Culture Maintenance of Wood Decay Fungal Isolates Used in Soil Block Tests

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ABSTRACT

The AWPA E10 soil block test is one of the most often used methods to screen wood protectants against pure cultures of wood degrading fungi in North America. The test methodology was first developed in the early 1900s and has been adapted to evaluate a wide range of chemically treated, modified, and naturally durable woods and wood-based materials. The history and basic requirements of this fundamental test, along with good laboratory practices for optimum fungal strain performance, are discussed with practical considerations to ensure optimal and reproducible results.

BRIEF HISTORY OF THE E10 SOIL BLOCK TEST

The following is not intended to be a thorough examination of the history of the soil bottle, although that work is currently in progress. The contemporary E10 soil bottle is in essence an adapted soil jar test used by forest pathologists in the early 1900s. Notable examples of these can be found in Zeller[1-4] and Humphrey[5]. An excellent literature review on the development of early soil bottle methods is presented in Colley[6]. Notable works by Leutritz[7], Richards[8,9], and Duncan[10] are additional essential reading for understanding the development of this test. Duncan proposed the soil bottle test as an ASTM test method in 1953 and it became an accepted evaluation method in 1964. The soil bottle test was adopted by the American Wood Preservers Association (now the American Wood Protection Association) in 1972 as standard M10, which was reorganized in 2008 to the current distinction as the E10 soil bottle test. The E10 soil bottle represents a core test in wood preservative evaluation methods and is widely used by industry, academics, and government researchers.

SOIL BLOCK 101: BASIC METHODOLOGIES

The scope of the E10 soil bottle defines it as a screening test to determine the resistance of wood-based materials to decay by select fungi under controlled laboratory conditions. In the following sections, any references to the current E10 standard are indicated by the appropriate section designation for ease of navigation. It also states that the test can be used to establish the minimum amount of preservative that is effective at preventing decay of selected species of wood by selected fungi under optimum laboratory conditions. This requires an actively growing and aggressive strain of wood rot fungi (Secs 6.2 and 6.3), test blocks that are representative of the material under consideration (Sec 5.2), a suitable soil substrate (Sec 7.2), and adequate moisture to arrive at a soil moisture content to approximately 130% of water holding capacity (Sec 7.2.1), which ensures adequate soil moisture to maintain a wood MC in the range of 40-80%, which is required for the growth of common decay fungi. The final ingredient is time, and it is critical to the planning of soil bottle tests. Fungi often need additional time to establish and ensure that the test materials are indeed subjected to worst case conditions for the test subject and best conditions for the invading fungus. It should be noted that this paper deals only with optimizing growth of fungal isolates to be used in test and that careful consideration should also be given to all other guidance contained in the standard. The next section will identify four underlying parameters that are referenced in the standard, and provide some background information as to why these parameters are critical to achieving optimal, reproducible, and relevant data.

BEST LABORATORY PRACTICES FOR MAINTAINING FUNGAL ISOLATES

1. Nuclear Condition (Sec. 8)

Ascomycete and basidiomycete fungi belong to subkingdom Dikarya because both groups can produce dikaryons, which is a condition in which two nuclei cohabitate a fungal cell without fusing. This condition is maintained through the production of croziers in ascomycetes and clamp connections in basidiomycetes to allow nuclei to migrate from cell to cell[11]. In basidiomycetes, the dikaryon is considered the dominant condition but monokaryons (single nuclei) do form under various circumstances. In most cases, monokaryons are considered weaker in pathogenicity and maintenance of dikaryotic stocks is specifically mentioned in the standard[12]. The literature around this issue is not conclusive. Certain studies indicate that mono- and dikaryons have similar capabilities[13], while others indicate either increased or decreased decay capacity between the two[14-17]. The underlying fact remains that decay capacity can vary between mono- and dikaryons and nuclear condition should be documented or at least confirmed, especially in the event of errant results being obtained. Nuclear condition can be confirmed microscopically (shown below for *G. trabeum*) or weak sectors in a petri dish should be assumed to be monokaryotic and avoided. It has also been observed that repeated subculturing of decay fungi can lead to the occurrence of monokaryons, which should also be considered.

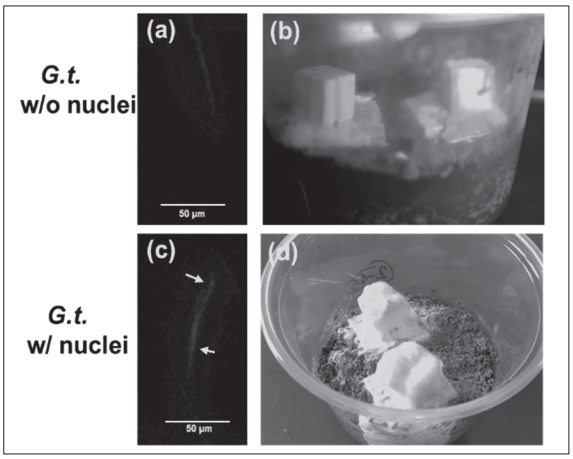

Figure 1. Examples of mono- and dikaryotic forms of *Gloeophyllum trabeum* (Mad 617): Images (a and c) fluorescent microscopic images showing hyphae representative of mono and dikaryons, respectively. Images (b and d) are comparative images to illustrate fungal vigor between mono- and dikaryotic strains.

2. Media Selection (Sec 7.1)

The composition of artificial media used to culture basidiomycete isolates can influence isolate vigor and colonization rate[18-21]. Interestingly, the physiological differences noted on deficient media are similar to those of monokaryotic isolates (thin aerial hyphae, reduced growth, and vigor). The current E10 standard recommends 0.5% yeast extract added to 2% Malt extract and 1.5% agar[22]. The images below (Figure 2) illustrate growth differences with and without the 0.5% yeast extract for three different, but commonly used, soil bottle fungi. Note the dense appressed hyphal mats on the petri dishes with the recommended yeast extract; this yields much more suitable inoculum in the form of mycelial plugs used to seed feeders in the test.

3. Moisture/Temperature Optimums (Sec 3.2, 15.1)

Decay fungi have variable optimal ranges for growth regarding both temperature and moisture[23,24]. The current standard specifies a temperature range of 22°C to 28°C, but perhaps greater attention should be paid to optimum ranges for each individual species and even isolate. An example of this would be *Serpula larymans*, which exhibits optimum growth and performance at 20°C, and at FPL we now have a separate incubator specifically for *Serpula*.

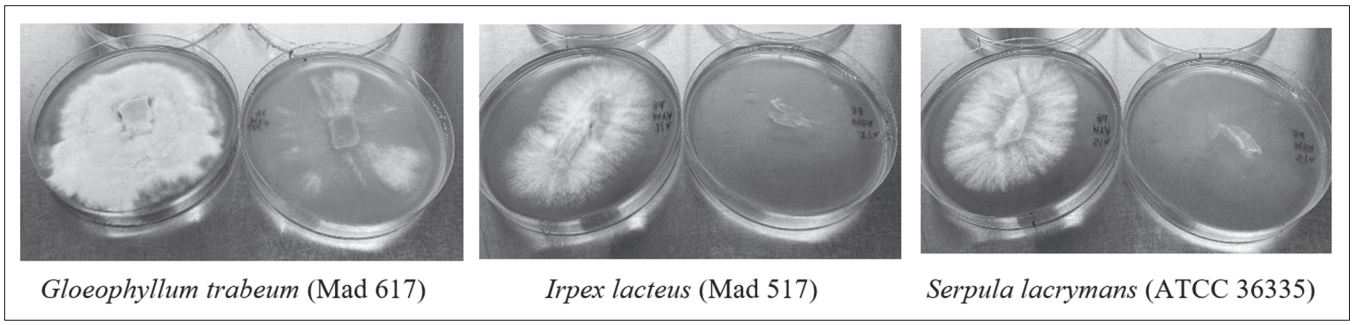

Figure 2. Illustrated examples of differences in cultural growth on different media types. For each isolate, the left dish is using the current AWPA recommendation of including 0.5% yeast extract, while the petri dish on the right is without. Note the reduced growth and lack of aerial hyphae.

4. Long Term Storage (Sec. 8)

Decay isolates do not function indefinitely, as repeated and routine subculturing can decrease isolate vigor and yield inconsistent results. Wood decay fungi can be stored for several years on media slants amended with sterilized wood sawdust. This has been found to maintain decay capacity, presumably due to continued activation of genes that control wood metabolic pathways. However, media slants are not an effective long term storage solution, so other methods should be considered for archiving isolates[25,26].

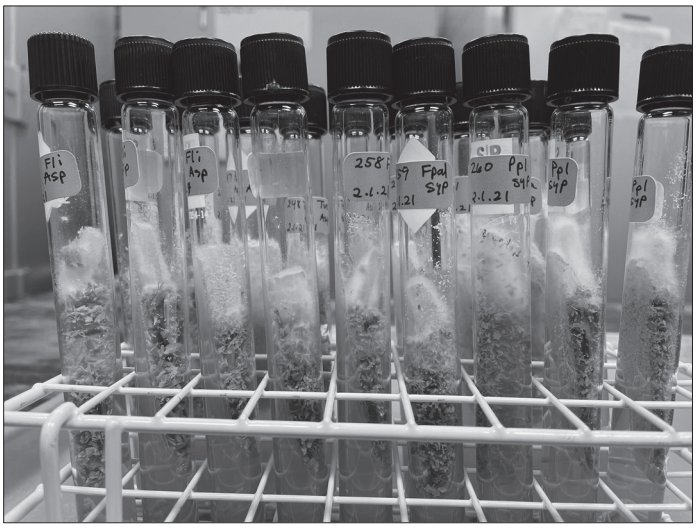

Figure 3. Maintenance of fungal cultures on media slants amended with sterilized wood sawdust.

CONCLUSION

The E10 soil bottle is a screening tool to assess relative efficacy of wood treatments to pure cultures of basidiomycete fungi. The goal of this presentation is to highlight some important considerations that might be overlooked or underemphasized when conducting routine E10 testing. Genetic condition, culture media recipe, incubation conditions, and long-term storage of fungi are important factors that are listed within the text of the standard but could be emphasized or amended with additional reference material in the future.

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