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Short Communication

Some observations on fungal succession during decomposition of wool in soil

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Fungal succession on woolen baits was studied under laboratory conditions for more than one year. It was found that the initial colonizers on woolen baits are non-keratinophilic fungi, while the late colonizers are keratinophilic fungi. Six phases in total were observed during fungal succession. The successional trends obtained during decomposition of wool in soil samples collected from plain and hilly areas were almost the same, except for the dominant colonization in the last phase, which was constituted by *Chrysosporium tropicum* for the plain, but *Microsporum gypseum* and *M. fulvum* for the hilly area.

Key Words——*Chrysosporium tropicum*; fungal succession; *Microsporum fulvum*; *Microsporum gypseum*; woolen bait.

In natural environments, a variety of factors, abiotic as well as biotic, are responsible for the decomposition of keratinous materials. These materials are quite resistant to decomposition until and unless they are attacked or utilized by substrate-specific microbes known as keratinophilic fungi. In addition, other microbes may interact and enhance the degradation of keratinous material.

Degradation of keratinous material has been studied by various workers (Kunert, 1973, 1976; Kushwaha and Agrawal, 1981; Safranek and Goos, 1981; Wainright, 1982; Nigam and Kushwaha, 1992) but none of them reported the involvement of other soil microbes in the degradation of keratin or the successional trends of microbes which take place. A little information is available on the succession of fungi on keratinous material (Griffin, 1960; Evans and Hose, 1975), but sufficient information on fungal succession on woolen baits is still lacking. The present study aims at providing some clue to the fungal succession of substrate-specific and non-specific fungi and their involvement in the decomposition of wool.

The fungal succession was studied in soil samples collected from Agra (U.P.), Dholpur (Rajasthan) and Mandi (H.P.), India. The soil samples were collected with a sterile plastic spoon from the superficial layer of soil (2–5 cm). Soil types were alluvial, grey-brown alluvial and submontane. Textures of soil were loamy sand, sandy loam and silty clay respectively. The dominant vegetation is formed by species of *Accacia*, *Euphorbia* and grasses in the plain area, while in the hilly area it is formed by *Pinus* trees mixed with other angiosperms.

A portion of 50 g moist weight of each soil sample was taken in sterile Petri dishes. Wool was purchased from British India Co. (Lal Imli), Kanpur, India. The wool was washed with sterilized water, cut into 2-cm pieces, autoclaved at 103.5×10^3 Pa. for 10 min, then used as a bait. For each type of soil, 10 soil samples were taken and amended with sterilized woolen bait. In the Petri dish, the soil and bait were mixed together, and were incubated at $27 \pm 2°C$. The samples were observed microscopically for the appearance of fungi at regular intervals of 15 d for more than 1 yr (one and a half years). Throughout the experiment, the soil moisture was maintained by the addition of sterilized water.

When fungi were detected on the woolen bait, they were isolated by direct transfer of fungal mycelia from the invaded bait onto Sabouraud's dextrose agar (dextrose 40 g, peptone 10 g, agar 15 g, and distilled water 1,000 ml). The fungi isolated were tested for their capability to decompose the wool. For this purpose, keratinolytic activity of fungi was measured by the methods of Carmichael (1962) and Wawrizkiewicz et al. (1991). Fungi that responded positively in both tests were judged to be keratinophilic, and those that responded negatively in either test to be non-keratinophilic.

Frequency of fungi appearing on bait was defined as:

$$\% \text{ Frequency} = \frac{\text{No. of soil samples in which fungus appeared on bait}}{\text{Total no. of soil samples incubated with bait}} \times 100$$

The duration of each phase of colonization was decided on the basis of observation of how long the fungi in a particular colonization were observed together. It was counted from the first day of appearance of visible mycelial growth on bait.

Table 1. Types of fungi appearing on woolen bait.

Keratinophilic fungi[a)]	Non-keratinophilic fungi
Aspergillus niger	*Rhizopus* sp.
Acremonium sp. 1	*Fusarium* sp.
Acremonium sp. 2	*Penicillium* sp.
Acremonium sp. 3	*Aspergillus fumigatus*
Acremonium sp. 4	*Aspergillus* sp.
Chrysosporium tropicum	
Chrysosporium indicum	
Chrysosporium sp. 1	
Chrysosporium sp. 2	
Chrysosporium sp. 3	
Microsporum gypseum	
Microsporum fulvum	
Trichophyton simii	

a) Keratinolytic activity based on their utilization of keratin after the method of Carmichael (1962) and Wawrzkiewicz et al. (1991).

Regular microscopic examination of soil samples baited with wool revealed a successional colonization of keratinophilic and non-keratinophilic fungi (Table 1). *Aspergillus niger* and 12 other keratinophilic species belonging to *Acremonium*, *Chrysosporium*, *Microsporum* and *Trichophyton* were isolated from the samples as decomposers of the wool. On the other hand, only 5 species were non-keratinophilic, appearing during the early period of the decomposition of woolen baits.

The fungal succession on woolen baits with their percentage frequencies is summarized in Table 2. In first phase, four non-keratinophilic fungi appeared, together with *A. niger* as the only keratinophilic species. The constituent fungi of phase 1, *Rhizopus* sp., *A. niger*, *Aspergillus fumigatus*, *Penicillium* sp. and *Fusarium* sp., were observed together for up to 30 d. In second phase, which continued for only 15 d, both keratinophilic and non-keratinophilic species grew together, but keratinophilic ones such as *Acremonium* sp. 1 (43%) and *A. niger* (10%) dominated. *Fusarium* sp., which dominated the first phase declined in the second phase.

The third phase (duration of 45 d) was dominated mainly by *Acremonium* spp. 1, 2 and 3. In this phase, only one non-keratinophilic fungus, *Fusarium* sp., was observed. The change of decomposers of woolen baits from non-keratinophilic to keratinophilic was almost complete in this phase. In the fourth phase, *Acremonium* sp. 3 was still prevalent, occurring in 10% of the sample. However, the occurrence of *Chrysosporium* spp. as late colonizers was particularly characteristic. This phase was found to continue for up to 60 d.

In the fifth phase, species of *Acremonium* disappeared fully and other fungi like *Chrysosporium tropicum* (33%), *Chrysosporium* sp. 3 (7%) and *Trichophyton simii* (3%) were observed on woolen baits. This phase was observed for a duration of 180 d. The sixth phase was occupied only by *C. tropicum* (47%) in the case of soils from plains, and persisted for more than 1 yr. In the sample collected from a hilly area (Mandi, H. P.), the successional trends observed were almost the same except the last decomposing phase, in which *Microsporum gypseum* and *M. fulvum* were encountered in 17 and 13% of the samples respectively (Table 2).

Table 2. Different phases constituted by the fungi during fungal succession on woolen bait.

Type	Duration of phase (d)	Fungal species (% appearance frequency)
Phase 1	30	*Rhizopus* sp. (17), *Aspergillus niger* (27), *Aspergillus fumigatus* (27), *Penicillium* sp. (23) and *Fusarium* sp. (33)
Phase 2	15	*Aspergillus niger* (10), *Fusarium* sp. (27), *Acremonium* sp. 1 (43) and *Aspergillus* sp. (3)
Phase 3	45	*Fusarium* sp. (7), *Aspergillus niger* (3), *Acremonium* sp. 1 (13), *Acremonium* sp. 2 (10) and *Acremonium* sp. 3 (20)
Phase 4	60	*Acremonium* sp. 3 (10), *Acremonium* sp. 4 (3), *Chrysosporium* sp. 1 (10), *Chrysosporium* sp. 2 (3) and *Chrysosporium indicum* (7)
Phase 5	180	*Trichophyton simii* (3), *Chrysosporium tropicum* (33) and *Chrysosporium* sp. 3 (7)
Phase 6[a)]	More than 1 yr	*Chrysosporium tropicum* (47)

a) The soil samples collected from hilly area (Mandi, H. P.) in Phase 6 were dominated by *Microsporum gypseum* (17%) and *M. fulvum* (13%).

The periodical analysis of woolen baits for more than 1 yr indicates that the fungal colonization was initiated by non-keratinophilic fungi (saprophytic fungi), which grew luxuriously on the baits and utilized only non-keratinized part of the substrate. This observation coincides with that of English (1965), who found that saprophytic fungi are able to utilize, as sole source of nutrient, at least some part of various keratinized substrata supplied to them. Non-keratinized intercellular structures such as medullary trichohyalin and soft keratin might undergo digestion. Therefore, in the early period of the incubation, non-keratinophilic fungi formed the dominant colonizers and continued to do so until they had consumed all of the non-keratinized material. In a similar way, Griffin (1960) provided the nutritional background for fungal succession on keratinous material. He stated that the initial colonizers were common soil fungi of high general competitive saprophytic ability, such as *Fusarium* spp., which were finally replaced by slow-growing keratinolytic fungi: geophilic species of *Trichophyton* and *Microsporum*. Exactly the same pattern of succession occurred in our experiment, except that the dominant colonization in the later phases was constituted by the single genus of *Chrysosporium*. The late dominancy of *C. tropicum* may be attributed to its antagonistic activities, which are known to produce a variety of enzymes

and secondary metabolites, and its inhibitory effect against growth of other fungi (Nigam and Kushwaha, 1990). For this reason it was the only species growing on the woolen baits even after 1 yr of incubation.

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