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# Comparative Cellulolytic Ability of Microfungi Inhabiting Various Types of Litter

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Relative cellulolytic ability of seventy three different litter decomposing microfungi was measured *in vitro* by column clearance technique based on the clearing of acid swollen cellulose. All the fungi have been divided into four different groups on the basis of the nature of clearing of cellulose. Maximum depth of clearing has been shown by *Trichoderma harzianum* and species of Fusaria, Aspergilli and Penicillia. Pioneer colonizers of different types of litter had good cellulolytic ability.

## Introduction

Cellulose is the major constituent of plant cell walls and cellulolysis plays a vital role in nature, such as in germination of seeds, nutrition of ruminant animals, and conversion of billion of tons of plant materials to organic matter which remains in a bound and complex form, and in releasing energy rich compounds in soil. In fact, cellulolysis maintains the essential balance in nature and greatly contributes to sustenance of life on earth. There are numerous reports of fungal colonization of different types of litter from different parts of the globe, which have recently been reviewed in Biology of Plant Litter Decomposition (Dickinson & Pugh 1974) and Mehrotra and Aneja (1979), but there are a few reports on the capacity of microfungi to utilize cellulose *in vitro* (Garrett 1962, 1963 a, 1966, Hogg 1966, Reese & Levinson 1952, Rai 1970, Dwivedi & Singh 1974 and Sharma 1974). Garrett (1963b) suggested that the success of a fungus in competitive saprophytic colonization of any particular substrate is dependent, in its first place on its intrinsic biochemical ability to exploit or decompose that substrate i.e. the production of enzymes. The second requirement is the ability to succeed in competitive saprophytic colonization and which is conditioned by three factors: (i) competitive saprophytic ability of the particular fungus, (ii) its inoculum potential and (iii) environmental conditions, including the population of competing fungi and other soil microorganisms. The present study compares the relative cellulolytic ability of microfungi isolated from different types of litter by column clearance technique (Rautela & Cowling 1966).

## Materials and Methods

Isolation of the microflora from the litter of *Chenopodium album, Desmostachya bipinnata* and mixed litter of grasses which were kept at different positions i.e. soil surface, below the soil surface at 5 to 10 cm depth and

mid-canopy height (½ meter above the soil surface) in the grassland of Kurukshetra University campus was made as described earlier (Mehrotra & Aneja 1979).

In column clearance technique (Rautela & Cowling 1966) the composition of the medium employed (Tansey 1971) was as follows: cellulose suspension obtained from 6 g of air-dried Whatman Column Chromedia Cellulose Powder CFII, swollen in 85% orthophosphoric acid (160 ml) for 2 hr at 4°C was regenerated, washed in the cold with distilled water followed by 1% $Na_2CO_3$ and then with distilled water again until neutral and added to the medium of following composition:

| | |
|---|---|
| $NH_4H_2PO_4$ | 2.0g |
| $KH_2PO_4$ | 0.4g |
| $MgSO_4.7H_2O$ | 0.89g |
| Yeast extract | 0.5g |
| Adenine | 4.0mg |
| Adenosine | 8.0mg |
| Thiamine HCl | 100.0µg |
| Agar | 17.0g |
| Distilled water | 1 litre |

Sterilized vertical columns of the medium (6 cm in height) were made in culture tubes (20 × 150 mm). Discs of inoculum (a culture on agar media) were placed on the surface of the medium and the culture tubes were incubated upright in beakers containing water, and were sealed in polyethylene bags. The depth of clearing was measured at seven-day intervals.

**Results and Discussion**

All the litter-colonizing fungi tested for cellulolytic ability have been categorized into four groups on the basis of the nature of clearance of cellulose: (1) those which formed a zone of clearance with a clearly defined front (table 1); (2) those with diffused clearance (table 2); (3) those which formed distinct clearance but the zonal front was not sharp enough for precise measurement (table 3); and (4) those which did not clear acid swollen cellulose at all [for example, *Alternaria humicola* Oudemans, *Aspergillus niger* Van Tiegham *Bipolaris tetramera* (Mc Kinney) Shoemaker, *Coniothyrium palmarum* Corda, *Epicoccum purpurascens* Ehrenb ex. Schlecht, *Myrothecium striatisoporum* Preston, *Periconia minutissima* Corda, *Thielavia minor* (Rayss & Borut) Malloch & Cain, *Trichothecium roseum* (Pers.) Link ex Fries, *Trichurus spiralis* Hass., *Phoma glomerata* (Corda) Wollenw. & Hochapf., *Phoma* spp. I & II and Hyaline sterile form A].

Various species of fungi tested above, differed in the rate of clearing of cellulose. Several members of Deuteromycotina have exceedingly high cellulolytic rates in comparison to Ascomycetous species. *Trichoderma harzianum* and *Aspergillus flavus* dissolved the test cellulose almost twice as rapidly as the most vigorously cellulolytic Ascomycetes tested *(Chaetomium erectum)* (figure 1). Several other species of Deuteromycotina had high cellulolytic rates (tables 1 and 2).

Cellulolytic species showed differences in completeness of clearing of cellulose. There was no correlation of these differences with rates of clearing. For example, although many species of *Curvularia, Fusarium solani, Drechslera australiensis, D. rostrata, Trichoderma harzianum, Aspergillus flavus* were highly cellulolytic, clearing was quite complete in *T. harzianum, F. solani* whereas clearing was diffused in others and even in others scattered small particles of undissolved cellulose remained.

Different isolates of the same species usually showed similar cellulolytic rates (table 1) but two isolates of the *Trichoderma harzianum* tested here differed greatly in cultures and the major difference in depth of

Table 1 *Relative cellulolytic activity of litter inhabiting fungi forming clear columns in agar-diffusion assay*

| Species | Depth of clearing of acid swollen cellulose suspension in agar column | | | | |
|---|---|---|---|---|---|
| | Depth of clearing (mm) (Average of three replicates) | | | | |
| days | 7 | 14 | 21 | 28 | 35 |
| *Trichoderma harzianum* Rifai (Strain I) | 8 | 13.5 | 19 | 22.5 | 30 |
| *Fusarium solani* (Mart.) Sac. | 6.5 | 10 | 17 | 17 | 23 |
| *Penicillium piceum* Raper & Fennell (Strain I) | 7 | 13 | 17 | 20 | 23 |
| *Aspergillus rugulosus* Thom & Raper | 7 | 11 | 13 | 20 | 22 |
| *A. terreus* Thom. | 7 | 11.5 | 14 | 18 | 22 |
| *Aspergillus* sp. | 5.5 | 11 | 15.5 | 18 | 22 |
| *Penicillium piceum* Raper & Fennell (Strain II) | 7.5 | 12 | 16 | 19 | 22 |
| *Penicillium indicum* Sandhu & Sandhu | 7 | 11 | 15 | 18 | 21 |
| *P. oxalicum* Curr et Thom. | 6 | 11 | 14.5 | 17 | 20 |
| *P. funiculosum* group | 7 | 10 | 14 | 17 | 20 |
| *Aspergillus sulphureus* (Fres.) Thom. & Church | 6.5 | 11 | 16 | 18 | 19 |
| *A. niveus* Blochwitz | 4 | 7 | 8 | 12 | 19 |
| *A. fumigatus* Fresenius | 4.5 | 8 | 11.5 | 16 | 18 |
| *Fusarium lateritium* Nees ex Fries | 6 | 9 | 13 | 15 | 18 |
| *Aspergillus awamori* Nakazawa | 4.5 | 8 | 10 | 13 | 17 |
| *A. japonicus* Saito | 5 | 9.5 | 12 | 15 | 17 |
| *Chaetomium erectum* Skolko & Groves | 4 | 7.5 | 12.5 | 14 | 17 |
| *Penicillium funiculosum* group | 4.5 | 8 | 12 | 14 | 17 |
| *Stachybotrys atra* Corda | 2 | 8 | 9 | 13 | 17 |
| *Trichoderma harzianum* Rifai (Strain II) | 6 | 8 | 11 | 12 | 17 |
| *Aspergillus luchuensis* Inui | 6 | 9.5 | 12 | 15 | 16 |
| *Fusarium semitectum* Berk & Rev. | 5.2 | 9 | 9 | 13.5 | 16 |
| *Aspergillus nidulans* (Eidam) Winter (Strain II) | 4 | 9 | 10.5 | 13.5 | 15 |
| *Phoma sorghina* Sacc. | 5 | 9 | 11 | 13 | 14 |
| *Stachbotrys atra* Corda var. *microspora* Mathur & Sankhla | 1.5 | 6 | 8 | 12 | 14 |
| *Penicillium variabile* Sopp. | 3 | 6 | 9 | 12 | 14 |
| *Aspergillus candidus* Link | 4 | 7 | 9 | 12 | 13 |
| *Hyalostachybotrys bisbyi* Srinivasan | 2 | 5 | 6 | 10 | 13 |
| *Penicillium varians* G. Smith | 3.5 | 7 | 8.5 | 11 | 12 |
| *Sporothrix cyanescens* de Hoog & de Vries. | 6 | 9 | 10 | 11 | 12 |
| *Aspergillus terreus* var. *aureus* | 4 | 5 | 6 | 8 | 10 |
| *Chaetomium globosum* Kunze | 3.5 | 4.5 | 7 | 9 | 10 |
| *Pithomyces atro-olivaceus* (Cooke & Harkness) M. B. Ellis | 3.7 | 6 | 6 | 9 | 10 |
| *Acremonium implicatum* (Gilm. & Abbott) W. Gams=*A. terricola* (Millar al) W. Gams | 2.3 | 5 | 5 | 9 | 9 |
| *Colletotrichum dematium* (Fr.) Grove | 2.5 | 4 | 5,5 | 8 | 9 |
| *Verticillium dahliae* Kleb | 2 | 4 | 4.5 | 6 | 7 |
| *Memnoniella echinata* (Riv.) Gallowig | 0 | 0 | 2 | 5 | 6.5 |
| *Acrophialophora fusispora* (Saksena) Samson | 0.5 | 0.5 | 1 | 1 | 2 |

**Table 2** *Relative cellulolytic activity of litter inhabiting fungi showing diffused column in agar-diffusion assay*

| Species | Depth of clearing of acid swollen cellulose suspension in agar column | | | | |
|---|---|---|---|---|---|
| | Depth of clearing (mm) (Average of three replicates) | | | | |
| days | 7 | 14 | 21 | 28 | 35 |
| *Aspergillus flavus* Link. (Strain II) | 11 | 17.5 | 21.5 | 27 | 29 |
| *A. flavus* Link. (Strain I) | 9 | 15.5 | 21 | 24.5 | 27 |
| *Curvularia penniseti* (Mitra) Boedijn | 6 | 12 | 16 | 20 | 25 |
| *Drechslera australiensis* (Bugnicourt) Subram. & Jain ex M. B. Ellis | 5 | 11 | 15 | 17 | 22 |
| *Curvularia indica* Subram. | 4 | 7 | 10 | 15 | 20 |
| *C. lunata* (Wakker) Boedijn | 4 | 10 | 13 | 18 | 20 |
| *C. pallescens* Boedijn | 5 | 11 | 12 | 15 | 20 |
| *C. clavata* Jain | 6 | 9 | 12 | 15 | 19 |
| *Tiarosporella madreeya* (Subram. & Ram) Nag Raj | 5 | 11 | 14 | 15 | 17 |
| *Aspergillus nidulans* (Eidam) Winter (Strain I) | 7 | 9 | 12 | 12 | 12 |
| *Paecilomyces varioti* Bainier | 2 | 6 | 8 | 9 | 12 |
| *Myrothecium verrucaria* (Abb. & Schw.) Ditm ex Fr. | 2 | 5 | 5 | 9 | 10 |
| *Penicillium islandicum* Sopp | 5 | 6.5 | 7 | 8 | 10 |
| *Humicola fuscoatra* Traaen | 2 | 6 | 6 | 7.5 | 9 |
| *Aspergillus nidulans* (Eidam) Winter var. *acristatus* | 4.5 | 6 | 8 | 9 | 9 |
| *Achaetomium strumarium* Rai & al | 0 | 1 | 3.5 | 6 | 8.5 |
| Hyaline sterile form A | 2 | 4 | 4 | 7 | 8 |
| *Myrothecium roridum* Tode ex Fr. | 0 | 1 | 3 | 4.5 | 7 |
| *Cladosporium cladosporioides* (Fresen) de Vries | 2.5 | 3.5 | 5 | 6 | 6 |
| *Thielavia sepedonium* Emmons=*Coryanauscus sepedonium* (Emmons) V. Arx. | 0 | 0 | 0 | 3 | 3 |
| *Acremonium persicinum* (Nicot) W. Gams | 0.5 | 0.5 | 1.5 | 1.5 | 1.5 |

clearing at 35 days between Ist (30 mm) and 2nd (17 mm) isolate might reflect a species level difference. The rate of clearing found for second isolate (17 mm in 35 days) agrees (15 mm and 16 mm) with that obtained by Rautela and Cowling (1966) and Tansey (1971) for *T. viride* respectively.

The pattern of fungal succession followed was almost that of Hudson (1968) which can be represented as follows:

Common primary saprophytes (Deuteromycotina) → Restricted primary saprophytes → Secondary saprophytes (Ascomyotina and Deuteromycotina)

The ability to utilize cellulose is often regarded as an essential for saprophytic fungi (Melin 1948). According to the scheme proposed by Hudson (1968) the common primary saprophytes of different types of litter after senescence stage included *Cladosporium herbarum*, *Alternaria tenuis*, *Epicoccum nigrum*, *Aureobasidium pullulans* and *Botrytis cinerea*. On some substrates all are present while on others only one. These saprophytes isolated from various substrates (White et al. 1948, Siu 1951, Reese & Levinson 1952, Hancock et al. 1964) have

**Table 3** *Relative cellulolytic activitiy of litter inhabiting fungi showing no clear columns in agar-diffusion assay*

| Species | Depth of clearing of acid swollen cellulose suspension in agar column | | | | |
|---|---|---|---|---|---|
| | Depth of clearing (mm) (average of three replicates) | | | | |
| days | 7 | 14 | 21 | 28 | 35 |
| *Curvularia inaequalis* (Shear) Boedijin | 9 | 14 | 18 | 25 | a* |
| *Drechslera rostrata* (Drechsler) Richardson & Fraser | 5 | 10 | 14 | a | a |
| *Alternaria alternata* (Fr.) Keissler=*A. tenuis* C. G. Nees | a | a | a | a | a |
| *A. longissima* Deighton & McGarvie | 7 | 12 | 17 | 20 | a |
| | | | approx. | approx | |
| *A. tenuissima* (Kunze ex Pers.) Wiltsh | a | a | a | a | a |
| *Aspergillus nidulans* (Eidam) Winter (a typical strain) | 8.5 | 15 | 18.5 | 23 | a |
| *Bipolaris spicifera* (Bainier) Subram. | a | a | a | a | a |
| *Drechslera hawaiiensis* (Bugnicourt) Subram. & Jain ex M. B. Ellis | a | a | a | a | a |
| *D. halodes* (Drechsler) Subram. & Jain | a | a | a | a | a |
| *Humicola grisea* Traaen | a | a | a | a | a |
| *Pithomyces sacchari* (Speg.) M. B. Ellis | a | a | a | a | a |

a=definite clearing but the front not enough for precise measurement.

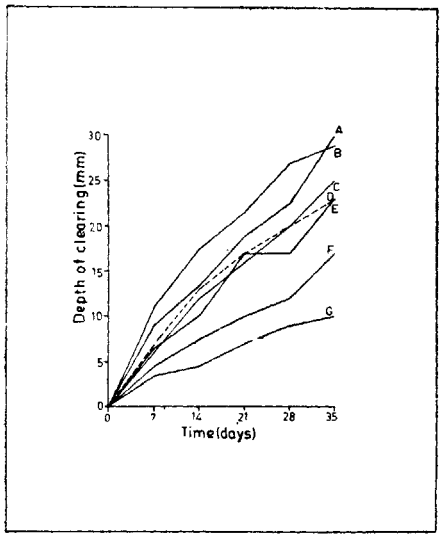


**Figure 1** Depth of clearing of acid-swollen cellulose by selected species of Deuteromycotina and Ascomycotina. *A, Trichoderma harzianum; B, Aspergillus flavus; C, Curvularia penniseti; D, Penicillium piceum; E, Fusarium solani; F, Chaetomium erectum;* and *G, Chaetomium globosum.*

observed variable cellulolytic ability i.e. from strongly cellulolytic to non-cellulolytic. Macauley and Thrower (1966) found that the fungi capable of utilizing cellulose or pectin are the important initial colonizers. Our results indicate that the primary saprophytes of different types of litters (i.e., *Chenopodium album, Desmotachya bipinnata* and mixed litter of grasses) in general have good cellulolytic ability as exemplified by the depth of clearing of cellulose. For example, the depth of clearing shown was 27–29 mm in *Aspergillus flavus*, 23 mm in *Fusarium solani*, 22 mm in *Aspergillus terreus*, 20 mm in *Penicillium oxalicum*, 19 mm in *Aspergillus niveus*, 18 mm in *A. fumigatus*, 16 mm in *A. luchuensis*, 14 mm in *Phoma sorghina* and 13 mm in *Aspergillus candidus* while in the case of *Alternaria alternata (=A. tenuis), A. tenuissima* and *Drechslera hawaiiensis*, although clearing was definitely there but due to the dark colour of the mycelium it was not easy to detect the exact zone of clearance.

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