

Isolating basidiomycetes from organic and mineral soil horizons

using selective media and red maple and white pine wood baits.

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Introduction

The wood decaying basidiomycete fungi are essential participants in the decomposition of coarse woody debris and are believed to contribute to the nutritional balance of forest soils. These fungi may form a bridge between felled decaying wood and the contiguous soil horizons as a means of transporting nutrients used in the decay process. They rarely appear in lists of fungi isolated from litter or soil.

Other researchers isolated microfungi and sterile mycelium from soils but rarely identified them as basidiomycete fungi. Basidiomycete fungi were isolated from roots, rhizomorphs, sclerotia, and the base of wheat plants, but not directly from soil (5); only 22 of 111 isolates were identified as saprophytic basidiomycete fungi from soil (4).

We used three previously developed selective media for isolation of basidiomycete fungi from decaying logs and soil: lignin guaiacol benomyl agar LGBA (4), lignin guaiacol benomyl dichloran agar LGBDA (1, 3), and malt yeast extract benomyl dichloran agar MYBDA (1, 3). Spodic soil horizons in a red maple-beech-balsam fir forest were classified and cultured.

We developed successful techniques for selective isolation and identification of decay basidiomycetes from organic and mineral soil, and three new culture methods:

Direct Soil

Soil Pit Baits, using red maple wood

Jar Baits, using red maple and white pine wood

Our methods resulted in the identification of basidiomycete fungi in 100% of soil pit and jar bait cultures, 68% of direct organic soil cultures and 59% of direct mineral soil cultures.

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LGBA

Thorn, R.G., C. A. Reddy, D. Harris, and E.A. Paul. 1996. Isolation of saprophytic basidiomycetes from soil. *Appl. Environ. Microbiol.* 62 (11): 4288-4292.

water	482 ml
KH_2PO_4	0.25 gm
$\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$	0.1 gm
NH_4NO_3	0.05 gm
KCl	0.05 gm
$\text{FeSO}_4 \cdot 7\text{H}_2\text{O}$	0.01 gm
$\text{Ca}(\text{NO}_3)_2 \cdot 4\text{H}_2\text{O}$	0.025 gm
malt extract	1.0 gm
agar	7.5 gm

After autoclaving and cooled to 55°C, add the following, with sterile equipment:

1 M KOH	2.5 ml	(5.6 gm in 100 ml sterile water)
guaiacol	0.2 ml	
indulin AT	(0.5 gm	suspended and partially dissolved in 5 ml dioxane)
chlortetracycline-HCl	30 mg	
streptomycin sulfate	15 mg	
penicillin G	15 mg	
* Benlate solution	1 ml	

* from Stock solution:

benomyl 0.2 g benomyl in 50 ml acetone & 50 ml 70 % ETOH

Final concentrations:

chlortetracycline-HCl	60 mg/l
streptomycin sulfate	30 mg/l
penicillin G	30 mg/l
benomyl	2 mg/l

Culture method	Soil Horizon	Identification	Genebank Accession #	# of Isolates
Basidiomycete:				
Direct Soil	organic	<i>Inonotus glomeratus</i>	AF247968	1
"	"	<i>Armillaria gallica</i>	AY190248	2
"	"	<i>Hypholoma</i> species	AF335450	4
"	"	<i>Thanatephorus cucumeris</i>	AF455461, AF455463	2
"	"	<i>Phellinus igniarius</i>	AF455434	1
"	"	<i>Tricholoma robustum</i>	AF455529	1
"	"	<i>Mycena aff. murina</i>	AF335444	1
"	"	<i>Armillaria ostoyae</i>	AJ250055	1
"	mineral	<i>Mycena aff. murina</i>	AF335444	2
"	"	un-cultured Basidiomycete	AY241670	1
"	"	<i>Thanatephorus cucumeris</i>	AF455463	1
"	"	<i>Armillaria gallica</i>	AY190248	1
"	"	<i>Hyphodontia flavipora</i>	AF455399	2
"	"	<i>Bullera variabilis</i>	AF444403	1
"	"	<i>Irpex lacteus</i>	AB079265	1
"	"	<i>Polyporus brumalis</i>	AB070875	1
"	"	<i>Echinodontium taxodii</i>	AF455458	1
"	"	<i>Stereum</i> species	AY207328	1
"	"	<i>Skeletocutis nivea</i>	AJ006679	1
Soil Pit Bait	organic	<i>Megacollybia platyphylla</i>	AF498289	1
"	"	<i>Hypholoma</i> species	AF335450	1
"	mineral	<i>Ceriporiopsis gilvescens</i>	AJ006684	1
"	"	<i>Perenniporia subacida</i>	AY089739	1
"	"	<i>Hypholoma</i> species	AF335450	3
Spring Jar Bait	mineral	<i>Hypholoma</i> species	AF335450	2
"	"	<i>Ceriporiopsis gilvescens</i>	AJ006684	3
Fall Jar Bait	organic	<i>Hypholoma</i> species	AF335450	1
"	"	un-identified Basidiomycete	AF241332	1
"	mineral	<i>Hypholoma</i> species	AF335450	5
Non-Basidiomycete:				
Direct Soil	organic	<i>Hypoxylon fragiforme</i>	AJ390403	1
"	"	leaf litter Ascomycete	AF502761	1
"	"	<i>Myrothecium inundatum</i>	AJ302005	2
"	"	<i>Verticillium bulbillosum</i>	AJ292410	2
"	mineral	<i>Cladosporium elatum</i>	AF393699	1
"	"	<i>Verticillium bulbillosum</i>	AJ292410	2
"	"	<i>Gibberella avenacea</i>	AY147285	1
"	"	<i>Myrothecium inundatum</i>	AJ302005	4
"	"	ectomycorrhizae	ER0430403	1

LGBDA

Thompson, T. A. 1998. Development of techniques for the selective isolation of basidiomycetes from coarse woody debris and contiguous soil horizons. *M.S. thesis, University of New Hampshire*

water	482 ml
KH ₂ PO ₄	0.25 gm
MgSO ₄ · 7H ₂ O	0.1 gm
NH ₄ NO ₃	0.05 gm
KCl	0.05 gm
FeSO ₄ · 7H ₂ O	0.01 gm
Ca(NO ₃) ₂ · 4H ₂ O	0.025 gm
malt extract	1.0 gm
agar	7.5 gm

After autoclaving and cooled to 55°C, add the following, with sterile equipment:

1 M KOH	2.5 ml (5.6 gm in 100 ml sterile water)
guaiacol	0.2 ml
indulin AT	(0.5 gm suspended and partially dissolved in 5 ml dioxane)
* chlortetracycline-HCl	5 ml
* streptomycin sulfate	5 ml
* penicillin G	5 ml
* Benlate solution	1 ml
* dichloran solution	1 ml

* from Stock solutions:

chlortetracycline-HCl	0.6 g in 100 ml sterile water
streptomycin sulfate	0.3 g in 100 ml sterile water
penicillin G	0.3 g in 100 ml sterile water
benomyl	0.2 g in 50 ml acetone & 50 ml 70 % ETOH
dichloran	0.1 g in 100 ml 50 % ETOH

Final concentrations:

chlortetracycline-HCl	60 mg/l
streptomycin sulfate	30 mg/l
penicillin G	30 mg/l
benomyl	2 mg/l
dichloran	2 mg/l

MYBDA

Thompson, T. A. 1998. Development of techniques for the selective isolation of basidiomycetes from coarse woody debris and contiguous soil horizons. *M.S. thesis, University of New Hampshire*

malt extract	5 gm
yeast extract	1 gm
agar	10 gm
water	483 ml

After autoclaving and cooled to 55 °C, add the following, with sterile equipment:

* chlortetracycline-HCl	5 ml
* streptomycin sulfate	5 ml
* penicillin G	5 ml
* Benlate solution	1 ml
* dichloran solution	1 ml

* from Stock solutions:

chlortetracycline-HCl	0.6 g in 100 ml sterile water
streptomycin sulfate	0.3 g in 100 ml sterile water
penicillin G	0.3 g in 100 ml sterile water
benomyl	0.2 g in 50 ml acetone & 50 ml 70 % ETOH
dichloran	0.4 g in 100 ml 50 % ETOH

Final concentrations:

chlortetracycline-HCl	60 mg/l
streptomycin sulfate	30 mg/l
penicillin G	30 mg/l
benomyl	2 mg/l
dichloran	8 mg/l