Studies on Soil Fungi from Teak Forests of Gorakhpur IV. Isolation of Fungi by Soil Plate and Soil Dilution Plate Methods

By Kamal and K. S. Bhargava

Department of Botany, University of Gorakhpur, Gorakhpur, U. P. India

Introduction

The study of soil fungi presents peculiar problems due to the nature of their habitat. Soil being opaque, one can not see the fungi, in situ, as to how they multiply and grow. A soil fungus to be studied has to be isolated and cultured in the laboratory in a petridish, an environment which is least comparable with its natural habitat. Garett (1956) has aptly remarked that "with the plate count method one identifies what one cannot see, where as with the direct method one sees what one cannot identify".

Direct microscopic observation methods have been used by several mycologists like Conn (1922), Jones and Mollison (1948), Kubiena (1938), Burges and Nicholas (1961), Nicholas, Parkinson and Burges (1965) and Hering (1966). Use of the methods is rather limited specially because the species cannot usually be identified and quantitative estimation is rather difficult. Inspite of this drawback, the information which is collected by these methods, cannot be made available by any other means.

Soil dilution plate method, first developed by bacteriologists to count bacterial numbers, was adopted by Waksman (1927) to study the soil fungi. Since then it has been used by various workers and a large number of fungi has been isolated by employing different media which act as selective substrate for the different groups. Besides, this method has been widely used for comparison of microflora of different groups of soils, specially in quantitative amounts. There are, however, certain disadvantages of this method. In adilution plate, one is usually counting only the fungal spores, since proportionaltey very few colonies arise from fungal hyphae in the plate. Here profusely sporing types are always better represented in the counts. Moreover, some of the heavier parts of the soil along with fungal material sink down rapidly to the bottom of the vessel even before the transferring of the suspension is accomplished. These carry some of the species which thus fail to be represented in the plate.

Warcup (1950) designed the soil plate method which has reduced

some of these disadvantages. Here, one deals with the entire pinch of the soil used for the isolation and nothing is left behind as in the soil dilution plate technique. Papavizas and Davey (1959) proposed a modification in Warcup's medium and substituted the use of acid by a broad apectrum antibiotic, Chlor-tetracycline, to check the growth of bacteria in the isolation plate. They also added Sodium propionate and ox-gall to reduce the colony size of the fast growing fungi so as to make the count and isolation easier.

Material and method

A. Site selected:

Three teak stands of different ages (15, 30, and 45 years) at a distance of about 10 Kms. from the laboratory were selected for the study. These are situated in the South Ramgarh Block of Tilkonia range in Gorakhpur division. The stands are mentioned as Road side teak stand, All India teak stand and Campbell teak stand respectively. These stands are adjacent to each other, about 500 meter apart. Road side teak stand as evident by its name is situated just by the side of Gorakhpur-Kasia road. All India teak stand is surrounded on three sides by tall and dense stands of *Shorea robusta* and is open on its easten side approached by a path linking with Gorakhpur-Kasia road. Campbell teak stand is situated by the side of a seasonal stream known as Turah Nala on its eastern side. On the north side the stand is facing Gorakhpur-Kasia road and is completely open. On the west and south side the neighouring forest of *Shorea robusta* has been thinned up for reafforestation.

B. Soil sampling method:

18 inch deep pits were dug at the site of soil collection. The sides of the pits were scraped by a sterilised spatula to avoid contamination. Half an inch soil from the ground surface was also removed so as to eliminate litter and other fallen materials. For taking soil samples, a sterilized cork borer was pushed horizontally into the prepared face of the pits and the sample thus caught was emptied into the sterilised polythene bottles. Ten samples were taken from each site and the soil samples collected from the same depth of these pits were mixed together. Isolation of fungi and determination of chemical and physical properties of the soil were made from this composite soil sample. The soil samples were invariably plated on the day of collection and the pH and moisture content of the soil determined. The residual soil samples were kept in bottles for the analysis of other constituents.

C. Timing number and horizons of soil sampling:

Soil samples were collected thrice a season i. e. in the begining, middle and end, from each of the three teak stands under investiga-

tion. Each time, 2 soil samples were collected, first representing upper six inches of the soil profile and the second 7—12 inches. The number of sample collected within a season from the three teak stands, was thus 18, the total number of samples collected during the two years of investigation being 108.

D. Investigation of Fungal flora:

Of the 41 media tried, Papavizas and Davey (1959) recommended a modification of Warcup's medium for the isolation of fungi from soil. They added, to the original ingredients, ox-gall and sodium propionate so as to check the spread of fast growing colonies and a broad spectrum antibiotic (Chlortetracycline) for checking the bacteria. In the present study also, modified Warcup's medium (Papavizas and Davey, 1959) was used for the isolation. Soil dilution plate (Waksman, 1927) and Soil plate method (Warcup, 1950) were employed for the isilation. The purification of culture was done either by single spore isolation or by cutting of the hyphal tips. The pure cultures were transferred to fresh agar slants of various media viz Czapek's malt extract, potato dextrose, synthetic mucor and oat meal according to need.

All the cultures were stored in a refrigerator running at 8—10° C. Cultures of new taxa have been deposited in one or more culture collections of the world.

Aspergilli and Penicillia were identified on the basis of standardised procedure of Raper and Fennell (1965) and of Raper and Thom (1949). Members of mucorales were diagnosed on synthetic mucor agar (Hasseltine, 1954 and 1955). Chaetomiaceae were studied and identified with the help of a recent monograph by Mazzucchetti (1965). For others, several books and monographs were consulted.

Two cultures producing spores could not be identified with the available resources and have been referred to as UK-1 and UK-2. Four isolates designated as SK-1, SL-2, SK-3 and SK-4 remained sterile.

Observations and results

Fungi isolated by two techniques from three teak stands of different ages are being listed in the following tables. It is to be noted that the isolation was made from two horizons as mentioned in the text but in the table the fungi have been presented irrespective of their space of horizontal distribution. Although the isolation was made in different seasons but the seasonal occurrence of fungi has also not been taken in to account. A consolidated account of the frequency of fungi isolated by two techniques is presented in table 1.

Discussion

As noted earlier soil dilution plate and soil plate methods were used for the isolation of fungi during the present course of investigation. An analysis of the table with the fungi isolated, reveals that the total number of species isolated by dilution plate method from all the three teak stands is 313 as compared to 338 isolated by soil plate method. This shows that soil plate method has a slight edge over the dilution plate method and justifies the view of Warcup (1960). It is also clear that most of the forms which sporulate heavily, are more abundant on dilution plate.

It is clear that two species viz., *Penicillium charlesii* and *Circinella mucoroides* could be isolated by dilution plate method only. There are other species, however, which could be isolated only by soil plate method. These were *Chaetomium apiculatum, C. bostrychodes C. biapiculatum, Scopulariopsis bravicaulis, S. brumptii, Torula herbarum* and *T. herbarum* f. *quaternella*. This is rather significant because all these fungi are cellulose decomposers and it appears that possibly small particles of the substance might have been adhering to the soil particles and would have given a fillip to the intitial growth of these forms and helped them to grow.

Acknowledgement

Authors feel pleasure in expressing their grateful thanks to the State Council of Scientific and Industrial Research, Uttar Pradesh, for the financial help which made this work possible.

Table 1

Abundance of fongal species isolated by two techniques

Species isolated	Tecniques DP	SP
Absidia ramosa	++++	++++
A. spinosa	+++	+++++
Gongronella butleri	++	+++
Rhizopus arrhizus	+	+
R. stolonifer	+	++
Circinella mucoroides	+++	−
C. muscae	++	++
C. umbellata	++	+++
Mucor bainieri	++	+
M. dispersus	++	++
M. genevensis	+++	+
M. hiemalis	+++++	+++++
M. luteus	+++	+
M. racemosus	+++	+
M. subtilissimus	−	++

Species isolated	Techniques DP	SP
Mortierella sp.	+	++
Choanephora cucurbitarum	+	+
Cunnighamella bertholletiae	++	++
C. echinulata	++	++
C. elegans	+	+
Syncephalastrum racemosum	+++	++
Zygorhynchus moelleri	+	+
Thielavia terricola	−	++
Chaetomium abuense	+	+++
C. apiculatum	−	−
C. biapiculatum	−	+++
C. bostrychodes	−	++
C. globosum	+	++
C. indicum	+	+
C. spirale	−	+
Chaetomium sp.	+	++
Gelasinospora sp.	+	++
Neocosmospora vasinfecta	++	+++
Phoma hibernica	++	++
Geotrichum candidum	+	+
Monilia candida	+++	+
M. sitophila	+	++
Cephalosporium curtipes	+++	++
C. acremonium	+++	++
C. coramioides	+	++
Cephaliophora irregularis	+	++
Trichoderma viride	+++	+++
Aspergillus aculeatus	++	++
A. amstelodami	+	+
A. avenaceus	++	+
A. awamori	+++	++
A. candidus	+++	+
A. carbonarius	++	+
A. cervinus	+	+
A. chevalieri	++	++
A. clavatus	+	+
A. carneus	++	++
A. fischeri	++	++
A. flavus	+++++	+++
A. flavipes	+++	++
A. foetidus	++	++
A. fumigatus	++++	++++
A. giganteus	++	+
A. gorakhpurensis	+	+
A. japonicus	+++	++
A. lanosus	+	+
A. montevidensis	++	++
A. niger	+++	+++
A. nidulans	++	++
A. niveus	++	+
A. ochraceous	++	+++
A. oryzae	+	+

Species isolated	Techniques DP	SP
A. panamensis	+	+
A. paradoxus	+	+
A. phoenicis	++	+
A. puniceus	++	++
A. proliferans	+	+
A. quadrilineatus	++	+++
A. ruber	+	+
A. rugulosus	+	++
A. sclerotiorum	++	++
A. subolivaceus	++	+
A. sulphureus	+	+++
A. sydowi	+++	+++
A. tamarii	+	+
A. terreus	++++	++++
A. terreus var. *africanus*	+	+
A. terreus var. *aureus*	+	+
A. ustus	+	+
A. ustus (AK 1)	+	+
A. variecolor	+	++
A. versicolor	++	++
A. wentii	++	+
Penicillium brefeldianum	+	+
P. charlesii	+	−
P. chysogenum	++	++
P. digitatum	+	+
P. expansum	+	+
P. frequentans	+++	++
P. funiculosum	+	++
P. humicola	+	++
P. luteum	+	+
P. nigricans	+	+
P. notatum	++	++
P. oxalicum	++	+
P. rocqufortii	++	++
P. spiculisporum	+++	+
P. steckii	+	+
P. terrestre	+++	++
P. variabile	+++	++
Penicillium (PK 1)	+	+
Penicillium (PK 2)	+	+
Penicillium (PK 3)	+	+
Penicillium (PK 4)	+	+
Penicillium (PK 5)	+	+
Scopulariopsis brevicaulis	−	+
S. brumptii	−	+
S. chartarum	+	++
Gliocladium roseum	+	+++
Botrytis cinerea	+	++
Acremonium sp.	++	+
Verticillium terrestre	+++	+++
Paecilomyces fusisporus	++	++
P. punctonii	+++	++

Species isolated	Techniques DP	SP
P. varioti	+	+
Trichothecium roseum	++	++
Torula herbarum	−	++
T. herbarum f. *quaternella*	−	+
T. herbarum f. *quaternella* (TK 4)	+	+
Bahusandhika caligans	+	+
Torula terrestris	+	+
Stachybotris atra	+	++
S. echinata	+	+
S. aurantia	+	+
Sporotrichum sp.	+	+
Nigrospora sphaerica	+	+++
Humicola grisea	++	++
Scolecobasidium constrictum	+	+
S. terreum	++	+
Cladosporium herbarum	+++	+++
Staphylotrichum coccosporium	+	+
Curvularia lunata	++	++
C. geniculata	+	+
C. palescens	+	+
Helminthosporium sativum	+	+
Helminthosporium (HK 1)	+	+
Helminthosporium (HK 2)	+	+
Helminthosporium (HK 3)	+	+
Helminthosporium (HK 4)	+	+
Tetracoccosporium paxianum	+	++
Stemphylium sp.	+	+
Alternaria tenuis	+++	++++
Stysanus medius	+	+
Fusarium avenaceum	++	+
F. chlamydosporum	++	++
F. moniliforme	+	+
F. nivale	+	+
F. oxysporum	+	+
F. poae	+	+
Fusarium sp.	+	+
Cylindrocladium sp.	+	+
Cylindrocarpon radicicola	+	+
Myrothecium roridum	+	++
Pestalotia monorhinca	+	+
Epicoccum duriaenum	++	+++
Unidentfied sp. (UK 1)	+	+
Unidentified sp. (UK 2)	+	+
Black sterile mycelium (SK 1)	+	+++
Black sterile mycelium (SK 2)	+	+++
Black sterile mycelium (SK 3)	+	++
White sterile mycelium (SK 4)	++	+

\+ = rare
++ = common
+++ = very common
++++ = abundant
+++++ = very abudantn

References

Burges, N. A. and Nicholas, D. P. (1961: Use of soil sections in sutdying amount of fungal hyphae in soil. *Soil Science*, 92: 25—29.

Conn, H. J. 1922: A microscopic method for demonstrating fungi and actinomycetes in soil. *Soil Science*, **14**: 149—151.

Garrett, S. D. 1964: Biology of root infecting fungi. Cambridge University Press, 293 pp.

Hering, T. F. 1966: An automatic soil washing apparatus for fungal isolation. Plant and soil, 25: 195—200.

Hesseltine, C. W. (1954): The section Genevensis of the genus *Mucor*. *Mycologia*, **46**: 358—366.

— 1965: Genera of Mucorales with notes on their synonymy. *Mycologia*, **47**: 344—363.

Jones, P. C. T. and Mollison, J. E. A.: A technique for the quantitative estimation of soil micro-organisms. *J. Gen. Microbiol.* **2**: 54—69.

Kubiena, W. L. 1938: *Micropedology*. Iowa Collegiate Press, Inc., Iowa, USA.

Nicholas, D. P., Parkinson, D. and Burges, N. A. (1965): Studies on fungi in Podsol-soil. II Application of soil sectioning technique to the study of amount of fungal mycelium in soil. J. Soil. Science, **16**: 258—269.

Mazzucchetti, G., 1965: Il Genero „Chaetomium", Roma 1965.

Papavizas, G. C. and Davey, C. B., 1959: Evaluation of various media and antimicrobial agents for isolation of soil fungi. Soil Science, 88: 112—117.

Raper, K. B. and Fennell, D. I., 1965: *The Genus Aspergillus*. Williams and Wilkins Co., Baltimore, Md.

— and Thom, C., 1949: A manual of the Penicillia. Williams and Wilkins Co., Baltimore, Md.

Waksman, S. A., 1927: Principles of soil microbiology, Williams and Wilkins Co., Baltimore, Md.

Warcup, J. H., 1950: The soil pate method for isolation of fungi from soil. *Nature, Lond.*, 166: 117—118.