



Středoevropský technologický institut  
BRNO | ČESKÁ REPUBLIKA

Research programme 4: Genomics and Proteomics of Plant systems  
**Core Facility - Proteomics**

# MALDI-TOF MS profiling of bacteria: how far can we go?

**Ondrej Šedo**

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EVROPSKÁ UNIE  
EVROPSKÝ FOND PRO REGIONÁLNÍ ROZVOJ  
INVESTICE DO VAŠÍ BUDOUCNOSTI



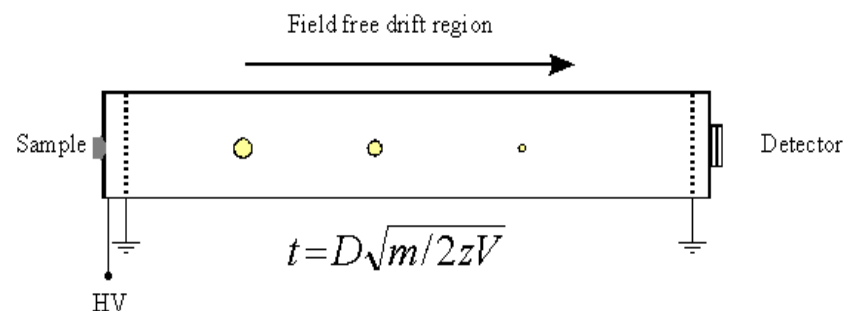
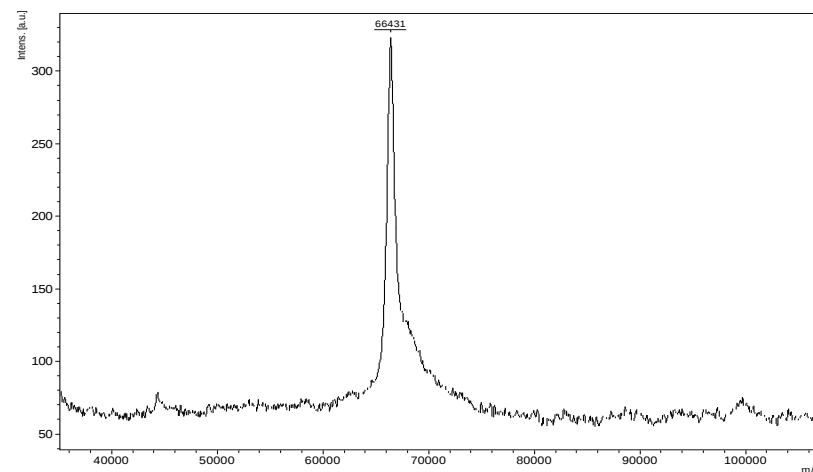
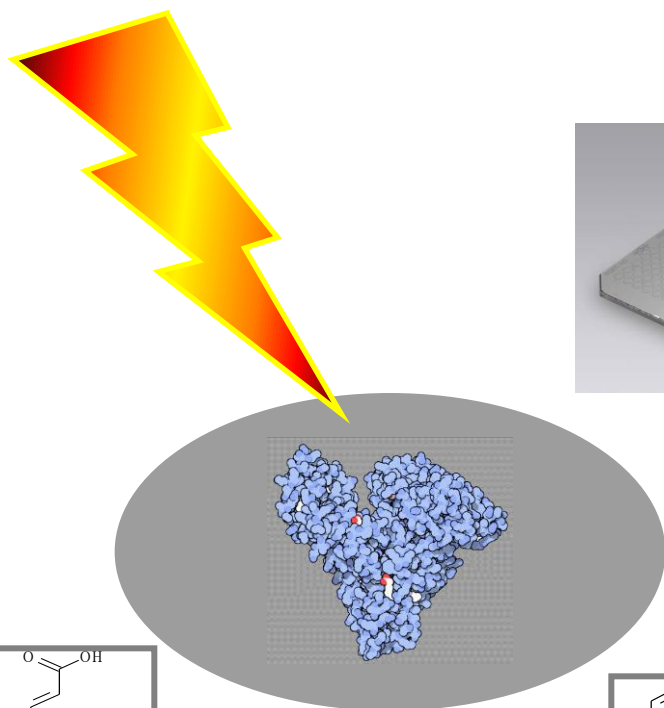
OP Výzkum a vývoj  
pro inovace



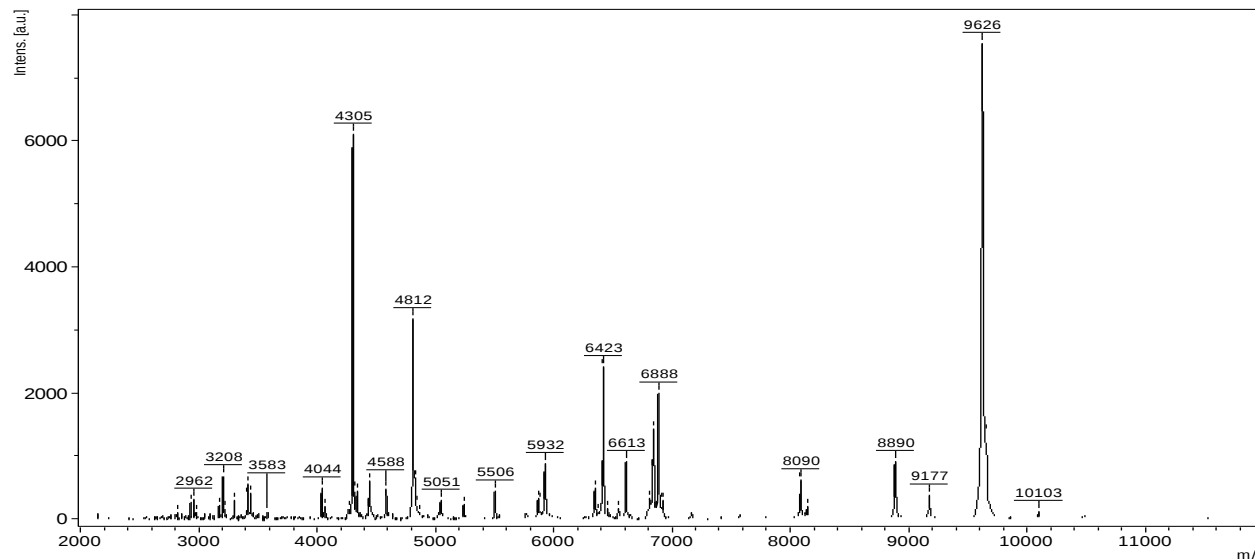
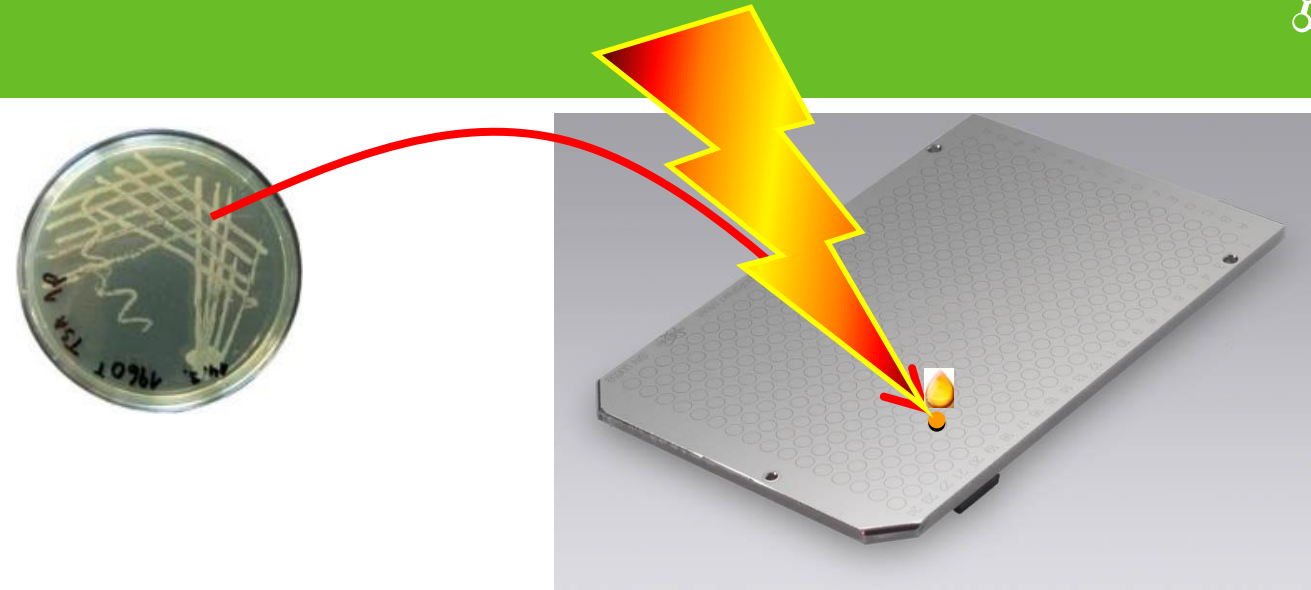
# MALDI-TOF MS



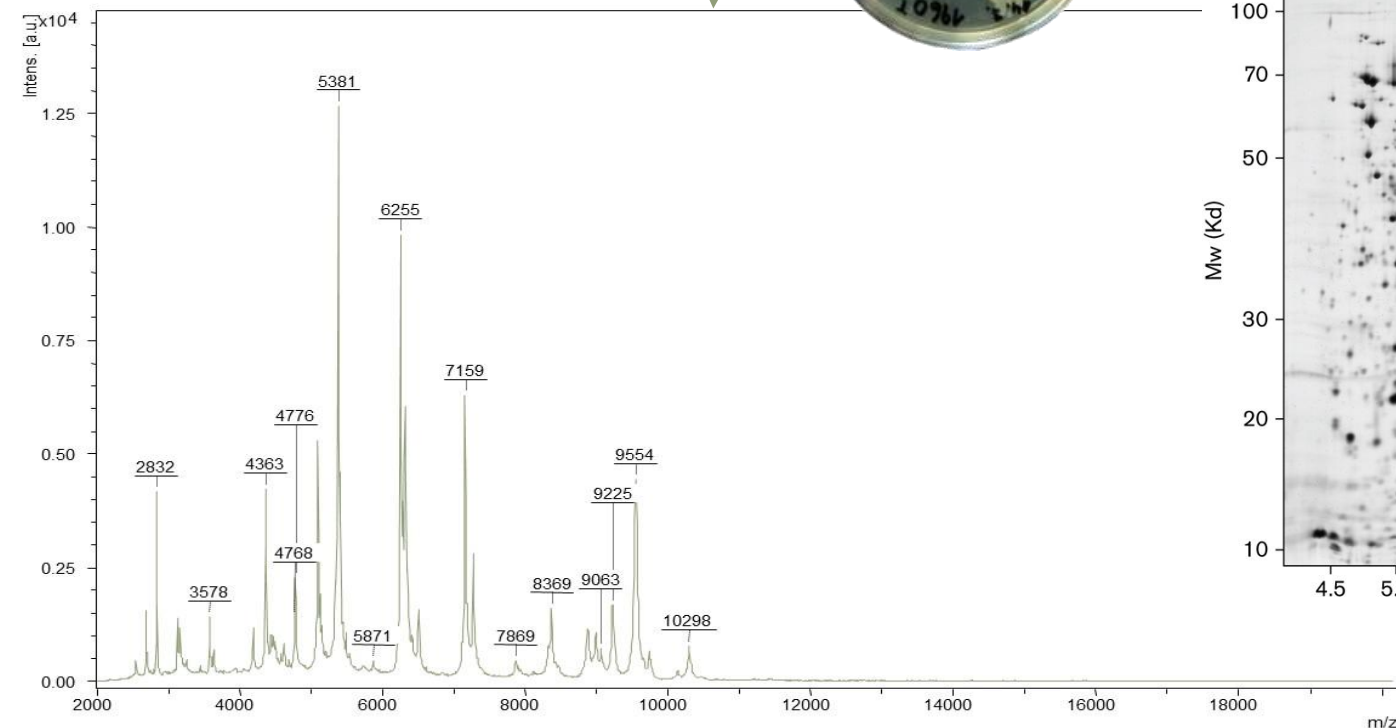
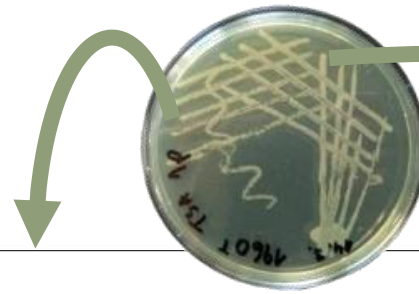
- **MALDI MS = Matrix-Assisted Laser Desorption/Ionization Mass Spectrometry**



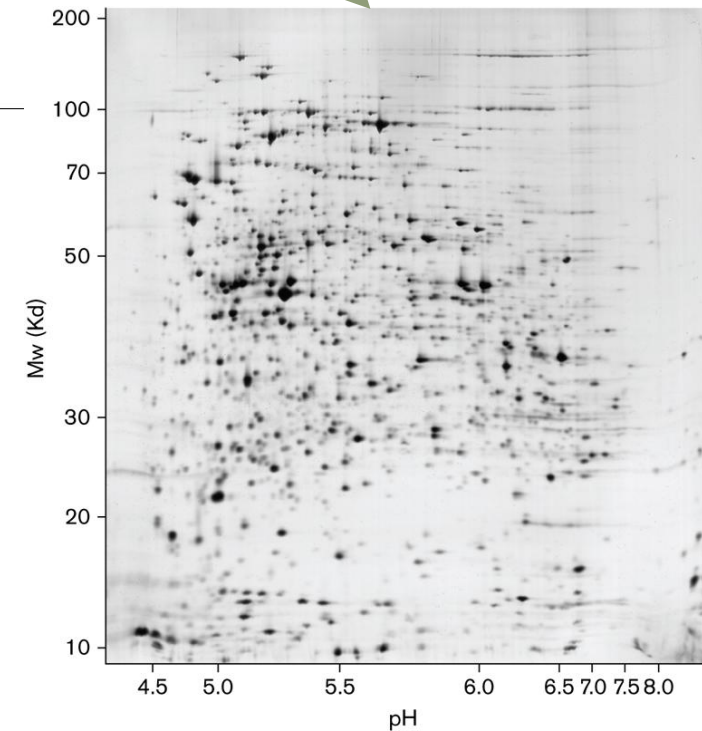
# MALDI-TOF MS profiling of bacteria



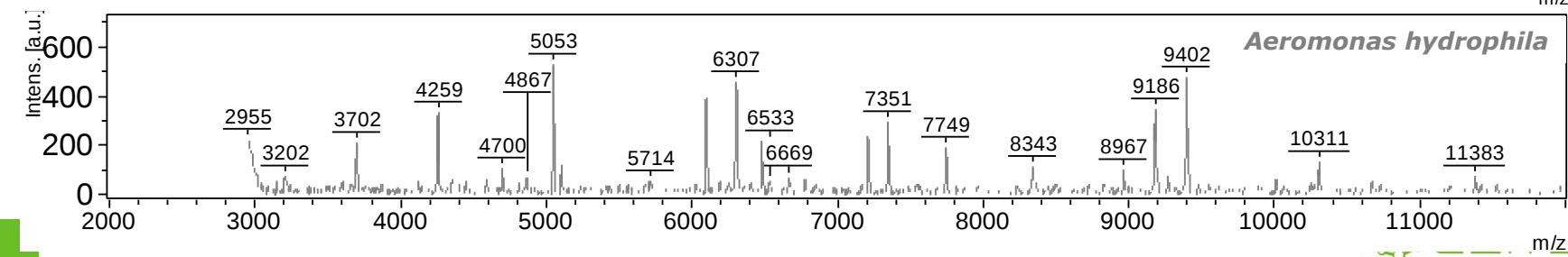
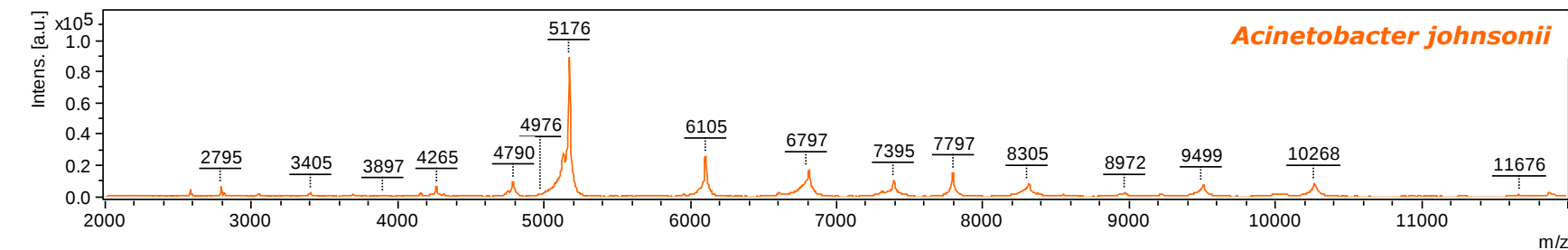
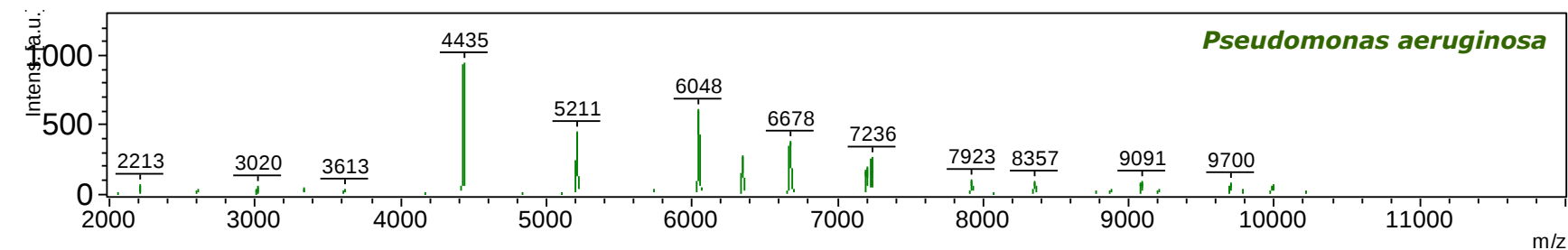
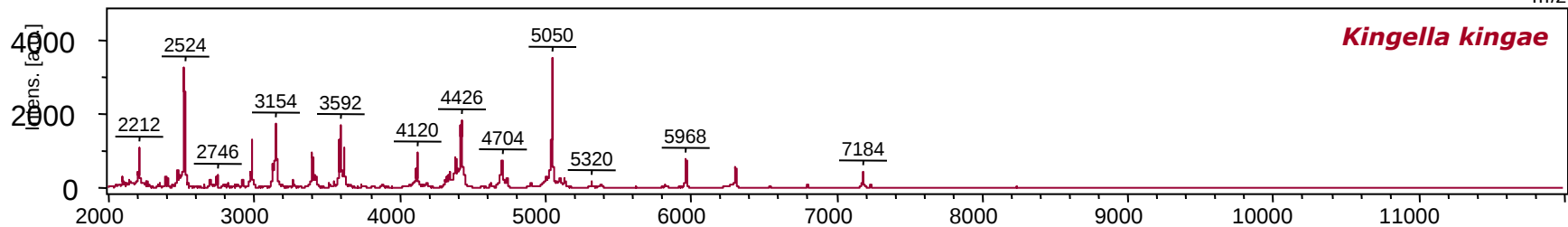
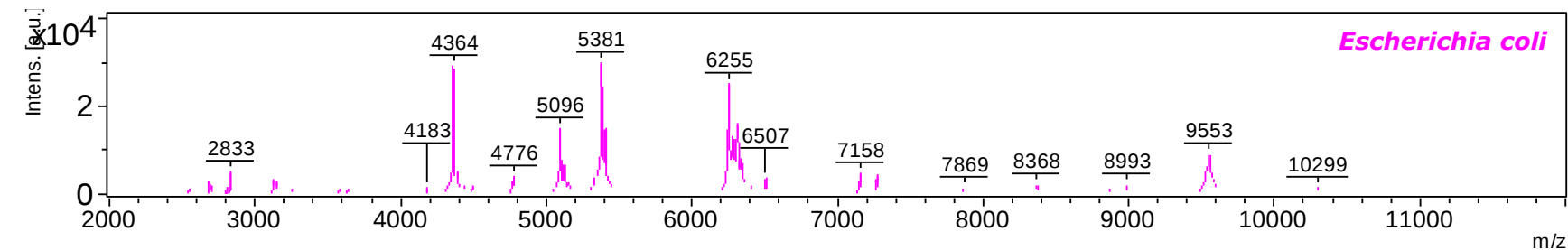
# MALDI-TOF MS profiling of bacteria

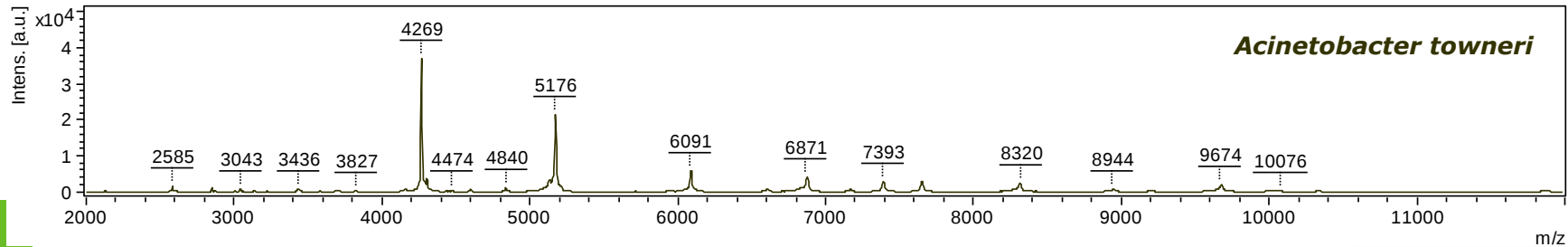
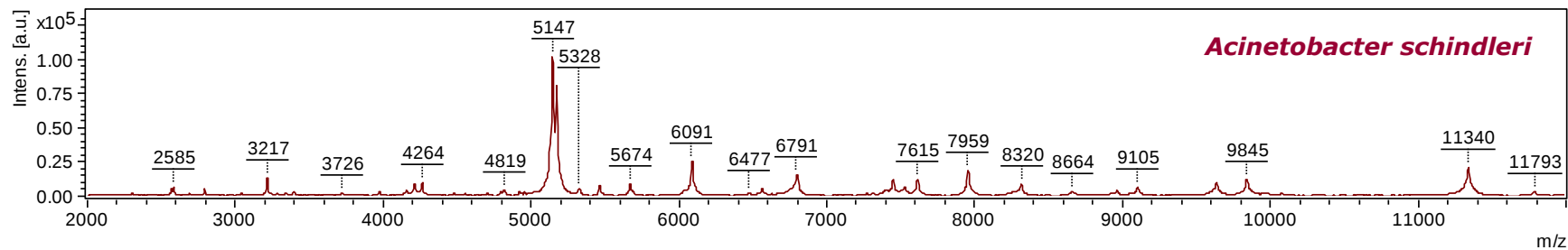
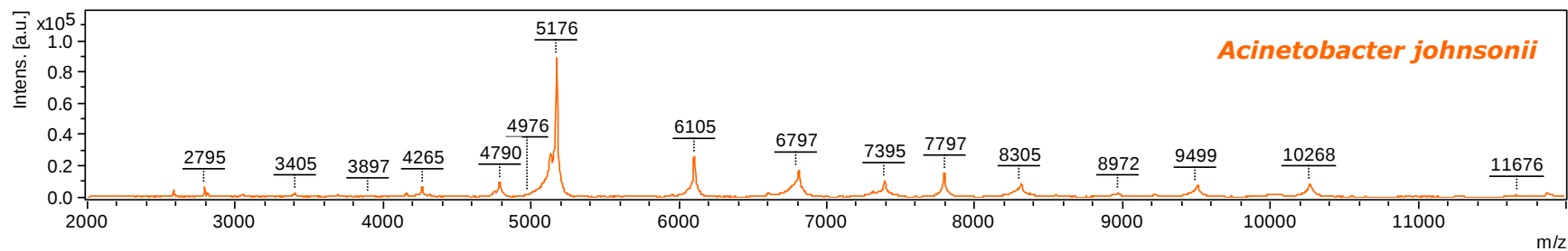
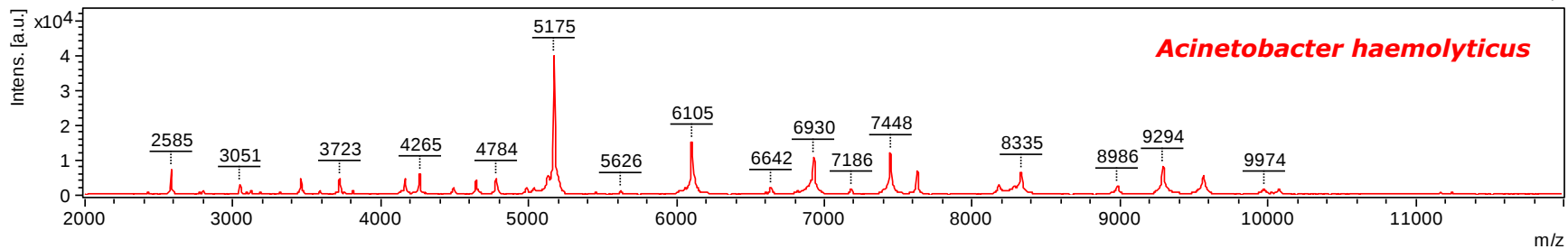
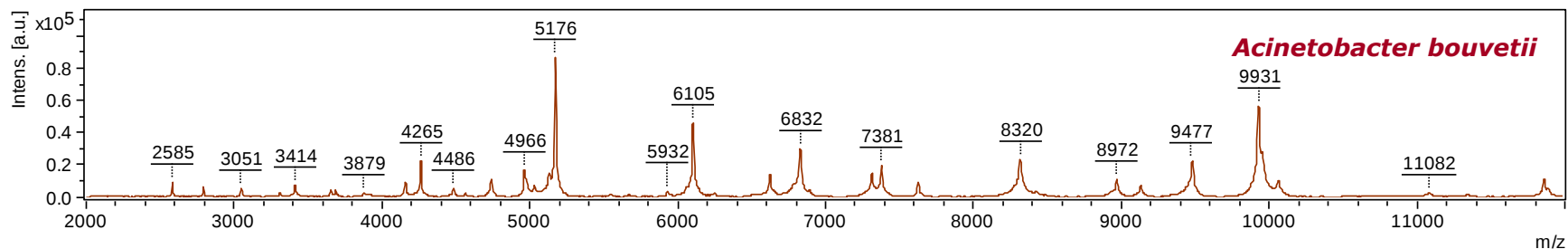


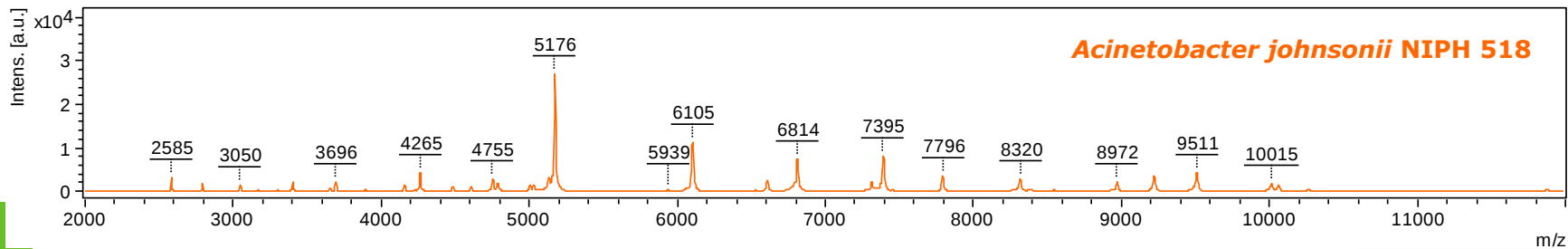
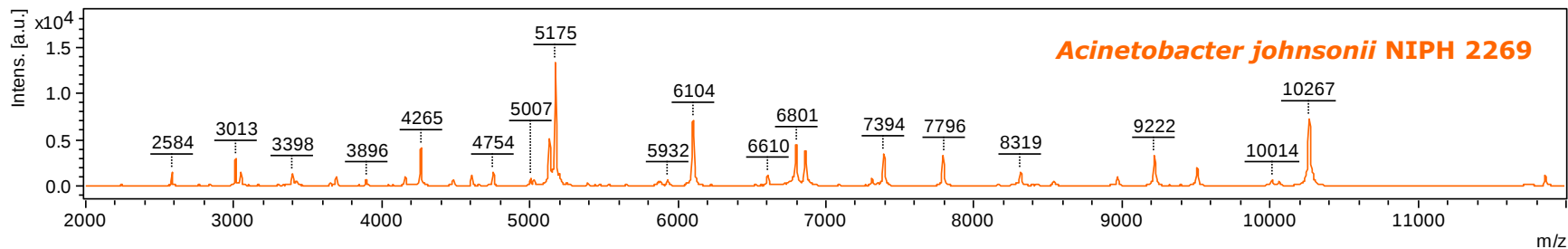
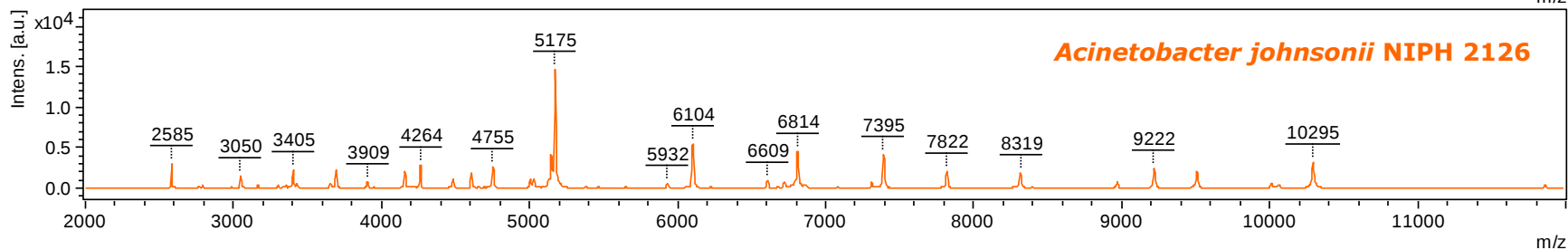
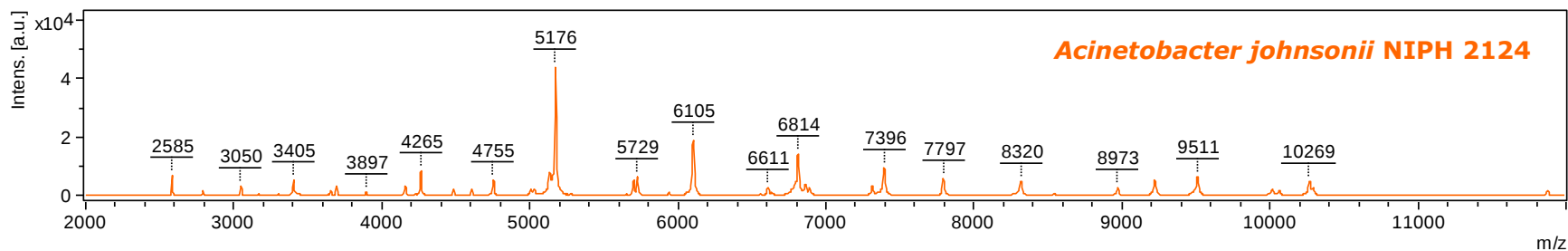
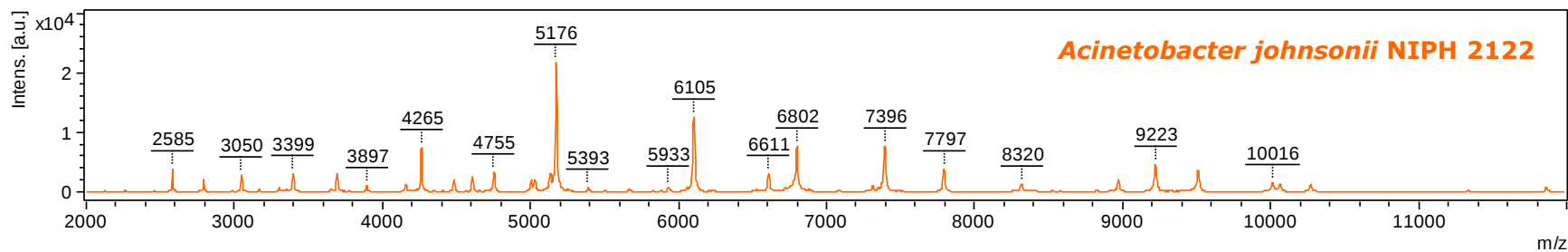
MALDI-TOF MS



2D GE



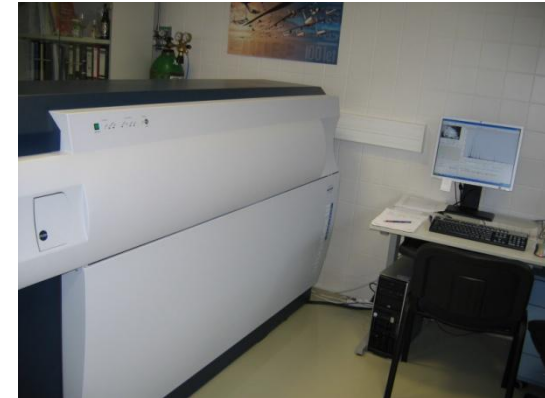




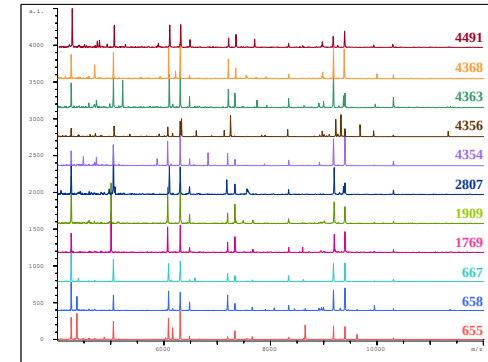
# MALDI-TOF MS profiling of bacteria



## I. Mass spectrometer



## II. Database of reference spectra



## III. Data treatment software

Result Overview

| Analyte<br>Name | Analyte<br>ID | Organization<br>(best match)                  | Score<br>Value | Organization<br>(second best match)          | Score<br>Value |
|-----------------|---------------|-----------------------------------------------|----------------|----------------------------------------------|----------------|
| 101<br>(+++)    | reference     | Escherichia coli J15449p188                   | 100            | Escherichia coli ATCC 25922 TBL              | 99.9           |
| 102<br>(+++)    | Profile 1     | Stenotrophomonas (ST) 2106089_MCD             | 100            | Stenotrophomonas (sp. nov.) DSM 98113<br>TBL | 99.9           |
| 103<br>(+++)    | Profile 2     | Stenotrophomonas (sp. nov.) DSM 98113<br>TBL  | 100            | Stenotrophomonas (ST) 2106089_MCD            | 99.9           |
| 104<br>(+++)    | Profile 3     | Morganella morganii (sp. nov.) DSM 1534_1 CDB | 100            | Morganella morganii DSM 1534_1 CDB           | 99.9           |
| 105<br>(+++)    | Profile 4     | Morganella morganii (sp. nov.) DSM 1534_1 CDB | 100            | Morganella morganii DSM 1534_1 CDB           | 99.9           |
| 106<br>(+++)    | Profile 5     | Acinetobacter (sp. nov.) DSM 1534_1 CDB       | 100            | Acinetobacter (sp. nov.) DSM 1534_1 CDB      | 99.9           |
| 107<br>(+++)    | Profile 6     | Acinetobacter (sp. nov.) DSM 1534_1 CDB       | 100            | Acinetobacter (sp. nov.) DSM 1534_1 CDB      | 99.9           |
| 108<br>(+++)    | Profile 7     | Acinetobacter (sp. nov.) DSM 1534_1 CDB       | 100            | Acinetobacter (sp. nov.) DSM 1534_1 CDB      | 99.9           |
| 109<br>(+++)    | Profile 8     | Acinetobacter (sp. nov.) DSM 1534_1 CDB       | 100            | Acinetobacter (sp. nov.) DSM 1534_1 CDB      | 99.9           |
| 110<br>(+++)    | Profile 9     | Acinetobacter (sp. nov.) DSM 1534_1 CDB       | 100            | Acinetobacter (sp. nov.) DSM 1534_1 CDB      | 99.9           |

# MALDI-TOF MS profiling of bacteria



## **Main advantages:**

- few minutes required for identification**
- identification on the species level for > 90% of clinical isolates**
- low analysis costs**

# MALDI-TOF MS profiling of bacteria: How far can we go?



1. Identification of lactobacilli from dairy products
2. Identification of microbial contaminants of meat products
3. Identification of African wildlife microflora
4. Differentiation between *Acinetobacter* spp. (ACB complex)
5. Identification of *Aeromonas* spp.
6. Differentiation between strains within *L. acidophilus* group
7. Differentiation between *S. aureus* strains

# 1. Identification of lactobacilli from dairy products:

148 isolates from milk, fermented products and cheese



**Species identification  
success rate:**

**PCR: 77%**

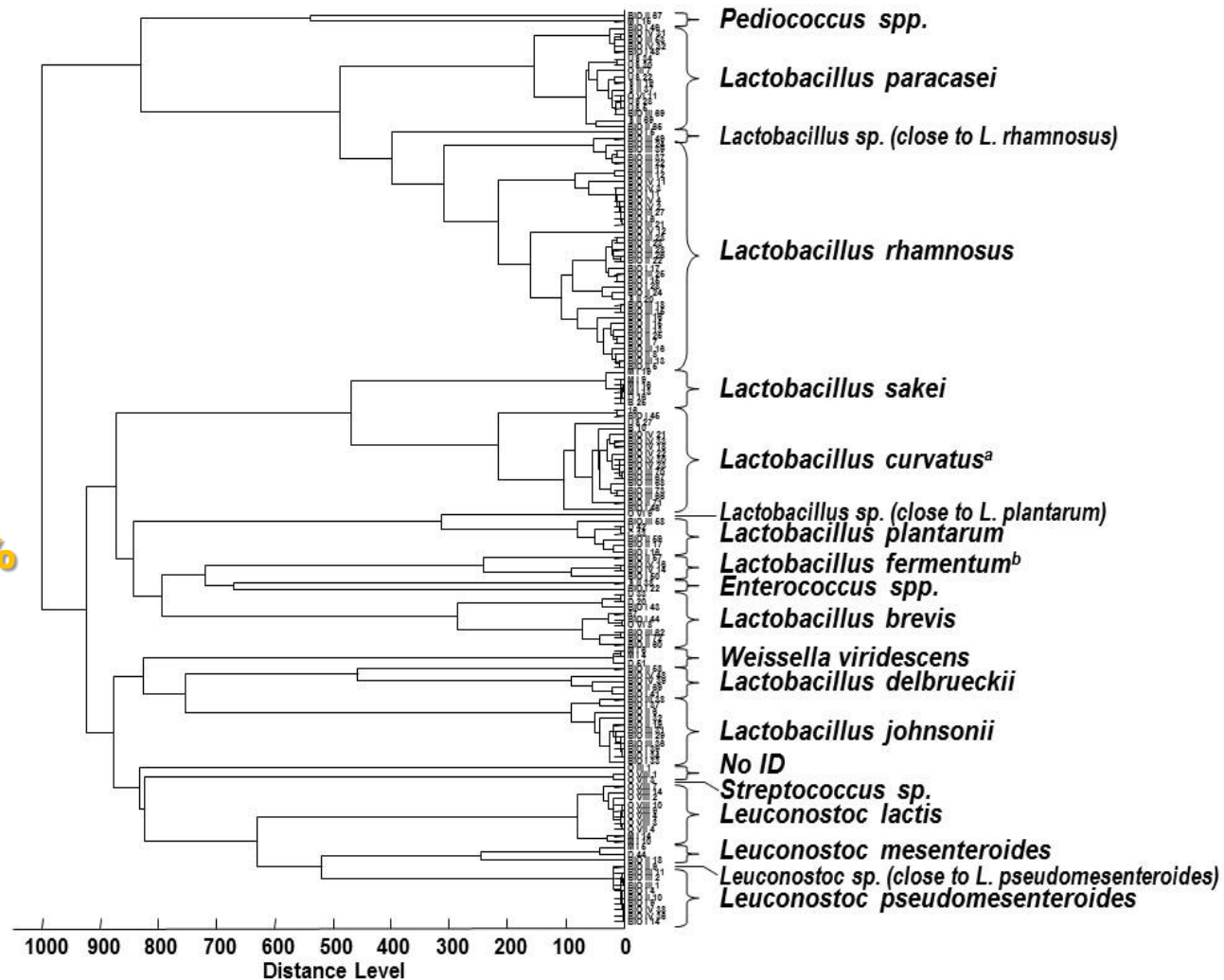
**MALDI MS: 93%**

**MALDI MS**

**genus identification: 5 %**

**MALDI MS**

**not identified: 2 %**



## 2. Identification of microbial contaminants of meat products:

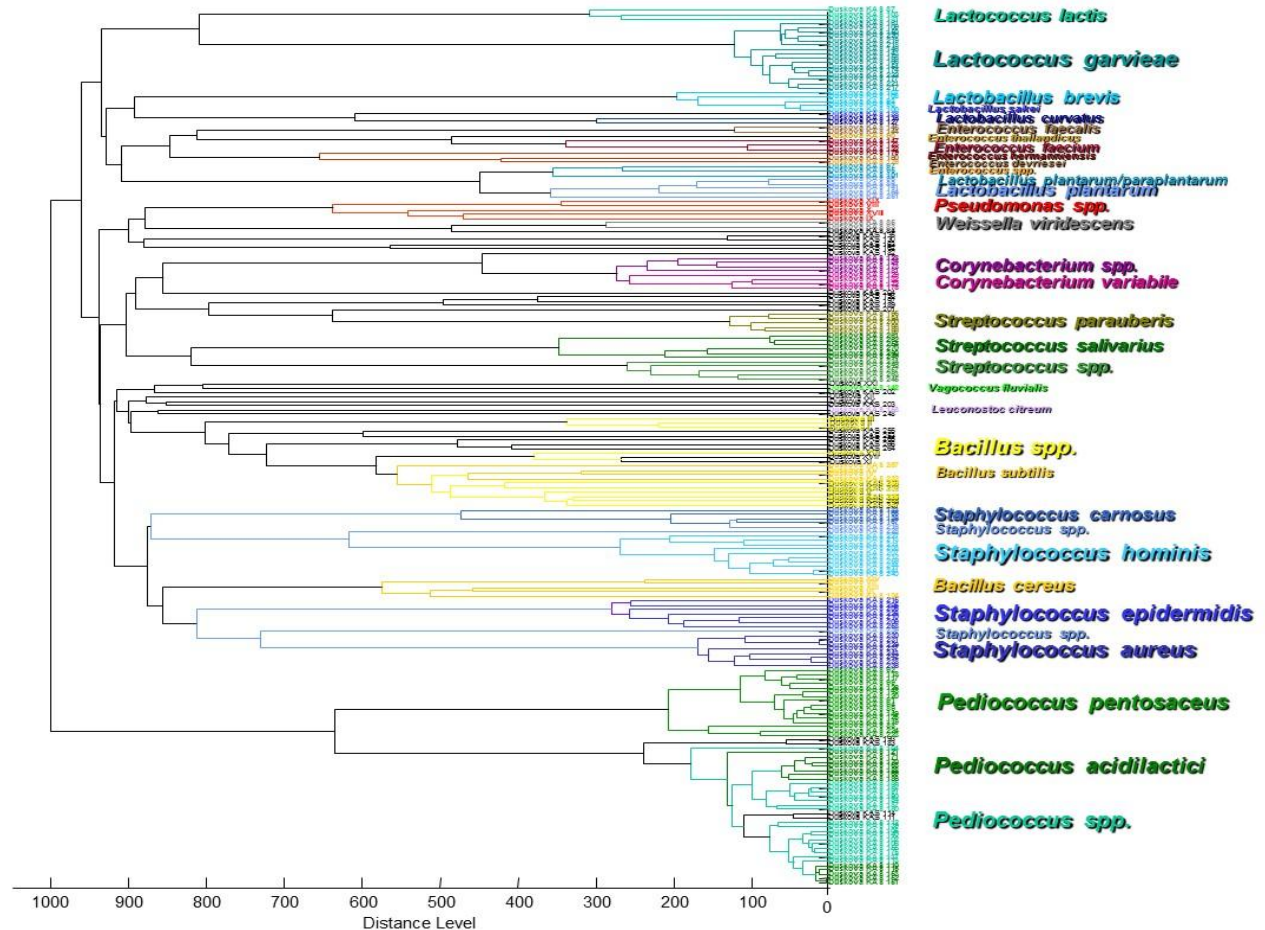
2035 isolates from meat products  
and meat processing facilities



**MALDI MS species  
identification: 64 %**

**MALDI MS genus  
identification: 21 %**

**MALDI MS not  
identified: 15 %**



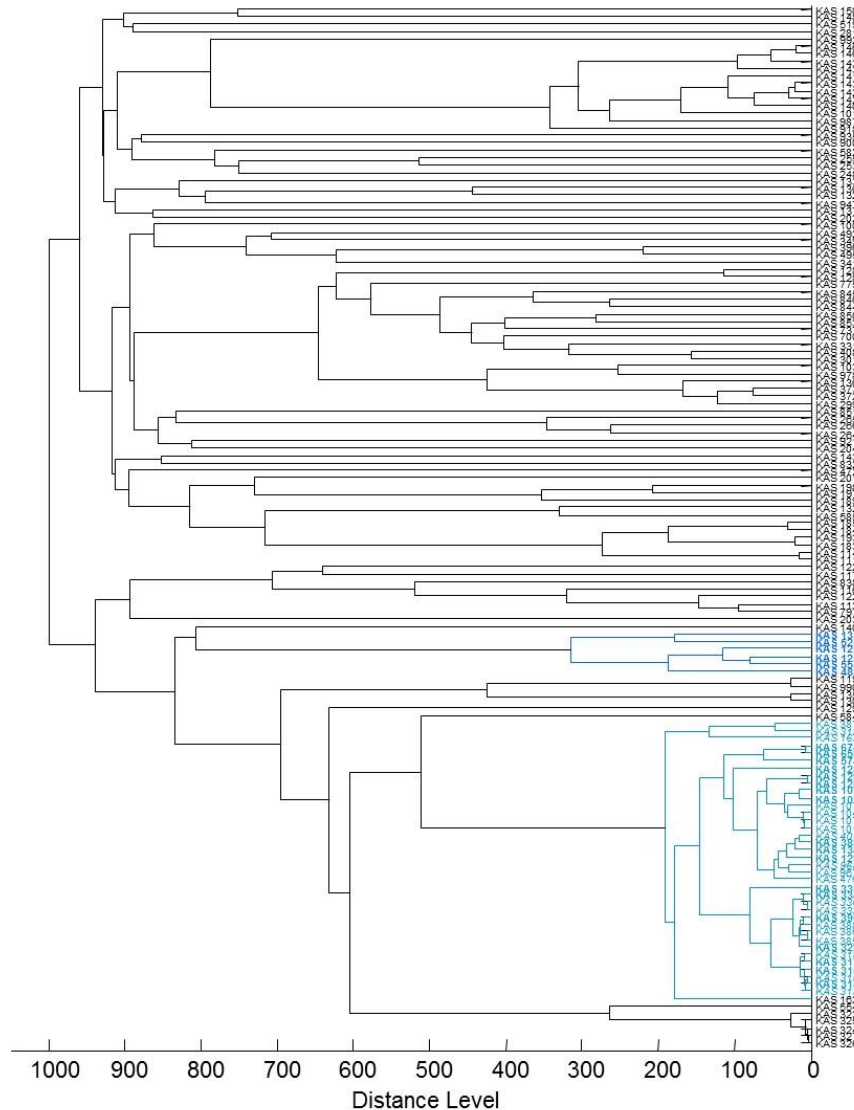
Kameník J., Dušková M., Saláková A., Šedo O. *Acta Vet. Brno* 82 (2013) 181.

Dušková M., Kameník J., Šedo O., Saláková A., Zdráhal Z., Karpíšková R., Lačanin I. *Food Control* 50 (2015) 804.

Kameník J., Dušková M., Šedo O., Saláková A., Pavlík Z., Zdráhal Z., Karpíšková R. *LWT Food Sci. Technol.*, 61 (2015) 492.

## 2. Identification of microbial contaminants of meat products:

Identification of unmatching isolates  
by database extension of the reference database



*Weissella confusa*

*Weissella hellenica*

### 3. Identification of African wildlife microflora:

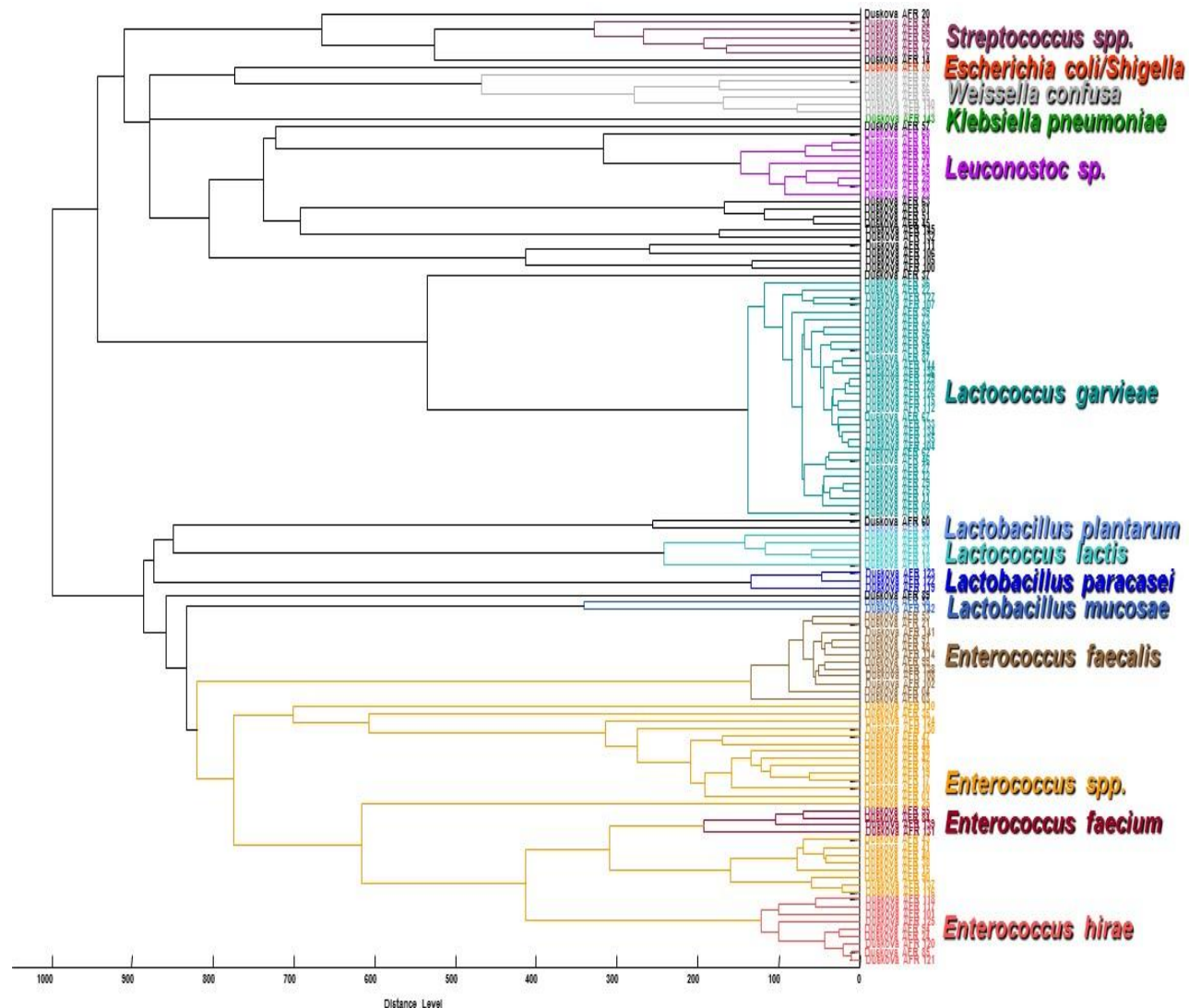
144 isolates from excrements of monkeys, elephants and other african animals



**MALDI MS species  
identification: 75 %**

**MALDI MS genus  
identification: 8 %**

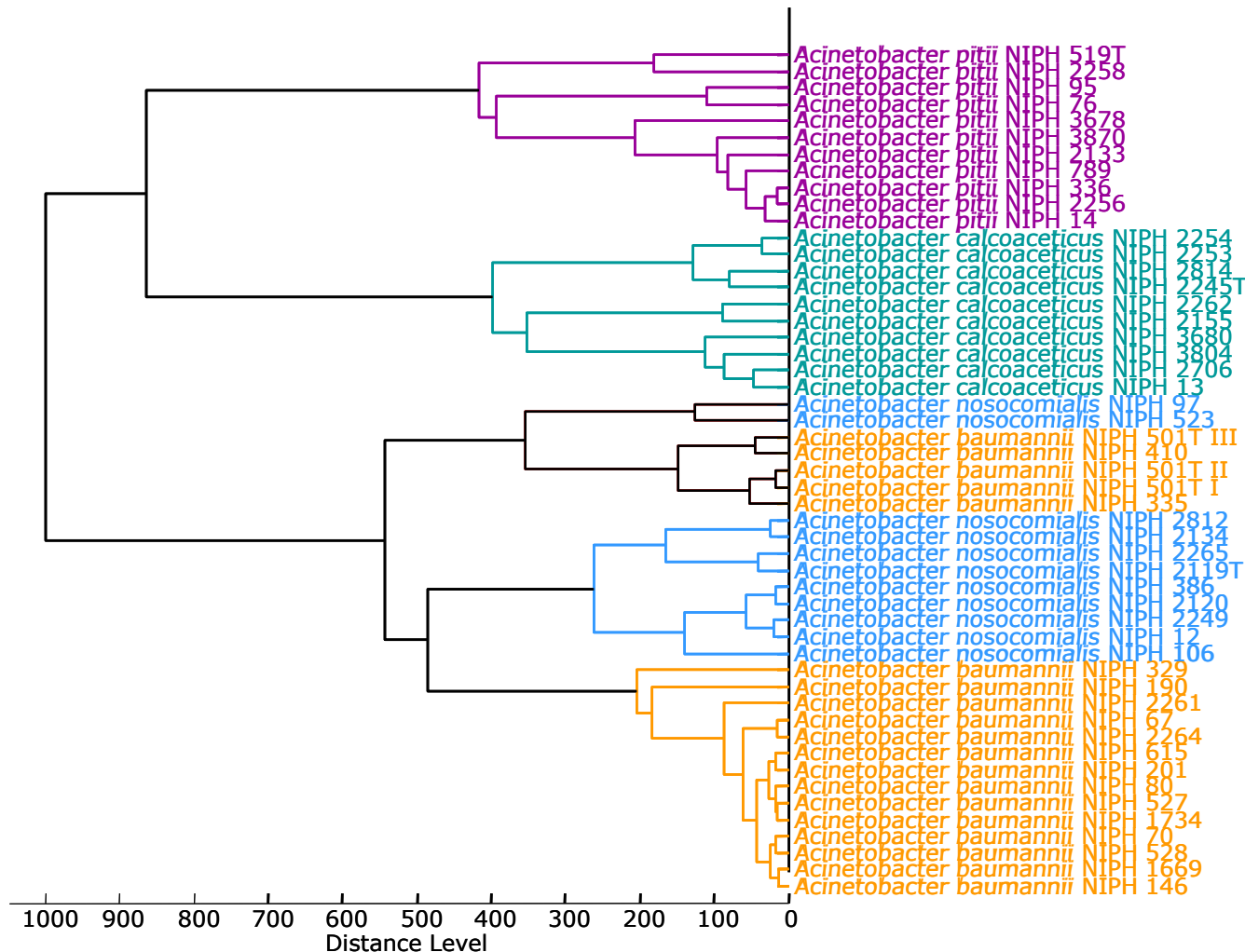
**MALDI MS not  
identified: 17 %**



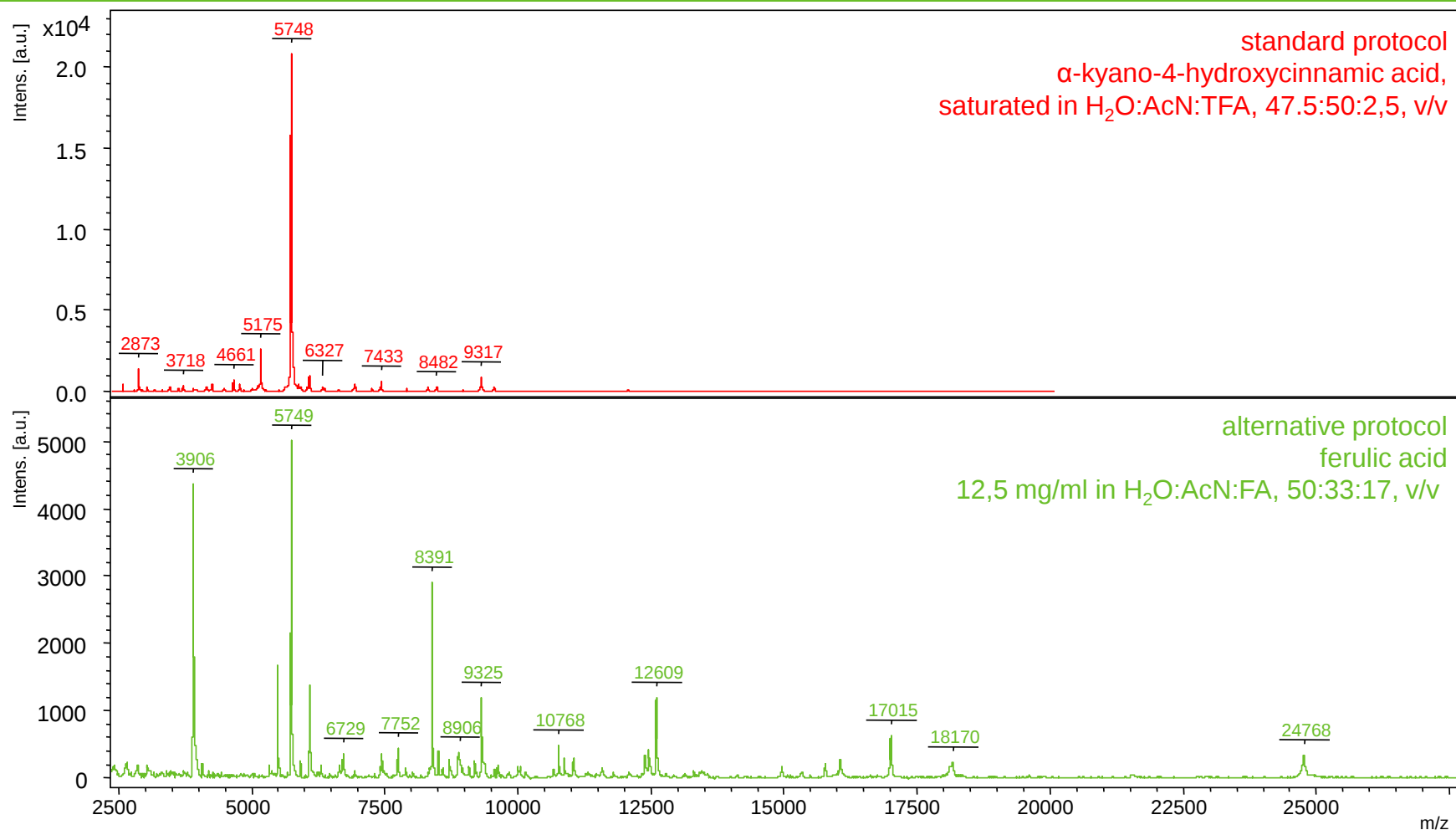
#### 4. Differentiation between *Acinetobacter* spp. (*A. baumannii* – *A. calcoaceticus* complex)



#### standard sample preparation protocol



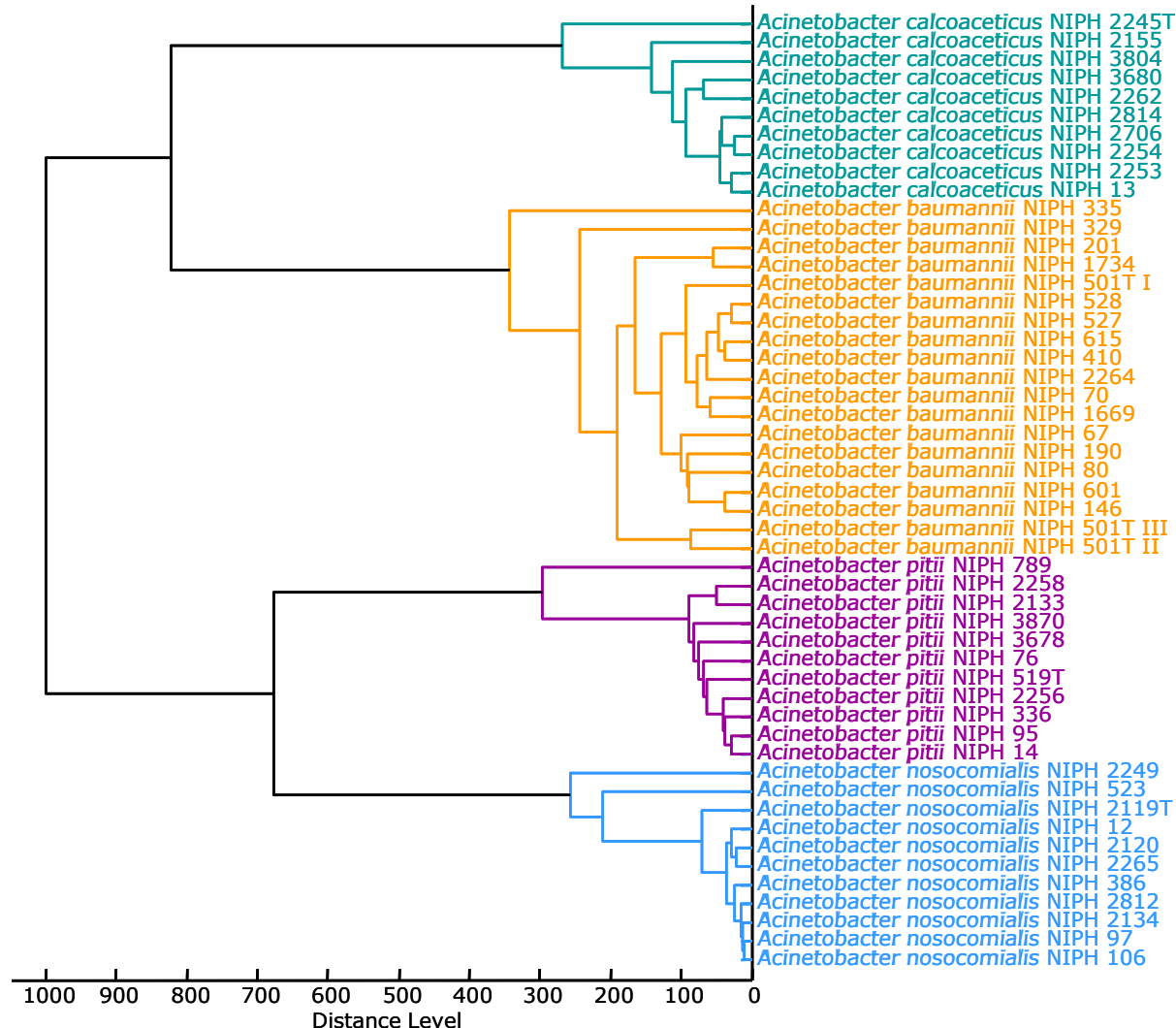
#### 4. Differentiation between *Acinetobacter* spp. (*A. baumannii* – *A. calcoaceticus* complex)



#### 4. Differentiation between *Acinetobacter* spp. (*A. baumannii* – *A. calcoaceticus* complex)



##### alternative sample preparation protocol



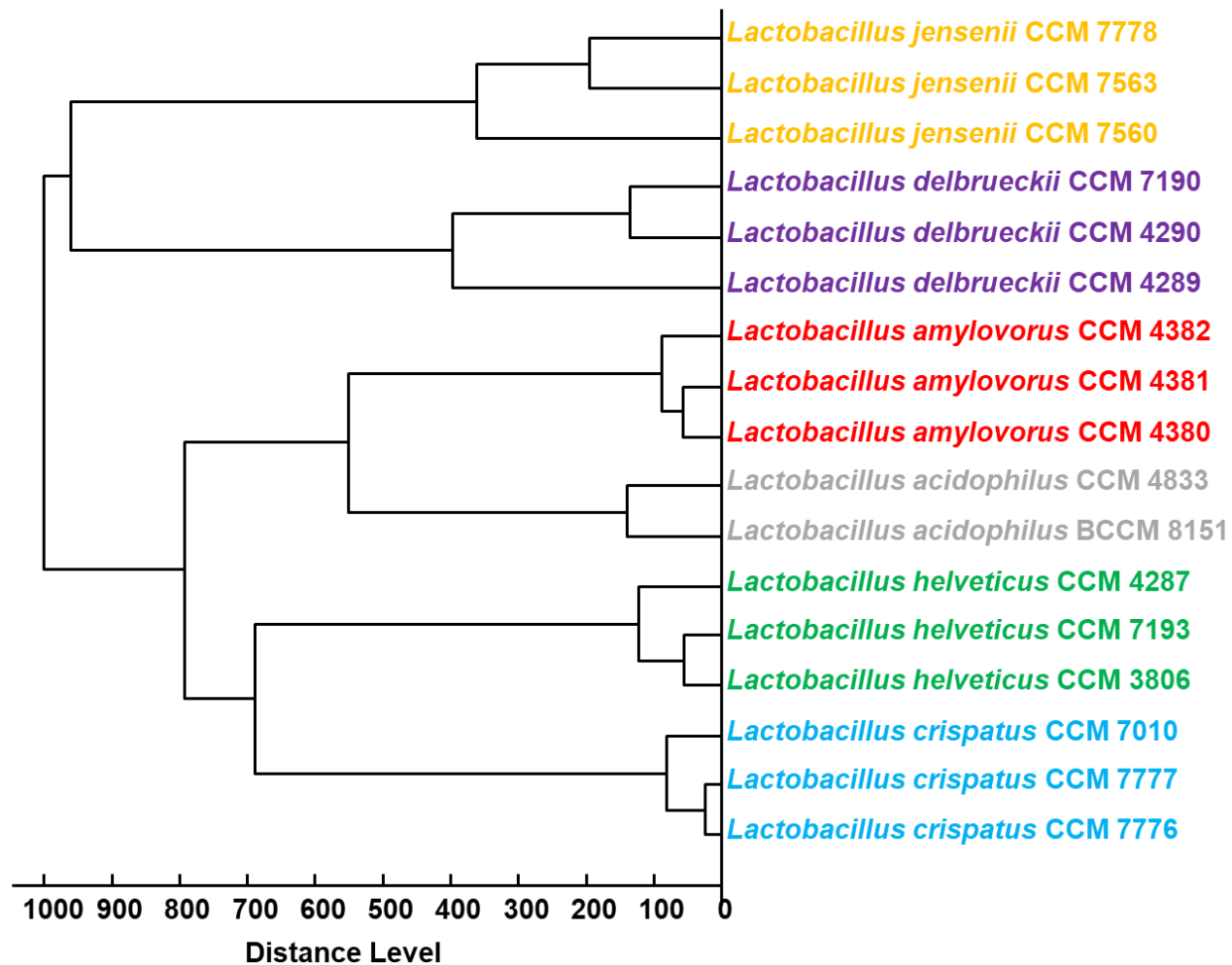
## 5. Identification of *Aeromonas* spp.

11 novel species absent from the database

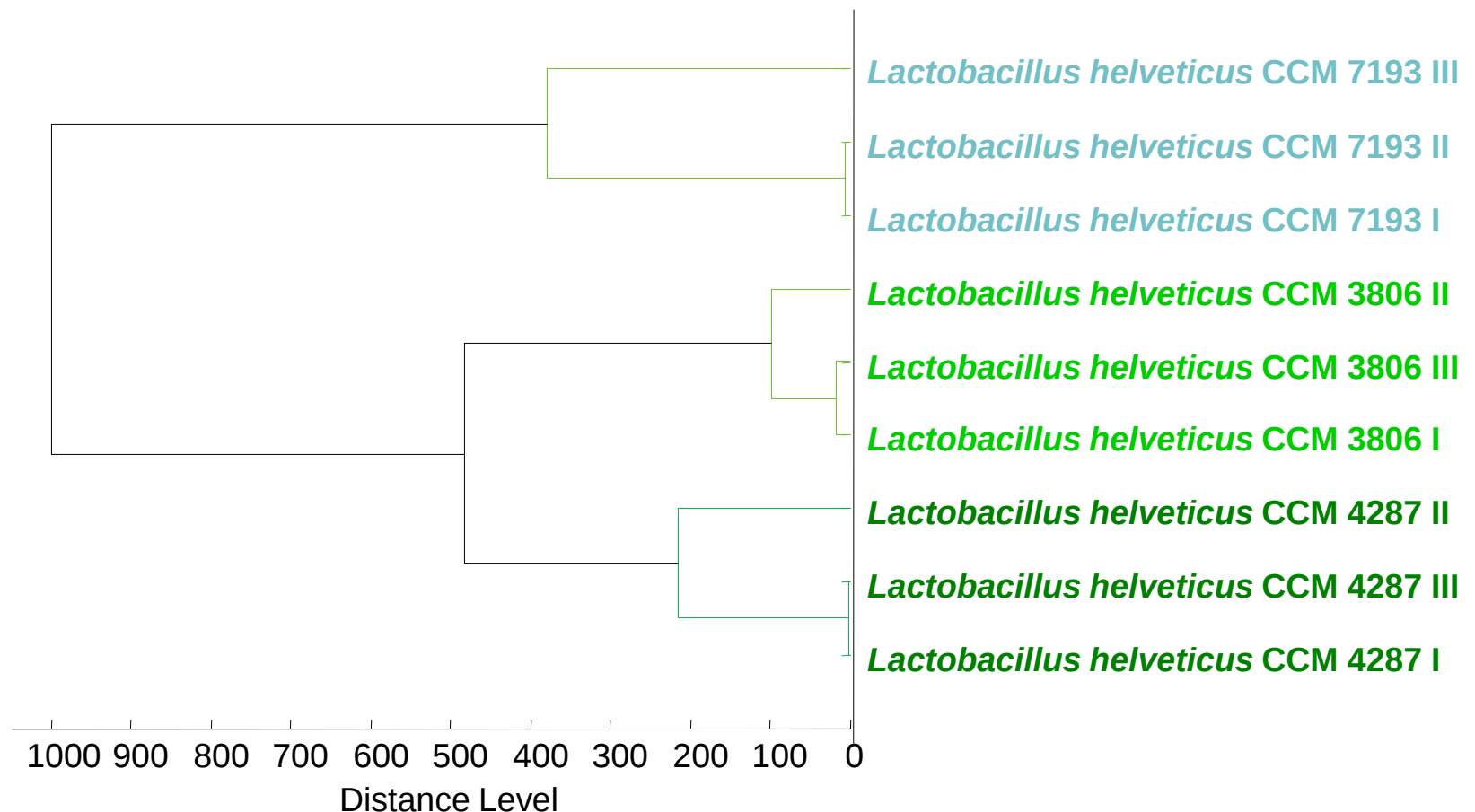


| <i>Aeromonas</i> spp.   | CCM No.           | Identification against database                                      | Biotyper log(score) |
|-------------------------|-------------------|----------------------------------------------------------------------|---------------------|
| <i>A. aquariorum</i>    | 7695 <sup>T</sup> | <i>A. jandaei</i><br><i>A. hydrophila</i>                            | 2.369<br>2.337      |
| <i>A. australiensis</i> | 8484 <sup>T</sup> | <i>A. jandaei</i><br><i>A. hydrophila</i>                            | 2.376<br>2.357      |
| <i>A. bivalvium</i>     | 7467 <sup>T</sup> | <i>A. veronii</i>                                                    | 2.216               |
| <i>A. cavernicola</i>   | 7641 <sup>T</sup> | <i>A. media</i><br><i>A. hydrophila</i>                              | 2.144<br>2.123      |
| <i>A. diversa</i>       | 7325 <sup>T</sup> | <i>A. schuberti</i>                                                  | 2.388               |
| <i>A. fluvialis</i>     | 8437 <sup>T</sup> | <i>A. veronii</i><br><i>A. hydrophila</i>                            | 2.389<br>2.381      |
| <i>A. piscicola</i>     | 7715 <sup>T</sup> | <i>A. bestiarum</i><br><i>A. salmonicida</i> ssp. <i>salmonicida</i> | 1.954<br>1.870      |
| <i>A. rivuli</i>        | 8438 <sup>T</sup> | <i>A. molluscorum</i>                                                | 2.362               |
| <i>A. sanarellii</i>    | 7886 <sup>T</sup> | <i>A. caviae</i><br><i>A. hydrophila</i>                             | 2.441<br>2.406      |
| <i>A. taiwanensis</i>   | 7885 <sup>T</sup> | <i>A. caviae</i><br><i>A. hydrophila</i>                             | 2.381<br>2.378      |
| <i>A. tecta</i>         | 7605 <sup>T</sup> | <i>A. eucrenophila</i>                                               | 2.425               |

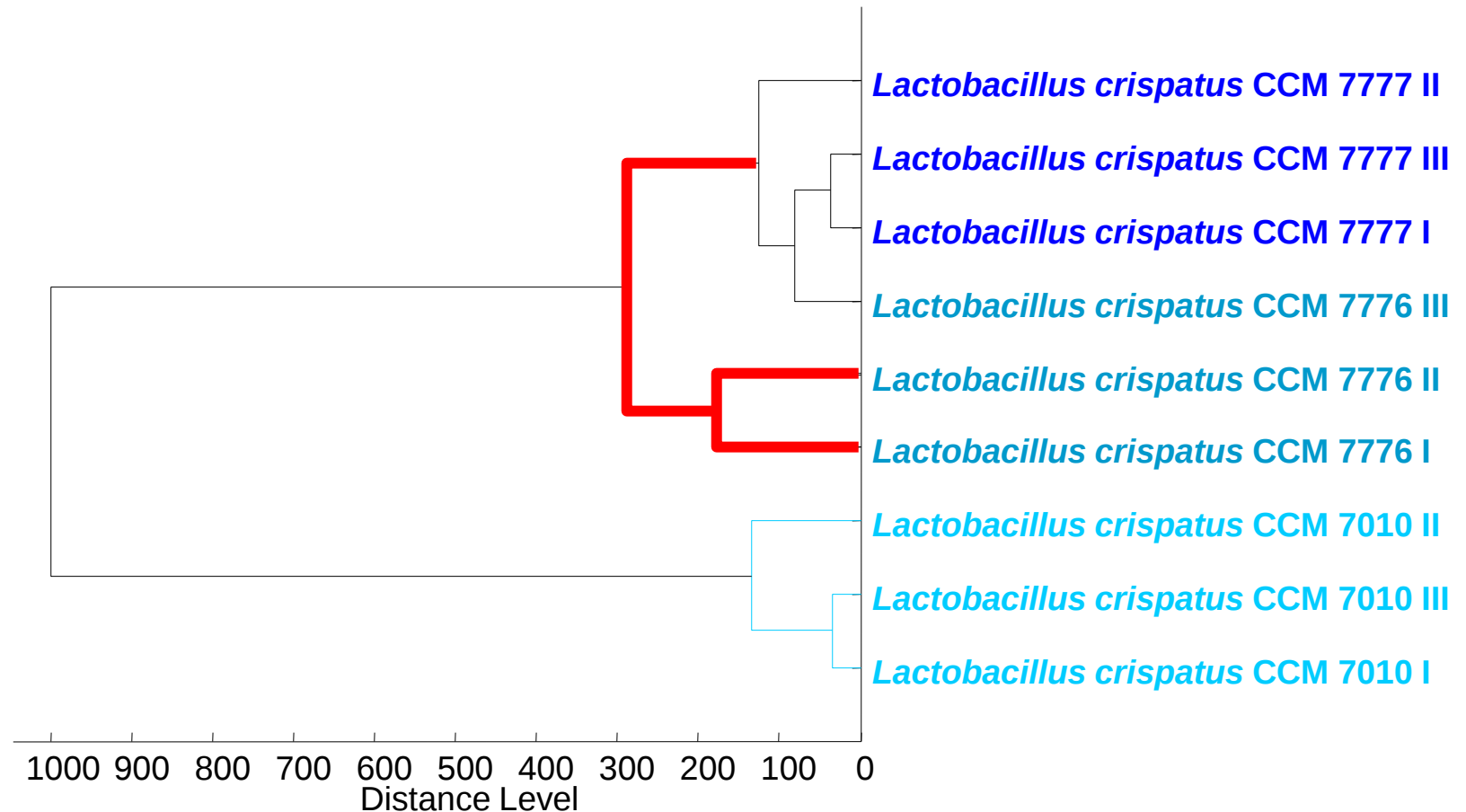
## 6. Differentiation between strains within *L. acidophilus* group



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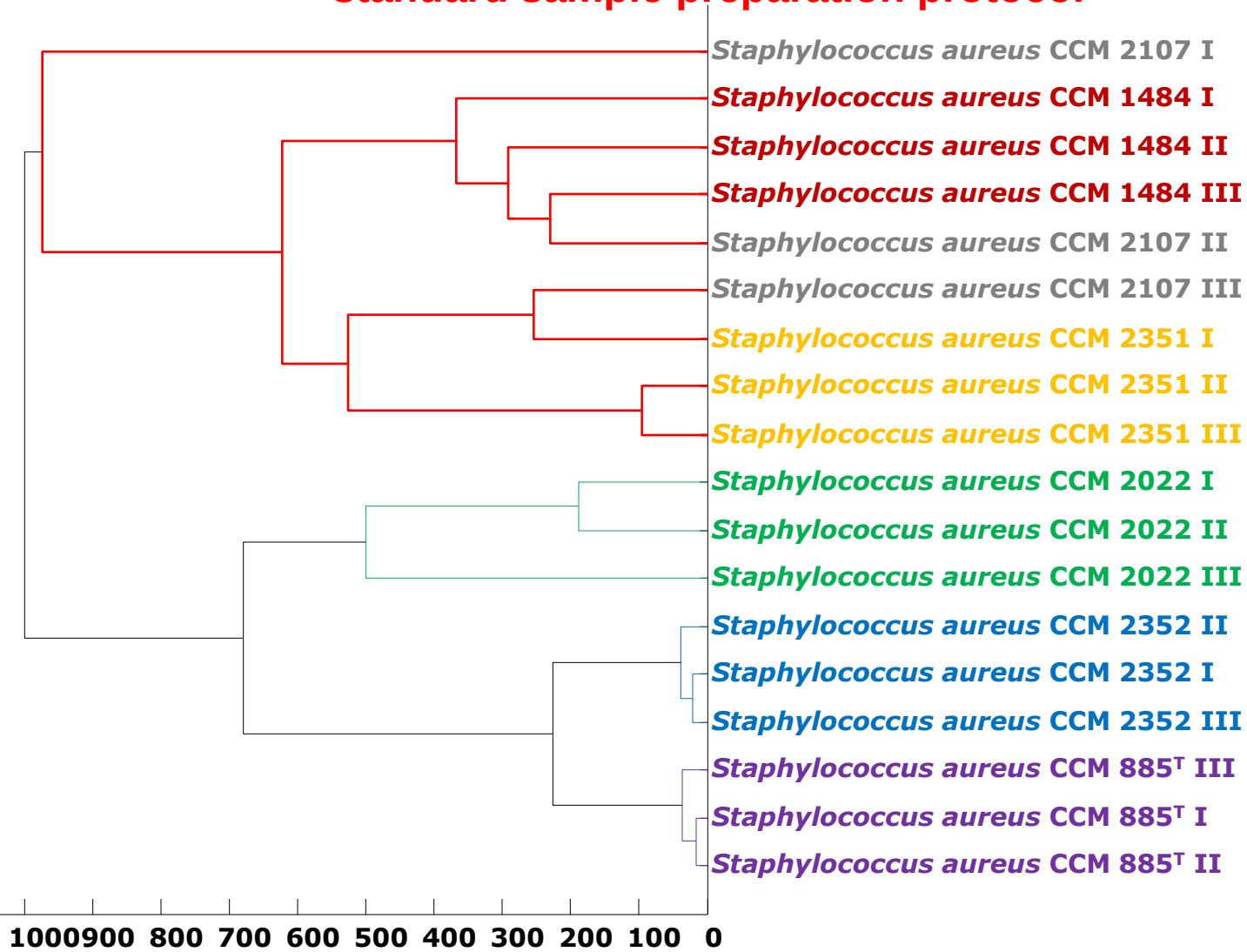


## 6. Differentiation between strains within *L. acidophilus* group

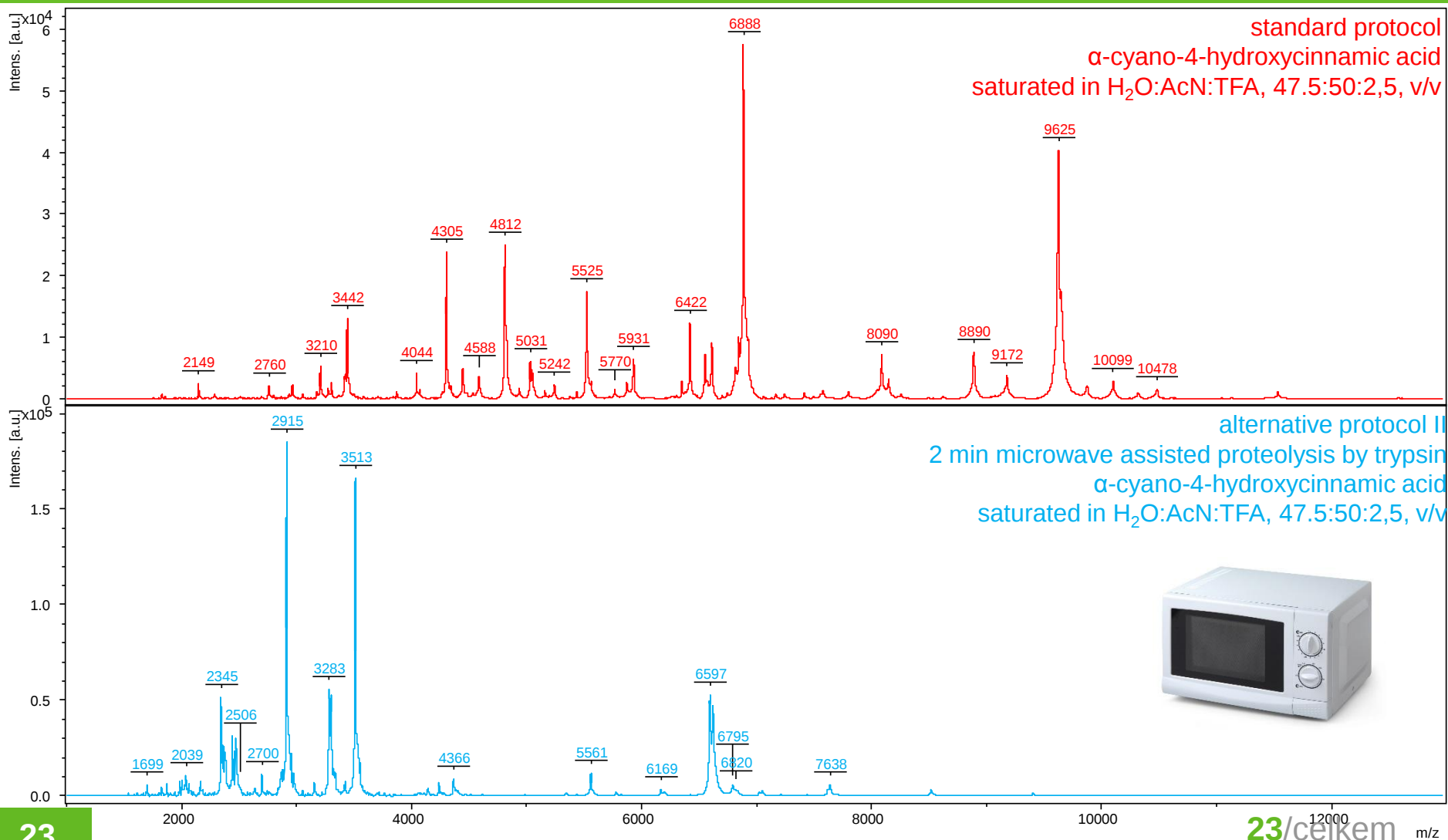


## 7. Differentiation between *S. aureus* strains

### standard sample preparation protocol



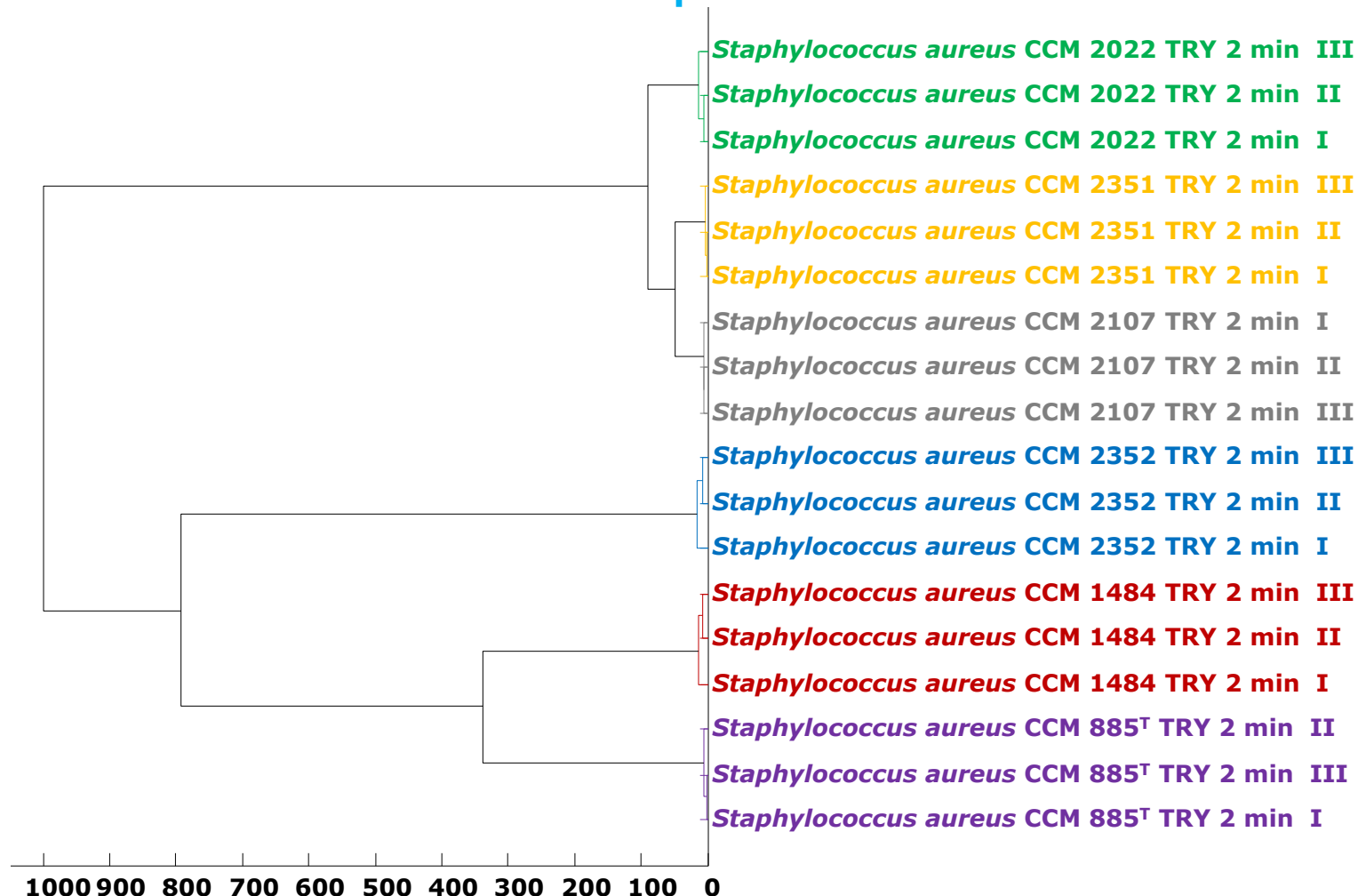
## 7. Differentiation between *S. aureus* strains



## 7. Differentiation between *S. aureus* strains



### alternative protocol II



# MALDI-TOF MS profiling of bacteria: How far can we go?



1. Identification of lactobacilli from dairy products

2. Identification of microbial contaminants of meat products

3. Identification of African wildlife microflora

4. Differentiation between *Acinetobacter* spp. (ACB complex)

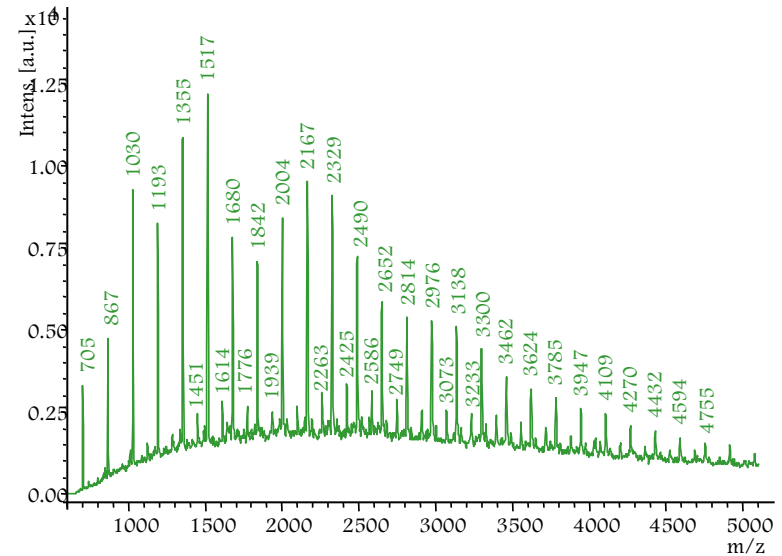
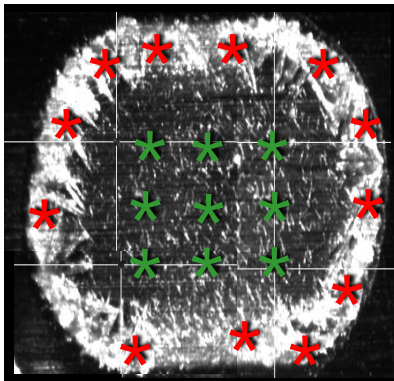
5. Identification of *Aeromonas* spp.

6. Differentiation between strains within *L. acidophilus* group

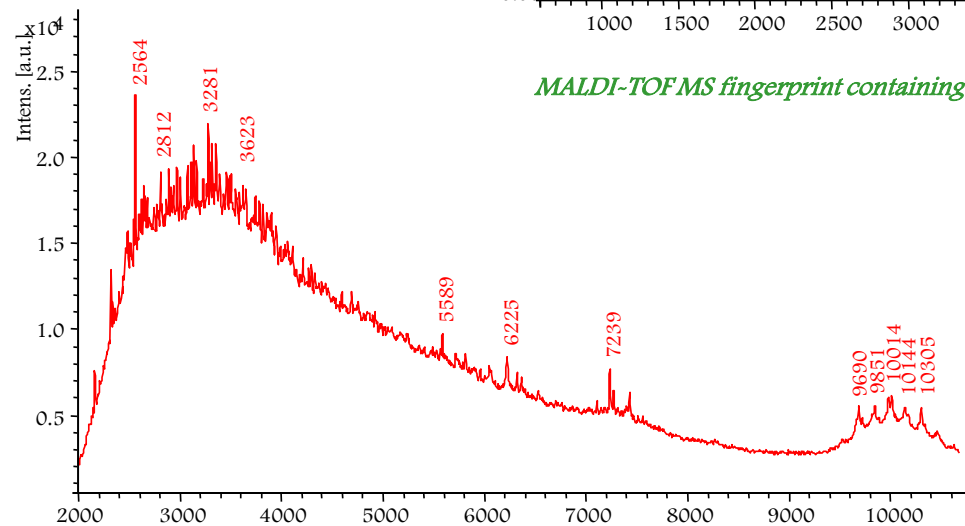
7. Differentiation between *S. aureus* strains

**8. Beer fingerprinting by MALDI-TOF MS**

# 8. Beer fingerprinting by MALDI-TOF MS



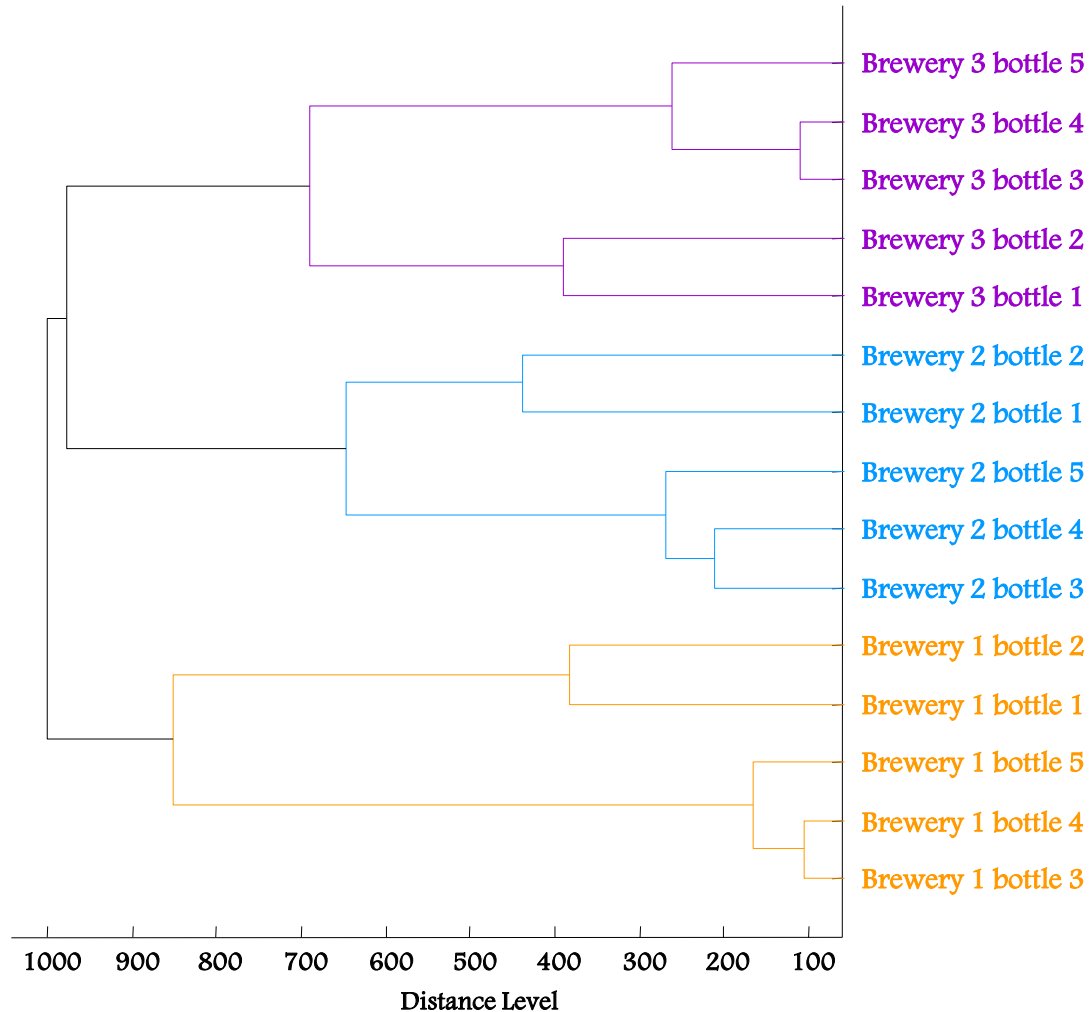
*MALDI-TOF MS fingerprint containing maltooligosaccharides*

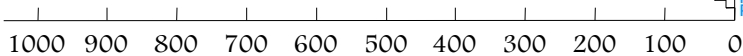


*MALDI-TOF MS fingerprint containing proteins*

# 8. Beer fingerprinting by MALDI-TOF MS

Five bottles from three breweries





# Acknowledgements

## People:

Tereza Balážová, Michal Boháč, David Burian, Marta Dušková,  
Libor Feigerl, Magdaléna Kačalová, Josef Kameník,  
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