

Use of MALDI-TOF MS to identify *Bacteroides* strains on species level

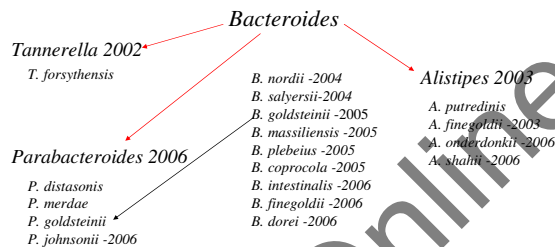
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This study was a part of the 3rd ESGARAB *Bacteroides* resistance evaluation (Nagy E., *et al.* CMI 15: 796-802, 2009)

Species determination of strains belonging in *Bacteroides* (*Parabacteroides*) genus

- Discrepancies between the species determination in the original lab and in the central lab
- In some isolating laboratories the identification was carried out only on genus level
- Differences can be in the susceptibility to different antibiotics according to the species
- Possibilities for collaboration with Bruker GmbH (Leipzig) Hamburg to use the MALDI-TOF MS (measurements was done at the beginning of 2008)
- To improve the data base of MALDI-TOF for species determination in genus *Bacteroides*
- In case of discrepant results 16S rRNA gene sequencing was performed

New genera and novel or renamed species (since 2002) in the genus *Bacteroides* isolated from humans



Today all together 23 species of *Bacteroides/Parabacteroides* should be determined

Chattopadhyay P, Jousimies-Salmela H. CID 35: S17-21 (2002)
 Song YL, Liu CX, McFarlane M. Fungal SM. JCM 42: 5565-5570 (2004)
 Song Y, *et al.* JCM 43: 4522-4527 (2005)
 Sakai T, *et al.* Int. J. Syst. Evol. Microbiol. 56: 1599-1605 (2006)

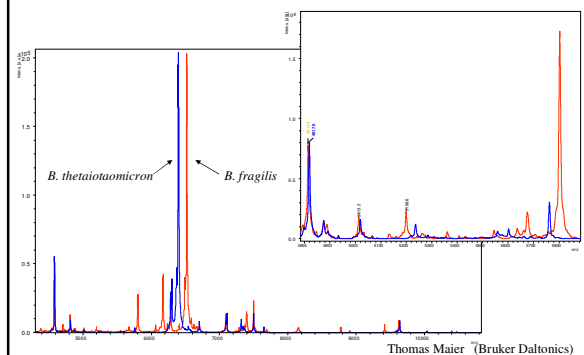
Collaborative study

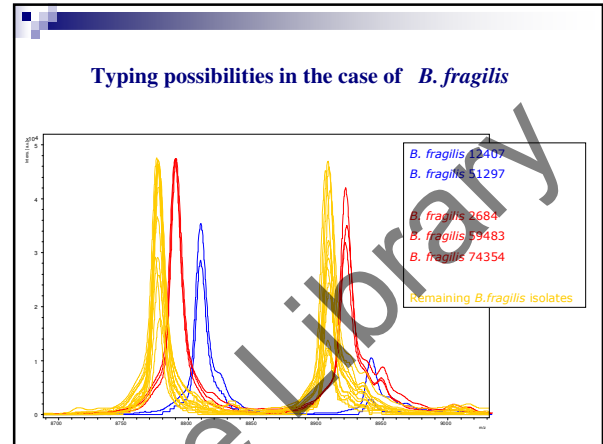
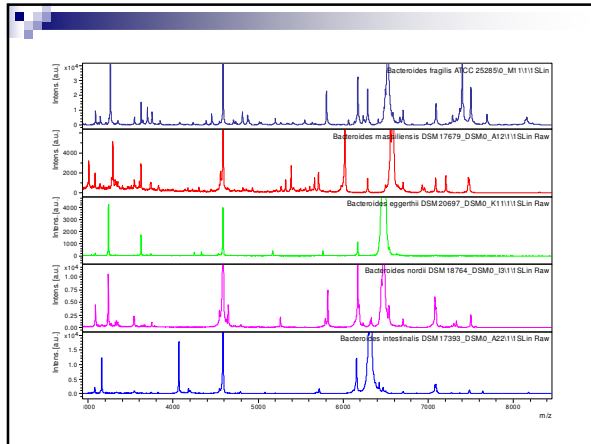
- A subset of the ESGARAB isolates – 277 *Bacteroides* strains were cultured
 - Columbia agar base supplemented with 5% cattle blood, haemin and vitamin K1 for 24h
 - Incubation in anaerobic chamber (Bactron)
- Sample preparation:
 - One colony was suspended in 300 µl distilled water
 - 900 µl ethanol was added and mixed thoroughly
 - Kept in -20°C till the transport to Leipzig

Collaborative study

- Sample preparation (continued in Leipzig)
 - Centrifugation for 2 min – dry the pellet
 - Resuspend in 50 µl of 70% aqueous formic acid and 50 µl of acetonitrile
 - Centrifugation and 1 µl of the supernatant was spotted on the MALDI target plate and dried at room temperature
 - 2 µl of MALDI matrix was added to the spot and dried
 - Microflex LT or ultraflex TOF/TOF MALDI-TOF (Bruker) was used for the measurements
 - Recoded mass spectra were processed with the MALDI Biotyper 2.0 software package
 - Control strain at the beginning, cultured in both places:
 - *B. fragilis* ATCC25285
 - *B. fragilis* DSM2151
 - *B. thetaiotaomicron* ATCC 29742

The MS of *B. fragilis* and *B. thetaiotaomicron* can be distinguished easily





Species determination of 277 *Bacteroides* strains by MALDI-TOF MS

Species	Number of isolates		
	with an identification score ≥ 2.0 by MALDI-TOF MS	with discrepant species identification by biochemical tests	selected for 16S rDNA sequencing*
<i>Bacteroides fragilis</i>	179	5	2
<i>Bacteroides thetaiotaomicron</i>	43	5	4
<i>Bacteroides ovatus</i>	15	6	1
<i>Bacteroides vulgatus</i>	20	1	1
<i>Bacteroides uniformis</i>	5	3	2
<i>Bacteroides eggerthii</i>	1	0	0
<i>Bacteroides nordii</i>	4	3	1
<i>Bacteroides salyersiae</i>		1	0
<i>Bacteroides massiliensis</i>		2	0
Inconclusive identification by MALDI-TOF MS		7	7

Log(score) <1.7 is not acceptable identification
 >1.7 identification on genus level
 >2.0 acceptable identification on species level

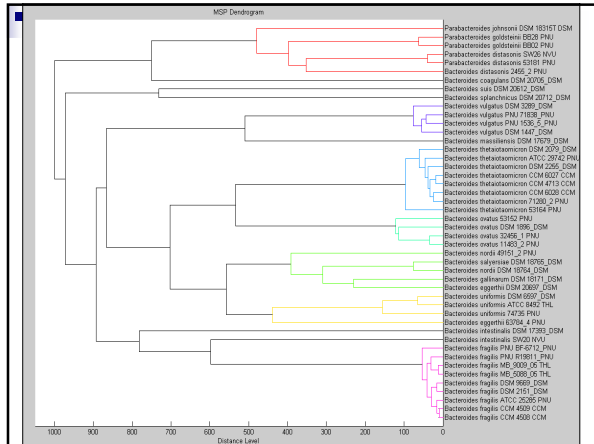
Only strains with log(score) of 2.0-2.5 were selected for sequencing

Sample number	Species determined by		
	biochemical tests	MALDI-TOF MS	16S rDNA sequencing
1	<i>B. ovatus</i>	<i>B. fragilis</i>	<i>B. fragilis</i>
2	<i>B. uniformis</i>	<i>B. fragilis</i>	<i>B. fragilis</i>
3	<i>B. ovatus</i>	<i>B. thetaiotaomicron</i>	<i>B. thetaiotaomicron</i>
4	<i>B. uniformis</i>	<i>B. thetaiotaomicron</i>	<i>B. thetaiotaomicron</i>
5	<i>B. uniformis</i>	<i>B. thetaiotaomicron</i>	<i>B. thetaiotaomicron</i>
6	<i>B. ovatus</i>	<i>B. thetaiotaomicron</i>	<i>B. thetaiotaomicron</i>
7	<i>B. uniformis</i>	<i>B. ovatus</i>	<i>B. ovatus</i>
8	<i>B. thetaiotaomicron</i>	<i>B. vulgatus</i>	<i>Bacteroides</i> sp (new?)
9	<i>B. ovatus</i>	<i>B. uniformis</i>	<i>B. uniformis</i>
10	<i>B. ovatus</i>	<i>B. uniformis</i>	<i>B. uniformis</i>
11	<i>B. ovatus</i>	<i>B. nordii</i>	<i>B. nordii</i>
12	<i>B. thetaiotaomicron</i>	II* (log(score)>2.5)**	<i>P. (B.) distasonis</i>
13	<i>P. (B.) distasonis</i>	II (log(score)>2.5)	<i>P. (B.) distasonis</i>
14	<i>P. (B.) distasonis</i>	II (log(score)>2.5)	<i>P. (B.) distasonis</i>
15	<i>P. (B.) distasonis</i>	II (log(score)>2.5)	<i>P. (B.) distasonis</i>
16	<i>B. thetaiotaomicron</i>	II	<i>B. eggerthii</i>
17	<i>P. (B.) merdae</i>	II	<i>P. (B.) goldsteini</i>
18	<i>Bacteroides</i> sp	II	<i>B. intestinalis</i>

* II inconclusive identification
 **Achieved log(score) after adding to the data base the MS of the sequenced *P. (B.) distasonis*

- ### List of *Bacteroides*/*Parabacteroides* strains included to the Bruker's data base
- B. caecae* DSM 19024T DSM
 - B. coagulans* DSM 20705T DSM
 - B. eggerthii* 63784_4 PNU
 - B. eggerthii* DSM 20697T DSM
 - B. finegoldii* DSM 17565T DSM
 - B. fragilis* ATCC 25285T PNU
 - B. fragilis* CCM 4508 CCM
 - B. fragilis* CCM 4509 CCM
 - B. fragilis* DSM 2151T DSM
 - B. fragilis* DSM 9669 DSM
 - B. fragilis* MB_5088_05 THL
 - B. fragilis* MB_9009_05 THL
 - B. fragilis* PNU BF_6712_PNU
 - B. fragilis* PNU R19811_PNU
 - B. gallinarum* DSM 18171T DSM
 - B. intestinalis* DSM 17393T DSM
 - B. intestinalis* SW20 PNU
 - B. massiliensis* DSM 17679T DSM
 - B. nordii* 49151_2 PNU
 - B. nordii* DSM 18764T DSM
 - B. ovatus* 11483_2 PNU
 - B. ovatus* 32456_1 PNU
 - B. ovatus* 53152 PNU
 - B. ovatus* DSM 1896T DSM
 - B. salyersiae* DSM 18765T DSM
 - B. suis* DSM 20612T DSM
 - B. thetaiotaomicron* 53164 PNU
 - B. thetaiotaomicron* 71280_2 PNU
 - B. thetaiotaomicron* ATCC 29742 PNU
 - B. thetaiotaomicron* CCM 4713 CCM
 - B. thetaiotaomicron* CCM 6027 CCM
 - B. thetaiotaomicron* CCM 6028 CCM
 - B. thetaiotaomicron* DSM 2079T DSM
 - B. thetaiotaomicron* DSM 2255 DSM
 - B. uniformis* 74735 PNU
 - B. uniformis* ATCC 8492T THL
 - B. uniformis* DSM 6597T DSM
 - B. vulgatus* DSM 1447T DSM
 - B. vulgatus* DSM 3289 DSM
 - B. vulgatus* PNU 1536_5 PNU
 - B. vulgatus* PNU 71838 PNU

- ### List of *Bacteroides*/*Parabacteroides* strains included to the Bruker's data base
- Parabacteroides distasonis* 53181 PNU
 - Parabacteroides distasonis* DSM 20701T DSM
 - Parabacteroides distasonis* RV 412_0209_5 LBK
 - Parabacteroides distasonis* RV_00005_09 ERL
 - Parabacteroides distasonis* SW26 PNU
 - Parabacteroides goldsteini* BB02 PNU
 - Parabacteroides goldsteini* BB28 PNU
 - Parabacteroides goldsteini* DSM 19448T DSM
 - Parabacteroides johnsonii* DSM 18315T DSM
- 15 *Bacteroides* species and 3 *Parabacteroides* species are represented



MALDI-TOF results of two measurements of different ethanol pellets of some Bacteroides strains in different time points:

Organism (05.04.2008)	Analyte ID	Organism (07.10.09)	Score (07.10.09)	Analyte ID	Organism (27.01.10)	Score (27.01.10)
Bacteroides ovatus	Francia_29	Bacteroides fragilis	1.981	HU100127_1	Bacteroides fragilis	2.400
Bacteroides thetaiotaomicron	Francia_30	Bacteroides fragilis	2.521	HU100127_2	Bacteroides fragilis	2.400
Bacteroides ovatus	Francia_33	Bacteroides fragilis	2.49	HU100127_3	Bacteroides fragilis	2.403
Bacteroides vulgatus	Francia_7235	Bacteroides fragilis	2.405	HU100127_4	Bacteroides fragilis	2.547
Bacteroides thetaiotaomicron	Hovlat_25	Bacteroides fragilis	2.593	HU100127_5	Bacteroides fragilis	2.440
Bacteroides vulgatus	Hovlat_30	Bacteroides fragilis	2.465	HU100127_6	Bacteroides fragilis	2.66
Bacteroides vulgatus	Hovlat_37	Bacteroides fragilis	2.58	HU100127_7	Bacteroides fragilis	2.686
Bacteroides uniformis	Goeroeg_34	Bacteroides fragilis	2.251	HU100127_8	Bacteroides fragilis	2.626
Parabacteroides merdae	Goeroeg_63	Bacteroides fragilis	2.165	HU100127_9	Bacteroides fragilis	2.713
Bacteroides vulgatus	Goeroeg_64	Bacteroides fragilis	2.591	HU100127_10	Bacteroides fragilis	2.632
Bacteroides vulgatus	Goeroeg_4	Bacteroides fragilis	2.523	HU100127_11	Bacteroides fragilis	2.640
Bacteroides vulgatus	Goeroeg_75	Bacteroides fragilis	2.57	HU100127_12	Bacteroides fragilis	2.644
Bacteroides thetaiotaomicron	Olazc_805	Bacteroides thetaiotaomicron	2.599	HU100127_13	Bacteroides thetaiotaomicron	2.151
Bacteroides thetaiotaomicron		Bacteroides fragilis	2.59	HU100127_14	Bacteroides thetaiotaomicron	2.151

The second MALDI analysis of these strains (always separate ethanol pellets were analysed and not the same sample) confirmed the previous MALDI Biotyper analysis results in 12 out of 14 samples.

MALDI BioTyper results for sample no. 13 and no. 14

Analyte Name	Organism (best match)	Score Value	Organism (second best match)	Score Value	sample information
xxx HU100127_13_direct (***)	Bacteroides fragilis	2.400	Bacteroides fragilis	2.400	aliquot first prep. direct transfer
xxx 14_Extra1 (***)	Bacteroides fragilis	2.400	Bacteroides fragilis	2.400	aliquot second prep. extraction
xxx 13_direct_original (***)	Bacteroides fragilis	2.400	Bacteroides fragilis	2.400	original sample tube, direct transfer
xxx HU100127_14_direct (***)	Bacteroides thetaiotaomicron	2.151	Bacteroides thetaiotaomicron	2.151	aliquot first prep. direct transfer
xxx 14_Extra1 (***)	Bacteroides thetaiotaomicron	2.151	Bacteroides thetaiotaomicron	2.151	aliquot second prep. extraction
xxx 14_direct_original (***)	Bacteroides thetaiotaomicron	2.151	Bacteroides thetaiotaomicron	2.151	original sample tube, direct transfer

To summarise

- MALDI-TOF MS provides a rapid species determination in case of *Bacteroides*/*Parabacteroides*
- Culture media (suitable for their isolation) is not influencing the result
- The same isolates cultured and prepared for the MALDI-TOF MS in different laboratories gave the same result
- Classical species identification of the many species needs at least 2 to 6 days incubation. In this case same day result is possible.
- Rapid identification systems need high inoculums and the result is influenced by the composition of the media used for isolation.
- MALDI-TOF may be rapid alternative of any present species identification
- Sequencing from the same ethanol extract of the cells is possible

