Applied and Environmental Microbiology

Isolation of saprophytic basidiomycetes from soil.

R G Thorn, C A Reddy, D Harris and E A Paul
Appl. Environ. Microbiol. **1996, 62(11):4288.**

Updated information and services can be found at:
http://aem.asm.org/content/62/11/4288

These include:

CONTENT ALERTS

Receive: RSS Feeds, eTOCs, free email alerts (when new articles cite this article), more»

Information about commercial reprint orders: **http://journals.asm.org/site/misc/reprints.xhtml**
To subscribe to to another ASM Journal go to: **http://journals.asm.org/site/subscriptions/**

Journals.ASM.org

Downloaded from http://aem.asm.org/ on March 17, 2014 by COLARADO STATE UNIV

APPLIED AND ENVIRONMENTAL MICROBIOLOGY, Nov. 1996, p. 4288–4292 Vol. 62, No. 11
0099-2240/96/$04.00+0
Copyright © 1996, American Society for Microbiology

Isolation of Saprophytic Basidiomycetes from Soil

R. GREG THORN,[1]* C. ADINARAYANA REDDY,[1,2] DAVID HARRIS,[1,3] AND ELDOR A. PAUL[1,3]

Center for Microbial Ecology,[1] Department of Crop and Soil Science,[3] and Department of Microbiology,[2] Michigan State University, East Lansing, Michigan 48824-1325

Received 13 May 1996/Accepted 2 September 1996

A method with the combined advantages of soil particle washing, selective inhibitors, and an indicator substrate was developed to isolate saprophytic basidiomycetes from soil. Organic particles were washed from soil and plated on a medium containing lignin, guaiacol, and benomyl, which reduced mold growth and allowed detection of basidiomycetes producing laccase or peroxidase. The 64 soil samples yielded 67 basidiomycete isolates, representing 51 groups on the basis of morphology and physiology. This method should facilitate investigations into the biodiversity of soil basidiomycetes and yield organisms that are useful in bioremediation of soils contaminated with pesticides or other recalcitrant aromatic compounds.

Turnover of the plant residue fraction of soil organic matter plays a dominant role in global carbon cycling and is fundamental to soil structure and fertility. Saprophytic basidiomycetes are likely to be the dominant recyclers of plant wastes in soil since they are the main producers of lignin-modifying enzymes: lignin and manganese peroxidases and laccases (39). Degradation of the lignin component of organic matter is the rate-limiting step for rapid and efficient decomposition of the associated cellulose. Knowledge of the physiology of lignin degradation by basidiomycetes is limited to a few wood-inhabiting species such as *Phanerochaete chrysosporium* and *Trametes versicolor* (9). These species are not known to be important members of the soil community, and little is known about which soil fungi are important in lignin degradation.

Estimates of the presence and diversity of soil-inhabiting basidiomycetes are usually based on surveys of their fruiting bodies, which are of ephemeral and irregular occurrence. In temperate areas, several hundred to several thousand species of mushrooms and related basidiomycetes can be found fruiting on field or forest soils (15, 24). Despite this known diversity, only seven species of basidiomycetes are listed in a recent worldwide compendium of fungi isolated from soil (18) and few others have been reported in the literature (44, 45, 49, 52–57). In 1949, Chesters (14) labeled the basidiomycetes the "missing link" in soil mycology and this statement is still largely true today.

Evolution of the technique. Saprophytic basidiomycetes have rarely been isolated from soil by the soil dilution method because ascomycetous and zygomycetous molds produce abundant asexual spores in soil that greatly outnumber the reproductive propagules of basidiomycetes and they typically outgrow saprophytic basidiomycetes on culture media (6, 14, 20, 50). Because of this, Warcup (51) developed a technique for isolating fungi from vegetative hyphae present in soil by careful manipulation and plating of hyphae observed under a high-power dissecting microscope. Although Warcup and Warcup and Talbot (52–56) isolated a number of saprophytic basidiomycetes from soil using this method, it has not come into widespread use because of its painstaking nature. The soil plate method (48), in which 5 to 15 mg of soil is spread in petri dishes before being cooled and molten agar is added, has been used with success for isolation of some soil basidiomycetes (44, 45, 49). Basidiomycete isolates must be distinguished among the variety of ascomycetes and zygomycetes which are also recovered by this method.

Plant debris in soil serves as the major reservoir of saprophytes and facultative pathogens with saprophytic phases, including basidiomycetes (13, 22, 34). Chesters (13) developed a complex apparatus for aseptically washing plant debris particles from soil, and Boosalis and Scharen (10) used this method to selectively isolate the basidiomycete plant pathogen *Rhizoctonia solani* (*Thanatephorus cucumeris*) from infested soil. Particle washing has since become a standard technique of soil mycologists (2, 7, 20, 21). A modification of this technique was used for the analysis of fungal biodiversity in samples of tropical leaf litter and produced an exceptional yield of 78 to 134 fungal species per litter sample (4). However, with the exception of *Rhizoctonia solani*, the fungi reported to have been recovered by the particle-washing technique on nonselective media have included ascomycetes and zygomycetes, but not basidiomycetes. The selective, indicator medium used in this study not only enabled the isolation of basidiomycetes but also allowed the use of a much simpler washing apparatus than those described by other investigators (2, 4, 7, 13, 21, 37).

Selective isolation media primarily rely on selective inhibitors of nontarget organisms and complex or chromogenic substrates catabolized specifically by the target organisms (29, 41). Benomyl is a semiselective fungicide to which many basidiomycetes show some degree of tolerance, whereas most ascomycetes, except members of the Pleosporales, are sensitive (8, 19). It has been used in media for the selective isolation of basidiomycetes from soil (17, 58), leaf litter (60), mycorrhizal plant roots (23), and decaying wood (26, 59). Guaiacol, a chromogenic substrate of laccase and peroxidase, was used in a medium also containing lignin in a survey for laccase-producing fungi in Indian soils (1). This survey used the soil dilution-plating method and recovered 12 laccase-positive isolates, including 2 basidiomycetes, from 150 soil samples (1). Nishida et al. (35) also used guaiacol as an indicator in an agar medium containing fine particles of beech wood for the isolation of basidiomycetes from wood.

We have taken advantage of the particle-washing technique (2, 4, 7, 13, 21, 37) to remove spores of ascomycetous and zygomycetous molds, and we plated the washed particles on a medium made selective for basidiomycetes by the incorporation of benomyl (8, 17, 19, 23, 26, 58–60). In addition to benomyl, the medium contained lignin, to encourage selection

* Corresponding author. Present address: Botany Department, University of Wyoming, Laramie, WY 82071-3165. Phone: (307) 766-6378. Fax: (307) 766-2851. Electronic mail address: thorn@uwyo.edu.

Downloaded from http://aem.asm.org/ on March 17, 2014 by COLARADO STATE UNIV

TABLE 1. Number of basidiomycete isolates from different sampling sites

Sample habitat	No. of isolates/no. of morphologically distinct groups (no. of samples) from indicated replicate plot				
	1	2	3	4	Total
Traditional tilled, corn-soybean	0/0 (1)	7/5 (2)	2/2 (2)	ND[a]	9/7 (5)
No-till, corn-soybean	3/3 (2)	0/0 (1)	8/7 (2)	ND	11/10 (5)
Low-input, organic-based, wheat-corn-soybean	0/0 (1)	0/0 (1)	0/0 (1)	ND	0/0 (3)
Zero-input, wheat-corn-soybean	0/0 (1)	0/0 (1)	0/0 (1)	ND	0/0 (3)
Hybrid poplar plantation	3/3 (2)	2/2 (2)	0/0 (1)	ND	5/5 (5)
Perennial alfalfa	0/0 (1)	0/0 (1)	0/0 (1)	ND	0/0 (3)
Old field succession	9/5 (3)	0/0 (2)	9/9 (3)	4/3 (1)	22/17 (9)
Never-tilled meadow	2/2 (2)	0/0 (2)	0/0 (2)	2/2 (3)	4/4 (9)
Hardwood forest	2/2 (2)	5/5 (3)	1/1 (3)	ND	8/7 (8)
Conifer forest	0/0 (2)	4/4 (3)	3/3 (3)	ND	7/7 (8)
Old field	0/0 (2)	1/1 (2)	0/0 (2)	ND	1/1 (6)
Total	NA[b]	NA	NA	NA	67/51 (64)

[a] ND, not determined.
[b] NA, not applicable.

of ligninolytic fungi, and guaiacol, which acts as a colorimetric indicator of the lignin-modifying enzymes laccase or peroxidases (1, 35). Except as indicated, all chemicals were obtained from Sigma Chemical Co., St. Louis, Mo. The basal medium contained (per liter of distilled water): 0.5 g of KH_2PO_4, 0.2 g of $MgSO_4 \cdot 7H_2O$, 0.1 g of NH_4NO_3, 0.1 g of KCl, 0.02 g of $FeSO_4 \cdot 7H_2O$, 0.05 g of $Ca(NO_3)_2 \cdot 4H_2O$, 2.0 g of Bacto malt extract (Difco Laboratories, Detroit, Mich.), and 15 g of Bacto agar (Difco). Basal medium was autoclaved in flasks with magnetic stir bars and cooled to approximately 55°C, when the following were added aseptically: 5 ml of 1 M KOH; 0.4 ml of guaiacol; 1.0 g of indulin AT (alkali lignin; Sigma), which was suspended and partially dissolved in 10 ml of dioxane; 60 mg of chlortetracycline-HCl; 30 mg of streptomycin sulfate; 30 mg of penicillin G (Na salt); 4 mg of benomyl (as Benlate 50 WP; DuPont, Wilmington, Del.) in 2 ml of 1:1 acetone–70% ethanol (to yield 2 $\mu g\ ml^{-1}$). The medium was stirred and poured into sterile 100-mm-diameter petri dishes.

Malt-yeast agar (5 g of malt extract, 1 g of yeast extract, and 15 g of agar per liter of distilled water) with 60 $\mu g\ ml^{-1}$ of chlortetracycline-HCl, 30 $\mu g\ ml^{-1}$ of streptomycin sulfate, and 30 $\mu g\ ml^{-1}$ of penicillin G was used for the first subculture of chosen isolates before transfer to media without these antibacterial antibiotics.

Soil particle washing and plating. Soil samples were collected from 11 habitat types and agricultural treatments in the Kellogg Biological Station Long-Term Ecological Research site in Kalamazoo County, Michigan (Table 1). Three to four replicate plots of each habitat were sampled from one to three times (June and July 1993 and April 1994). Soil cores of 2.5-cm diameter were taken to 25-cm depth after the litter layer was removed. Samples for each plot were pooled from four or more cores per plot. Samples were stored at 4°C until processing, in most cases within 2 days. Approximately 5 g fresh weight (2.5 to 4.5 g dry weight) of each soil sample was added to 500 ml of sterile 0.1% (wt/vol) sodium pyrophosphate in 1-liter mason jars. These were gently shaken end-to-end on a platform shaker for 1 h at 4°C to disperse soil clumps and colloids (5). The entire suspension was poured through stacked 20-cm-diameter soil sieves (Newark wire cloth) of 250 μm (no. 60) and 53 μm (no. 270) mesh and rinsed through with a brief shower of cold tap water. Particles remaining on the 53-μm-mesh sieve were then washed for 5 min under this shower at a flow rate of approximately 20 liters/min. Remaining solids were collected at one edge of the sieve, and the sieve was tilted to separate suspended organic particles from settled mineral particles. One milliliter of a dense suspension of the organic particles was picked up in a sterile broad-bore pipette tip (Gilson P-1000). This suspension was diluted in sterile distilled water to 10^{-2}, and 0.4 ml of this dilution was spread onto each of 20 petri dishes of lignin-guaiacol-benomyl agar. Sieves were rinsed with water and sterilized in 70% ethanol between samples. Tap water controls from the washed, sterilized 53-μm sieve yielded no fungal or bacterial isolates on either lignin-guaiacol-benomyl agar or malt-yeast agar with antibiotics.

Isolation and identification of basidiomycetes. Microscopic observation of washed soil particles revealed fragments of plant debris colonized by fungal hyphae as well as hyphae free from debris (Fig. 1). Although these particles were plated at a high density (ca. 25 cm^{-2}), few colonies grew on the lignin-guaiacol-benomyl agar. After 1 day, the petri dishes were packed in their plastic sleeves and incubated at room temperature for 2 weeks before making isolations. At this time, plates were scanned for colonies that caused reddening of the guaiacol by the action of laccase or peroxidase (Fig. 2). These colonies were examined microscopically (at ×40 and ×100) for the presence of conidia or clamp connections. Plates were screened again after 4 and 6 weeks. At each screening, colonies of putative basidiomycetes were isolated onto malt-yeast agar containing antibiotics. A total of 111 such fungi were selected and isolated from 64 soil samples.

In order to identify putatively basidiomycete isolates, induction of fruiting bodies on various mushroom media was attempted (43). In the absence of identifiable sexual fruiting bodies or asexual spores, cultures were considered to be basidiomycetes if they showed clamp connections at septa (Fig. 1b; diagnostic for basidiomycetes if present, but absent in many groups of them) or positive staining with diazonium blue B ($ZnCl_2$ complex of tetrazotized *o*-dianisidine; Sigma) (25, 46). Only 22 of the 111 isolates selected provided definite evidence of basidiomycete identity; 13 isolates possessed clamp connections, and 9 others produced basidiomycete fruiting bodies. However, these and an additional 45 isolates gave positive, red to purple reactions to diazonium blue B following pretreatment of their mycelia with 1 M KOH and were identified as basidiomycetes on that basis (46). The remaining 44 isolates were identified as ascomycetes by their production of identifiable asexual spores (conidia) or by negative staining with diazonium blue B.

Fruiting bodies were produced in vitro by species of *Clitopilus* (Entolomataceae) and *Coprinus* (Coprinaceae) of the Agaricales, *Irpex* (Steccherinaceae) of the Hericiales, and *Ceratobasidium* (Ceratobasidiaceae) of the Tulasnellales. Of these, *Clitopilus* (?) *scyphoides* was isolated from five different sites, including old field succession, never-tilled meadow, and hardwood and coniferous forests. A polypore tentatively identified as *Irpex lacteus* was isolated from old field succession and hardwood forest. A species of *Armillaria*, identified by its production of rhizomorphs in culture, was isolated from a conventionally tilled corn-soybean rotation plot.

Diversity and distribution. The 67 basidiomycete isolates were recovered from 40 of the 64 soil samples and from 8 of the 11 habitats and 17 of the 35 plots sampled (Table 1). Fifty-one macromorphological, micromorphological, and physiological characters of the isolates in culture (36) were used for numerical taxonomic analyses of the isolates, using NTSYS-PC

Downloaded from http://aem.asm.org/ on March 17, 2014 by COLARADO STATE UNIV

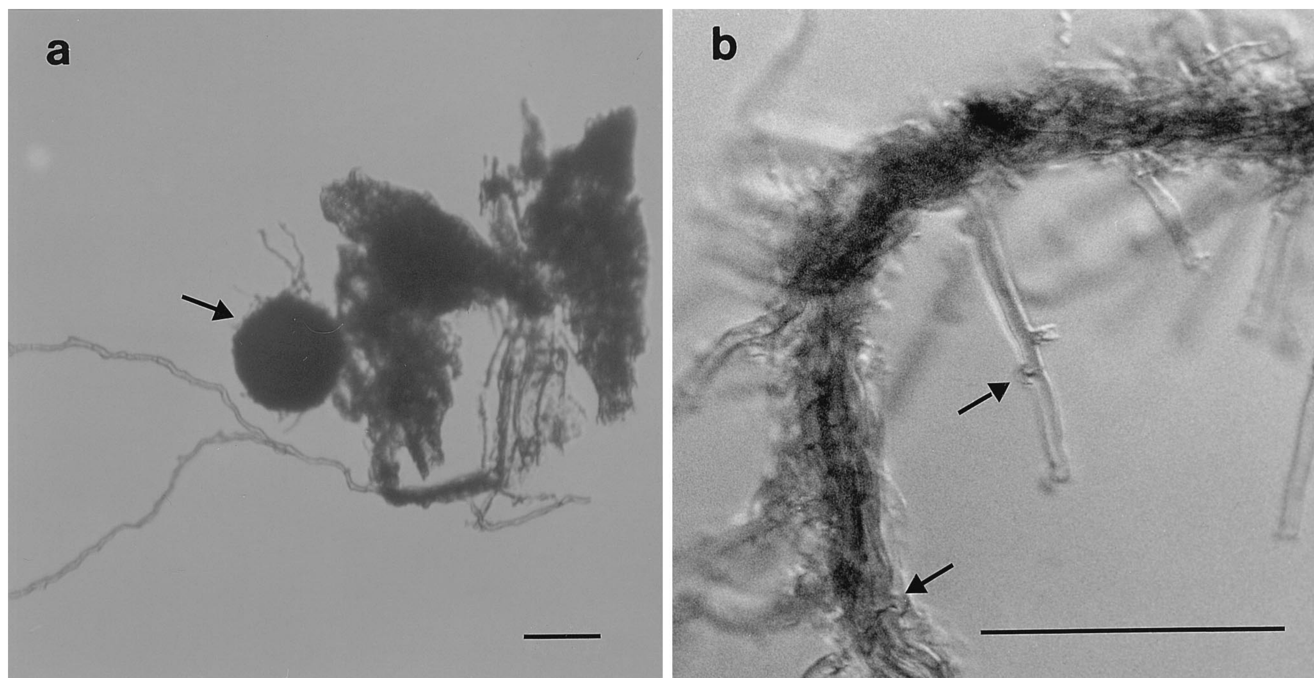

FIG. 1. Particles washed from Michigan soils in 0.1% sodium pyrophosphate and recovered on a 53-μm-mesh sieve. (a) Fragment of decaying plant material with hyphae and a fungal sclerotium (arrow). (b) Basidiomycete rhizomorph; note hyphae with clamp connections (arrows). Bars, 100 μm.

(40), and to derive keys to morphologically recognizable groups of isolates, using INTKEY (38) and DELTA (16). This analysis sorted the 67 isolates into 7 major groups and 51 recognizable taxa (data not shown). These could not be identified using traditional taxonomic characters. Available keys for the identification of lignin-degrading basidiomycete cultures (33, 36, 42) include only a small proportion of the species occurring on wood and almost none that inhabit soil. Therefore, we were unable to identify most of the 51 morphotaxa found in our study since we lacked matching reference strains of known identity. We are obtaining genes coding for rRNA (rDNA) nucleotide sequence data from the 5′ end of the large subunit nuclear rDNA by which these unknown isolates may be placed in a phylogeny built with sequences from known strains (unpublished data). This method has been used for the identification and phylogenetic analysis of the morphologically undistinguished fungal symbionts of attine ants (12, 27).

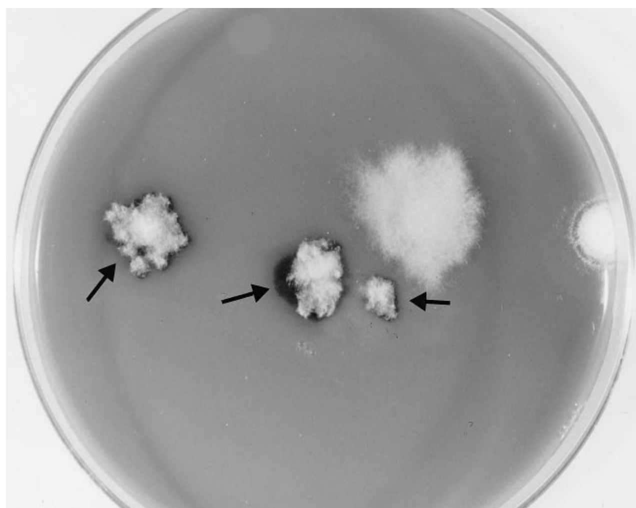

FIG. 2. Isolates producing laccase or peroxidase on lignin-guaiacol-benomyl agar (arrows) are readily located by the bright red zone beneath their colonies caused by the quinone oxidation product of guaiacol (dark in this black and white photo).

Forty-three morphotypes were represented by only one isolate each. This indicates both the inadequacy of our sampling and the potential existence of a great diversity of ligninolytic basidiomycetes in these soils. The total sampling effort for 35 plots in 11 habitats was just 320 g of soil; more samples would undoubtedly yield more isolates and greater diversity. A range of zero to nine morphologically distinct isolates was obtained from individual sample plots, and from zero to 17 morphologically distinct isolates were found in different habitat types. Greatest diversity was found in the old field succession and no-till corn-soybean plots. No basidiomycetes were found in the low-input and zero-input corn-soybean-wheat or perennial alfalfa plots, and only one was found in the old field plots. However, these plots were sampled less frequently than the others. Better estimates of the yield and diversity of basidiomycetes in these soils must await more systematic sampling of plots and identification of the isolates using molecular means. The 67 isolates recovered undoubtedly do not represent the full diversity present in these soils, but they represent an improvement on the two basidiomycetes recovered from 150 soil samples by Arora and Sandhu (1). Our method is fast and technically unchallenging, is not dependent on discovery of basidiomycete fruiting bodies, and produces more basidiomycete isolates than any previous method or study reported.

Some saprophytic soil basidiomycetes may not be recovered by the method described here. Warcup (49) collected soil beneath fruiting bodies of some soil basidiomycetes, such as *Agaricus arvensis* (*Psalliota arvensis*), *Lepista nuda* (*Tricholoma nudum*), and *Marasmius oreades*, and found that they will grow out from undisturbed soil blocks or in soil plates. During this

Downloaded from http://aem.asm.org/ on March 17, 2014 by COLARADO STATE UNIV

study, soil was collected below fruiting bodies of *Lepista nuda*. This species could not be recovered from washed particles plated on lignin-guaiacol-benomyl agar but was isolated from hyphae growing out of small soil crumbs sprinkled on water agar. Thus, it seems that the particle-washing technique should be combined with other methods such as the soil plate method (48) or the soil crumb or sprinkle plate methods (3, 47) for a more complete estimate of the diversity of saprophytic basidiomycetes. Isolations made at temperatures other than room temperature (e.g., 5 and 37°C) may also increase the diversity of fungi isolated (11). Isolations made at 5°C have been used to obtain basidiomycetes from litter in Finland (28).

No attempt was made to isolate ectomycorrhizal basidiomycetes, which are present in the poplar, conifer, and hardwood forest sites at the Kellogg Biological Station. Most ectomycorrhizal fungi do not give positive reactions for laccase or peroxidase (30) and thus would not have been chosen for isolation even if they were capable of growth on lignin-guaiacol-benomyl agar. These fungi are more simply isolated from washed mycorrhizal roots (23). Isolation of these more fastidious fungi from soil might require the use of a specialized medium for mycorrhizal fungi (31) modified with benomyl and dichloran (59). Washed particles containing hyphae and sclerotia of ectomycorrhizal fungi or basidiospores recovered from soil by sucrose-gradient centrifugation (32) could be used as inoculum.

This research was supported by NSF grant BIR 9120006 to the Center for Microbial Ecology, Michigan State University.

We thank Gerald Bills (Merck, Sharp & Dohme, Rahway, N.J.) for valuable discussions and Sandy Halstead (Kellogg Biological Station Long-Term Ecological Research site) for collection of soil samples.

REFERENCES

1. **Arora, D. S., and D. K. Sandhu.** 1985. Survey of some Indian soils for laccase producing fungi and their lignin degrading ability. Proc. Indian Acad. Sci. Plant Sci. **94:**567–574.
2. **Bååth, E.** 1988. A critical examination of the soil washing technique with special reference to the size of the soil particles. Can. J. Bot. **66:**1566–1569.
3. **Barron, G. L.** 1977. The nematode-destroying fungi. Canadian Biological Publications, Guelph, Ontario.
4. **Bills, G. F., and J. D. Polishook.** 1994. Abundance and diversity of microfungi in leaf litter of a lowland rain forest in Costa Rica. Mycologia **86:**187–198.
5. **Bingle, W. H., and E. A. Paul.** 1986. A method for separating fungal hyphae from soil. Can. J. Microbiol. **32:**62–66.
6. **Bisby, G. R., M. I. Timonin, and N. James.** 1935. Fungi isolated from soil profiles in Manitoba. Can. J. Res. Sect. C Bot. Sci. **13:**47–65.
7. **Bissett, J., and P. Widden.** 1972. An automatic, multichamber soil-washing apparatus for removing fungal spores from soil. Can. J. Microbiol. **18:**1399–1404.
8. **Bollen, G. J., and A. Fuchs.** 1970. On the specificity of the in vitro and in vivo antifungal activity of benomyl. Neth. J. Plant Pathol. **76:**299–312.
9. **Boominathan, K., and C. A. Reddy.** 1992. Fungal degradation of lignin: biotechnological applications, p. 763–822. *In* D. K. Arora, R. P. Elander, and K. G. Mukerji (ed.), Handbook of applied mycology, vol. 4. Fungal biotechnology. Marcel Dekker, New York.
10. **Boosalis, M. G., and A. L. Scharen.** 1959. Methods for microscopic detection of *Aphanomyces euteiches* and *Rhizoctonia solani* associated with plant debris. Phytopathology **49:**192–198.
11. **Carreiro, M. M., and R. E. Koske.** 1992. Room temperature isolations can bias against selection of low temperature microfungi in temperate forest soils. Mycologia **84:**886–900.
12. **Chapela, I. H., S. A. Rehner, T. R. Schulz, and U. G. Mueller.** 1994. Evolutionary history of the symbiosis between fungus-growing ants and their fungi. Science **266:**1691–1694.
13. **Chesters, C. G. C.** 1948. A contribution to the study of fungi in the soil. Trans. Br. Mycol. Soc. **30:**100–117.
14. **Chesters, C. G. C.** 1949. Concerning fungi inhabiting soil. Trans. Br. Mycol. Soc. **32:**197–216.
15. **Clark, M. C.** 1980. A fungus flora of Warwickshire. British Mycological Society, London.
16. **Dallwitz, M. J., and T. A. Paine.** 1986. User's guide to the DELTA system—a general system for processing taxonomic descriptions, 3rd ed. CSIRO Aust. Div. Entomol. Rep. **13:**1–106.
17. **Dietrich, D. M., and R. T. Lamar.** 1990. Selective medium for isolating *Phanerochaete chrysosporium* from soil. Appl. Environ. Microbiol. **56:**3088–3092.
18. **Domsch, K. H., W. Gams, and T.-H. Anderson.** 1993. Compendium of soil fungi. IHW Verlag, Eching, Germany.
19. **Edgington, L. V., K. L. Khew, and G. L. Barron.** 1971. Fungitoxic spectrum of benzimidazole compounds. Phytopathology **61:**42–44.
20. **Gams, W.** 1992. The analysis of communities of saprophytic microfungi with special reference to soil fungi, p. 183–223. *In* W. Winterhoff (ed.), Fungi in vegetation science. Kluwer Academic Publishers, Dordrecht, The Netherlands.
21. **Gams, W., and K. H. Domsch.** 1967. Contributions to the soil washing technique for the isolation of soil fungi. Arch. Mikrobiol. **58:**134–144.
22. **Garrett, S. D.** 1956. Biology of root-infecting fungi. University Press, Cambridge, England.
23. **Genua, J. M.** 1985. A technique for isolating the mycorrhizal fungus *Laccaria laccata* from conifer seedlings. Plant Dis. **69:**1080–1081.
24. **Gourley, C. O.** 1983. An annotated index of the fungi of Nova Scotia. Proc. Nova Scotia Inst. Sci. **32:**75–295.
25. **Hagler, A. N., and D. G. Ahearn.** 1981. Rapid diazonium blue B test to detect basidiomycetous yeasts. Int. J. Syst. Bacteriol. **31:**204–208.
26. **Hale, M. D. C., and J. G. Savory.** 1976. Selective agar media for the isolation of basidiomycetes from wood—a review. Int. Biodeterior. Bull. **12:**112–115.
27. **Hinkle, G., J. K. Wetterer, T. R. Schultz, and M. L. Sogin.** 1994. Phylogeny of the attine ant fungi based on analysis of small subunit ribosomal RNA gene sequences. Science **266:**1695–1697.
28. **Hintikka, V.** 1964. Psychrophilic basidiomycetes decomposing forest litter under winter conditions. Commun. Inst. Forest. Fenn. **59**(2)**:**1–20.
29. **Hunter-Cevera, J. C., M. E. Fonda, and A. Belt.** 1986. Isolation of cultures, p. 3–23. *In* A. L. Demain and N. A. Solomon (ed.), Manual of industrial microbiology and biotechnology. American Society for Microbiology, Washington, D.C.
30. **Hutchison, L. J.** 1990. Studies on the systematics of ectomycorrhizal fungi in axenic culture. III. Patterns of polyphenol oxidase activity. Mycologia **82:**424–435.
31. **Marx, D. H.** 1969. The influence of ectotrophic mycorrhizal fungi on the resistance of pine roots to pathogenic infections. I. Antagonism of mycorrhizal fungi to root pathogenic fungi and soil bacteria. Phytopathology **59:**153–163.
32. **Miller, S. L., P. Torres, and T. M. McClean.** 1994. Persistence of basidiospores and sclerotia of ectomycorrhizal fungi and *Morchella* in soil. Mycologia **86:**89–95.
33. **Nakasone, K. K.** 1990. Cultural studies and identification of wood-inhabiting Corticiaceae and selected Hymenomycetes from North America. Mycologia Memoir 15. J. Cramer, Berlin.
34. **Neate, S. M.** 1987. Plant debris in soil as a source of inoculum of *Rhizoctonia* in wheat. Trans. Br. Mycol. Soc. **88:**157–162.
35. **Nishida, T., Y. Kashino, A. Mimura, and Y. Takahara.** 1988. Lignin biodegradation by wood-rotting fungi. Mokuzai Gakkaishi **34:**530–536.
36. **Nobles, M. K.** 1965. Identification of cultures of wood-inhabiting Hymenomycetes. Can. J. Bot. **43:**1097–1139.
37. **Parkinson, D., and S. T. Williams.** 1961. A method for isolating fungi from soil microhabitats. Plant Soil **13:**347–355.
38. **Partridge, T. R., M. J. Dallwitz, and L. Watson.** 1988. A primer for the DELTA system on MS-DOS and VMS, 2nd ed. CSIRO Aust. Div. Entomol. Rep. **38:**1–17.
39. **Rayner, A., and L. Boddy.** 1988. Fungal decomposition of wood. John Wiley & Sons, New York.
40. **Rohlf, F. J.** 1988. NTSYS-pc: Numerical taxonomy and multivariate analysis system, version 1.50. Exeter Publishing Ltd., Setauket, N.Y.
41. **Seifert, K. A.** 1990. Isolation of filamentous fungi, p. 21–51. *In* D. P. Labeda (ed.), Isolation of biotechnological organisms from nature. McGraw-Hill Publishing Co., New York.
42. **Stalpers, J. A.** 1978. Identification of wood-inhabiting Aphyllophorales in pure culture. Stud. Mycol. **16:**1–248.
43. **Stamets, P., and J. S. Chilton.** 1983. The mushroom cultivator. Agarikon Press, Olympia, Wash.
44. **Steiman, R., P. Guiraud, L. Sage, F. Seigle-Murandi, and J.-L. Lafond.** 1995. Mycoflora of soil around the Dead Sea. I. Ascomycetes (including *Aspergillus* and *Penicillium*), Basidiomycetes, Zygomycetes. Syst. Appl. Microbiol. **18:**310–317.
45. **Stenton, H.** 1953. The soil fungi of Wicken Fen. Trans. Br. Mycol. Soc. **36:**304–314.
46. **Summerbell, R. C.** 1985. The staining of filamentous fungi with diazonium blue B. Mycologia **77:**587–593.
47. **Waksman, S. A.** 1916. Do fungi live and produce mycelium in the soil? Science **44:**320–322.
48. **Warcup, J. H.** 1950. The soil-plate method for isolation of fungi from soil. Nature (London) **146:**117.
49. **Warcup, J. H.** 1951. Studies on the growth of basidiomycetes in soil. Ann. Bot. **15:**305–317.
50. **Warcup, J. H.** 1955. On the origin of colonies of fungi developing on soil

Downloaded from http://aem.asm.org/ on March 17, 2014 by COLARADO STATE UNIV

dilution plates. Trans. Br. Mycol. Soc. **38:**298–301.
51. **Warcup, J. H.** 1955. Isolation of fungi from hyphae present in soil. Nature (London) **175:**953–954.
52. **Warcup, J. H.** 1957. Studies on the occurrence and activity of fungi in a wheat-field soil. Trans. Br. Mycol. Soc. **40:**237–262.
53. **Warcup, J. H.** 1959. Studies on basidiomycetes in soil. Trans. Br. Mycol. Soc. **42:**45–52.
54. **Warcup, J. H., and P. H. B. Talbot.** 1962. Ecology and identity of mycelia isolated from soil. Trans. Br. Mycol. Soc. **45:**495–518.
55. **Warcup, J. H., and P. H. B. Talbot.** 1963. Ecology and identity of mycelia isolated from soil. II. Trans. Br. Mycol. Soc. **46:**465–472.
56. **Warcup, J. H., and P. H. B. Talbot.** 1965. Ecology and identity of mycelia isolated from soil. III. Trans. Br. Mycol. Soc. **48:**249–259.
57. **Watanabe, T.** 1994. A pictorial atlas of soil and seed fungi. Lewis Publishing, Boca Raton, Fla.
58. **Windham, A. S., and L. T. Lucas.** 1987. A qualitative baiting technique for selective isolation of *Rhizoctonia zeae* from soil. Phytopathology **77:**712–714.
59. **Worrall, J. J.** 1991. Media for selective isolation of Hymenomycetes. Mycologia **83:**296–302.
60. **Young, C. S., and J. H. Andrews.** 1990. Recovery of *Athelia bombacina* from apple leaf litter. Phytopathology **80:**530–535.

Downloaded from http://aem.asm.org/ on March 17, 2014 by COLARADO STATE UNIV