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Scientific letter

A simple MALDI-TOF-MS method for the identification of non-mycobacterial aerobic actinomycetes growing on blood agar subcultures from positive MGIT cultures

Un método sencillo para la identificación mediante MALDI-TOF-MS de actinomicetos aerobios no micobacterianos crecidos en subcultivo en agar sangre a partir de medio MGIT positivo

Dear Editor,

In this study, we tested the performance of the Bruker Biotyper MALDI-TOF MS system for identifying aerobic actinomycetes (AA) that were prospectively isolated on blood agar subcultures from mycobacterial culture media, using a simple and quick method that did not require any pre-treatment step. In the first stage of our evaluation (from September 2019 to July 2021), we examined the correlation between this simple MALDI-TOF MS identification method and 16S rRNA sequencing results. Additionally, during a subsequent time frame (from August to December 2023), we conducted a comparative analysis between the outcomes obtained using this simplified methodology and those recommended by the manufacturer, as well as with the reference standard (16S rRNA sequencing).

When the automated system detected a positive culture signal in MGIT medium, the culture broth was examined for the presence of acid-fast bacilli (AFB). A portion of the broth was placed on a Lowenstein-Jensen medium and on a sheep blood agar plate. When no AFB were observed, the subcultures on the blood agar plate were incubated for 1–7 days at 37 °C and inspected every day for bacterial growth in a Class 1 biological safety cabinet. Most bacteria, yeast, and filamentous fungi that grew on sheep blood agar plates showed a different morphology from the suspicious actinomycetes and were therefore not included in this study. Only colonies suspected of belonging to the phylum *Actinobacteria* were assayed for identification.

Once minimal visible growth was observed on blood agar plates, in the simple method protocol colonies were directly processed for MALDI-TOF MS analysis by placing one colony on two wells of a steel target plate using a disposable wooden stick. When following the manufacturer's recommended protocol, colonies were processed as indicated by Martin et al. (2023).¹ In both protocols, the overlay solution was air-dried at room temperature and

then analysed using the MALDI-TOF Biotyper[®] spectrophotometer (Bruker, Daltonik, Bremen, Germany). The plate was placed into MALDI-TOF MS instrument, and the captured spectra were analysed using versions 6 (first period) or 7 (second period) of the BDAL Mycobacteria Biotyper library with the simple method protocol and with the manufacturer protocol, respectively. The highest score value of both identifications was recorded. Bacteria were processed for DNA extraction and partial 16S rRNA sequencing as described previously.²

The genus-level identification by MALDI-TOF was found to be consistent with 16S rRNA sequencing in both periods, with no observable discrepancies. Initially, species-level differences were observed in 24.4% (11/45) of the isolates when comparing the simple method with the reference method and species-level agreement with a score of 1.7 or higher was achieved in 64.4% (29/45). We found that these results were unequally distributed, and affected particularly *Streptomyces* spp. genera, showing the lowest median values, 1.42. In the second study period, species-level differences were observed in 23.8% (5/21) and 33.3% (7/21) of the isolates when comparing the simple and manufacturer methods with the reference method, respectively (Table 1).

Several important findings were disclosed in our analysis of this performance of the Bruker Biotyper MALDI-TOF MS system. First of all, our approach is the first publication which has yielded very satisfactory results without any pretreatment step. We have also found, in the first study period, that the discriminative capacity of the proposed method depends on the type of AA. In our study, it seemed very useful, except for *Streptomyces* spp. This could be related to the greater cell wall hardness of this genus. *Tsukamurella tyrosinosolvens* was the most common species observed, which is in line with previous findings.³ We had better *Nocardia* spp. identification results than those described with the previous version of the BDAL database.⁴ In the second phase, we aimed to take into account updates to the Bruker database and compare with the manufacturer's proposed methodology. We observed that the simple method yielded even better results than the manufacturer's one, achieving accurate species-level identification in a greater number of included strains. Therefore, we recommend its implementation in the daily routine, taking into account its limitations with certain genera. However, our study has several limitations. Mainly, the study is conducted based on MALDI-TOF results, which sometimes do not yield acceptable scores. Ideally, the study should be based on molecular results.

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Table 1

Comparative identification with MALDI-TOF (simple and manufacturer's methods) and 16S rRNA sequencing of all clinical isolates of AA included in the study.

Simple method	Score	Manufacturer's protocol	Score	Result
<i>Brevibacterium</i> spp.				
<i>B. casei</i>	1.955			<i>B. casei</i>
<i>B. casei</i>	1.872			<i>B. casei</i>
<i>B. casei</i>	2.46			<i>B. casei</i>
<i>Corynebacterium</i> spp.				
<i>C. amycolatum</i>	2.27			<i>C. jeikeium</i>
<i>C. argenteoratense</i>	2.315			<i>C. argenteoratense</i>
<i>C. bovis</i>	2.112			<i>C. bovis</i>
<i>C. amycolatum</i>	2.144			<i>C. jeikeium</i>
<i>Gordonia</i> spp.				
<i>G. archiensis</i>	1.803			<i>G. archiensis</i>
<i>G. bronchialis</i>	1.32			<i>G. bronchialis</i>
<i>G. bronchialis</i>	2.375			<i>G. bronchialis</i>
<i>G. rubripunctata</i>	1.79			<i>G. terrae</i>
<i>G. sp.</i>	1.67			<i>G. sp.</i>
<i>G. sp.</i>	1.609			<i>G. sp.</i>
<i>G. sp.</i>	1.905			<i>G. sp.</i>
<i>G. sp.</i>	1.991			<i>G. sp.</i>
<i>G. bronchialis</i>	1.81	<i>G. bronchialis</i>	1.91	<i>G. bronchialis</i>
<i>G. bronchialis</i>	1.95	<i>G. bronchialis</i>	1.85	<i>G. bronchialis</i>
<i>G. sp.</i>	1.71	NO-ID		<i>G. sp.</i>
<i>G. terrae</i>	2.07	<i>G. terrae</i>	1.59	<i>G. terrae</i>
<i>Nocardia</i> spp.				
<i>N. abscessus</i>	2.06			<i>N. asiatica</i>
<i>N. carnei</i>	1.511			<i>N. carnei</i>
<i>N. carnei</i>	1.459			<i>N. ramosissima</i>
<i>N. carnei</i>	1.426			<i>N. asteroides</i>
<i>N. carnei</i>	2.105			<i>N. carnei</i>
<i>N. carnei</i>	2.39			<i>N. carnei</i>
<i>N. cyriacigeorgica</i>	1.634			<i>N. cyriacigeorgica</i>
<i>N. cyriacigeorgica</i>	1.762			<i>N. cyriacigeorgica</i>
<i>N. cyriacigeorgica</i>	1.857			<i>N. cyriacigeorgica</i>
<i>N. cyriacigeorgica</i>	1.862			<i>N. cyriacigeorgica</i>
<i>N. cyriacigeorgica</i>	2.488			<i>N. cyriacigeorgica</i>
<i>N. nova</i>	2.257			<i>N. nova</i>
<i>N. carnei</i>	1.5	<i>N. carnei</i>	1.55	<i>N. ramosissima</i>
<i>N. cyriacigeorgica</i>	1.64	<i>N. cyriacigeorgica</i>	1.61	<i>N. cyriacigeorgica</i>
<i>N. cyriacigeorgica</i>	1.58	<i>N. cyriacigeorgica</i>	1.54	<i>N. cyriacigeorgica</i>
<i>N. cyriacigeorgica</i>	1.48	<i>N. cyriacigeorgica</i>	1.71	<i>N. cyriacigeorgica</i>
<i>N. farcinica</i>	1.55	<i>N. carnei</i>	1.27	<i>N. carnei</i>
<i>N. farcinica</i>	2.11	<i>N. cyriacigeorgica</i>	1.87	<i>N. farcinica</i>
<i>N. nova</i>	1.82	<i>N. nova</i>	1.96	<i>N. nova</i>
<i>N. nova</i>	1.48	NO-ID		<i>N. nova</i>
<i>N. nova</i>	2.17	<i>N. nova</i>	1.25	<i>N. nova</i>
<i>N. veterana</i>	1.68	<i>N. veterana</i>	1.27	<i>N. veterana-nova</i>
<i>Rhodococcus</i> spp.				
<i>R. erythropolis</i>	2.022			<i>R. erythropolis</i>
<i>R. erythropolis</i>	2.114			<i>R. erythropolis</i>
<i>R. erythropolis</i>	2.127			<i>R. erythropolis</i>
<i>R. erythropolis</i>	2.225			<i>R. erythropolis</i>
<i>R. hoagii</i>	1.707			<i>R. hoagii</i>
<i>R. hoagii</i>	1.747			<i>R. hoagii</i>
<i>R. hoagii</i>	2.092			<i>R. hoagii</i>
<i>R. erythropolis</i>	1.49	<i>R. erythropolis</i>	1.7	<i>R. erythropolis</i>
<i>R. erythropolis</i>	2.07	<i>R. erythropolis</i>	1.91	<i>R. ginsengii</i>
<i>Streptomyces</i> spp.				
<i>S. violaceoruber</i>	1.81			<i>S. maritimus</i>
<i>S. badii</i>	1.66			<i>S. drzewieckii</i>
<i>S. violaceus</i>	1.783			<i>S. maritimus</i>
<i>S. marinus</i>	1.79	<i>S. chartreusensis</i>	1.72	<i>S. heteromorphus</i>
<i>Tsukamurella</i> spp.				
<i>T. paulametaloba</i>	1.631			<i>T. tyrosinosolvens</i>
<i>T. tyrosinosolvens</i>	1.73			<i>T. tyrosinosolvens</i>
<i>T. tyrosinosolvens</i>	1.821			<i>T. tyrosinosolvens</i>
<i>T. tyrosinosolvens</i>	1.88			<i>T. tyrosinosolvens</i>
<i>T. tyrosinosolvens</i>	1.917			<i>T. tyrosinosolvens</i>
<i>T. tyrosinosolvens</i>	1.951			<i>T. tyrosinosolvens</i>
<i>T. tyrosinosolvens</i>	2.1			<i>T. tyrosinosolvens</i>
<i>T. tyrosinosolvens</i>	2.307			<i>T. tyrosinosolvens</i>
<i>T. paurometabola</i>	2.2	<i>T. tyrosinosolvens</i>	1.94	<i>T. tyrosinosolvens</i>
<i>T. tyrosinosolvens</i>	2.22	NO-ID		<i>T. tyrosinosolvens</i>
<i>T. tyrosinosolvens</i>	1.71	<i>T. tyrosinosolvens</i>	2.1	<i>T. tyrosinosolvens</i>
<i>T. tyrosinosolvens</i>	1.9	<i>T. tyrosinosolvens</i>	1.94	<i>T. tyrosinosolvens</i>

NO-ID: no identification.

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Conflict of interest

We have no conflicts of interest to disclose.

Data availability statement

The 16S rRNA sequencing datasets analysed during the current study were compared to those deposited in the Sample GenBank Record – NCBI repository, (<https://www.ncbi.nlm.nih.gov/genbank/>).

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