Occurrence and Species Distribution of Pathogenic Mucorales in Unselected Soil Samples from France

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Introduction:

Mucormycosis is a life threatening invasive fungal disease that affects a variety of patient groups. Although Mucorales are mostly opportunistic pathogens, originating from soil or decaying vegetations, there is currently few data on prevalence of this group of fungi in the environment. The aim of the present study was to assess the prevalence and diversity of species of Mucorales from soil samples collected in France.

Material and Methods:

Soil samples (fig 1) were collected in different regions. Two grams of soil were homogenized in 8mL of sterile saline containing 0.05% Tween 80. Suspensions were plated on Sabouraud dextrose agar (SDA) and RPMI agar supplemented with itraconazole (4 mg/L) or voriconazole (1mg/L). Both media contained chloramphenicol and gentamicin. The plates were incubated at 35±2°C and checked daily for fungal growth for a maximum of 7 days. Mucorales were subcultured to purity. Each strain was identified phenotypically and molecular identification was performed by ITS sequencing.

Results

A total of 136 soil samples were analyzed. Twenty eight (20.5%) samples were positive for Mucorales. Among these isolates, there were 19 *Rhizopus oryzae,* 8 *Mucor circinelloides* and one *Cunninghamella bertholletiae*. ITS sequencing confirmed the phenotypic identification in all cases (fig 2). Positive soil samples came from cultivated fields (including fields of maize, sunflower and oilseed rape) but also from other type of soil such as flower beds (fig 3). Mucorales were retrieved from samples obtained in different geographical regions of France. Culture media supplemented with voriconazole enhanced the recovery of Mucorales (fig 4).

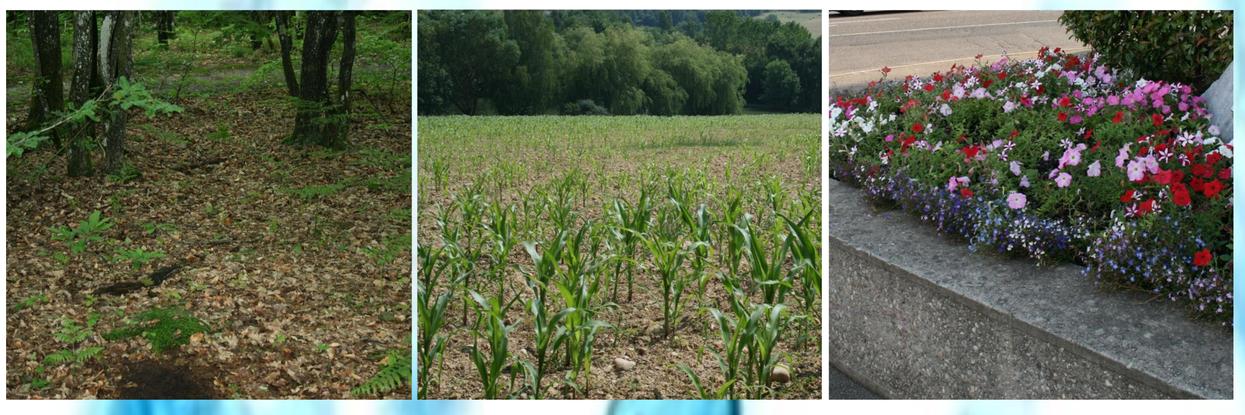

Fig 1. Examples of sampling sites: left to right: Forest, cultivated field and flower bed

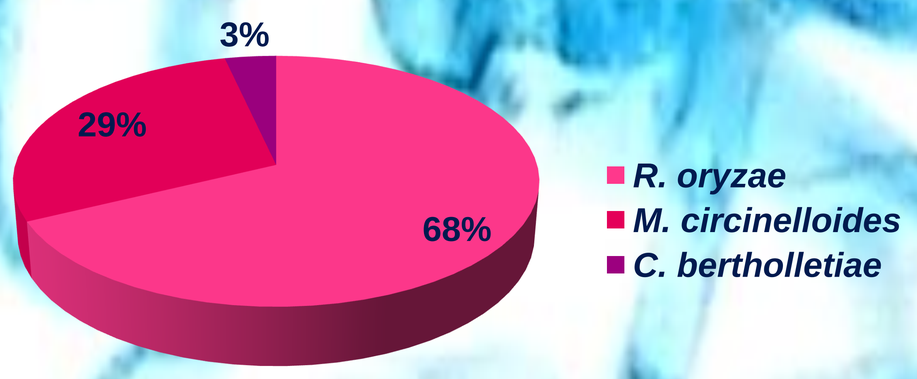

Fig 2. Species distribution: *Rhizopus oryzae: 68% , Mucor circinelloides 29%* and *Cunninghamella bertholletiae 3%*

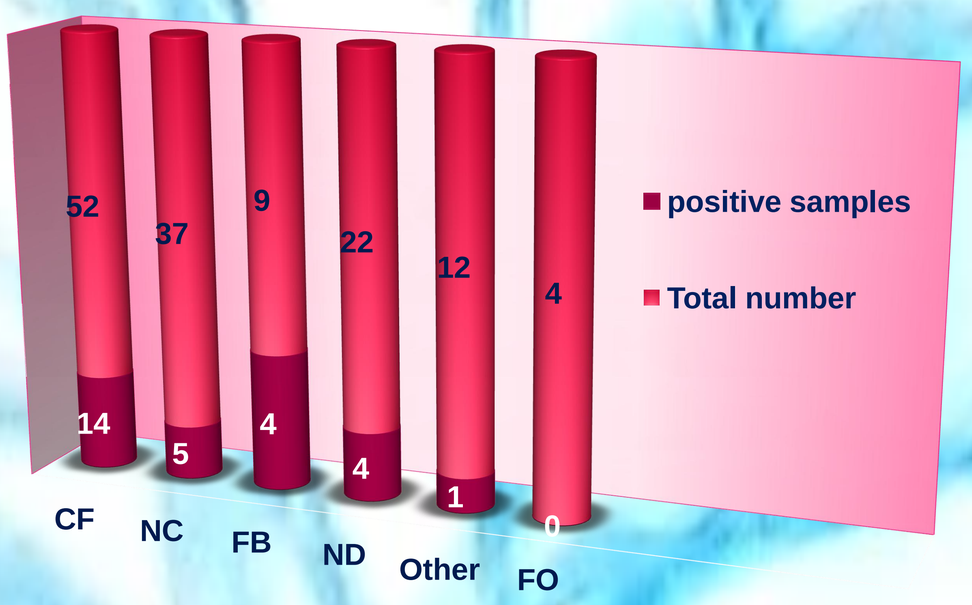

Fig 3. Number of positive samples by type of soil; CF: Cultivated field. NC: Non cultivated; FB: Flower bed; ND: Not determined; FO: Forest

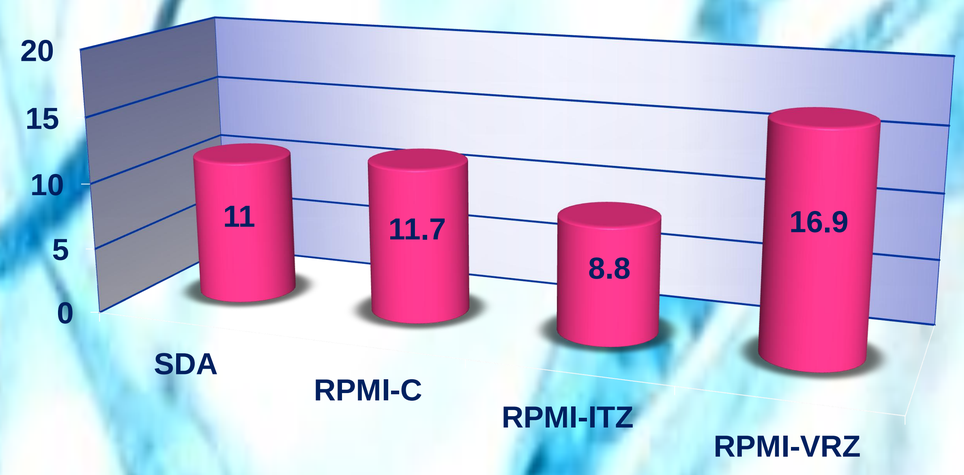

Fig 4. Percentage of positive samples for Mucorales on different media. Sabouraud dextrose agar (SDA), RPMI agar without antifungals (C) or supplemented with itraconazole (ITZ) or voriconazole (VRZ).

Conclusion:

The present study showed that human-pathogenic Mucorales are frequently recovered from soil samples in France. Species diversity should be further analyzed on a larger number of soil samples from different geographic areas in France and in other countries.

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