*Mycologia*, 86(2), 1994, pp. 275-280.

# Preserving cultures of wood-decaying Basidiomycotina using sterile distilled water in cryovials

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**Abstract**: Prior to 1985, cultures at the Center for Forest Mycology Research were maintained on 1.5% malt extract agar test-tube slants. This system not only made it necessary to transfer the entire collection every year but also permitted genetic change because continual growth occurred. In 1985, the method of storing fungal cultures in sterile distilled water in cryovials was introduced. This study reports on the use of this method for long-term fungal storage. For varying periods up to 7 years, 151 miscellaneous species of wood-decaying Basidiomycotina were stored in sterile distilled water. Water storage has numerous advantages: culture viability or growth rate is not significantly influenced; isolates can be stored longer; genetic stability is greater; the method is quick, easy, and inexpensive, and requires less space.

**Key Words**: Basidiomycotina, cryovial, culture collection, water storage

The Center for Forest Mycology Research (CFMR) is home to the largest culture collection of wood-rotting Basidiomycotina in the world. The collection, started in 1932, contains more than 11000 secondary mycelium isolates of more than 1600 species and more than 3200 monobasidiosporous isolates taken from many of the same specimens as the dikaryons. Prior to 1985, the cultures were maintained on 1.5% malt extract agar test-tube slants, making it necessary to transfer the entire collection at least once every year. This system, because it allowed continual growth, also permitted genetic change to occur during years of storage. A back-up collection was maintained on malt extract agar under sterile mineral oil. In 1985, a new, more convenient method of fungal preservation was introduced at CFMR, storing fungal cultures in sterile distilled water in cryovials.

Preserving fungal cultures in water is not a new concept. Castellani (1939, 1962) described the basic technique when studying various ways to avoid pleomorphism in human pathogenic fungi. Figueiredo (1967) and Figueiredo and Pimentel (1975) preserved pathogenicity in 22 plant pathogenic fungi using a water storage technique. Boesewinkel (1976) successfully stored 53 cultures representing Ascomycetes, Basidiomycetes, Deuteromycetes, and phycomycetes *(fide* Boesewinkel) from either 1968 or 1969 to January 1975. Boesewinkel also successfully stored more than 650 plant pathogenic and saprophytic fungi in sterile water. All retained viability. Ellis (1979) worked with a group of fungi that had not survived Iyophylization and preserved the viability of these fungi using water storage. Marx and Daniel (1976) reported the storage of 64 isolates representing 14 species of ectomycorrhizal fungi in sterile distilled water. Richter and Bruhn (1989) reported that cultures of saprobic and of mycorrhizal basidiomycetes survived cold storage in sterile water. Some of these fungi exhibited varying degrees of viability, which seemed to depend on the family they represent. Onions (1983) and Smith (1984, 1988) described the advantages and disadvantages of preserving different groups of fungi by various methods including water storage. Jones et al. (1991) detailed a miniaturized system for storage of fungal cultures in water. They stored more than 4000 cultures, mostly Hyphomycetes and Zygomycetes, for 2 yr. Their method was very similar to the one described here, but we describe its use for long-term storage (at least 7 yr) of Basidiomycotina.

*Methods.*—To preserve basidiomycetous wood-decay fungi with the sterile distilled water method, the fungus is plated onto a suitable medium; our choice for maintenance of these fungi is 1.5% malt extract agar (Difco Bacto Malt Extract agar). However, some of these fungi (e.g., *Phellinus* spp.) grow poorly on this medium. In these cases, potato dextrose agar is used as an alternative, although other media may be suitable.

Petri plates with 60- or 90-mm diameters are inoculated at the center and incubated until sufficient growth occurs (at least 3 cm in diam). The fungal

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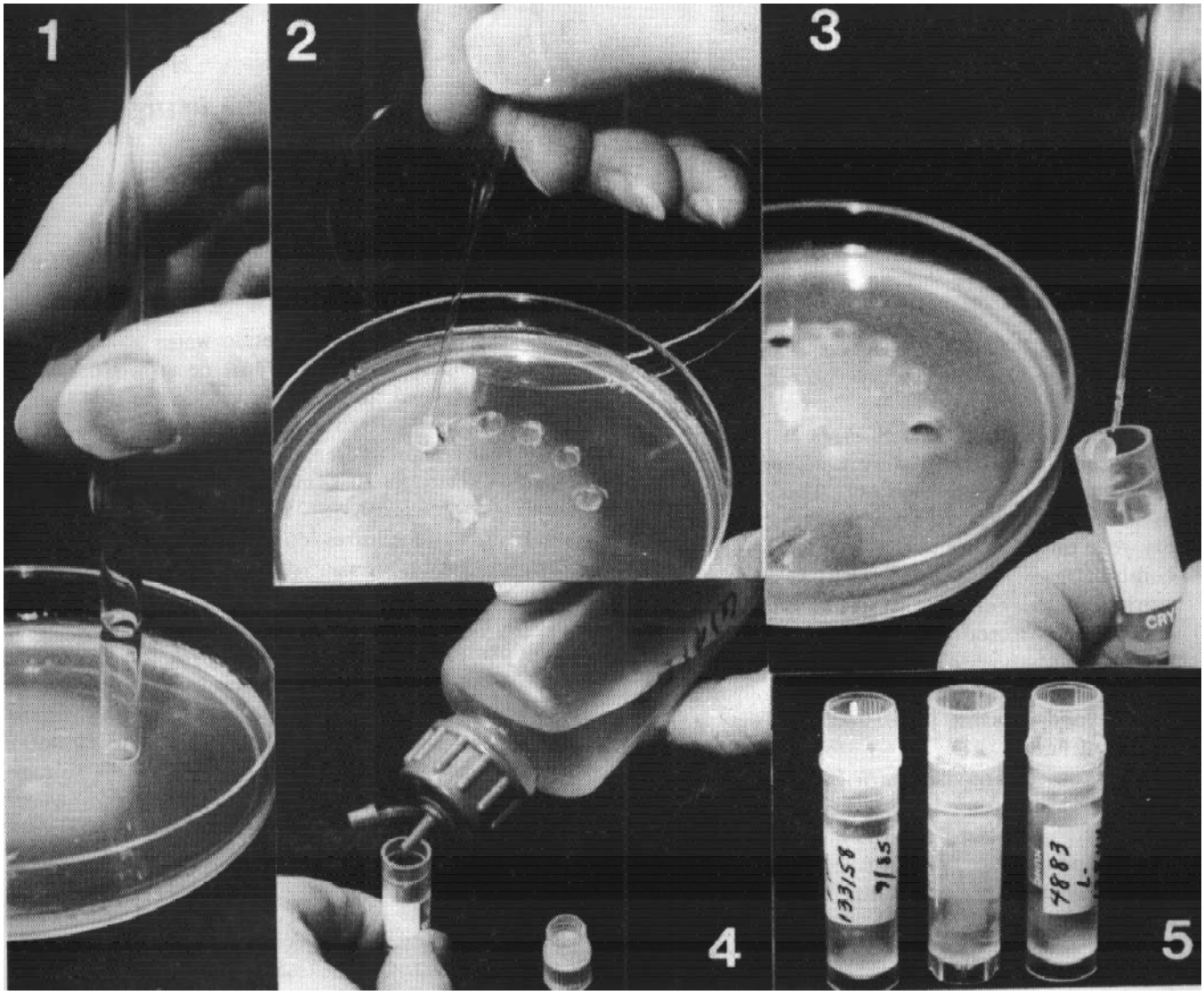
[2] The Forest Products Laboratory is maintained in cooperation with the University of Wisconsin. This article was written and prepared by U.S. Government employees on official time, and it is therefore in the public domain and not subject to copyright.

Accepted for publication November 17, 1993.




FIGS. 1-5. Illustrated technique for transferring cultures for distilled water storage. 1. Cutting mycelium/agar plugs with sterile pipet. 2. Transferring mycelium/agar plugs with sterile pipet. 3. Placing mycelium/agar plugs into cryovial using sterile pipet. 4, Filling cryovial with sterile distilled water. 5. Filled and labelled cryovials ready for storage.

culture is cut with the blunt end of a 14-cm-long, disposable, sterilized pipet (FIG. 1), resulting in a 6-mm mycelial agar plug. These plugs are taken from the growing edge of the mycelial mat (FIG. 2) to ensure that young, actively growing hyphae become part of future cultures. Five or six plugs are transferred to a sterile 2-ml cryovial using the pointed end of the same pipet (FIGS. 2, 3). When the vial has been filled to slightly below the fill line, indicated on the vial, sterile distilled water is squeezed from a plastic bottle that has an elongated delivery tube (FIG. 4) until the fill line covering the plug is reached. The vial is capped and ready for storage. However, we usually allow the vials to set at room temperature for 7 days to observe any contamination. We recommend storing in a vertical position to prevent leakage. We keep each vial in duplicate. Isolate information is recorded on each vial, including the field or collection number, the accession number, and the month and year of transfer (FIG. 5). The vials are plated in numerical order by the accession number, stored in boxes 12.7 by 12.7 by 5 cm (Revco Div., Revco Scientific, Inc., Asheville, North (Carolina[3]), divided into 100 vial-size compartments, and held at 5 C. Refrigeration may not be necessary. Boesewinkel el (197 Smith th (198 and Jones et al. u. (19 have maintained isolates at room temperature. When a culture is required, the vial is opened using a sterile technique preferably in a laminar airflow hood. The

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TABLE I. Growth rate of cultures of basidiomycetous wood-decay fungi before and after storage in sterile distilled water in cryovials

| Fungus | Accession number | Length of storage (years) | Growth rate (mm) | |
|---|---|---|---|---|
| | | | Prior to water storage | After water storage |
| *Agrocybe praecox* (Pers.: Fr.) Fayod | 00058 | 6.5 | TR[a], 21 | |
| *Agrocybe* sp. | 00064 | 7.0 | 12, 30 | |
| *Amylocystis lapponica* (Rom.) Bond. & Singer | 00130 | 7.0 | TR, 61 | 13, 37 |
| *Anomoporia albolutescens* (Rom.) Pouz. | 00145 | 7.0 | TR, 12 | TR, 10 |
| *Anomoporia bombycina* (Fr.) Pouz. | 00151 | 7.0 | TR, TR | TR, TR |
| *Antrodia oleracea* (Davids. & Lomb.) Ryv. | 00281 | 7.0 | 13, 43 | 14, 63 |
| | 00289 | 6.0 | 41, 88 | 32, 72 |
| *Antrodia serialis* (Fr.) Donk | 00305 | 7.0 | 31, 68 | 14, 38 |
| | 00312 | 7.0 | 26, 64 | 23, 63 |
| *Antrodia sinuosa* (Fr.) P. Karst. | 00261 | 7.0 | 23, 34 | |
| *Antrodiella romellii* (Donk) Niemala | 00393 | 7.0 | 28, 74 | 22, 73 |
| *Antrodiella semisupina* (Berk. & Curt.) Ryv. | 00418 | 7.0 | 46, 88 | 29, 88 |
| | 00429 | 7.0 | 32, 86 | 21, 63 |
| *Armillaria tabescens* (Scop.: Fr.) Dennis et al. | 00517 | 7.0 | 21, 85 | |
| *Athelia galzinii* (Bourd.) Donk | 00585 | 7.0 | 48, 65 | |
| *Athelia munda* (Jacks. & Deard.) M. P. Christ. | 00587 | 7.0 | 33, 59 | |
| *Auricularia mesenterica* (L.: Fr.) Underw. | 00707 | 7.0 | 24, 55 | |
| *Bjerkandera fumosa* (Pers.: Fr.) P. Karst. | 00681 | 7.0 | 42, 63 | 28, 54 |
| *Bondarzewia berkeleyi* (Fr.) Bond. & Singer | 00696 | 7.0 | 21, 58 | 32, 70 |
| | 00701 | 7.0 | 18, 68 | 32, 51 |
| *Boreostereum radiatum* (Pk.) Parm. | 00709 | 7.0 | TR, 48 | 33, 90 |
| *Cerrena unicolor* (Bull.: Fr.) Murr. | 00903 | 6.5 | 57, 90 | |

necessary plugs are transferred to the appropriate medium for fungal growth. The vial with the remaining plugs may be returned to storage.

Two-milliliter polypropylene screw cryovials, which are used for cryogenic storage in liquid nitrogen, were used for the water storage method. Each vial was complete with a teflon seal that restricted evaporation in the use proposed here. Several brands of vials were tested. All were found to be equally effective for use in this manner and can be ordered from many laboratory supply companies. To evaluate the effectiveness of this storage method, 151 vials, stored for different lengths of time, were randomly selected and plated onto 1.5% malt extract agar. The diameters of the fungus mats were measured and recorded after 1 and 2 wk incubation at 25 C.

Of the 151 isolates taken from water storage (after storage up to 7 yr), 94% were viable. For those isolates with previously recorded growth rates, the growth rates were not consistently affected by the distilled water storage method (TABLE I). For those for which previous information was not available, the growth rates were as expected (TABLE I). Thus, water storage did not have an obvious influence on culture viability or growth rate.

An unanticipated result of the water storage method was that some of the cultures, usually polypores, that were plated after water storage, developed the ability to produce fruit bodies in culture within approximately 6 wk. Because fruiting is rare in transfers from agar-slant test-tube storage and more frequent in cultures revived from sterile distilled water storage, we believe that the fruiting is affected by this method of storage.

Conclusions.—The water storage technique has numerous advantages compared with agar-slant test-tube storage. One advantage is the length of time isolates can be stored without transfer (at least 7 yr), which reduces media volume and expensive labor costs. The length of storage, lack of frequent transfer, and the slow growth rate under water increase genetic stability. Another advantage is that fungi that cannot be stored through lyophylization (e.g., most wood-decay Basidiomycetes) or through liquid nitrogen methods can frequently be stored in sterile distilled water.

This method is quick, relatively easy, and inexpensive. Considerably less space is required to store these cultures compared with that for test-tube storage. Most contamination is easy to discern if the vials are observed at room temperature for at least 7 days before storing. Mites cannot enter the screw-capped vials.

TABLE I. Growth rate of cultures of basidiomycetous wood-decay fungi before and after storage in sterile distilled water in cryovials (Continued)

| Fungus | Accession number | Length of storage (years) | Growth rate (mm) | |
|---|---|---|---|---|
| | | | Prior to water storage | After water storage |
| *Climacodon septentrionalis* (Fr. : Fr.) P. Karst. | 00977 | 7.0 | 30, 64 | 23, 56 |
| | 00979 | 7.0 | 20, 54 | 18, 69 |
| *Clitocybe nuda* (Bull. : Fr.) Cke. | 00999 | 6.5 | 0, 0 | |
| *Corticium meridioroseum* Boid. & Lanq. | 03926 | 5.5 | 25, 46 | |
| *Gloeocystidiellum furfuraceum* (Bres.) Donk | 10110 | 5.5 | 0, 0 | |
| *Grandinia arguta* (Fr.) Jülich | 03812 | 6.5 | 15, 44 | 10, 36 |
| | 03837 | 6.5 | TR, 19 | |
| *Grandinia crustosa* (Pers. : Fr.) Fr. | 03848 | 6.5 | TR, 18 | |
| | 03849 | 6.5 | TR, 24 | 0, TR |
| *Grandinia hastata* (Litsch.) Jülich | 03858 | 6.5 | TR, TR | CONT |
| *Grandinia nespori* (Bres.) Cejp. | 03806 | 6.5 | 0, 0 | |
| *Grandinia spathulata* (Schrad. : Fr.) Jülich | 03872 | 6.5 | 22, 57 | 21, 58 |
| | 03876 | 6.5 | 27, 77 | 12, 42 |
| *Helicogloea farinacea* (Hoehn.) Rogers | 10178 | 2.5 | | |
| *Hericium abietis* (Weir *ex* Hubert) K. Harrison | 02717 | 6.5 | TR, 22 | |
| | 02720 | 2.0 | TR, 16 | |
| *Hericium coralloides* (Scop. : Fr.) S. F. Gray | 02805 | 4.5 | 7, 54 | |
| | 02810 | 5.0 | 12, 21 | TR, 20 |
| *Hyphoderma clavigerum* (Bres.) Donk | 03136 | 6.5 | TR, TR | |
| *Hyphodontia* sp. | 03432 | 4.0 | 8, 39 | |
| *Hypholoma capnoides* (Fr. : Fr.) P. Kumm. | 04430 | 5.5 | 19, 53 | |
| *Hypholoma* sp. | 04449 | 5.5 | 51, 90+ | |
| *Inonotus dryadeus* (Pers. : Fr.) Murr. | 03555 | 5.0 | 20, 40 | 33, 62 |
| *Junghuhnia luteoalba* (P. Karst.) Ryv. | 03752 | 6.5 | 19, 42 | |
| | 03757 | 6.5 | 12, 42 | |
| | 03772 | 5.5 | 19, 51 | |
| *Laeticorticium roseum* (Pers. : Fr.) Donk | 03926 | 5.5 | 12, 28 | |
| | 03927 | 6.5 | 20 | |
| *Laetisaria arvalis* Burds. | 03972 | 6.5 | 10, 40 | |
| *Lentinellus omphaloides* (Fr.) P. Karst. | 04046 | 6.5 | 34, 72 | |
| *Lentinellus ursinus* (Fr.) Kühner | 04067 | 6.5 | 32, 59 | |
| | 04070 | 6.0 | | |
| | 04071 | 6.5 | 10, 42 | |
| *Lentinellus vulpinus* (Fr.) Kühner & Maire | 04075 | 6.5 | 0-TR | |
| *Lentinus velutinus* (Fr.) Kühner | 04147 | 6.5 | 43, 90 | |
| *Lopharia cinerascens* (Schw.) Cunn. | 04157 | 6.5 | 68, 90 | 19, 58 |
| *Lyophyllum montanum* A. H. Smith | 04526 | 5.0 | 0, 0 | |
| *Marasmius oreades* (Bolt. : Fr.) Fr. | 04213 | 6.5 | 20, 30 | |
| *Merulius* sp. | 04315 | 6.5 | 30, 60 | 32, 64 |
| *Microporellus obovatus* (Jungh.) Ryv. | 04341 | 6.5 | 34, 90 | 43, 90 |
| *Mucronella aggregata* Fr. | 04347 | 6.0 | TR | |
| *Mycena leaiana* (Berk.) Sacc. | 04365 | 6.5 | 14, 46 | |
| | 04367 | 1.5 | 24, 65 | |
| *Mycena* sp. | 04383 | 6.5 | 0, 0 | |
| *Mycoacia uda* (Fr.) Donk | 04401 | 6.5 | 66, 90 | 65, 90 |
| | 04406 | 6.5 | 62, 90 | 64, 86 |
| *Mycoacia* sp. | 04420 | 5.5 | 66, 90+ | 27, 53 |
| *Neolentinus lepideus* (Fr. : Fr.) Redhead & Ginns | 04058 | 6.5 | 34, 88 | |
| *Nigroporus durus* (Jungh.) Murr. | 04454 | 6.5 | 20, 45 | |
| *Nivatogastrium nubigenum* (Harkness) Singer & Smith | 04463 | 6.5 | 31, 63 | |
| *Omphalotus olearius* (DC. : Fr.) Singer | 04490 | 6.5 | 0, 18 | |
| *Oudemansiella radicata* (Relh. : Fr.) Singer | 04538 | 6.5 | 34, 89 | 28, 75 |

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| Fungus | Accession number | Length of storage (years) | Growth rate (mm) | |
|---|---|---|---|---|
| | | | Prior to water storage | After water storage |
| *Oxyporus corticola* (Fr.) Domans. | 04549 | 6.5 | 37, 89 | 25, 60 |
| | 04556 | 6.5 | 30, 80 | 58, 65 |
| *Oxyporus latemarginatus* (Dur. & Mont.) Donk | 04558 | 6.5 | 43, 90 | 90, 90 |
| *Oxyporus populinus* (Schum. : Fr.) Donk | 04565 | 6.5 | 32, 87 | 27, 70 |
| *Panellus stipticus* (Bull. : Fr.) P. Karst. | 04598 | 6.5 | 16, 90 | 20, 90 |
| *Panus crinitus* (L. : Fr.) Singer | 04636 | 6.5 | 28, 63 | 15, 47 |
| *Panus fragilis* (O. K. Miller) | 04646 | 6.5 | 70, 90 | 44, 86 |
| | 04649 | 6.5 | 80, 90 | 69, 90 |
| *Panus kauffmanii* (A. H. Smith) Corner | 04651 | 5.5 | 84, 90 | 56, 90 |
| *Panus lecomtei* (Fr.) Corner | 04869 | 6.5 | 65, 90 | |
| | 04673 | 5.5 | 47, 90 | |
| *Panus levis* Berk. & Curt. | 04672 | 5.5 | 44, 80 | |
| *Panus strigosus* Berk. & Curt. | 04688 | 6.5 | TR, 27 | 15, 29 |
| *Paxillus atrotomentosus* (Batsch : Fr.) Fr. | 04693 | 6.5 | 15, 23 | 10, 23 |
| *Peniophora aurantiaca* (Bres.) v. Höhn & Litsch. | 04709 | 6.9 | TR, TR | |
| *Peniophora cinerea* (Fr.) Cke. | 04728 | 6.5 | 46, 90 | 54, 90 |
| | 04732 | 6.5 | 55, 90 | 30, 70 |
| *Peniophora pithya* (Pers.) J. Erikss. | 04742 | 1.0 | 90, 90 | 75, 90 |
| *Peniophora pseudopini* Weres. & Gibson | 04772 | 6.5 | 59, 90 | 68, 90 |
| *Peniophora* sp. | 04787 | 1.5 | 47, 90 | 69, 90 |
| *Peniophora* sp. | 04838 | 6.5 | 86, 90 | 60, 74 |
| *Peniophora* sp. | 04843 | 6.5 | 30, 60 | 47, 90 |
| *Peniophora* sp. | 04853 | 6.5 | 28, 50 | 50, 90 |
| *Peniophora* sp. | 04860 | 6.5 | 80, 90 | 80, 90 |
| *Peniophora* sp. | 04863 | 6.0 | 73, 90 | |
| *Peniophora* sp. | 04870 | 6.5 | 90+ | 42, 90 |
| *Peniophora* sp. *C* | 04883 | 6.5 | 74, 90 | 23, 48 |
| *Peniophora* sp. *E* | 04870 | 6.0 | 42, 90 | |
| *Peniophora violaceolivida* (Sommerf.) Massee | 04834 | 6.0 | 80, 90 | |
| *Perenniporia compacta* (Overh.) Ryv. & Gilbn. | 04825 | 6.5 | 0, 0 | |
| | 04900 | 6.0 | 40, 90 | |
| *Perenniporia ellisiana* (F. W. Anderson) Ryv. & Gilbn. | 04905 | 6.0 | 75, 90 | |
| *Perenniporia fraxinea* (Bull. : Fr.) Ryv. | 04914 | 6.0 | 0, 0 | |
| | 04924 | 2.0 | 56, 90 | |
| *Perenniporia tenuis* (Schw.) Ryv. | 04930 | 6.0 | 44, 90 | |
| *Perenniporia tenuis* var. *pulchella* (Schw.) Gilbn. & Ryv. | 05055 | 6.5 | 30, 65 | 0, 0 |
| | 05078 | 6.5 | 32, 33 | 35, 65 |
| *Phaeolus schweinitzii* (Fr.) Pat. | 05098 | 6.5 | 70, 90 | 48, 90 |
| | 05095 | 6.5 | 50, 90 | 25, 90 |
| *Phanerochaete chrysorhiza* (Torr.) Bud. & Gilbn. | 05099 | 6.5 | 50, 90 | 40, 90 |
| *Phanerochaete chrysosporium* Burds. | 05153 | 6.0 | 20, 43 | |
| | 05171 | 2.0 | 80, 90 | |
| | 05175 | 6.5 | 90+, − | 88, 90 |
| *Phanerochaete ericina* (Bourd.) Erikss. & Ryv. | 05175 | 7.0 | 90+, − | 85, 90 |
| *Phanerochaete joseferreirae* (Reid) Reid | 05119 | 6.0 | 0, 0 | |
| *Phellinus arctostaphyli* (Long) Niemala | 10393 | 6.0 | 40, 90 | 43, 78 |
| *Phellinus everhartii* (Ell. & Gal.) Pilat | 05344 | 5.0 | TR, 86 | |
| *Phellinus ferrugineofuscus* (P. Karst.) Bourd. | 05432 | 6.0 | 11, 40 | |
| | 05459 | 1.5 | 0–TR, 25 | |
| *Phellinus ferruginosus* (Schrad. : Fr.) Pat. | 05464 | 1.0 | 12, 42 | 25, 57 |
| | 05471 | 5.5 | TR, 19 | |

TABLE I. Growth rate of cultures of basidiomycetous wood-decay fungi before and after storage in sterile distilled water in cryovials (Continued)

| Fungus | Accession number | Length of storage (years) | Growth rate (mm) | |
|---|---|---|---|---|
| | | | Prior to water storage | After water storage |
| *Phellinus laevigatus* (Fr.) Bourd. & Galz. | 05629 | 6.5 | 52, 90 | 48, 90 |
| *Phellinus viticola* (Schw. *apud* Fr.) Donk | 05963 | 0.5 | 19, 61 | |
| *Phlebia queletii* (Bourd. & Galz.) M. P. Christ. | 04396 | 2.0 | 37, 90 | 20, 80 |
| | 04397 | 2.0 | 61, 90 | 56, 90 |
| | 04398 | 1.5 | 78, 90 | |
| *Pleurocybella porrigens* (Pers. : Fr.) Singer | 06463 | 6.0 | 0, 0 | |
| *Postia guttulata* (Pk.) Jülich | 06853 | 6.0 | 22, 48 | |
| *Postia obducta* (Berk.) Lombard & Larsen | 04526 | 5.0 | 0, 34 | |
| *Radulomyces confluens* (Fr.) M. P. Christ. | 03489 | 6.5 | 30, 47 | |
| *Trametes suaveolens* (L. : Fr.) Fr. | 08891 | 6.0 | 66, 90 | 54, 90 |
| *Tricholomopsis streetsii* Gilbn. | 09056 | 6.5 | 51, 90 | TR, TR |
| *Wolfiporia cocos* (F. A. Wolf) Ryv. & Gilbn. | 04186 | 6.0 | 12, 41 | |

[a] TR = trace.

**Heat from a hot, sterile, transfer needle, which can be detrimental to the fungus when cultures are transferred, is avoided by using a presterilzed, disposable pipet and a laminar airflow hood.**

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