Mycobiomics: Isolation and preliminary characterization of keratinophilic fungi from tropical soils of Kenya

HORIZON 2020 project "MYCOBIOMICS"
(MSCA-RISE-2020: Marie Sklodowska-Curie Research and Innovation Staff Exchange GA-No: 101008129)

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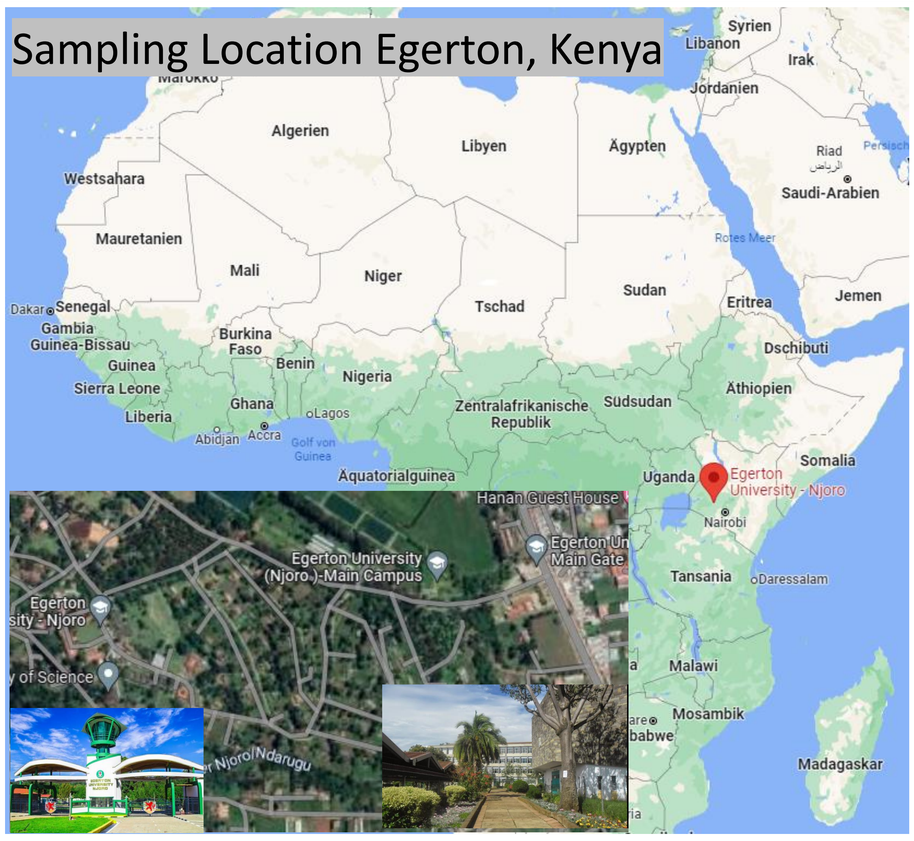
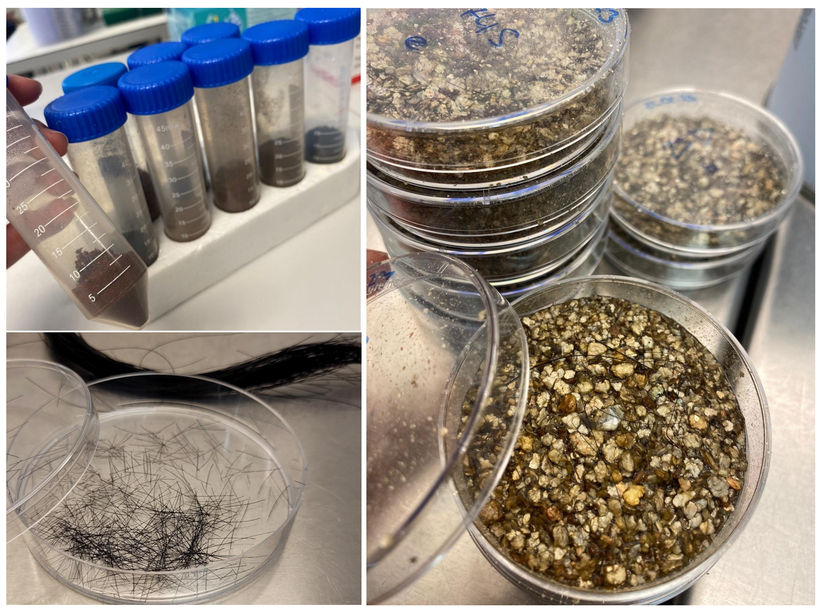
Sampling Location Egerton, Kenya



A total of **125 soil samples** were collected from **82 different spots** within **Egerton University campus** and the region of **Kakamega Forest** during **February-April 2022**. The samples were taken from the rhizospheric (5-10 cm) and deeper soil horizons (50-60 cm).

Isolation of keratinophilic Fungi and Results



Procedure:

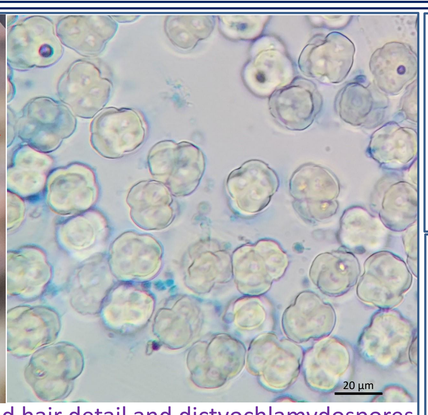
A sample was divided into 5 subsamples. The subsamples (5 g each) were poured into Petri dishes (50 mm in diameter) and mixed with 0.5 g Vermiculite, then soaked with 3-4 mL (depending on moiety of the sample) antibiotic solution containing 0.5 g/L cycloheximide and 0.1 g/L chloramphenicol. **A total of 24 samples were analyzed so far.**

Sterile defatted horse hair fragments (10 pieces of ca 2.0 cm per plate) were used as baits (**horse-hair baiting method**).

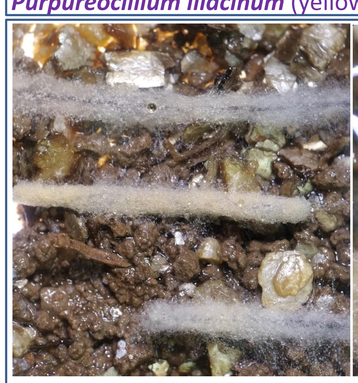
The Petri dishes were then incubated at laboratory temperature (23 ± 1 °C), in darkness, for a period of **2–3 months** and remoistened with sterile deionized water when necessary.

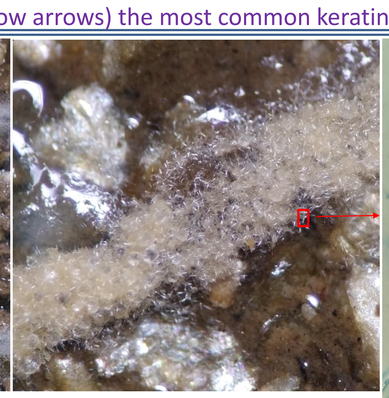
The Petri dishes were checked weekly for the presence of fungi, and isolates were cultured on Malt extract agar (MEA, Merck, Darmstadt, Germany) supplemented with 0.1 g chloramphenicol.

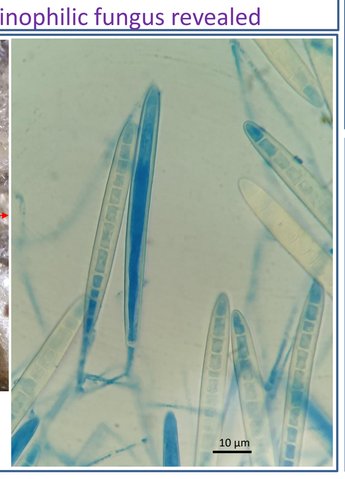
The fungi were identified **phenotypically** and by PCR (**ITS-** and **LSU-** rDNA regions).



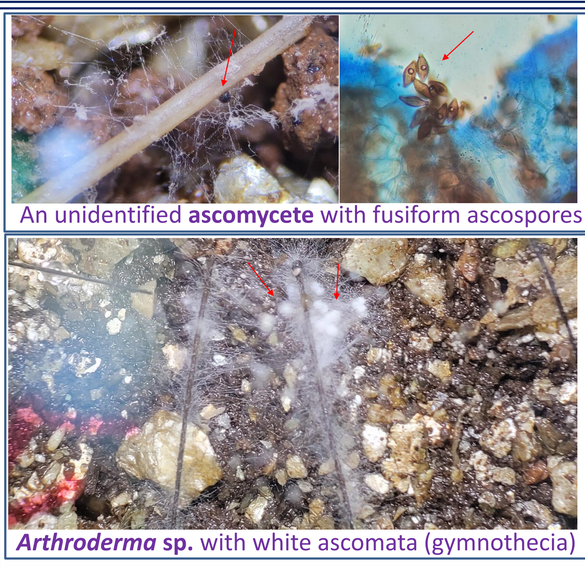
Pochonia chlamydosporia (red arrow/s) hair baits, colonized hair detail and dictyochlamydospores.
Purpureocillium lilacinum (yellow arrows) the most common keratinophilic fungus revealed





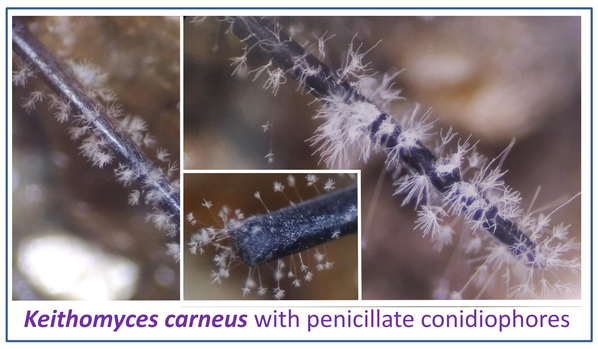


Arthroderma uncinatum = *Trichophyton ajelloi*
(hair baits, colonized hairs detail, cigar-shaped macroconidia)



An unidentified **ascomycete** with fusiform ascospores

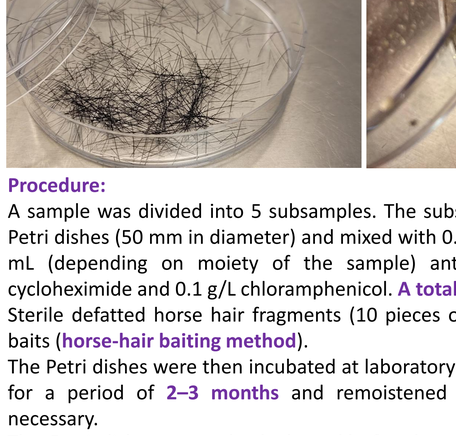
***Arthroderma* sp.** with white ascomata (gymnothecia)



Keithomyces carneus with penicillate conidiophores

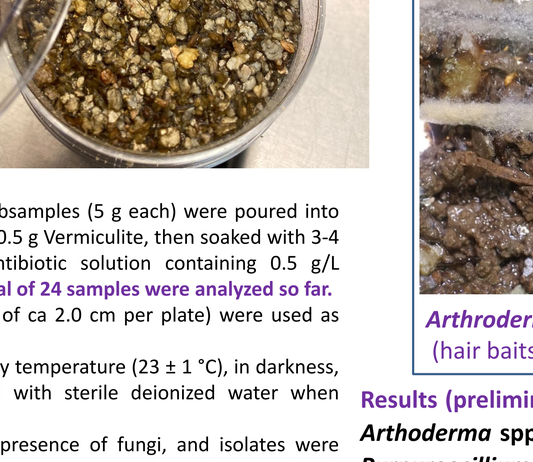
Results (preliminary): The following onygenalean fungi, ***Aphanoascus reticulisporus***, ***Nannizia fulva***, ***Arthroderma uncinatum*** (= ***Trichophyton ajelloi***), and two ***Arthoderma* spp**. were isolated on the hair fragments from **24 soil samples** so far. However, the other keratinophilic fungi (other than onygenaleans), such as ***Purpureocillium lillacinus***, ***Keithomyces carneus***, ***Pochonia chlamydosporia***, and/or ***Clonostachys rosea*** were dominant in nearly all samples investigated here. ***Purpureocillium lilacinum*** has been found in all 24 samples (100% abundance). One fungus was found to be strongly active against ***Candida albicans*** (see below).

ITS+LSU+BEN
(1000 repl.)



Maximum Likelihood tree based on a concatenated set of ITS, LSU and BeN loci for the new taxon (in violet) is compared with species from the same genus *Arthroderma*. *Ctenomyces serratus* CBS 187.61 was used as outgroup.

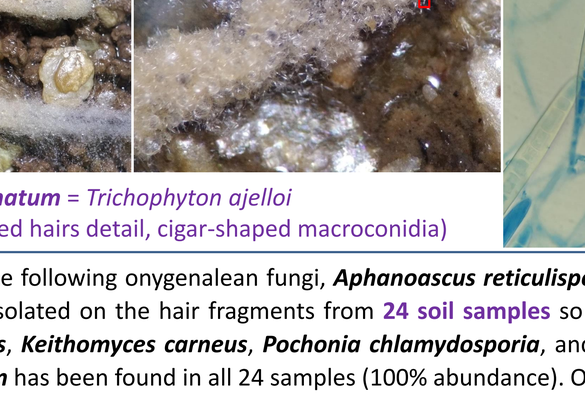
Arthroderma species BiMM-K79



Arthroderma sp. BiMM-K79 (colonies on SDA, at 25°C, in dark, after 18 days; pyriform aleurioconidia on fertile hyphae, stained with methylen blue)

A simple screening method to test anti-microbial activity (agar plug method)

An example of a simple direct screening for antimicrobial properties (vs 6 pathogens) of selected fungal strains (n = 9) grown **on SDA, at 25°C, in dark, for 18 days**.
A total of 54 reactions were observed, with 2 clear inhibitions revealed (vs. *Candida* and *Aspergillus*, see picture at right) by the fungal strain **#5** (**BiMM-K123**)



Aspergillus fumigatus + **activity**

Candida albicans +++ **activity**

Fungal strain #	*SA*	*PA*	*EC*	*KL*	*CA*	*Afu*
1 K-83						
2 K-119	+-					
3 K-118						
4 K-114						
5 K-123					+++	+
6 K-81	+-					
7 K-115	+-					
8 K-79	+-					
9 K-77	+-					

SA, *Staphylococcus aureus* ATCC 6538; ***PA***, *Pseudomonas aeruginosa* ATCC 9027; ***KL***, *Klebsiella pneumoniae* ATCC 10031; ***EC***, *Escherichia coli* ATCC 25922; ***CA***, *Candida albicans* ATCC 10231; ***Afu***, *Aspergillus fumigatus* RL 578.