

Hyphae in focus: comparison of fungal growth on agar with and without SpinAX sample preparation

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Background

In clinical tissue samples, molds typically appear in hyphal form, while spore formation (macro- and microconidia) is largely restricted to superficial growth. For culture-based diagnostics, mycologists fear that homogenization (bead-beating) of tissue could likely damage hyphae¹; therefore, it is recommended to chop tissue into small portions. The SpinAX system (AxonLab) offers automated, standardized, bead-free homogenization using a grinder, but its effect on hyphal integrity and fungal viability remains unclear.

Aim

To assess whether processing of tissue with the SpinAX system affects hyphal structure or growth of molds on agar.

Methods

Mold isolates were embedded in tubes containing liquid Sabouraud (SAB) agar (42 ° C) to inhibit sporulation. After solidification, tubes were incubated at 28 ° C for 1–2 days until fungal growth became visible (agar turbidity). The agar block was removed from the tube: one part was homogenized using SpinAX, the other manually ground. Both preparations were microscopically examined and plated onto SAB agar. After incubation at 28 ° C for 1–2 days, fungal growth and colony morphology were qualitatively assessed (Fig. 1).

Results

A total of 24 culture assays, including 3 negative controls and 14 Isolates (Tab. 1) were included in the study. All isolates showed growth on SAB agar after SpinAX homogenization. Six isolates were tested in duplicates with congruent results (Tab. 1, isolates indicated with (2)). Growth was comparable between conventional and SpinAX-prepared samples. Homogenization with the SpinAX showed a uniform distribution of colonies on the agar and a well separation of individual colonies in the fractions (Fig. 2).

Microscopic examination showed intact hyphal structures after SpinAX homogenization (Fig. 3).

Discussion

SpinAX offers a standardized alternative, potentially valuable for homogenization of spore-deficient clinical samples. Here, we established a reproducible protocol for induction of hyphal growth to simulate the processing of tissue containing fungal structures. We could show that hyphae were not damaged after SpinAX homogenization and that growth was comparable to manually processed 'tissue'. In this artificial setting, we certainly deal with a high density of hyphae, which may not perfectly represent patient samples. Therefore, it remains to be seen whether the method yields comparable results in real patient samples.

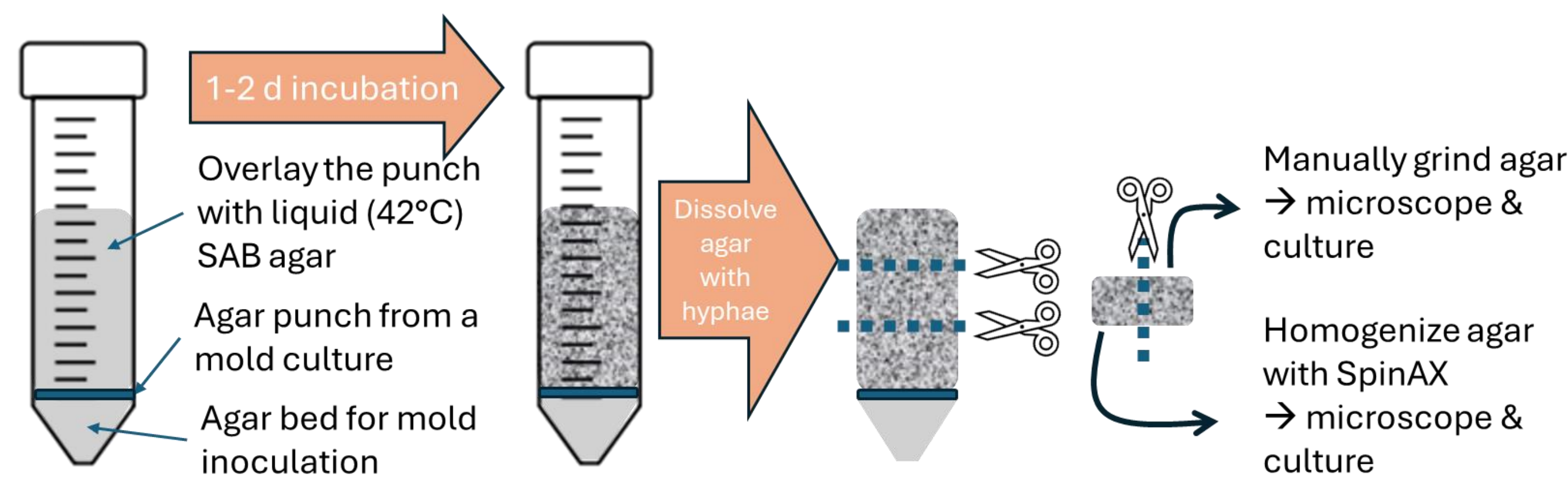
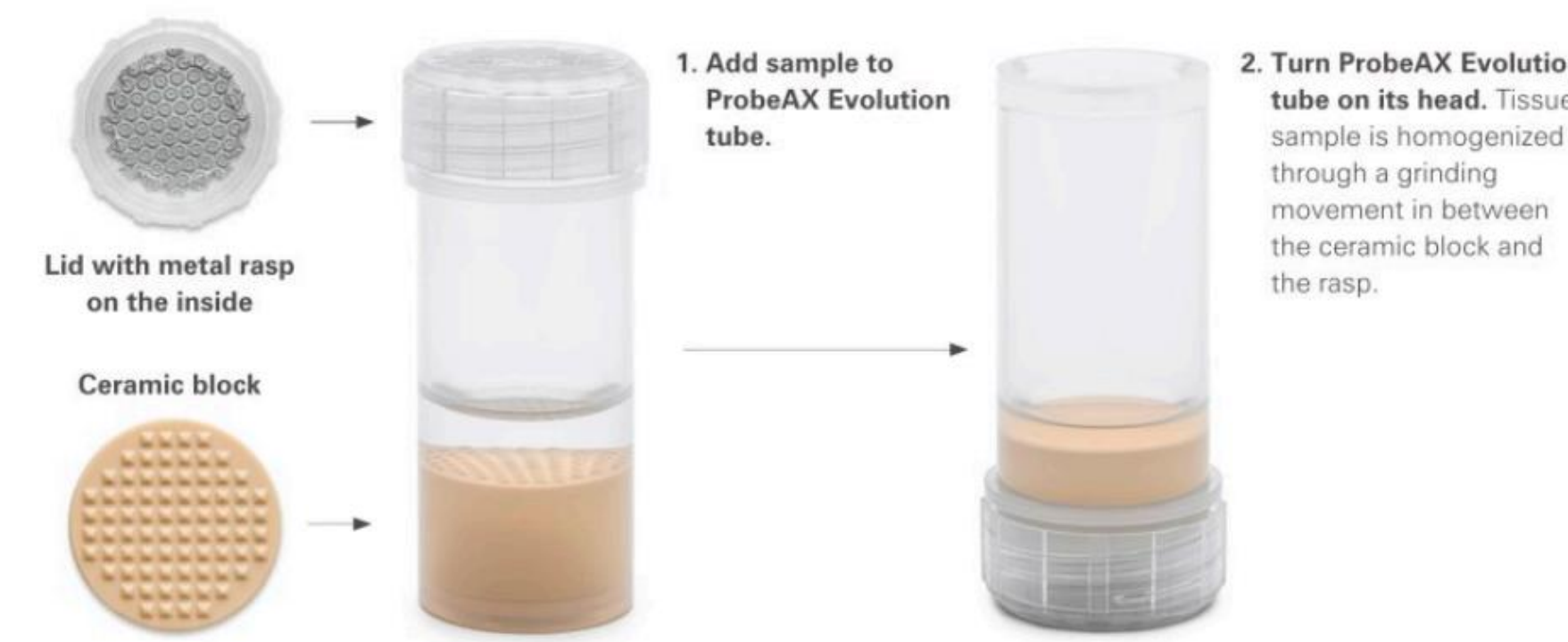


Fig. 1: Study design: An agar punch containing mold was embedded in SAB agar at 42 ° C, a temperature chosen to preserve fungal structures. After 1–2 days of incubation, the agar with grown-in hyphae was removed from the tube and a section was cut: one piece was homogenized using the SpinAX device, while the other served as a positive control and was manually ground before being re-inoculated onto SAB agar.

Species	No. of Isolates
Candida sp.	1
Aspergillus fumigatus	2
Aspergillus niger	1
Aspergillus brasiliensis	1
Aspergillus vitis (2)	1
Microsporum gypseum	1
Penicillium sp.	1
Fusarium solanie complex (2)	1
Trichophyton mentagrophytes complex (2)	2
Mucor cacinelloides (2)	1
Rhizomucor pusillus	1
Rhizops arrhizus (2)	1
total	14

Tab. 1: One Candida and 13 mold species of different origins were used for this study. Six Isolates were tested in duplicate (indicated with (2)).

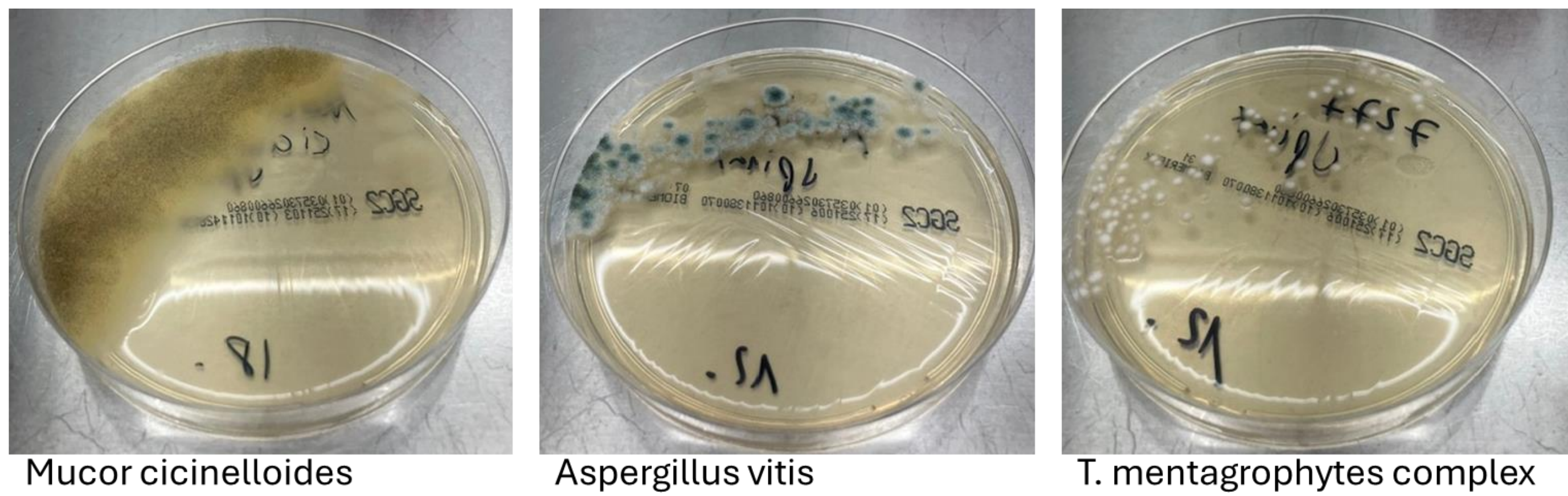
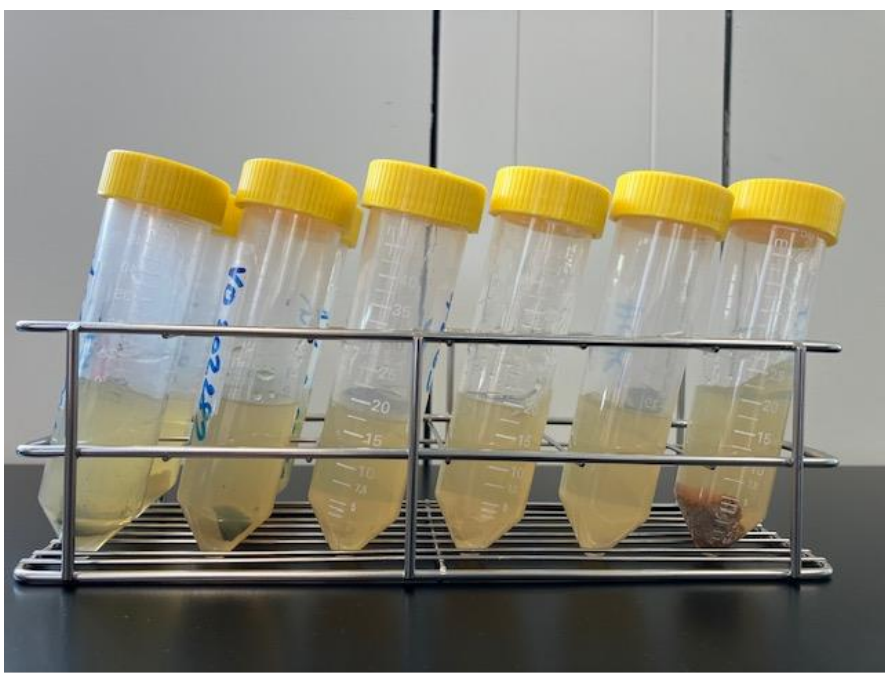


Fig. 2: Growth of mold on SAB after SpinAX homogenization, single colonies are detectable

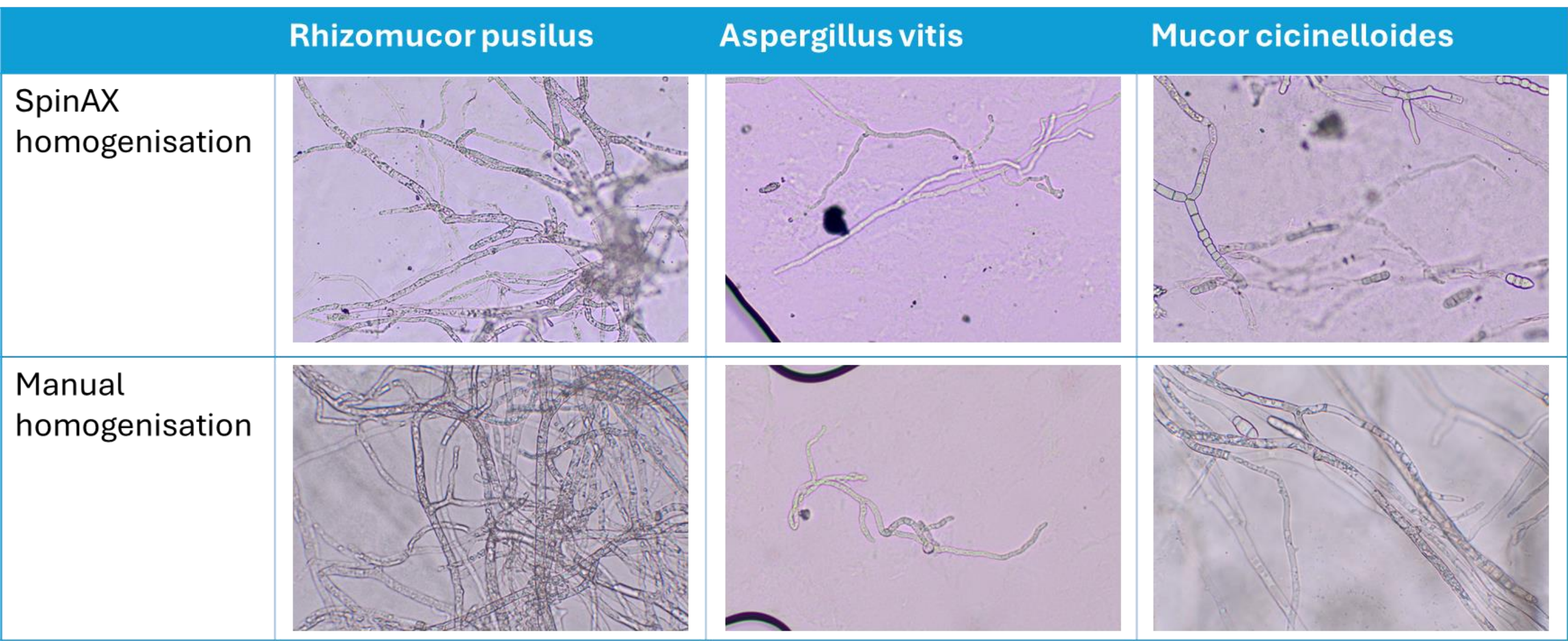


Fig. 3: Microscopic comparison of three mold species following manual and SpinAX homogenization. The images demonstrate that hyphae remain intact after both procedures, indicating that the SpinAX treatment does not cause structural damage.

¹Med Mycol. 2022 Dec 7;60(12):myac081. doi: 10.1093/mmy/myac081. <https://pubmed.ncbi.nlm.nih.gov/36477206/>