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BOOK OF ABSTRACTS

THP-25: USING MALDI-TOF MS FOR THE DETECTION OF PUTATIVE NEW ACINETOBACTER SPECIES FROM CATTLE FECES

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Question: *Acinetobacter* is a taxonomically diverse bacterial genus, with 72 validly named species and many provisional taxa [1]. The genus is ubiquitous, inhabiting plethora of natural ecosystems. *Acinetobacter* spp., with *A. baumannii* in particular, have emerged as opportunistic human pathogens, associated with multidrug resistance and epidemic spread [2]. As the knowledge on *Acinetobacter*s in non-human ecosystems is limited, we conducted a study on the impact of antibiotic use in livestock on the occurrence and spread of resistant and potentially pathogenic *Acinetobacter* spp. in cattle feces.

Methods: Samples of cattle feces were homogenized and cultured aerobically in a mineral medium supplemented with sodium acetate. The grown-up cultures were streaked onto both acetate agar and chrome agar plates. Up to 24 agar-grown colonies per sample were directly identified by MALDI-TOF MS using the current Bruker database supplemented with homemade entries of provisional *Acinetobacter* taxa. The spectra were further compared using cluster analysis to dereplicate isolates of the same strains.

Results: A total of 19 samples from 16 different cattle farms were analyzed. As many as 186 *Acinetobacter* isolates were obtained: *A. baumannii* (6), *A. courvalinii*/*A. vivianii* (2), *A. gandensis* (17), *A. haemolyticus* (1), *A. indicus* (26), *A. pseudolwoffii* (32), *A. variabilis* (13), Taxon 36 (9), genomic sp. 15/16 (1) and 79 unidentified isolates. Sixteen unidentified isolates formed a distinct and cohesive MALDI-TOF MS cluster, possibly representing a novel species.

Conclusion: We have applied an effective approach based on MALDI-TOF MS, which enables quick screening of multiple *Acinetobacter* isolates to assess their taxonomic diversity and to identify putative novel species.

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Using MALDI-TOF MS for the detection of putative new *Acinetobacter* species from cattle feces

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INTRODUCTION

Acinetobacter is a taxonomically diverse (74 validly named species) and ubiquitous genus, with some species being troublesome opportunistic human pathogens associated with multiresistance to antibiotics and epidemic spread (Nemeč, 2022). *Acinetobacter* taxonomy and ecology in non-human ecosystems is largely unknown and studies of the impact of antibiotic use in livestock on the occurrence and spread of *Acinetobacter* are required.

AIM

To design and evaluate an effective approach to recover taxonomically diverse and potentially antimicrobial-resistant *Acinetobacter* (AMRA) strains from cattle feces.

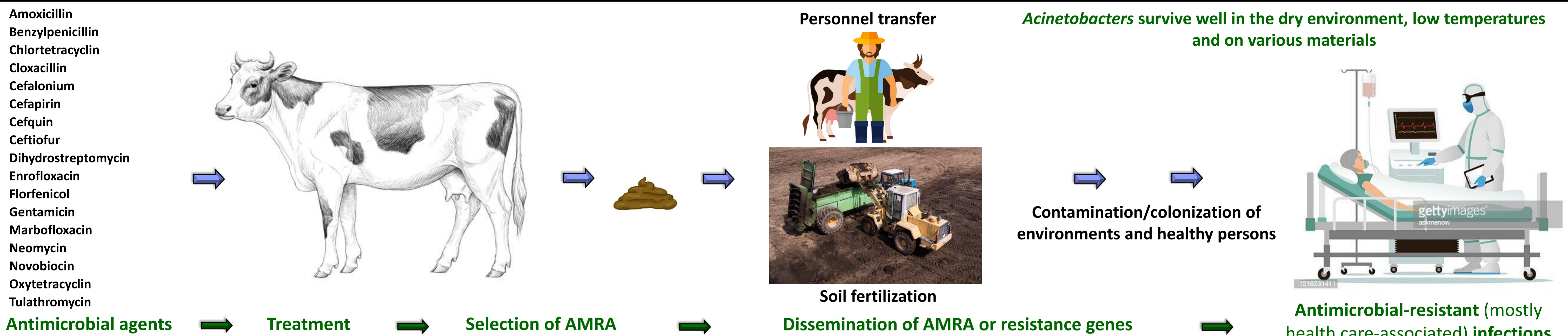


Fig. 1. Hypothetical spread of AMRA or AMRA-associated resistance genes.

SAMPLES

16 samples of cattle feces were collected during 2022, from 7 cattle farms in the Central Bohemian Region.

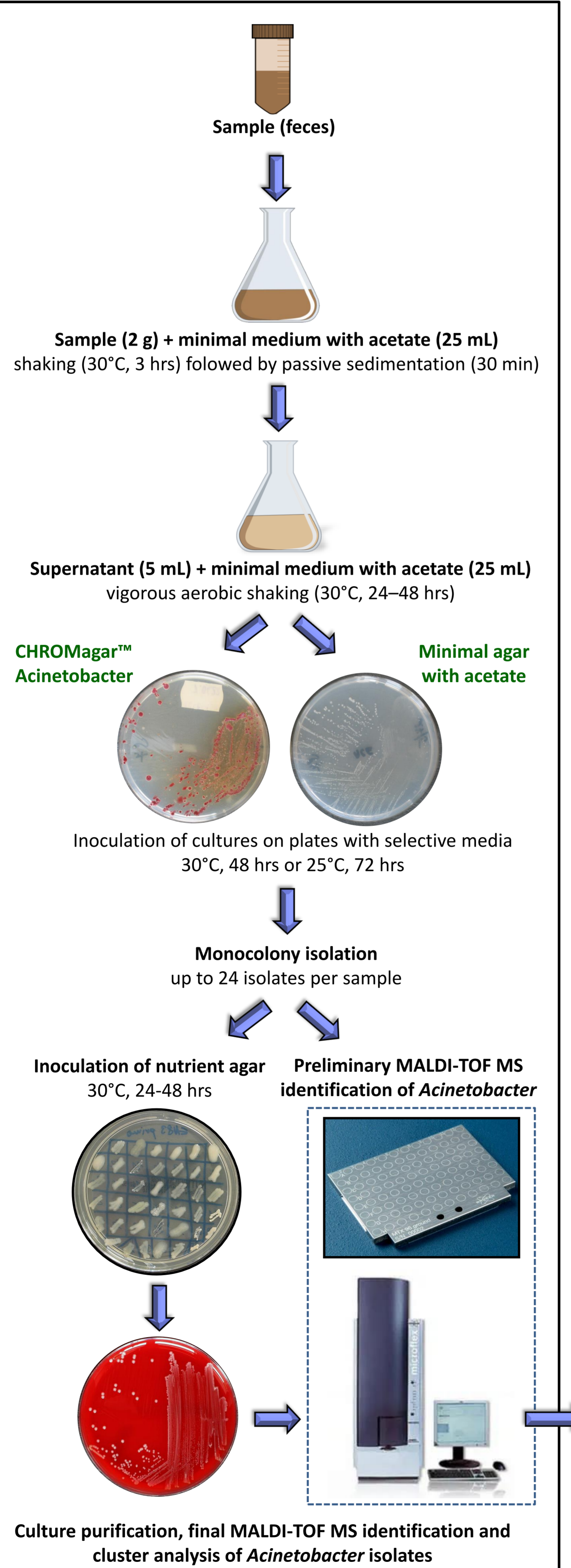
METHODS

Sample processing: depicted in Fig. 2.

Species identification based on MALDI-TOF MS (matrix-assisted laser desorption/ionization time-of-flight mass spectrometry): using the Microflex LT instrument and Biotyper RTC with Compass version 4.1.80 (Bruker Daltonics) in automatic mode, with the current Bruker database (v. 2022) supplemented with homemade entries of additional species and provisional taxa (Table 1).

Dereplication of isolates: cluster analysis of MALDI-TOF mass spectra to exclude isolates grouping at a distance of ≤ 50 (Fig. 3) followed by the verification of the genotypic uniqueness of selected isolates using DNA fingerprinting (Fig. 4).

Fig. 2. Isolation and identification of acinetobacters from cattle feces.



RESULTS

186 *Acinetobacter* isolates were obtained: *A. baumannii* (n=6), *A. courvalinii*/*A. vivianii* (2), *A. gandensis* (17), *A. haemolyticus* (1), *A. indicus* (26), *A. pseudowolfii* (32), *A. variabilis* (13), Taxon 36 (9), genomic sp. 15/16 (1) and 79 unidentified isolates; 14 unidentified strains formed a distinct and cohesive MALDI-TOF MS cluster (Fig. 5), possibly representing a novel species, provisionally termed **Taxon 7201** (Fig. 4 and 5).

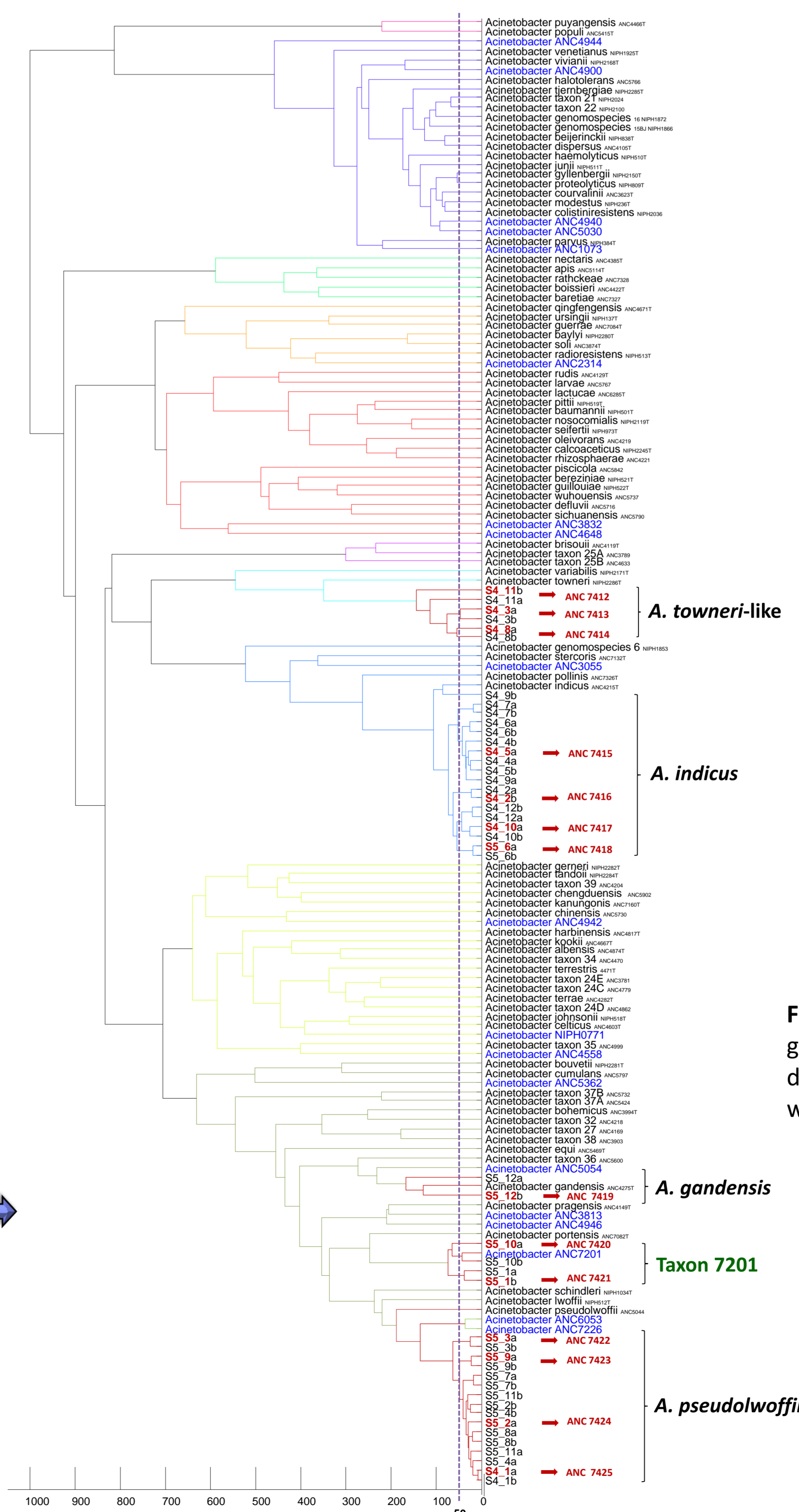


Fig. 3. MALDI-TOF MS-based dendrogram of isolates from samples RUD-F1 and POZ-F1 and reference strains of the known *Acinetobacter* spp. Arrows indicate isolates selected after dereplication at the threshold indicated by the vertical dashed line.

Table 1. Homemade reference spectra of the species and provisional taxa not included in the Bruker database

<i>Acinetobacter</i> genomospecies_15B _{NIPH1866}	<i>Acinetobacter</i> NIPH0771
<i>Acinetobacter</i> genomospecies_16 _{NIPH1872}	<i>Acinetobacter</i> NIPH1073
<i>Acinetobacter</i> genomospecies_6 _{NIPH1853}	<i>Acinetobacter</i> NIPH1454
<i>Acinetobacter</i> kyonggiensis _{ANC5109}	<i>Acinetobacter</i> NIPH2314
<i>Acinetobacter</i> lanii _{ANC5348}	<i>Acinetobacter</i> ANC2221
<i>Acinetobacter</i> marinus _{ANC5699}	<i>Acinetobacter</i> ANC3055
<i>Acinetobacter</i> oleivorans _{ANC4219}	<i>Acinetobacter</i> ANC3813
<i>Acinetobacter</i> rhizosphaerae _{ANC4221}	<i>Acinetobacter</i> ANC3832
<i>Acinetobacter</i> rongchengensis _{ANC5989}	<i>Acinetobacter</i> ANC4558
<i>Acinetobacter</i> taxon_21 _{NIPH2024}	<i>Acinetobacter</i> ANC4648
<i>Acinetobacter</i> taxon_22 _{NIPH2100}	<i>Acinetobacter</i> ANC4900
<i>Acinetobacter</i> taxon_24C _{ANC4779}	<i>Acinetobacter</i> ANC4940
<i>Acinetobacter</i> taxon_24D _{ANC4862}	<i>Acinetobacter</i> ANC4942
<i>Acinetobacter</i> taxon_24E _{ANC3781}	<i>Acinetobacter</i> ANC4944
<i>Acinetobacter</i> taxon_25A _{ANC3789}	<i>Acinetobacter</i> ANC4946
<i>Acinetobacter</i> taxon_25B _{ANC4633}	<i>Acinetobacter</i> ANC5030
<i>Acinetobacter</i> taxon_27 _{ANC4169}	<i>Acinetobacter</i> ANC5034
<i>Acinetobacter</i> taxon_32 _{ANC4218}	<i>Acinetobacter</i> ANC5054
<i>Acinetobacter</i> taxon_34 _{ANC4470}	<i>Acinetobacter</i> ANC5362
<i>Acinetobacter</i> taxon_36 _{ANC5600}	<i>Acinetobacter</i> ANC5367
<i>Acinetobacter</i> taxon_37A _{ANC5424}	<i>Acinetobacter</i> ANC6053
<i>Acinetobacter</i> taxon_37B _{ANC5732}	<i>Acinetobacter</i> ANC7201
<i>Acinetobacter</i> taxon_38 _{ANC3903}	<i>Acinetobacter</i> ANC7209
<i>Acinetobacter</i> taxon_39 _{ANC4204}	<i>Acinetobacter</i> ANC7384
<i>Acinetobacter</i> tianfuensis _{ANC5987}	<i>Acinetobacter</i> ANC7655

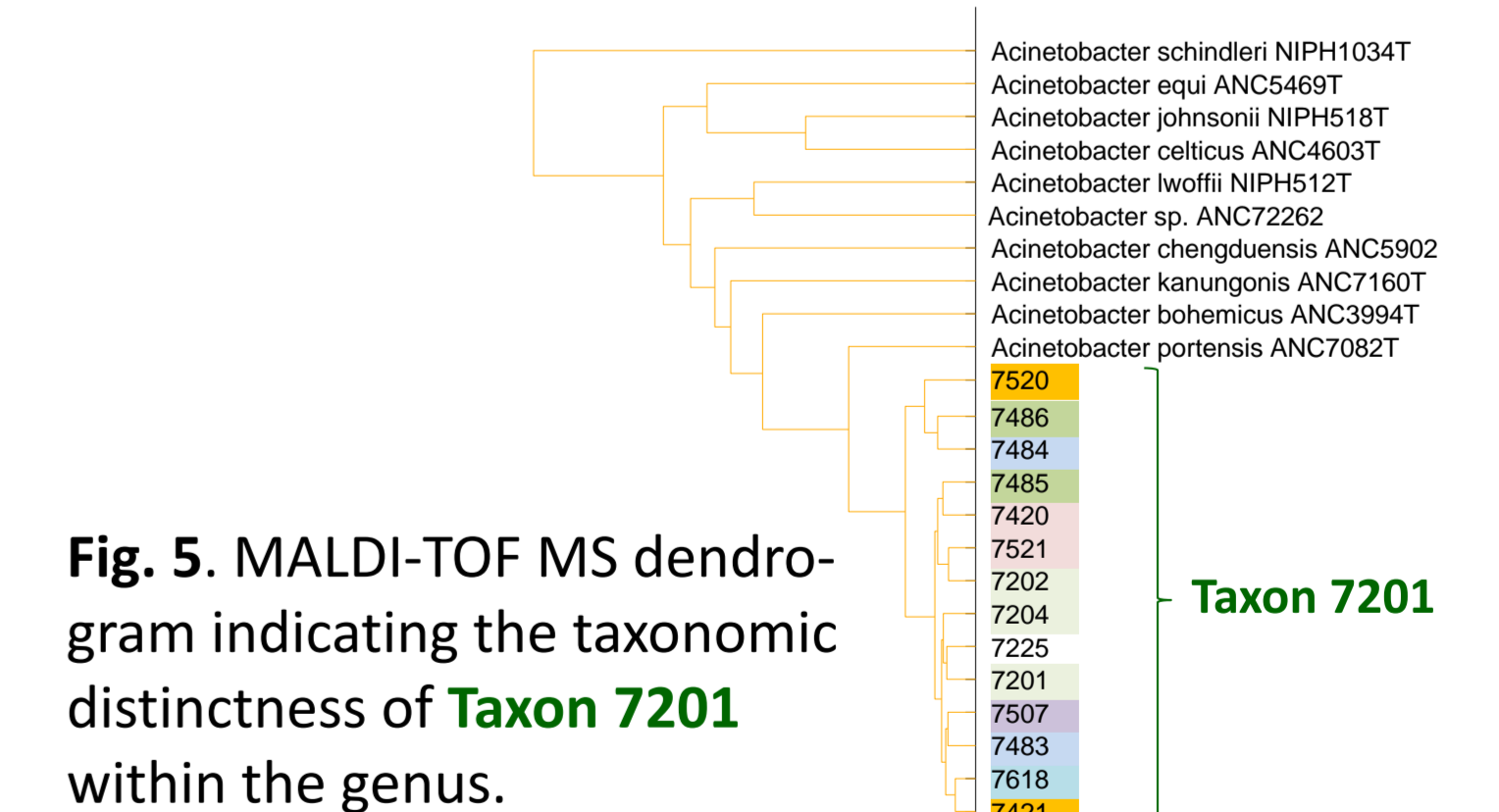


Fig. 4. Genomic DNA restriction patterns of 14 isolates of **Taxon 7201** obtained by ApI macro-restriction analysis/pulsed-field gel electrophoresis.

CONCLUSION

We propose a both powerful and efficient approach based on MALDI-TOF MS, which enables quick screening of multiple *Acinetobacter* isolates to assess their taxonomic diversity and to identify putative novel species.