

# IDENTIFICATION OF ISOLATES BELONGING TO THE *FUSARIUM* GENUS AND THE *MUCORALES* ORDER USING MALDI-TOF MS

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## BACKGROUND

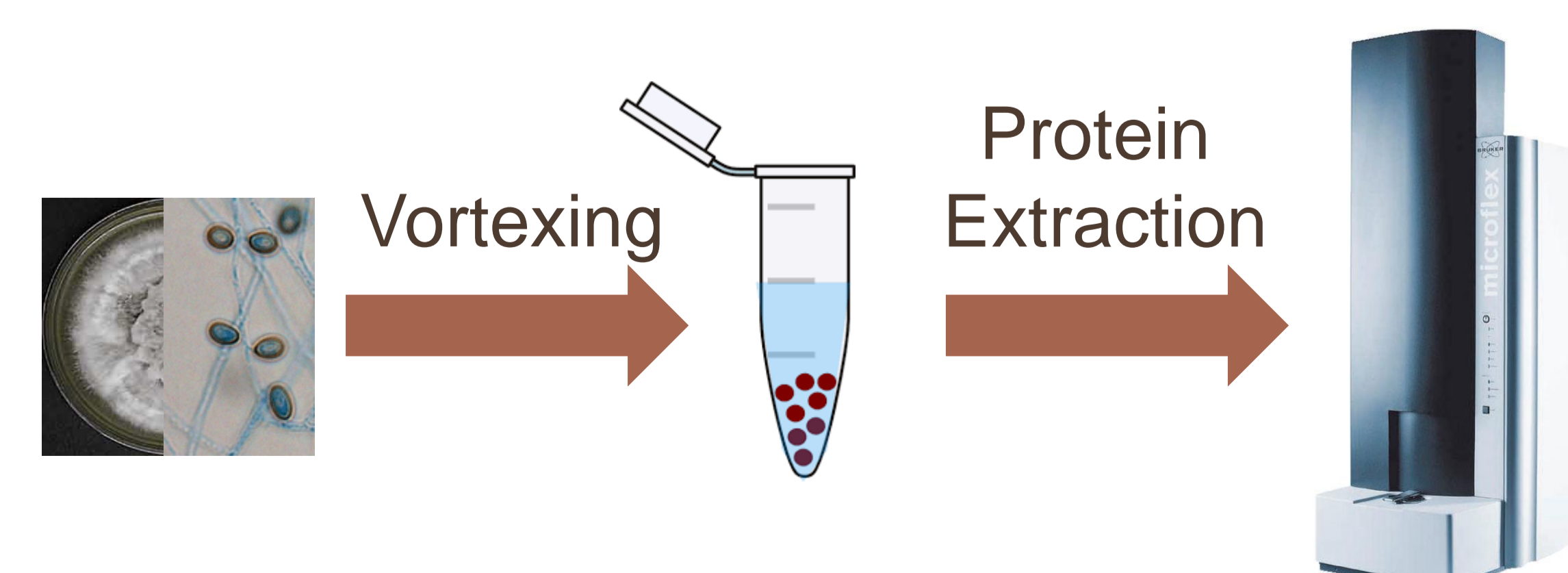
- Filamentous fungi causing fusariosis and mucormycosis are particularly difficult to identify and difficult to treat in clinical practice.
- Their identification still remain a challenge for MALDI-TOF MS
- Factors leading to this situation include 1) **sample preparation** producing poor quality spectra and 2) the availability of a **database** where clinically important species are sometimes underrepresented.
- In this study we assessed the ability of MALDI-TOF MS to identify filamentous fungi belonging to *Fusarium* genus and the *Mucorales* order using a simplified preprocessing protocol and the Filamentous Fungi Library 1.0 –FFL1.0- (Bruker Daltonics, Bremen, Germany) plus a home-enriched database.

## MATERIAL AND METHODS

- We studied **72 clinical isolates (32 *Fusarium* spp and 40 *Mucorales*)** previously characterized by amplification and sequencing of the ITS region and Elongation Factor 1- $\alpha$  gene. This method was considered our gold standard.
- The isolates were further identified by MALDI-TOF MS (Bruker Daltonics, Bremen, Germany) after a simplified preprocessing method consisting of a brief vortexing with 0.5 mm glass beads and standard protein extraction using ethanol, formic acid and acetonitrile. Samples were analyzed in duplicates. The Biotyper system 3.1 and the Filamentous Fungi Library 1.0 was used alone and in combination with our in-house database.

## RESULTS

- Out of the 32 *Fusarium* isolates analyzed, MALDI-TOF MS with the Filamentous Fungi Library 1.0 identified **27 isolates** to the species level (84.4%): 14 with a score value  $\geq 1.8$ , 6 isolates with score  $\geq 1.6$  and 7 isolates with score  $< 1.6$ .



## RESULTS II

- 5 isolates were identified only to the genus level: 1 isolate belonged to a species not present in the database (*F. keratoplasticum*) and the other 4 were identified as closely related species.
- On the other hand, MALDI-TOF MS with the Filamentous Fungi Library 1.0 failed to identify all *Mucorales*. However, when well-characterized isolates from this group were included in the database, MALDI-TOF MS provided identification at the species level for 18 isolates (85.7%), 14 of them with a score value  $\geq 1.8$ . Only in three cases the identification was not in agreement with the reference method.

IDENTIFICATION BY EF-1 SEQUENCING ANALYSIS	NUMBER OF ISOLATES	ID WITH FFL 1.0	ISOLATES ADDED TO IN-HOUSE DB	ID WITH FFL 1.0 + IN-HOUSE DB	SCORE VALUE $\geq 1.8$	SCORE VALUE $\geq 1.6$	SCORE VALUE $< 1.6$	No ID or MisID
<i>Fusarium dimerum</i>	1	1	0	1	1	0	0	0
<i>Fusarium keratoplasticum</i>	1	0	0	0	0	0	0	1
<i>Fusarium oxysporum</i>	4	4	0	4	1	3	0	0
<i>Fusarium proliferatum</i>	19	17	0	17	11	2	4	2
<i>Fusarium solani</i>	5	5	0	5	1	1	3	0
<i>Fusarium verticillioides</i>	2	0	0	0	0	0	0	2
<b>TOTAL</b>	<b>32</b>	<b>27</b>	<b>0</b>	<b>27</b>	<b>14</b>	<b>6</b>	<b>7</b>	<b>5</b>

<i>Cunninghamella bertholletiae</i>	1	0	2	0	0	0	0	1
<i>Lichtheimia corymbifera</i>	1	0	2	1	0	1	0	0
<i>Lichtheimia ramosa</i>	7	0	6	7	7	0	0	0
<i>Mucor circinelloides</i>	2	0	2	2	1	1	0	0
<i>Rhizomucor pusillus</i>	5	0	2	4	4	0	0	1
<i>Rhizopus arrhizus</i>	4	0	3	4	2	1	1	0
<i>Rhizopus microsporus</i>	1	0	2	0	0	0	0	1
<b>TOTAL</b>	<b>21</b>	<b>0</b>	<b>19</b>	<b>18</b>	<b>14</b>	<b>3</b>	<b>1</b>	<b>3</b>

## CONCLUSIONS

- Currently available MALDI-TOF MS database allowed a relatively good identification of *Fusarium* spp. isolates but not of *Mucorales* isolates.
- The use of an in-house database for filamentous fungi provided a rapid and accurate identification of a high percentage of mucormycosis-causing fungi.
- The simplified protocol for sample preparation yields high-quality spectra