Validation of MALDI Biotyper for the identification of *Enterococcus species*

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OBJECTIVES

Our laboratory is the Belgian Reference Centre for Enterococci. In the framework of these activities, the applicability of the microflex LT MALDI-TOF MS Biotyper (Bruker) was evaluated for the identification of *Enterococcus species*. Since for some *Enterococcus species* only 3 or less spectra were available in the MALDI Biotyper database version 3995, a large set of strains was investigated.

MATERIALS & METHODS

For the validation of the MALDI Biotyper, 136 enterococcal strains (76 reference strains (Table 1) and 60 clinical isolates identified by in-house validated ddl-PCR and species-specific PCR) were tested. The collection consisted of *E. faecalis* (n=20), *E. faecium* (n=21), *E. gallinarum* (n=20), *E. raffinosus* (n=2), *E. casseliflavus* (n=20), *E. avium* (n=12), *E. hirae* (n=21) and *E. durans* (n=20). Identification was done according to the instructions of the manufacturer using an overnight culture. A log-score >2 was used as cut-off. To solve discrepant identification results, 16S rDNA PCR and sequencing were applied.

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RESULTS

- Using the above mentioned cut-off, for 20/20 *E. faecalis* strains, 21/22 *E. faecium* strains, 1/2 *E. raffinosus* strains, 19/20 *E. gallinarum* strains, 20/20 *E. casseliflavus* strains, 19/20 *E. durans* strains, 20/21 *E. hirae* strains, 8/12 *E. avium* strains, concordant results were obtained between the MALDI Biotyper identification and the previous identification.
- In addition, the identification was confirmed for 1 *E. raffinosus*, 1 *E. gallinarum*, 1 *E. durans*, 2 *E. avium*, but with a log-score varying between 1.74 and 1.95.
- The following discrepant results were obtained: 1x *L. murinus* instead of *E. faecium*, 1x *E. faecium* instead of *E. hirae*, 2x *E. faecalis* instead of *E. avium*. 16S rDNA sequencing confirmed the MALDI Biotyper identification for 3/4 except for the *E. hirae* strain.

Table 1. Overview results with log-score <2 and discordant results

Species	Strain Nr	*MS-ID*	logscore	*16S result*
E. faecium	LMG17203	*L. murinus*	2,01	*L. murinus*
E. avium	16/5	*E. faecalis*	2,24	*E. faecalis*
E. avium	16/05	*E. faecalis*	2,10	*E. faecalis*
E. hirae	04SS05	*E. faecium*	2,47	*E. hirae*
E. gallinarum	004K	*E. gallinarum*	1,95	/
E. raffinosus	111-005886	*E. raffinosus*	1,93	/
E. avium	PT03-07	*E. avium*	1,84	/
E. avium	59097	*E. avium*	1,86	/
E. durans	LMG12285	*E. durans*	1,74	/

CONCLUSIONS

- The MALDI Biotyper is a fast, convenient and reliable method for the identification of *Enterococcus species*.

Table 1. Overview reference strains used

Species	Strain Nr
E. faecalis	LMG17123
E. faecalis	LMG17122
E. faecalis	LMG17121
E. faecalis	LMG16303
E. faecalis	LMG16302
E. faecalis	LMG16301
E. faecalis	ATCC29212
E. faecalis	BM4539
E. faecalis	BM4405
E. faecalis	BM4518
E. faecium	LMG15876
E. faecium	LMG16308
E. faecium	CCUG31387
E. faecium	CCUG32171
E. faecium	CCUG32655
E. faecium	CCUG34324
E. faecium	CCUG45539
E. faecium	CCUG29743
E. faecium	CCUG36838
E. faecium	LMG8148
E. faecium	LMG8149
E. faecium	LMG17204
E. faecium	LMG17200
E. faecium	LMG147188
E. faecium	LMG17179
E. faecium	LMG17178
E. faecium	LMG17175
E. faecium	LMG17174
E. faecium	BM4339
E. gallinarum	LMG12904
E. gallinarum	LMG12313
E. gallinarum	LMG13129
E. gallinarum	LMG14401
E. gallinarum	LMG14405
E. gallinarum	LMG16197
E. gallinarum	LMG16276

Species	Strain Nr
E. gallinarum	LMG16283
E. gallinarum	LMG16289
E. raffinosus	LMG12888T
E. casseliflavus	LMG12311
E. casseliflavus	LMG12314
E. casseliflavus	LMG120309
E. casseliflavus	LMG12901
E. casseliflavus	LMG12307
E. casseliflavus	LMG12310
E. casseliflavus	LMG12306
E. casseliflavus	CCUG13597
E. casseliflavus	LMG14406
E. casseliflavus	LMG16277
E. casseliflavus	LMG16282
E. casseliflavus	LMG16284
E. casseliflavus	LMG16285
E. casseliflavus	LMG16286
E. casseliflavus	LMG16287
E. casseliflavus	LMG16288
E. casseliflavus	LMG938
E. casseliflavus	LMG12311
E. casseliflavus	LMG10745
E. casseliflavus	LMG12314
E. avium	LMG10744T
E. hirae	LMG14200
E. hirae	LMG14201
E. hirae	LMG14488
E. hirae	LMG6399T
E. hirae	LMG11493
E. hirae	LMG11492
E. hirae	LMG14260
E. hirae	LMG14489
E. durans	LMG14763
E. durans	LMG10746T
E. durans	LMG14197
E. durans	LMG12903
E. durans	LMG12283
E. durans	LMG13064T2
E. durans	LMG14202