P 2345 The value of MALDI-TOF MS in the identification of clinically relevant anaerobic bacteria

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Introduction

Anaerobic bacteria are a major component of human microflora on mucosal membranes and several hundreds of different species of anaerobic organisms can be identified by using classical and molecular methods. They also predominate in many infections, particularly those arising from mucosal sites. Species identification of anaerobic bacteria by phenotypic methods is hindered by many factors. Several anaerobic species are inactive in biochemical tests. To detect preformed enzymes high inoculum is needed which is difficult to reach due to their poor growth on the surface of media. Classical biochemical tests need at least 5 days of incubation in anaerobic environment, which is not acceptable from the clinical point of view. Due to the wide-spread use of sequencing several taxonomic changes went on in the field of anaerobic bacteria during the past decades, together with the description of new species and genera among clinically relevant anaerobes.

During the past few years matrix-assisted laser desorption ionization-time of flight mass spectrometry (MALDI-TOF MS) proved to be a fast and inexpensive technology for routine identification of various clinically important bacteria including anaerobes (1-3). The aims of this study were to investigate: (i) what proportion of the anaerobic isolates obtained from clinically relevant materials can be identified on species or genus level by MALDI-TOF MS (MALDI Biotyper; Bruker Daltonik, Bremen, Germany), (ii) how classical biochemical tests perform for the same isolates and (iii) whether discrepant results could be confirmed by 16S rRNA gene sequencing.

Table 1. Number of Gram-negative anaerobic isolates identified on species or genus level by MALDI-TOF MS and by phenotypical methods

Bacterial strain	Number of strains identified by MALDI-TOF MS at log(score) ≥2.000	Number of strains identified by MALDI-TOF MS at log(score) ≥1.700	Number of strains identified by phenotypical methods
B. fragilis	37	0	22
B. thetaiotaomicron	9	1	3
B. ovatus	8	0	6
B. uniformis	2	0	6
B. vulgatus	3	0	5
B. urealyticus	2	5	7
B. capillosus	0	0	3
B. stercoris	0	0	2
B. caccae	0	0	3
B. eggerthii	0	0	1
Parabacteroides merdae	0	0	1
P. distasonis	4	0	3
Bacteroides sp	2	0	4
P. oralis	0	0	5
P. bivia	6	4	8
P. buccae	7	0	7
P. loescheii	0	0	1
P. intermedia	0	0	1
P. disiens	3	0	2
P. baronae	1	0	0
P. assacharolytica	0	0	1
Prevotella sp	0	0	2
Fusobacterium sp	2	1	0
F. necrophorum	4	1	3
F. nucleatum	0	1	5
F. varium	0	0	1
Capnocytophaga sputigena	0	1	0
Capnocytophaga sp	0	0	1
	90	14	104

Table 4. Phenotypic identification and 16S rRNA gene sequencing data of eight isolates for which no reliable identification was obtained by the MALDI-TOF MS (log(score) <1.700)

Sample number	Original designation of the strain	Log(score) obtained by MALDI-TOF MS	Phenotypic identification by rapid ID32A	Species identification obtained by 16S rRNA sequencing
1.	HU40261	1.564	*Anaerococcus prevotii*	*Anaerococcus vaginalis* (97.97%)
2.	HU43546_1	1.395	*Anaerococcus prevotii*	*Anaerococcus vaginalis* (97.97%)
3.	HU29644_1	1.238	*Finegoldia magna*	*Finegoldia magna* (100%)
4.	HU52042	1.406	*Clostridium clostridiiforme*	*Clostridium hathewayi* (99.33%)
5.	HU33416	1.313	*Clostridium fallax*	*Bacteroides ureolyticus* (100%)
6.	HU30296_1	1.417	*Anaerococcus prevotii*	*Finegoldia magna* (99.55%)
7.	HU29642_2	1.372	*Anaerococcus prevotii*	*Finegoldia magna* (100%)
8.	HU30410_3	1.277	*Prevotella loescheii*	*Prevotella baroniae* (99.56%)

References

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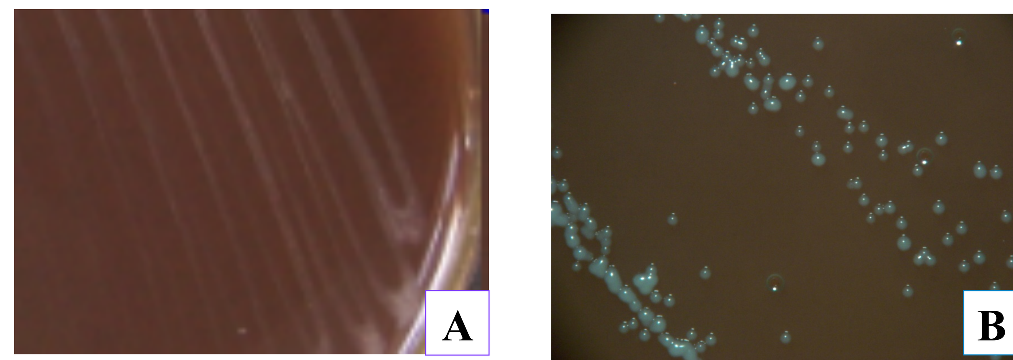
Materials and methods

During 2010 and 2011 non-duplicate clinical isolates (196) cultured in an anaerobic environment were identified by using the standard protocol with extraction of the MALDI-TOF MS (Microflex) and the spectra were imported into the Biotyper software (version 2.0 and later version 3.0). The isolates were tested if they formed a well defined colony. According to the manufacturer a log(score) ≥1.7 is indicative for a close relationship on genus level, while a log(score) ≥2.000 is set as threshold for a match on species level. Strains which had a log(score) ≥2.000 were accepted as a correct identification, however if the phenotypic identification was different, sequencing was carried out. All strains with a log(score) ≥1.700 were sequenced to confirm species identification, except those where the phenotypic method gave an unambiguous species level identification. Isolation and identification of anaerobic bacteria were carried out by standard laboratory methods (4). For phenotypic identification the rapid ID32A (BioMerieux) was used.

Table 2. Number of Gram-positive anaerobic isolates identified on species or genus level by MALDI-TOF MS and by phenotypical methods

Bacterial strain	Number of strains identified by MALDI-TOF MS at log(score) ≥2.000	Number of strains identified by MALDI-TOF MS at log(score) ≥1.700	Number of strains identified by phenotypical methods
Clostridium perfringens	31	2	31
C. difficile	3	0	1
C. innocuum	3	0	1
C. tertium	2	0	2
C. septicum	3	0	3
C. butyricum	0	1	1
C. ramosum	2	0	0
C. baratii	0	1	0
C. fallax	0	0	1
C. paraputrificans	0	0	1
C. bifermentans	0	0	1
Clostridium sp.	0	0	2
GPAC*		0	4
Finegoldia magna	2	0	2
Parvimonas micra	1	0	1
P. anaerobius	3	0	1
Peptostreptococcus sp	0	0	7
Gemella morbillorum	1	0	2
G. haemolysans	0	1	0
G. sanguinis	0	1	0
Streptococcus salivarius	0	1	0
Propionibacterium acnes	6	0	6
P. avidum	1	2	0
Propionibacterium sp	0	0	2
Actinomyces odontolyticus	2	5	5
Actinomyces sp	0	0	2
Lactobacillus gasseri	1	0	0
L. paracasei	1	0	0
L. jensenii	0	1	0
Lactobacillus sp	0	0	3
Granulicatella adiacens	1	2	0
	63	17	80

Figure 1. Growth of *Propionibacterium acnes* in an anaerobic environment (A) 24 h incubation time; (B) 72 h incubation time



Conclusions

MALDI-TOF MS seems to be a very promising identification method especially in the case of anaerobic bacteria, which need a special culture condition, a longer incubation time to get proper growth and are biochemically often inactive.

In the case of slow growing anaerobic bacteria the optimal incubation time before preparing the sample for MS-based identification in a routine laboratory should be tested.

The continuing development of the database by including new, clinically important species and optimizing the database by including more spectra of clinical isolates for certain genera and species is mandatory to increase identification levels.

Despite of the fact that 16S rRNA gene sequencing is considered as the "gold standard" for identification of anaerobic bacteria, it is still not applicable in most routine laboratories and it needs considerable time to get the result. The MALDI-TOF MS (Bruker MALDI Biotyper) in our study could identify 84.6% of randomly selected clinical anaerobic isolates at the present time simulating a routine situation. Besides being fast and inexpensive its advantage is that rapid development and optimization of the database, by adding further spectra of strains belonging to the same species and isolated from clinical specimens, can be achieved in local settings as well as by the provider. If more and more laboratories, interested in anaerobic bacteriology, will use the system, more knowledge will be accumulated about problematic species which need further development.

Results

Out of the 196 isolates 184 were identified by the MALDI-TOF MS with a log(score) ≥ 1.700 (Table 1 and 2). In the case of 12 strains the MALDI-TOF MS gave "no reliable identification". Out of the Gram-positive bacteria isolated in an anaerobic environment few were identified as aerobic isolates (*Streptococcus salivarius*, lactobacilli, *Granulicatella adiacens*). 17 Gram-positive bacteria and 14 Gram-negative anaerobes gave a log(score) >1.700 with an identical species determination by the phenotypic identification. Most of the *Actinomyces odontolyticus* (5 of 7 isolates) and *Bacteroides urealyticus* (5 of 7 isolates) had a log(score) >1.700 and < 2.000 with the first two or three best match of the same species. In all cases where the phenotypic identification gave a discrepant result the strain was identified by the 16S rRNA gene sequencing (Table 3). In 41 of the 44 strains the sequencing confirmed the identification of the MALDI-TOF MS. Two *Bacteroides xylanisolvens* for which reference is not present in the MS database were identified by MALDI-TOF MS as *Bacteroides ovatus.* Out of 12 strains with "no reliable" identification 8 were sequenced and compared with the results of the phenotypic identification (Table 4). The diverse results were partly explained by missing the species from the database of the rapid ID32A (*Anaerococcus vaginalis* and *Clostridium hathewayi*) or few differences in the biochemical tests between the species. *Prevotella baroniae* and *Finegoldia magna* were added to the MALDI-TOF MS database at this stage of the study.

Table 3. Sequencing data of those strains where the species identification by the MALDI-TOF MS and by the phenotypic methods gave discrepant results

Species identified by MALDI-TOF MS	Log (score)	Species identification by phenotypic methods	Species identification by 16S rRNS gene sequencing	Homology level (%)
C . difficile	2.392	*C. perfingens*	*C. difficile*	100
C. ramosum	2.317	*C. paraputrificans*	*C. ramosum*	100
C. perfingens	2.362	*C. bifermentans*	*C. perfingens*	100
Clostridium baratii	1.727	*Clostridium fallax*	*Clostridium sp*	99.77
Finegoldia magna	2.127	*Peptostreptococcus sp.*	*Finegoldia magna*	100
Gemella morbillorum	2.491	*Peptostreptococcus sp*	*Gemella morbillorum*	100
Str. salivarius	1.998	*Peptostreptococcus sp.*	*Str. salivarius*	99
G. adiacens	1.87	*Peptostreptococcus sp*	*G. adiacens*	99
G. adiacens	1.962	*Peptostreptococcus sp*	*G. adiacens*	99.13
G. adiacens	2.187	*Peptostreptococcus sp*	*G. adiacens*	99
B. fragilis	2.153	*B. capillosus*	*B. fragilis*	100
B. fragilis	2.537	*B. vulgatus*	*B. fragilis*	100
B. fragilis	2.399	*B. uniformis*	*B. fragilis*	99
B. fragilis	2.489	*B. uniformis*	*B. fragilis*	99
B. fragilis	2.454	*B. vulgatus*	*B. fragilis*	99
B. fragilis	2.576	*B. capillosus*	*B. fragilis*	99.56
B. fragilis	2.61	*P. merdae*	*B. fragilis*	99.56
B. fragilis	2.538	*B. thetaiotaomicron*	*B. fragilis*	99.56
B. fragilis	2.54	*B. caccae*	*B. fragilis*	99
B. fragilis	2.608	*B. caccae*	*B. fragilis*	99
B. fragilis	2.489	*B. caccae*	*B. fragilis*	100
B. fragilis	2.389	*P. loescheii*	*B. fragilis*	99.12
B. fragilis	2.61	*B. stercosis*	*B. fragilis*	100
B. fragilis	2.421	*B. stercosis*	*B. fragilis*	99.34
B. fragilis	2.449	*P. bivia*	*B. fragilis*	99.78
B. fragilis	2.386	*P. bivia*	*B. fragilis*	99.56
B. thetaiotaomicron	2.445	*P. distasonis*	*B. thetaiotaomicron*	100
B. thetaiotaomicron	2.406	*B. uniformis*	*B. thetaiotaomicron*	99
B. thetaiotaomicron	2.38	*B. fragilis*	*B. thetaiotaomicron*	99
B. thetaiotaomicron	2.15	*B. fragilis*	*B. thetaiotaomicron*	100
B. thetaiotaomicron	2.251	*B. ovatus*	*B. thetaiotaomicron*	99
B. thetaiotaomicron	2.366	*B. fragilis*	*B. thetaiotaomicron*	99.78
B. thetaiotaomicron	2.216	*B. eggerthii*	*B. thetaiotaomicron*	100
B. ovatus	2.261	*B. eggerthii*	*B. xylanisolvens*	99.78
B. ovatus	2.242	*B. thetaiotaomicron*	*B. xylanisolvens*	99.78
B. uniformis	2.637	*P. buccae*	*B. uniformis*	100
P. distasonis	2.453	*P. intermedia*	*P. distasonis*	98.89
P. distasonis	2.533	*B. fragilis*	*P. distasonis*	99.89
P. buccae	2.297	*P. asaccharolyticus*	*P. buccae*	99.78
P. buccae	2.309	*P. oralis*	*P. buccae*	99.13
P. disiens	2.194	*P. oralis*	*P. disiens*	98.89
P. bivia	2.348	*P. oralis*	*P. bivia*	100
P. bivia	2.236	*P. oralis*	*P. bivia*	100
P. baroniae	2.189	*P. loescheii*	*P. baroniae*	98.90

Clear differentiation of Division I and II of *B. fragilis* by MALDI-TOF MS