IDENTIFICATION OF *C. UPSALIENSIS* AND *C. HELVETICUS*

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C. UPSALIENSIS/C. HELVETICUS

- PT 2018: only 9 out of 31 labs identified ***C. upsaliensis*** correctly
- ***C. upsaliensis***: SVA, Sandstedt 1991
- ***C. helveticus***: Stanley et al. 1993
- Cats and dogs
- ***C. upsaliensis*** and ***C. helveticus….***

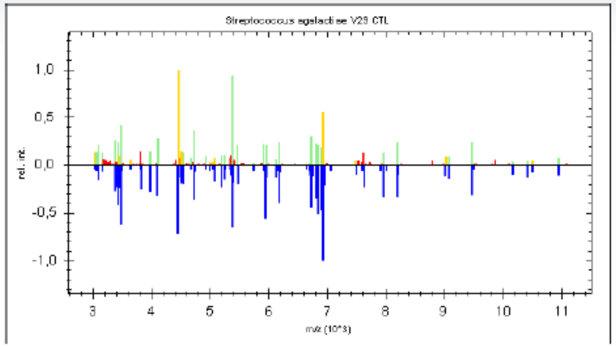
METHODS USED IN PT 22 FOR IDENTIFICATION OF *C. UPSALIENSIS*

- MALDI-TOF
- PCR (Wang et al. 2002, Yamazaki-Matsune et al. 2007)

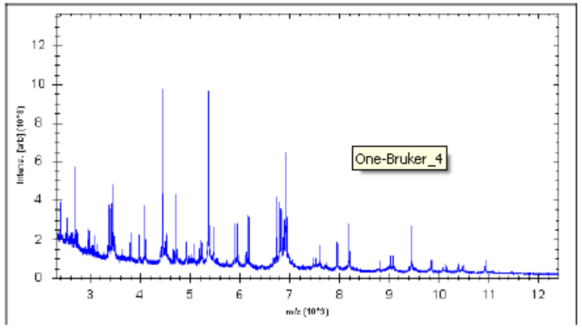
IDENTIFICATION WITH MALDI-TOF

MALDI-TOF MS microorganism identification

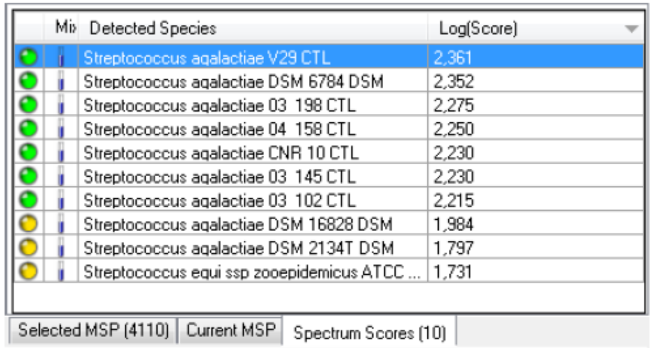
Creation of characteristic peak list



Match against database and ranking of results, threshold for identification

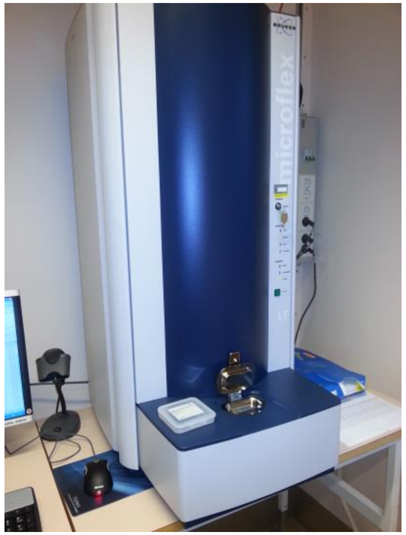


Profile mass spectrum



Mi	Detected Species	Log(Score)
🟢	Streptococcus agalactiae V29 CTL	2,361
🟢	Streptococcus agalactiae DSM 6784 DSM	2,352
🟢	Streptococcus agalactiae 03 198 CTL	2,275
🟢	Streptococcus agalactiae 04 158 CTL	2,250
🟢	Streptococcus agalactiae CNR 10 CTL	2,230
🟢	Streptococcus agalactiae 03 145 CTL	2,230
🟢	Streptococcus agalactiae 03 102 CTL	2,215
🟡	Streptococcus agalactiae DSM 16828 DSM	1,984
🟡	Streptococcus agalactiae DSM 2134T DSM	1,797
🟡	Streptococcus equi ssp zooepidemicus ATCC ...	1,731

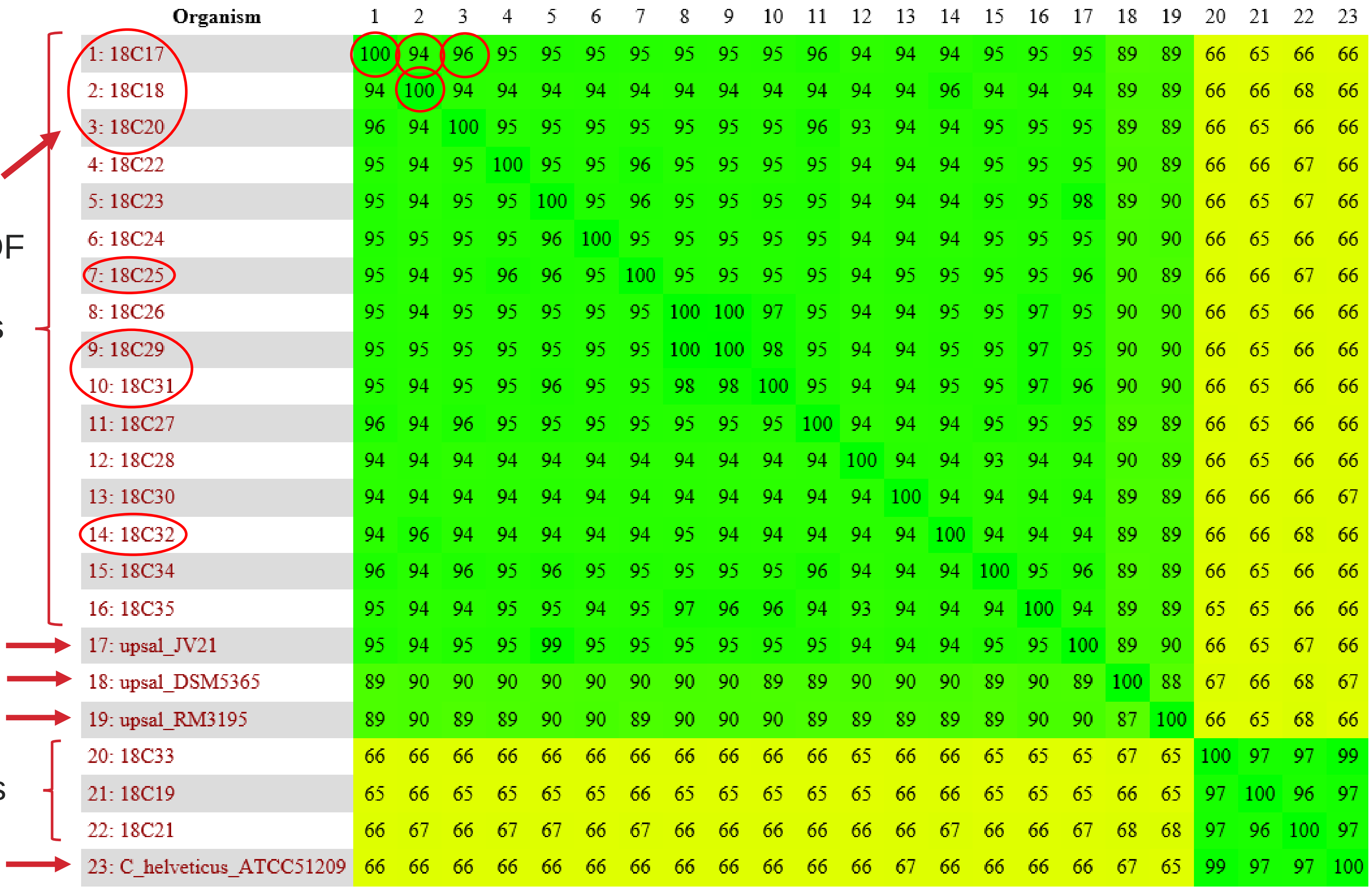
MALDI-TOF VS SEQUENCING



- 19 clinical strains from 2016-2018 were whole genome sequenced: **9 *C. upsaliensis*** and **10 *C. helveticus*** strains identified by MALDI-TOF (score >2).

- The sequence data were compared. 3 draft *upsaliensis* genomes and 1 complete *helveticus* genome from GenBank included as references in the comparison.

RESULTS: MALDI-TOF VS SEQUENCING



C. helveticus
by MALDI-TOF

Upsaliensis

Helveticus

Organism	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23
1: 18C17	100	94	96	95	95	95	95	95	95	95	96	94	94	94	95	95	95	89	89	66	65	66	66
2: 18C18	94	100	94	94	94	94	94	94	94	94	94	94	94	96	94	94	94	89	89	66	66	68	66
3: 18C20	96	94	100	95	95	95	95	95	95	95	96	93	94	94	95	95	95	89	89	66	65	66	66
4: 18C22	95	94	95	100	95	95	96	95	95	95	95	94	94	94	95	95	95	90	89	66	66	67	66
5: 18C23	95	94	95	95	100	95	96	95	95	95	95	94	94	94	95	95	98	89	90	66	65	67	66
6: 18C24	95	95	95	95	96	100	95	95	95	95	95	94	94	94	95	95	95	90	90	66	65	66	66
7: 18C25	95	94	95	96	96	95	100	95	95	95	95	94	95	95	95	95	96	90	89	66	66	67	66
8: 18C26	95	94	95	95	95	95	95	100	100	97	95	94	94	95	95	97	95	90	90	66	65	66	66
9: 18C29	95	95	95	95	95	95	95	100	100	98	95	94	94	95	95	97	95	90	90	66	65	66	66
10: 18C31	95	94	95	95	96	95	95	98	98	100	95	94	94	95	95	97	96	90	90	66	65	66	66
11: 18C27	96	94	96	95	95	95	95	95	95	95	100	94	94	94	95	95	95	89	89	66	65	66	66
12: 18C28	94	94	94	94	94	94	94	94	94	94	94	100	94	94	93	94	94	90	89	66	65	66	66
13: 18C30	94	94	94	94	94	94	94	94	94	94	94	94	100	94	94	94	94	89	89	66	66	66	67
14: 18C32	94	96	94	94	94	94	94	95	94	94	94	94	94	100	94	94	94	89	89	66	66	68	66
15: 18C34	96	94	96	95	96	95	95	95	95	95	96	94	94	94	100	95	96	89	89	66	65	66	66
16: 18C35	95	94	94	95	95	94	95	97	96	96	94	93	94	94	94	100	94	89	89	65	65	66	66
17: upsal_JV21	95	94	95	95	99	95	95	95	95	95	95	94	94	94	95	95	100	89	90	66	65	67	66
18: upsal_DSM5365	89	90	90	90	90	90	90	90	90	89	89	90	90	90	89	90	89	100	88	67	66	68	67
19: upsal_RM3195	89	90	89	89	90	90	89	90	90	90	89	89	89	89	89	90	90	87	100	66	65	68	66
20: 18C33	66	66	66	66	66	66	66	66	66	66	66	65	66	66	65	65	65	67	65	100	97	97	99
21: 18C19	65	66	65	65	65	65	66	65	65	65	65	65	66	66	65	65	65	66	65	97	100	96	97
22: 18C21	66	67	66	67	67	66	67	66	66	66	66	66	66	67	66	66	67	68	68	97	96	100	97
23: C_helveticus_ATCC51209	66	66	66	66	66	66	66	66	66	66	66	66	67	66	66	66	66	67	65	99	97	97	100

- upsaliensis/helveticus – two different species
- 7 *C. helveticus* strains misidentified by MALDI-TOF

REFERENCES IN BRUKER DATABASE

→

ID nr	Species ID by SVA	Species ID by Bruker	Species WGS/gegenees
Cb 370-01	C. helveticus	C. helveticus	C. upsaliensis
Cb 412-01	C. upsaliensis	C. upsaliensis	C. upsaliensis
Cb 451-01	C. upsaliensis	C. upsaliensis	C. upsaliensis
Cb 8174-01	C. helveticus	C. helveticus	
Bkt 7522-08	C. upsaliensis	C. upsaliensis	C. upsaliensis
Bkt 7986-08	C. upsaliensis	C. helveticus	C. helveticus
CCUG 14913	C. upsaliensis	C. upsaliensis	C. upsaliensis
CCUG30682	C. helveticus	C. helveticus	C. helveticus

RESULTS: PCR – WANG AND YAMAZAKI

Figure removed.

Yamazaki showed a better sensitivity than Wang

Wang et al. Journal of clinical microbiology, dec 2002, 4744-4747. Yamazaki-Matsune et al. Journal of medical microbiology (2007), 56, 1467-1473.

PCR FOR UPSALIENSIS AND HELVETICUS
IN SILICO TEST

	Kamei et al. 2016	ups
	Fontanot et al. 2014	ups
→	Chaban et al. 2009	ups/helv
	Jensen et al. 2005	ups
	Klena et al. 2004	ups
→	Lawson et al. 1997	ups/helv
→	Linton et al. 1996	ups/helv

FUTURE WORK

- Test Chaban, Lawson, and Linton PCRs in vitro.
- Provide Bruker with more strains to improve the database
- Complete genomes of *C. upsaliensis* and submit GenBank

THANK YOU FOR YOUR ATTENTION!

QUESTIONS?