Automating MALDI-TOF sample preparation protocols to facilitate bacterial identification.

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Abstract

Matrix-assisted laser desorption/ionisation-time of flight (MALDI-TOF) is a mass

level. However, MALDI-TOF requires rigorous sample processing methods to ensure the reproducibility of bacterial cells from MS data. In this application, we show that introducing automation into MALDI-TOF pipelines can reduce sample processing times while maintaining accurate bacterial identification. This document provides you with the blueprint to adopt automation into your MALDI-TOF pipelines with the PIXL colony picker.

Introduction

Identifying bacteria at the species level underpins clinical and environmental microbiology. The introduction of MALDI-TOF has greatly increased the resolution in bacterial profiling, largely replacing more traditional qualitative staining assays for characterising bacteria. In the clinic, MALDI-TOF is helping diagnose disease[1] and identify antibiotic-resistant bacteria[2]. Microbiologists also use MALDI-TOF to distinguish bacterial strains and analyse human and environmental microbiomes[3,4]. MALDI-TOF's success in

their ribosomal proteins to membrane proteins, that MALDI-TOF can distinguish.

Although MALDI-TOF can recognise bacterial species by its proteins, generating reproducible MS data for bacterial identification requires many steps. Variables within these steps, such as the sample preparation method, the amount of bacterial colony obtained, and the age of the colony, can affect the quality of the MS data[7]. With many experimental sources of variation present in MALDI-TOF, researchers have increasingly looked to automation to minimise technical errors.

The Question: Automation and Maldi-Tof

Integrating automation provides several benefits for researchers. Most notably, automating experimental workflows reduces human errors, increases reproducibility, and enhances researcher efficiency[8]. For MALDI-TOF MS workflows, automating the sample processing and species identification protocols

MALDI-TOF MS species identification.

Methods

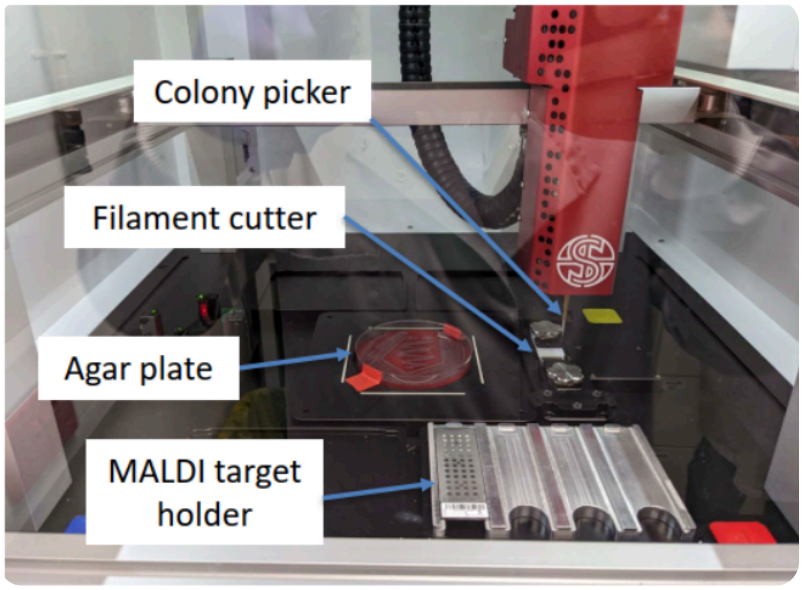
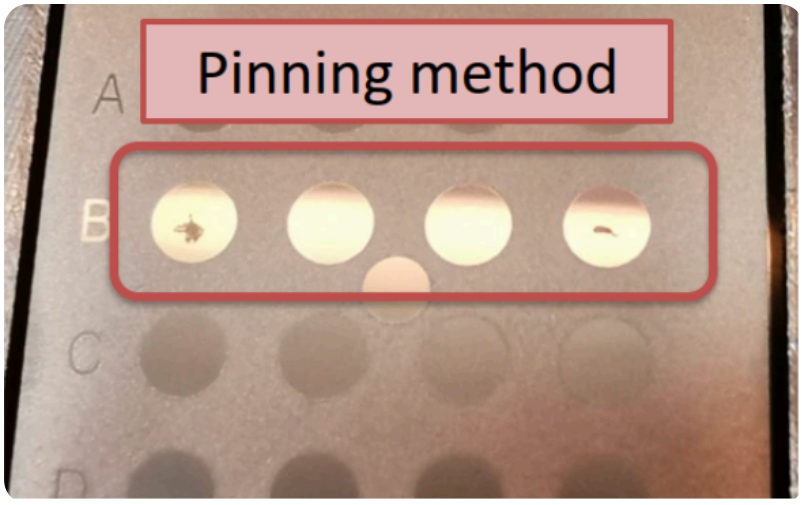


Figure 1. The PIXL colony picking robot [left] and its inner components [above], the automated system used for MALDI-TOF sample processing.

testing the automated method. In the automated pipeline, colonies were picked from cultured samples and deposited onto a disposable MALDI MS sample plate, such as the FlexiMass-DS slides (Shimadzu), using a PIXL precision microbial colony picker (Singer Instruments). The automated protocol was compared with manual preparations where an inoculation loop was used to spot the plates with sample material. The automation protocol was then further refined by determining whether it should feature a pinning method or a smearing method (Figure 2).



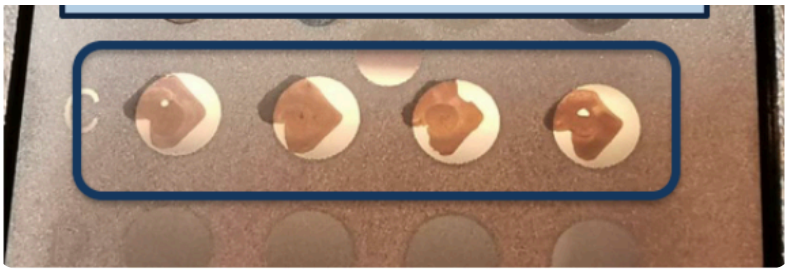


Figure 2. The two automated bacterial transfer methods were employed. The pin method (far left) 'dabs' the picked sample onto a spot on the target surface, while the smear method (left) forms a smear across the target surface.

To further evaluate the automated pipeline, different areas of the culture plates were also picked for each bacterial species (Figure 3).

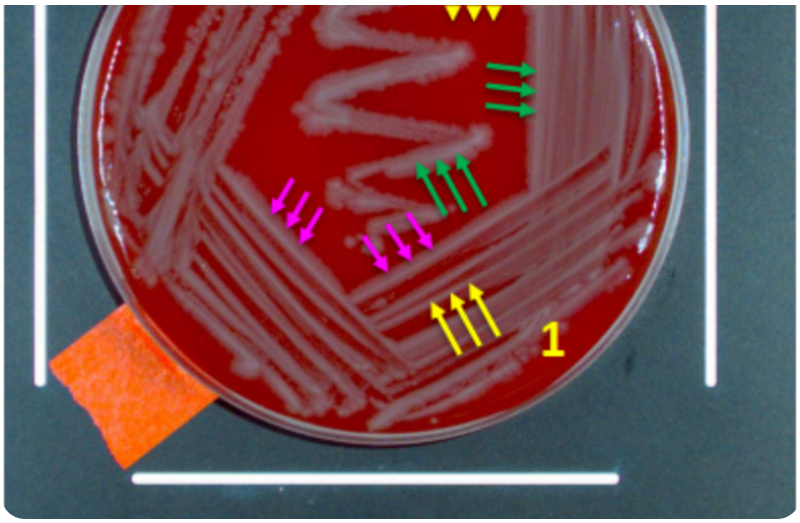


Figure 3. Three areas where bacteria were picked. Green refers to the thickest part of the culture growth, or “Deep”. Pink represents the “Edges” of the culture growth streak. Yellow refers to the material picked from the thickest part of the culture growth ‘steak’ (solid arrows, 1) and then dabbed onto an area of agar not containing any culture to remove excess material prior to depositing onto the MALDI target (dashed arrows, 2).

Once the samples were prepared, the pins and smears were analysed on an iDplus Performance MALDI-TOF mass spectrometer (Shimadzu) and submitted to a SARAMIS database for identification.

Results

To begin assessing the automated sampling method for MALDI-TOF, researchers tested it on three types of colonies across the culture plates. Doing so showed that the automated smear and automated single/double deposition of matrix solution were equally accurate in identifying *E. coli* with >99.9% confidence (Figure 4). In contrast, the manual method introduced plasticisers and other contaminants that obfuscated the *m/z* peaks for identifying microbial species.

With the automated protocol developed, researchers then determined whether the automated sample collection could identify three other bacterial species with high confidence. The automated protocol allowed two of the species, *P. agglomerans* and *C. uda*, to be identified with over 99.9% confidence. While there were red IDs for the *B. subtilis* identification, this was due to a mixed ID in the database, not the automated sampling method itself. Nonetheless, the most promising data was the MALDI-TOF MS spectra for *P. agglomerans* being nearly identical for both the PIXL automation and manual approaches (Figure 6.)

Figure 4. Identification results for *E. coli* samples were prepared with the automated smear pipeline for samples taken from all areas of the culture plate. Dark green = >99.9% confidence, light green = 90.0 – 99.8% confidence, red = mixed ID, white = no ID.

Figure 5. Identification results for the bacterial species *B. subtilis, C. uda, and P. agglomerans* when using the optimised automated method. Dark green = >99.9% confidence, light green = 90.0 – 99.8% confidence, red = mixed ID.

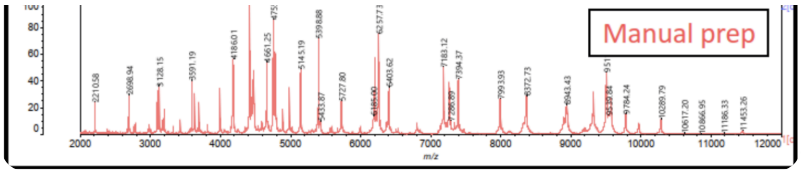


Figure 6. Representative MALDI-TOF MS spectra for *P. agglomerans* with a manually prepared smear sample (in red) and a smear sample prepared with the automated protocol (in blue).

Conclusions

Integrating automation into research pipelines can streamline research protocols and improve reproducibility in biomedical research. In microbiology, automation may also prove useful for identifying microbial species with MALDI-TOF. Here, we show that automation can facilitate sample collection and streaking protocols, both of which affect the accuracy and reproducibility of MALDI-TOF MS-based bacterial identification.

Using the PIXL to automate colony picking and matrix deposition allowed reference microbial species to be identified correctly and reproducibly. Furthermore, the automated

identification more reproducible.

With automated MALDI-TOF MS pipelines now possible, researchers can efficiently type and characterise microbes for a wide range of applications, from pathogen identification to antibiotic sensitivity testing.

Contact us to learn more about the PIXL colony picker and how it can be seamlessly integrated into your bacterial identification workflows.

References

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