra and the top three matches suggest this strain should be **reclassified as *Serratia rubidaea***, homotypic synonym *Serratia marnorubra* Figure 6.
- Three other NCTC strains, **3804**, **4619** and **9419** also deposited as *Serratia marcescens* and subsequently identified as *Serratia marnorubra* by NCTC have spectral patterns consistent with those above for *Serratia rubidaea*, homotypic synonym *Serratia marnorubra*, Figure 7.

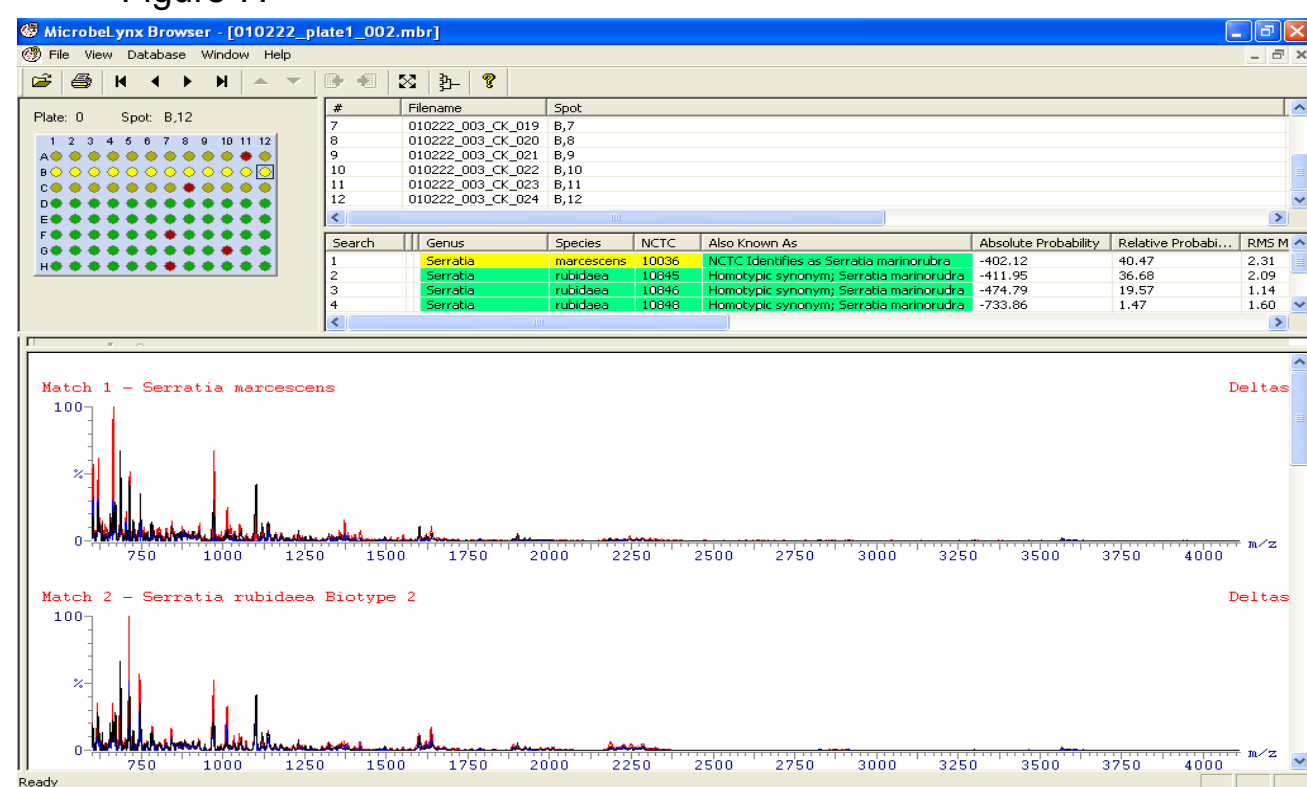


Figure 4: A browser display of the tabulated search results, the test spectrum *Serratia marnorubra* NCTC 10845 and the matching database spectrum are presented in a browser format.

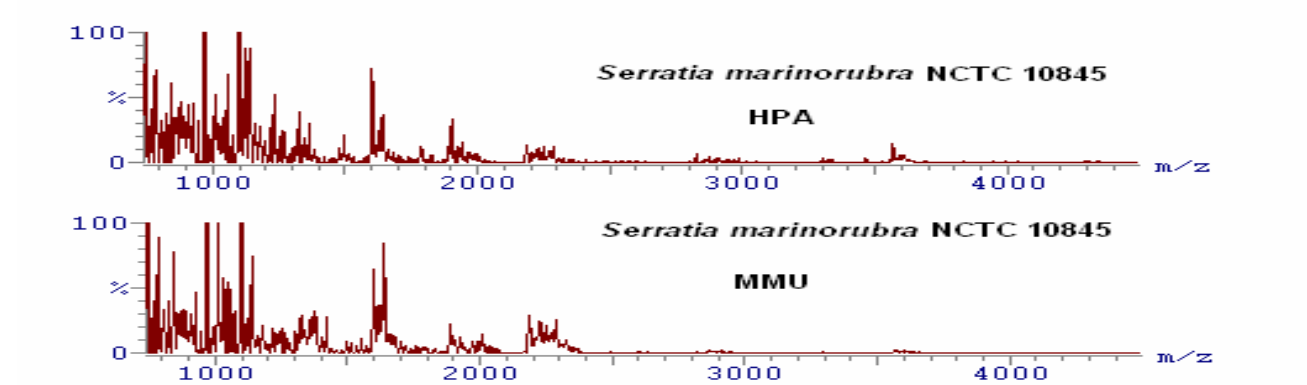


Figure 5: MALDI-TOF MS spectral fingerprints for two ampoules of *Serratia marnorubra* NCTC 10845, one ampoule cultured and analysed at HPA (top spectra) and the other cultured and analysed at MMU (bottom spectra), showing reproducibility of spectral pattern across two different sites, over m/z 500 to 4000.

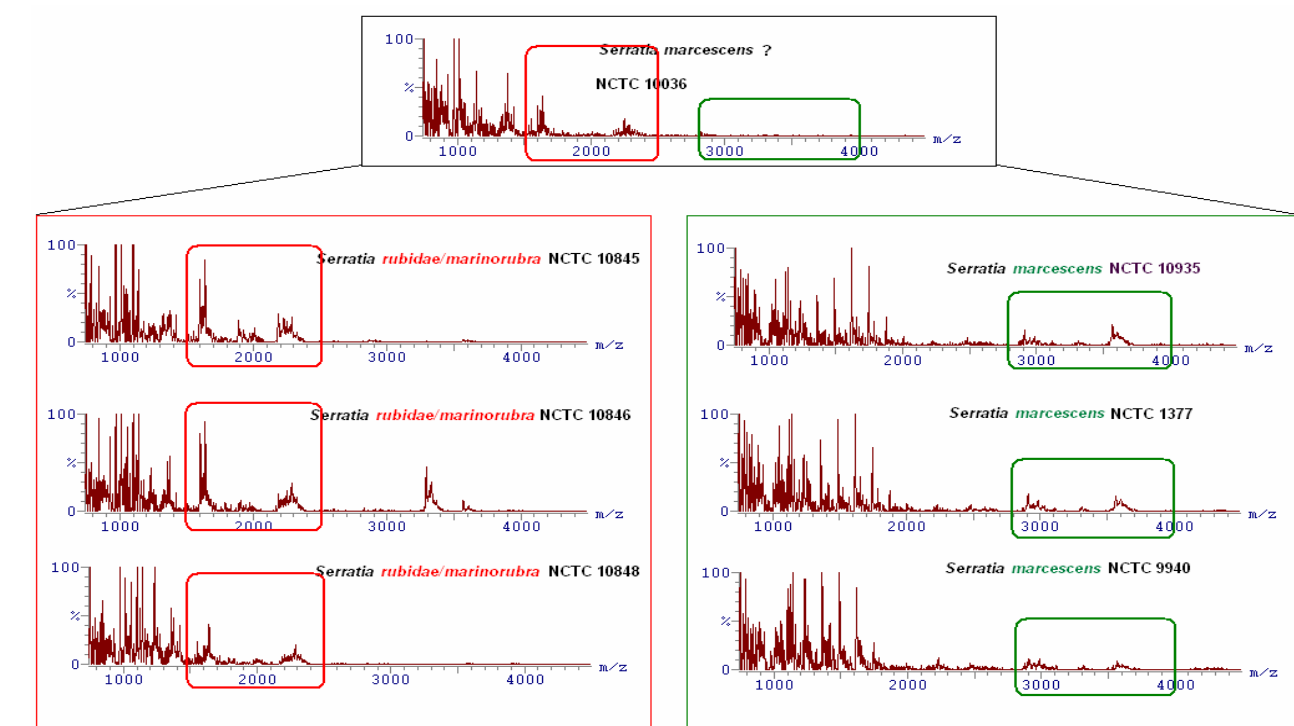


Figure 6: Comparison of MALDI-TOF MS spectral fingerprint of *Serratia marcescens* NCTC 10036, the first database match (Top) to the parallel test data for *Serratia marnorubra* NCTC 10845, with the second, third and fourth database matches of *Serratia rubidaea*, homotypic synonym *Serratia marnorubra* (bottom left, red box). The right hand spectra demonstrate the fingerprint pattern for a selection of database representatives for *Serratia marcescens* (bottom right, green box). Evaluation of the spectra suggests that the taxonomic classification of NCTC 10036 should be reassigned to *Serratia rubidaea*, homotypic synonym *Serratia marnorubra*.

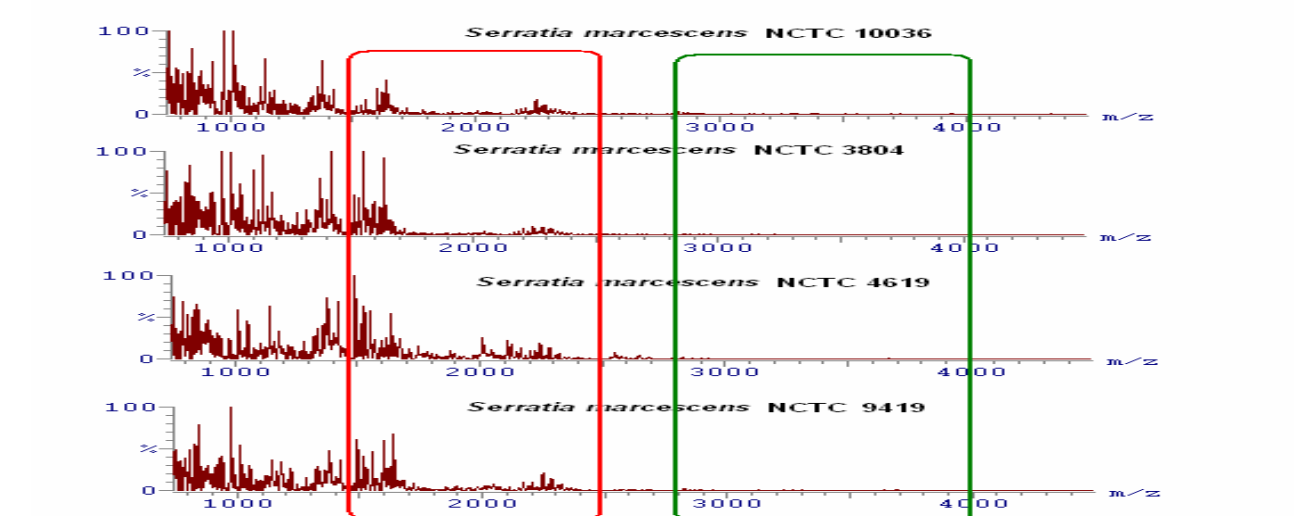


Figure 7: Comparison of MALDI-TOF MS spectral fingerprint of NCTC 10036 with those of NCTC 3804, 4619 and 9419. These were all received as *Serratia marcescens* and subsequently identified by the NCTC as *Serratia marnorubra*.

Conclusions

MALDI-TOF Mass spectral analysis of:

Bacillus subtilis NCTC 10452 suggest taxaomical **reclassification to *Bacillus licheniformis* NCTC 10452**.

Serratia marcescens NCTC 10036 suggest taxaomical **reclassification to *Serratia rubidaea***, homotypic synonym *Serratia marnorubra*.

Serratia marcescens NCTC 3804, 4619 and 9419 suggest taxaomical **reclassification to *Serratia rubidaea***, homotypic synonym *Serratia marnorubra*.

MALDI-TOF MS is a powerful tool to aid taxonomical classification of bacteria.

References

1. Keys C, Dare D, Sutton H, Wells G, Lunt M, McKenna T, McDowall M, Shah H., *Compilation of a MALDI-TOF mass spectral database for the rapid screening and characterisation of bacteria implicated in human infectious diseases*, *Infection, Genetics and Evolution*; 2004, Volume 4; Issue 3, 221 – 242
2. www.hpa.org.uk
3. www.bacterio.cict.fr/s/serratia.html