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Developing an automated preparation of microbiological samples for MALDI-TOF

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Master thesis
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INTRODUCTION

Bacteria play crucial roles in fields like food, environment, and human health. While some microorganisms are beneficial, such as those in the human gut aiding digestion, others are pathogenic, causing diseases like tuberculosis and Lyme disease [1,2]. Identifying bacteria at the species level is essential for an effective treatment, as susceptibility to antimicrobial agents can vary between species. However, clinical laboratories often rely on broader, less precise identifications, which can lead to a non effective treatment and/or the overuse of broad-spectrum antibiotics, contributing to antibiotic resistance [3]. This could be avoided with a proper sample preparation of the bacteria, which often takes more time. An automated process using a CTC PAL system would be cheap and quick, with the advantage to more precise identification of bacteria species.

BACTERIA CULTIVATION

The sample preparation starts with cultivating bacteria on agar plates. Because of phenotypic plasticity differences depending on the grow medium, agar plates with 5% blood were used. The bacteria were cultivated using a four-quadrant streaking method. This spreads out the bacteria evenly, so less extracellular matrix (ECM) is present.

Fig. 1: *E. coli* on a blood agar plate using the four-quadrant streaking method

3D DESIGN & PRINT

The custom rack design was created using Autodesk Fusion. The initial design featured a rectangular plate with indentations to hold the metal matrix-assisted laser desorption ionization (MALDI) plate, but vibrations from the vortex mixer caused the plate to slide out. To fix this, an overhanging edge was added so the plate could be slid into place, and a small groove was included to help remove the plate without scratching. The template was 3D printed using polylactic acid (PLA) on a Bambu Lab X1. PLA was chosen for its low cost and ease of use. This custom rack was then placed in a rack holder of the CTC PAL system, where it was taught as a new custom rack. This had to be done because of the individual spacing between the spots and the plates.

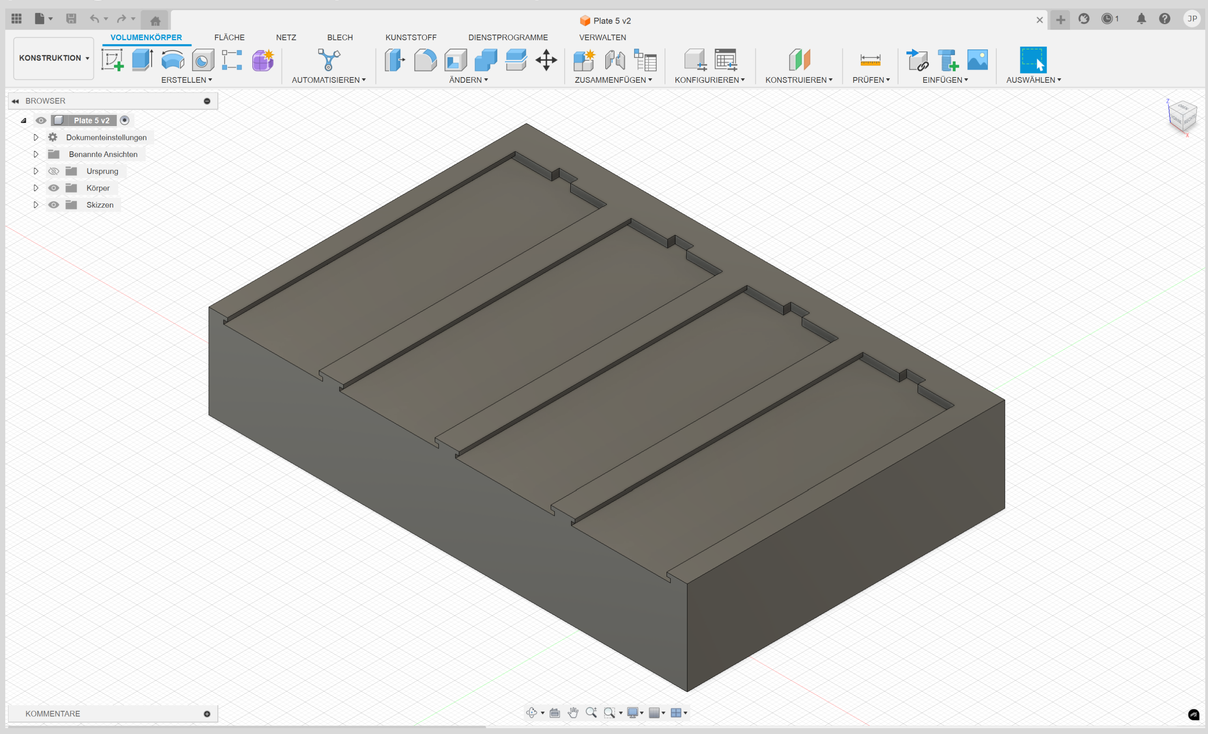


Fig. 2: Custom MALDI-Plate holder with four slots

AUTOMATION PROCESS

Two methods were automated, one using vials with inlets and formic acid (FA), to minimize the amount of bacteria needed. Also, a more complex procedure using glass beats and a shaker, was automated using specially shaped vials. Both methods allow adjustments such as the bacteria solution to matrix mixture or the amount of wash cycles, providing further flexibility for different bacterial types. In the end, the bacteria matrix solution is precisely placed on the metal MALDI plate. With a custom written script that avoids overlapping of the same spot, 1 µL of the solution is exactly placed in the marking. Afterwards, the solvent in the solution is evaporating and matrix crystal starts to from. These fully crystalized spots were lasered in the MALDI and afterwards analysed using a time-of-flight mass spectrometer (TOF MS).

Fig. 3: Placing liquid on MALDI plate

ANALYSIS WITH MALDI-TOF MS

In the end a spectrum is generated with masses of ribosomal proteins, which is specific for each individual bacteria species. Because the interpretation can be very complex mabritecCentral as an online database was used.

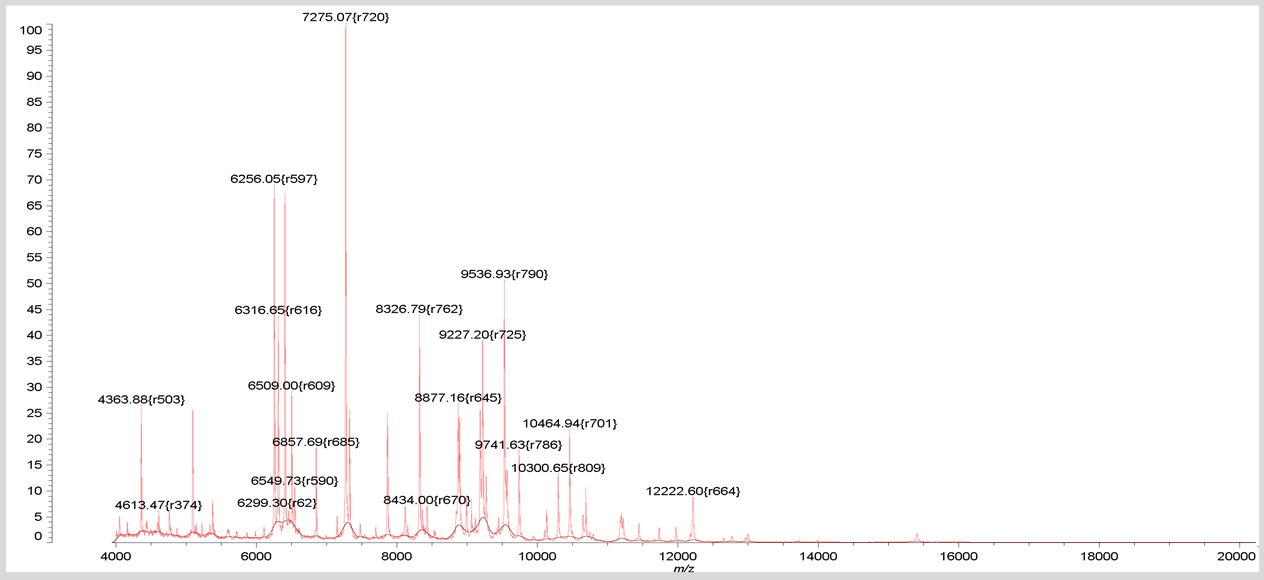


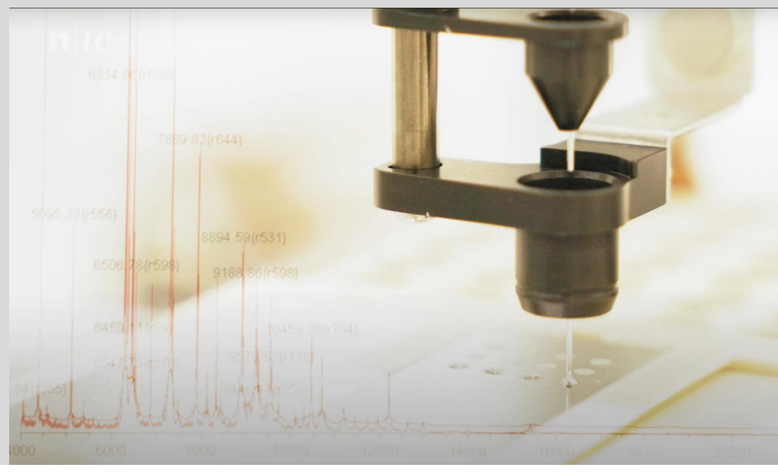
Fig. 4: Spectrum of *E. coli* with masses of ribosomal proteins, specific for its species

CONCLUSION

This thesis demonstrates the successful automation of sample preparation workflows using commonly available laboratory instruments, showcasing the feasibility of automating a sample preparation for microbiological samples.

Utilizing the FA method, it was shown that small bacterial quantities are enough to achieve species-level identification for various bacteria, including *E. coli*, *S. argenteus*, *A. radioresistens*, *A. venetianus*, *L. innocua*, *L. cossartiae subsp. cayugensis*, *L. immobilis*, *L. portnoyi*, *L. cossartiae subsp. cossartiae* and some strains of *L. faberi*. Additionally, a more complex method using glass beats could be showcased. A reliable MALDI-TOF database is critical for accurate identification, with the mabritecCentral database providing reliable species identification and compatibility, independent of the sample preparations. Furthermore, in combination with an automated sample preparation using a CTC PAL system, this offers a great potential in the field of clinical microbiology. Potentially cutting time and costs, by enhancing workflow consistency and reducing manual labour.

WATCH THE VIDEO



[1] M. T. Madigan et al., *Brock biology of microorganisms*, 2022
[2] N. A. Campbell et al., *Campbell Biologie*, 2016
[3] C. Walsh and T. Wencewicz, *Antibiotics: Challenges, mechanisms, opportunities*, 2016

mabritec

CTC Analytics