

MALDI-TOF MS for the identification of anaerobic bacteria

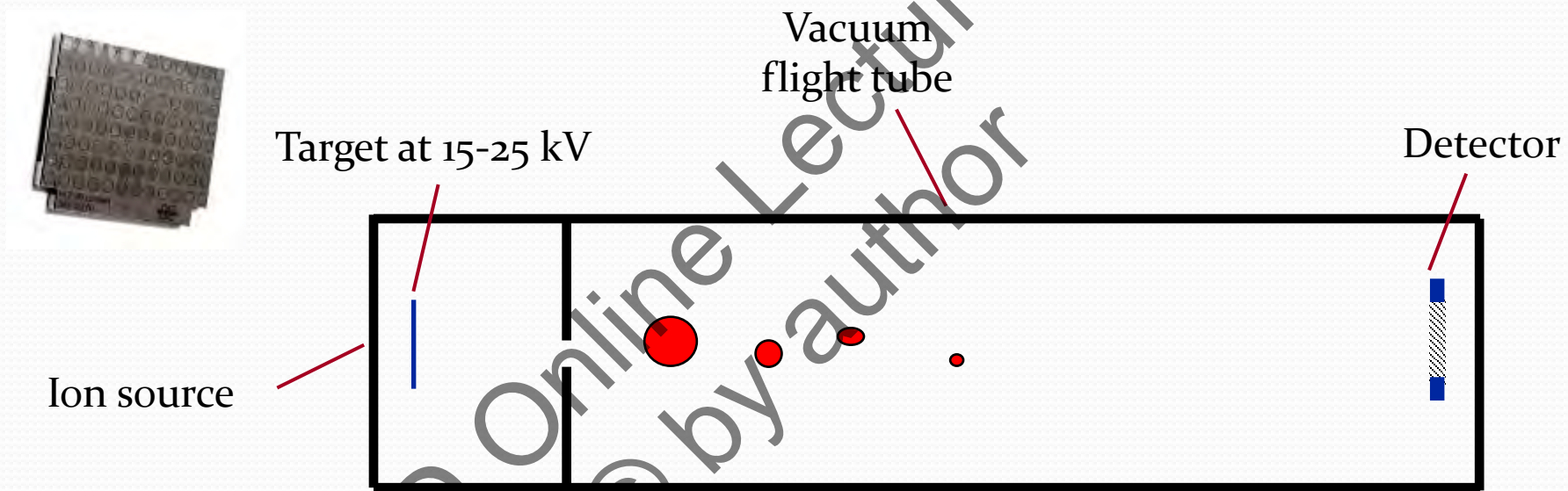
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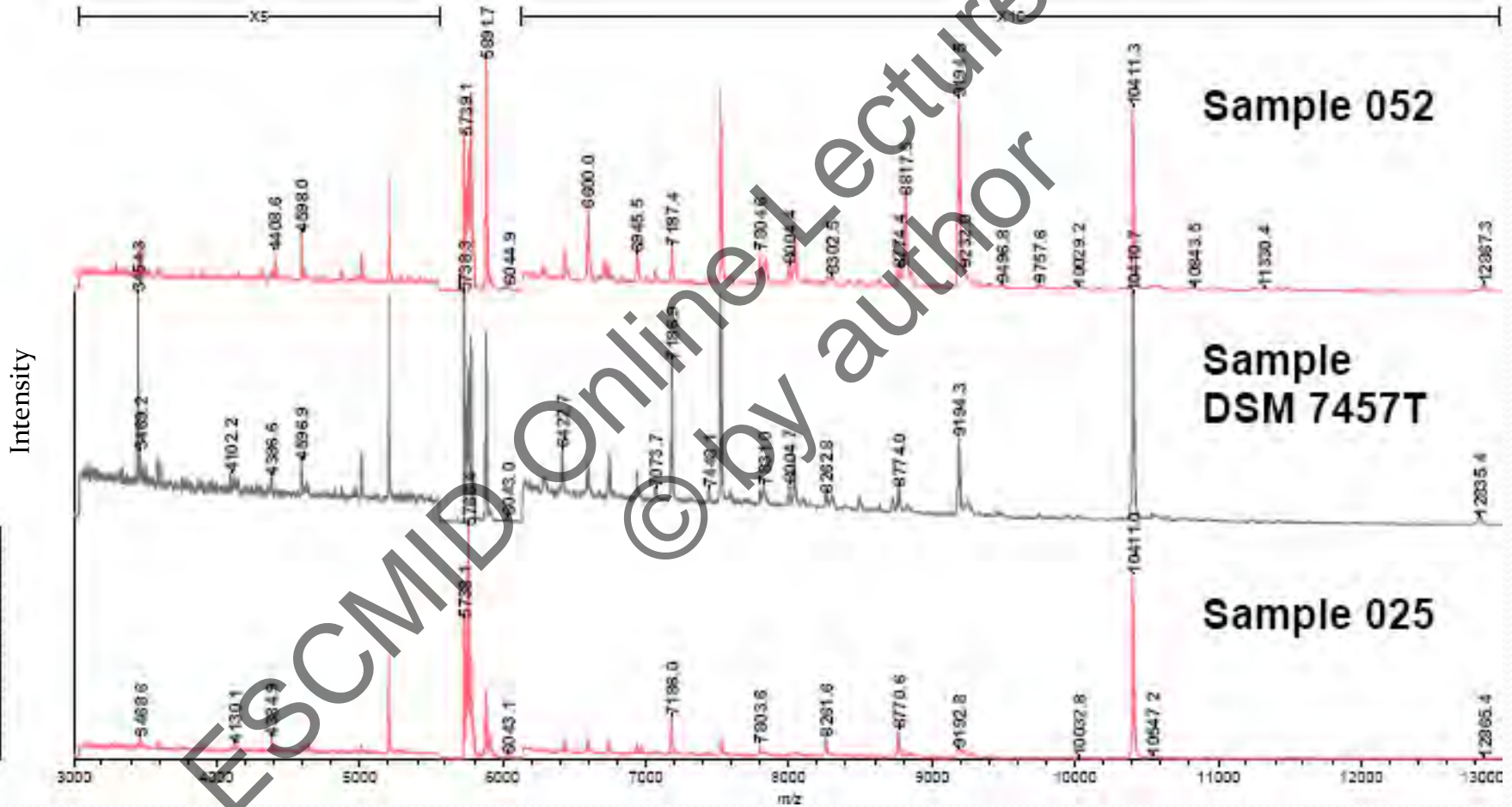


Matrix Assisted Laser Desorption/Ionization time-of-flight Mass Spectrometry (MALDI-TOF MS)

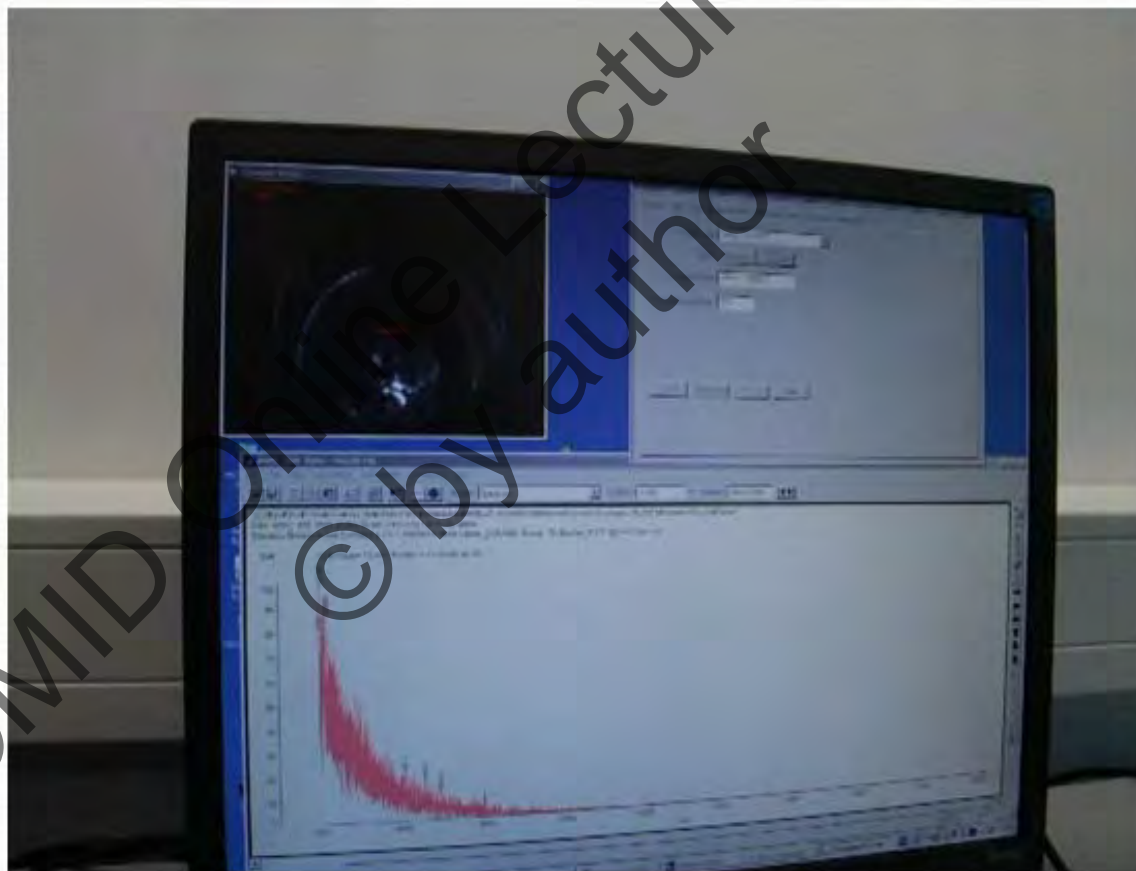


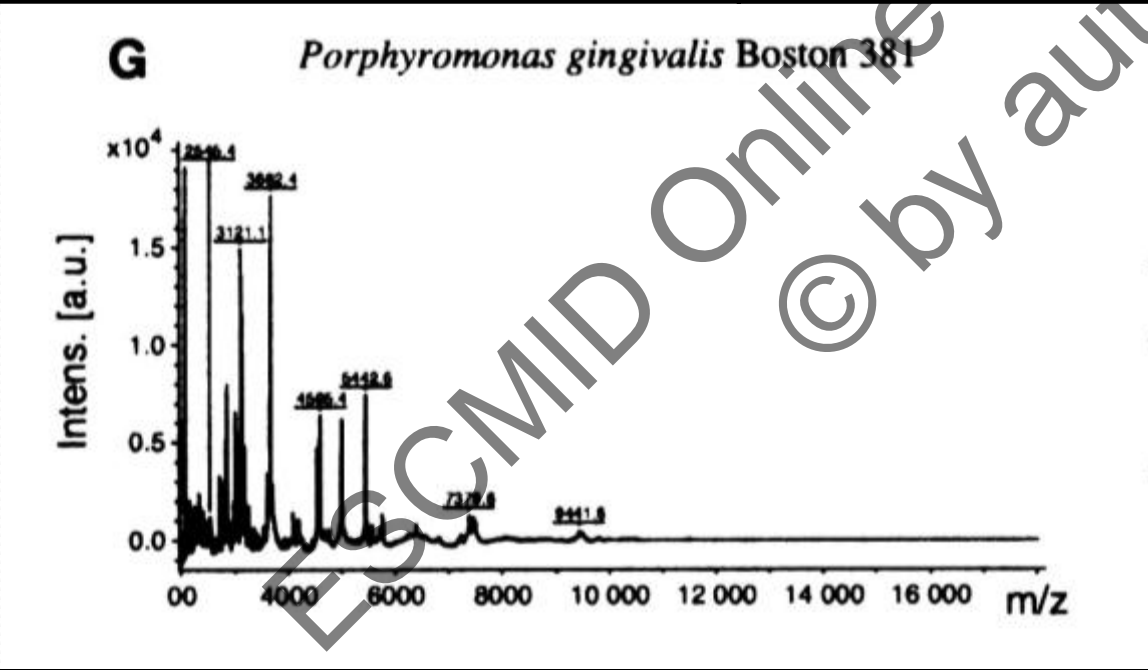
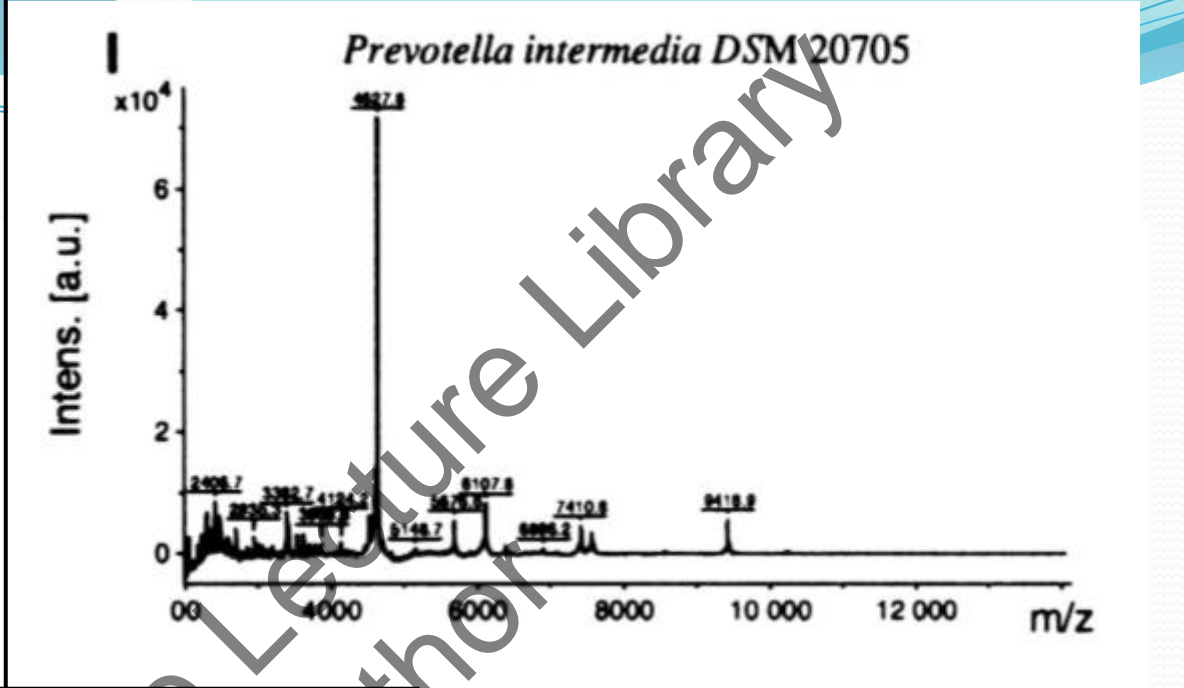
- “Time of flight” of individual proteins is converted into mass information.
- Spectrum is produced
- Database is built

Obtained spectrum is unique for bacterial species

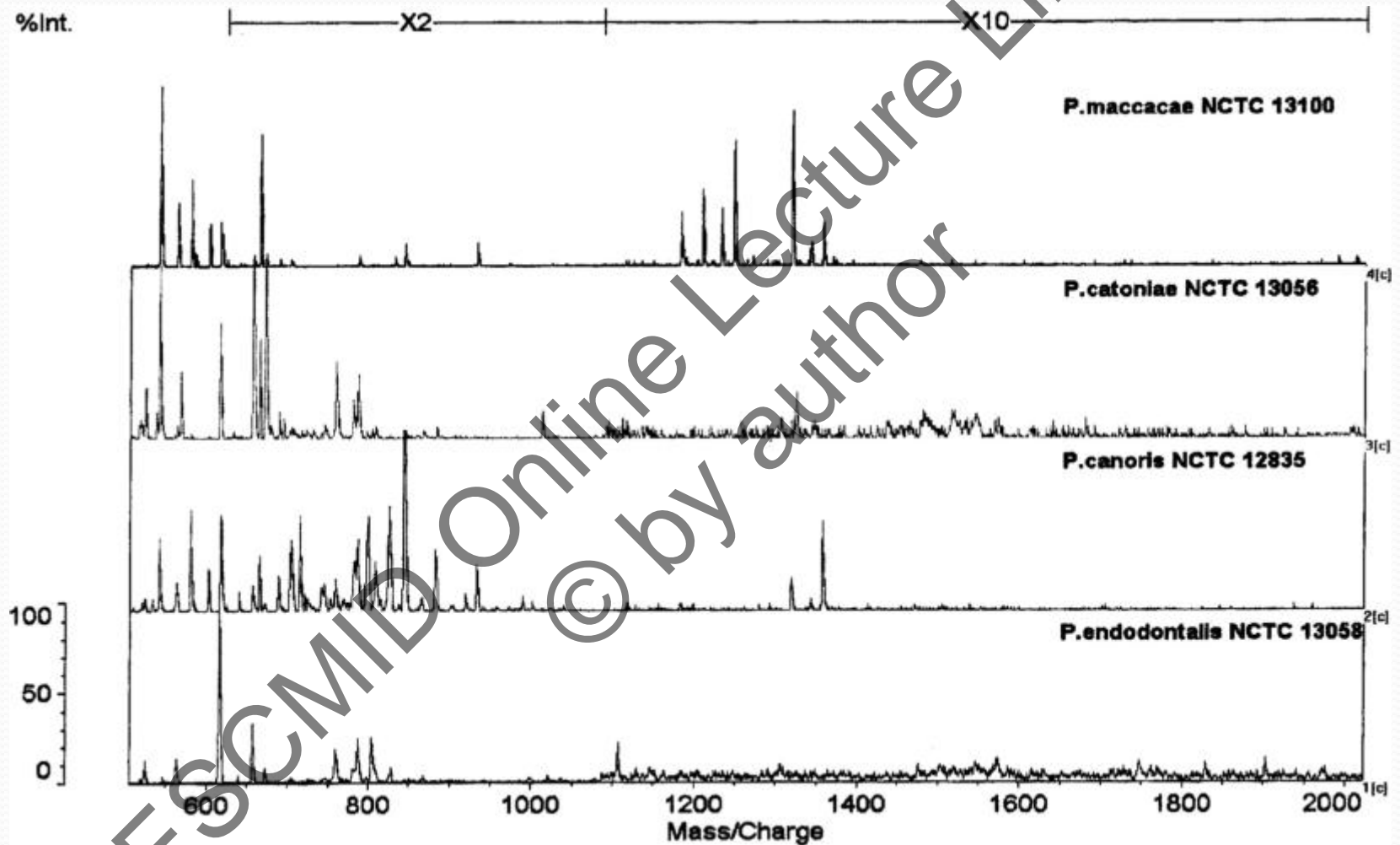


MALDI-TOF MS





Examples of the mass-spectral profiles of members of the genus *Porphyromonas*.



Shah H N et al. Clin Infect Dis. 2002;35:S58-S64

Anaerobic culture

Phenotypic

primary incubation
2-7 days

pure culture
2 days

aerotolerance
1 day

identification
2-14 days

MALDI-TOF MS

primary incubation
2-7 days

MALDI-TOF MS testing
minutes



Species identification of clinical isolates of *Bacteroides* by matrix-assisted laser-desorption/ionization time-of-flight mass spectrometry

Nagy et al. Clin. Microbiol .Infect. 2009; 15:796-802

Strain collection: 277 *Bacteroides* strains (phenotypically identified)

	Identical identification MALDI-TOF MS/phenotypic	
<i>B. fragilis</i>	174/179	(97 %)
<i>B. thetaiotaomicron</i>	38/43	(88 %)
<i>B. ovatus</i>	9/15	(60 %)
<i>B. vulgatus</i>	19/20	(95 %)
<i>B. uniformis</i>	2/5	(40 %)
<i>B. eggerthii</i>	1/1	(100 %)
<i>B. nordii</i>	1/4	(25 %)
<i>B. salyersiae</i>	1/1	(100 %)
<i>B. massiliensis</i>	2/2	(100 %)
Inconclusive identification by MALDI-TOF MS	7	

270/277 (97.5 %) of the
Bacteroides strains were
identified using MALDI-TOF MS

Rapid identification of clinical isolates of *Bacteroides* species by matrix-assisted laser-desorption/ionization time-of-flight mass spectrometry

Culebras et al. Anaerobe 2012; 18:163-165

	16S rRNA gene ID (no. of isolates)	Rapid ID 32A (no. of isolates)	MALDI-TOF MS (no. of isolates)
<i>B. fragilis</i>	58	33 (57 %)	58 (100 %)
<i>B. thetaiotaomicron</i>	53	31 (59 %)	53 (100 %)
<i>B. ovatus</i>	20	9 (45 %)	20 (100 %)
<i>B. vulgatus</i>	20	16 (80 %)	19 (95 %)
→ <i>B. dorei</i>	9	0	0
<i>P. distasonis</i>	8	6 (75 %)	8 (100 %)
<i>B. caccae</i>	5	4 (80 %)	5 (100 %)
→ <i>B. xylanisolvens</i>	3	0	0
<i>B. uniformis</i>	2	2 (100 %)	2 (100 %)
→ <i>B. faecis</i>	1	0	0
<i>B. intestinalis</i>	1	0	1 (100 %)
<i>B. salyersiae</i>	1	0	1 (100 %)
<i>P. goldsteinii</i>	1	0	1 (100 %)

→ Species not present in database.

Identification of Gram-positive anaerobic cocci by MALDI-TOF mass spectrometry

Veloo et al. Syst Appl Microbiol 2011; 34:58-62

Construction database:

sequenced clinical isolates (n=77) and reference strains (n=12)

reference strain *P. harei* is different compared to the clinical isolates *P. harei*

Validation of constructed database:

107 genotypically identified clinical isolates of GPAC

Validation database

Strain (n)	MALDI-TOF ID (n)	
	correct ID	no ID
<i>F. magna</i> (32)	29	3
<i>A. vaginalis</i> (3)	2	1
<i>P. ivorii</i> (3)	1	2
<i>P. octavius</i> (1)		1
<i>Bacterium</i> N14-24 (1)		1
GPAC (3)		3

1 strain sequence similarity < 98 %

sequence similarity < 98 %

Not present in database

3

All other GPAC were correctly identified:

- | | | |
|--------------------------|----------------------------|--------------------------|
| <i>P. micra</i> (29) | <i>P. gorbachii</i> (3) | <i>A. tetradius</i> (1) |
| <i>P. harei</i> (15) | <i>A. parvulum</i> (3) | <i>P. lacrimalis</i> (1) |
| <i>A. murdochii</i> (6) | <i>P. niger</i> (1) | |
| <i>P. anaerobius</i> (4) | <i>A. lactolyticus</i> (1) | |

Correctly identified: 96 strains

No identification: 11 strains

- 2 strains, no reference spectra present in database
- 6 strains, <98 % sequence similarity with closest relative
- 3 strains, unknown

90 % correctly identified

Species identification of clinical *Prevotella* isolates by Matrix-Assisted Laser Desorption Ionization-Time of Flight mass spectrometry

Wybo et al. J. Clin. Microbiol. 2012; 50:1415-1418

Strains: 102 sequenced clinical isolates

Identification MALDI-TOF MS:

1. using the commercial reference database
2. using the commercial reference database + the addition of main spectra of 23 reference strains and 7 sequenced clinical isolates

Commercial reference database:

- 63 % correct species identification
- 11 % correct genus identification
- 26 % no identification

Commercial database + additions:

- 83 % correct species identification
- 6 % correct genus identification
- 11 % no identification



14 % due to the addition of main spectra of species not yet present in database

6 % due to the addition of main spectra of species already present in database

Routine identification of clinical isolates of anaerobic bacteria: matrix-assisted laser desorption ionization-time of flight mass spectrometry performs better than conventional identification methods

Knoester et al. J. Clin. Microbiol. 2012; 50:1504

Parameter	No. of isolates (%)
Tested isolates	296
Similar ID species level	143 (48)
Similar ID genus level	82 (28)
Discrepant results	33 (11)
No reliable MALDI-TOF ID	38 (13)

UMCG 2011

Total no. of strains	375	
Species ID	251	67%
Genus ID	60	16%
No ID	63	17%

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Genus*	% species ID	% genus ID
<i>Clostridium</i> sp. (n=64)	95	5
<i>B. fragilis</i> sp. (n=82)	94	6
<i>Parabacteroides</i> sp. (n=7)	86	14
GPAC (n=49)	82	18
<i>Propionibacterium</i> sp. (n=36)	72	28
<i>Prevotella</i> sp. (n=30)	70	30
<i>Actinomyces</i> sp. (n=11)	55	45
<i>Fusobacterium</i> sp. (n=6)	50	50
<i>B. ureolyticus</i> (n=7)	43	57
<i>Bilophila</i> sp. (n=10)	10	90

* Only isolates which could be identified with MALDI-TOF MS

Conclusions

1. 60-70 % of the anaerobes can be identified using MALDI-TOF MS
2. Performance differs per species/genus
3. MALDI-TOF MS database needs optimizing for the identification of anaerobic bacteria
 - addition of spectra to existing database increases performance
 - reference strains
 - clinical isolates

ENRIA

European Network for the Rapid Identification of Anaerobes

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