

DIFFERENTIATION WITHIN THE BACILLUS CEREUS AND BACILLUS SUBTILIS GROUPS WITH MALDI-TOF

INTRODUCTION

Bacillus cereus group is composed of *B. anthracis*, *B. cereus*, *B. cytotoxicus*, *B. mycoides*, *B. pseudomycooides*, *B. thuringiensis* and *B. weihenstephanensis*.

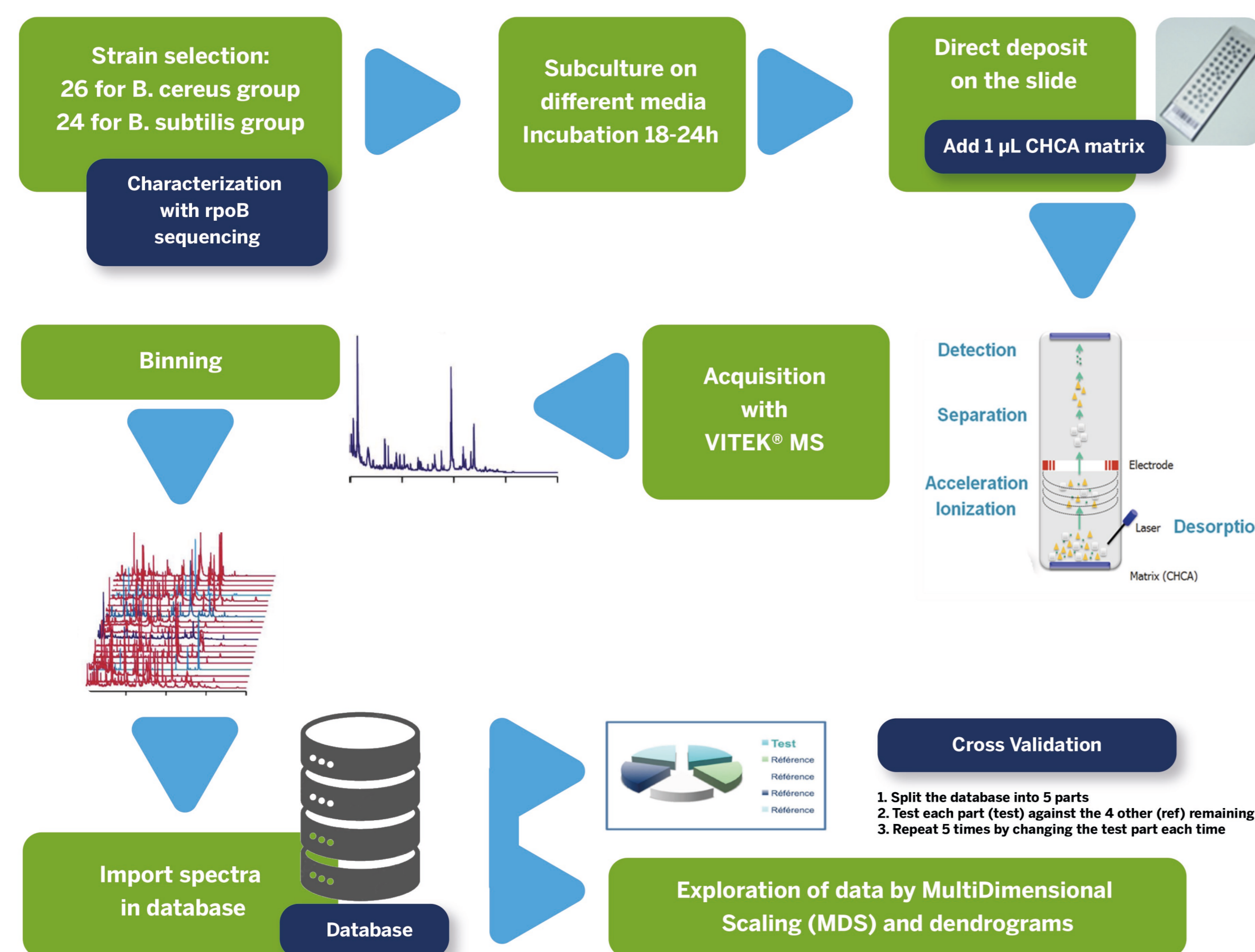
Bacillus subtilis group is composed of *B. amyloliquefaciens*, *B. subtilis*, *B. vallismortis*, *B. velezensis*.

They are well known pathogens among the *Bacillus* genus and can be a source of human infection as well as contaminants in agriculture and food industry. These species are very similar and their differentiation is cumbersome but essential notably in term of epidemiology.

Today, no mass spectrometry system is able to differentiate them in routine. The biochemical or molecular techniques are quite fastidious, long and expensive and not always conclusive.

The objective of this study was to develop a simple and user-friendly method to separate these species by MALDI-TOF.

MATERIALS AND METHODS



RESULTS

- Spectra analysis show different clusters corresponding to the species *Bacillus subtilis* group (Fig.1.a) and *Bacillus cereus* group (Fig.1.b).
- Even though a similarity near 65% is observed, species are still dissociable excepted for *B. weihenstephanensis* and *B. mycoides* (Fig.1.b).
- *B. velezensis* and *B. amyloliquefaciens* are very close (Fig.1.a) as well as *B. subtilis* ssp *subtilis* and *B. vallismortis*.
- The MDS analysis confirmed these observations (Fig.2) less than 48 hours.

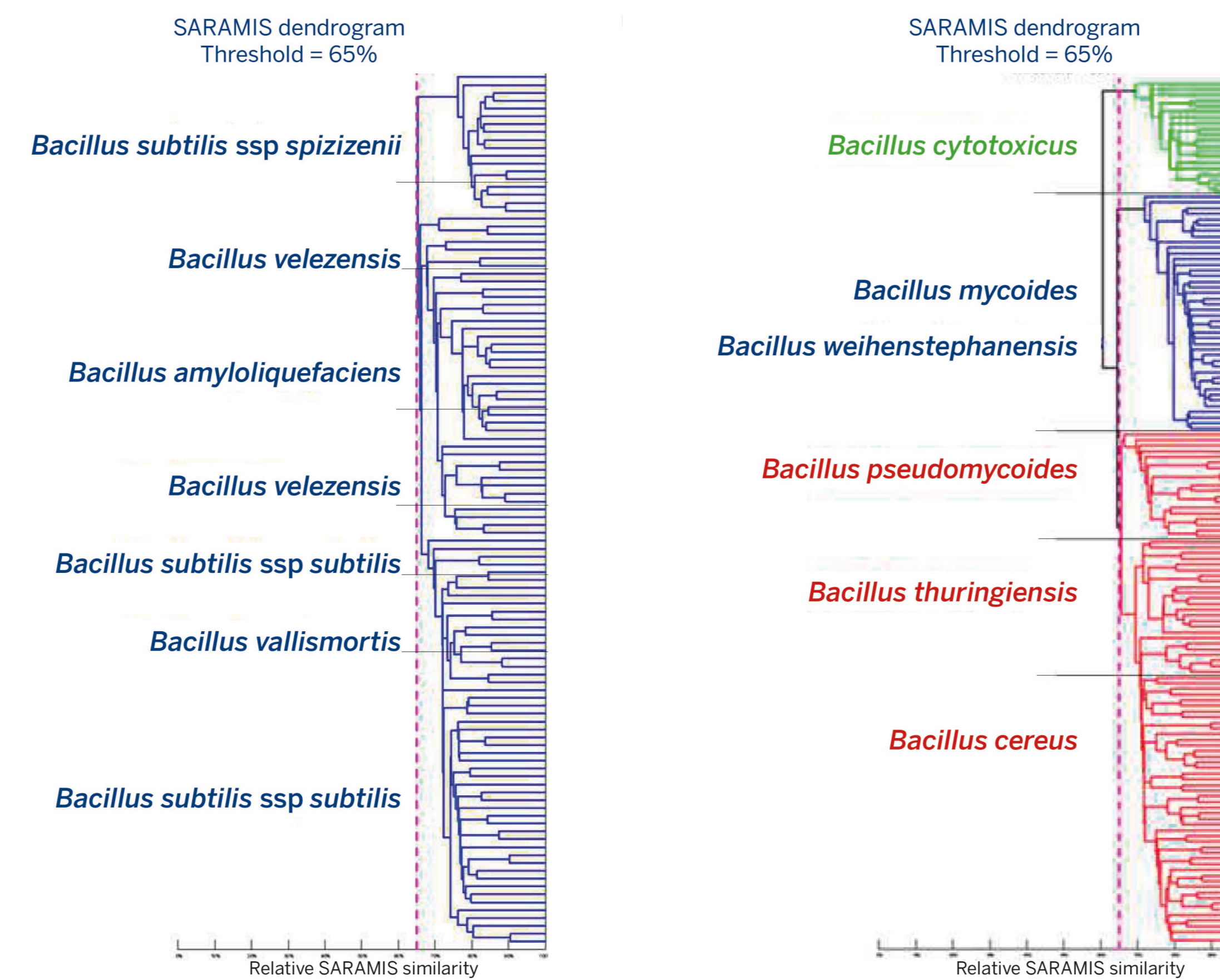


Figure 1 : Dendrograms based on relative similarity between spectra

All species of *B. subtilis* or *cereus* groups seem distinguishable (Fig.2.a & b), except *B. weihenstephanensis* and *B. mycoides* in the *B. cereus* group (Fig.2.b) which are overlapping.

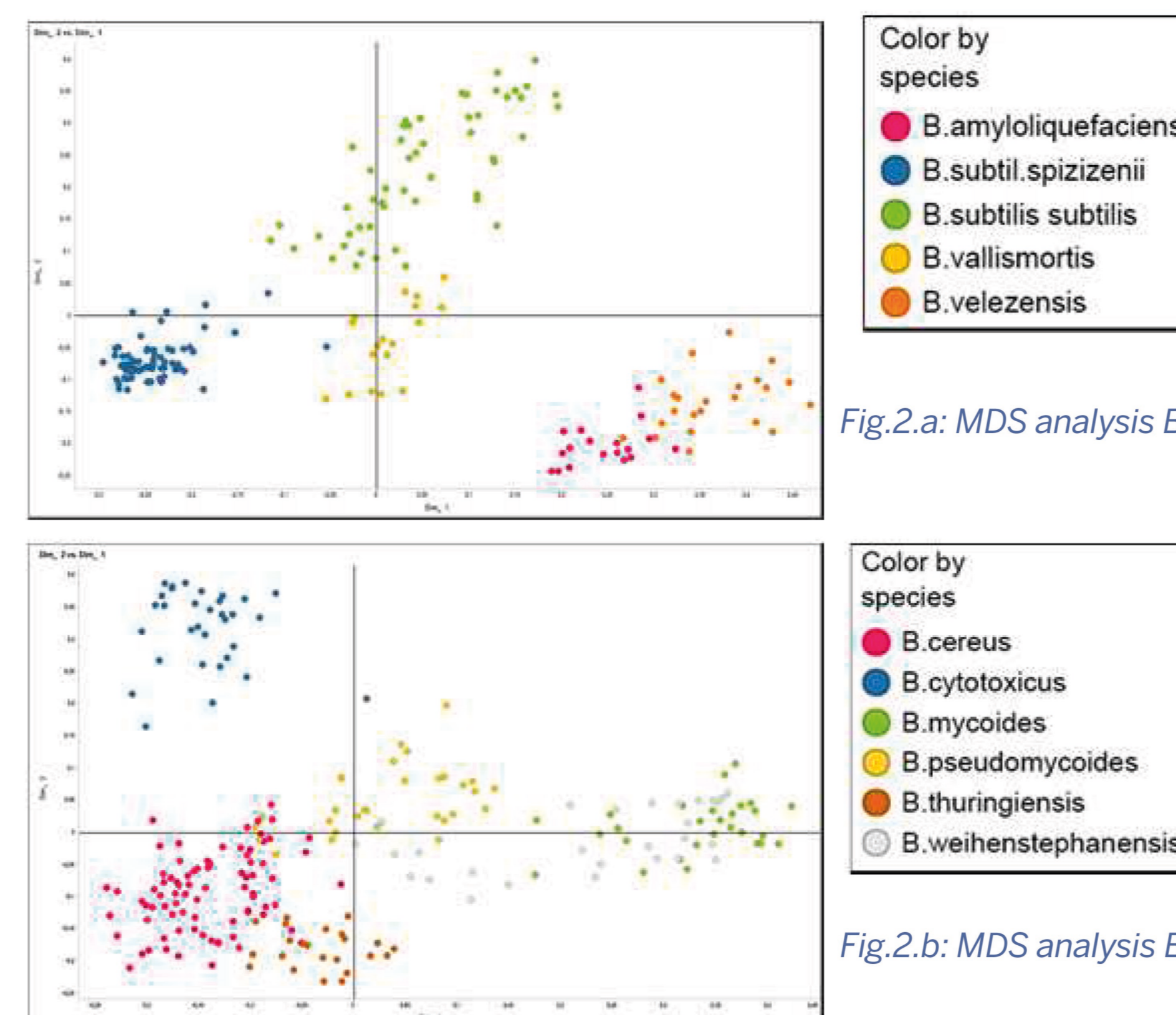


Fig.2.a: MDS analysis *Bacillus subtilis* group

Fig.2.b: MDS analysis *Bacillus cereus* group

Figure 2 : MDS analysis – distribution of all spectra on 2 dimensions.

Performance was estimated using the cross-validation method.

The database was created using 2745 reference spectra from 117 species among genus *Bacillus* and related species.

Cross validation results (Table 1) show that VITEK-MS algorithm allows the discrimination of all members for *B. cereus* and *B. subtilis* groups at species level with good performance from 98 to 100%, and confirm the proximity of *B. weihenstephanensis* and *B. mycoides* proposed in low discrimination.

<i>Bacillus subtilis</i> group	Nb spectra ⁽¹⁾	Correct ID ⁽²⁾	No ID ⁽³⁾	LD ⁽⁴⁾	Mis ID ⁽⁵⁾	Perf (%)
<i>B. amyloliquefaciens</i>	18	18	0	0	0	100 %
<i>B. subtilis</i> ssp <i>spizizenii</i>	66	66	0	0	0	100 %
<i>B. subtilis</i> ssp <i>subtilis</i>	58	58	0	0	0	100 %
<i>B. vallismortis</i>	24	24	0	0	0	100 %
<i>B. velezensis</i>	25	23	0	2 (6)	0	100 %
Total	191	189	0	2	0	100%

<i>Bacillus cereus</i> group	Nb spectra ⁽¹⁾	Correct ID ⁽²⁾	No ID ⁽³⁾	LD ⁽⁴⁾	Mis ID ⁽⁵⁾	Perf (%)
<i>B. cereus</i>	90	89	1	0	0	98.89%
<i>B. cytotoxicus</i>	36	36	0	0	0	100%
<i>B. mycoides</i>	33	0	0	33	0	100%
<i>B. pseudomycooides</i>	33	30	0	3(7)	0	100%
<i>B. thuringiensis</i>	24	24	0	0	0	100%
<i>B. weihenstephanensis</i>	30	0	0	30	0	100%
Total	246	179	1	66	0	99,6%

Table 1: Cross-validation - Identification results for *Bacillus subtilis* group and *Bacillus cereus* group per species.

(1) Number of spectra • (2) Good identification (single choice) • (3) Spectra not identified • (4) Low Discrimination (multiple choice) including the expected species • (5) Bad identification (identification to another species) • (6) LD: *B. amyloliquefaciens* • (7) LD: *B. cereus*

CONCLUSIONS

This study highlights the accuracy of the MALDI-TOF technology to differentiate close species such as members of the *Bacillus cereus* and *Bacillus subtilis* groups with a simple method without any extraction step.

These results open new prospects for epidemiology, food control and prevention of foodborne illness caused by *Bacillus* species.