Plant Health Care Inc.
440 William Pitt Way
Pittsburgh, PA 15238 USA

Technical Bulletin

024 08/16/06

MYCORRHIZAL FUNGI CANNOT BE COUNTED BY CFU'S

Occasionally, we are asked to provide counts of mycorrhizal fungi in our products in terms of CFU's, the standard unit for single-celled bacteria. This is not possible, since CFU's are not applicable to microbes of this kind. The reasons are discussed here:

The CFU: Colony Forming Unit

In industrial and research settings, there is a need to quantify the microbe content of microbial products. The method for doing this varies for different types of microbes. Traditionally, the first microbes to be used commercially were bacteria and yeasts. These are typically single-celled species that can be grown in natural and artificial media, and are well-suited to growth in agar gels on Petri plates. Using this method, individual cells or clumps of cells will form discrete colonies, which become visible to the naked eye as the colony grows. Counting the number of colonies provides a direct way to track the original number of discrete microbial units. A count determined this way been dubbed the number of "Colony-Forming Units" or "CFU" for short. CFU's are only applicable to single-celled microbes that can be grown on nutrient media, such as bacteria, yeasts, or spore-forming molds.

Mycorrhizal Fungi Spores Will Not Grow on Petri Plates

Several types of microbes cannot be counted using CFU's. This is due to a number of technical reasons. For example, spores of ecto- and VA mycorrhizal fungi will not grow in culture. These fungi are obligate symbionts. This means that they will only grow and proliferate in association with roots of a host plant. No one has ever devised a culture medium that will successfully grow the spores of these obligate symbionts. Therefore, no colonies can be grown from spores. Hence, the concept of a CFU is not applicable to these fungi.

Tissue Cultures of Ectomycorrhizal Fungi on Plates

Tissue cultures can be made from mushrooms or puffballs of ectomycorrhizal fungi, and these tissues will grow in artificial culture media on agar plants. (The spores of these fungi will not grow this way.) However, this is done by cutting off a macroscopic piece of multicellular tissue and placing it on a media plate. Here again, the CFU concept is not applicable, since this manual technique can only physically handle one multicellular piece at a time, and has no bearing on the size or cell count of the original piece of tissue. Also, these higher fungi grow by forming long, multicellular strands, like spagetti. There is no way to relate colony counts to the number of cells originally involved. Therefore, the concept of CFU cannot be used with these fungi. Finally, the tissues of these fungi will not remain alive for long if allowed to dry out. Therefore, only spores are used in quality commercial mycorrhizal products.

Tissue Cultures of VA Mycorrhizal Fungi

VA Mycorrhizal fungi do not form mushrooms or puffballs, or any complex macroscopic tissues. Neither spores nor vegetative cells of VA mycorrhizal fungi have been successfully grown independently of a host root. Hence, Petri plate colonies do not form, and no counts can be made.

How Are Mycorrhizal Fungi Mass-Produced?

Mycorrhizal fungi can only be mass-produced by mass-producing host plants first.

VA Mycorrhizal Fungi

VA Mycorrhizal fungi are mass-produced in greenhouses, where host plants are grown in pots. One common host plant often used is Sudan grass. The potting soil is inoculated with spores of a particular species of VA mycorrhizal fungus, and the plants are grown for several months. Environmental conditions are controlled in proprietary ways to maximize spore production in the potting soil. (For phytosanitary reasons, this is actually a soil-less horticultural potting medium.) At harvest time, the leaves and stem of the grass is cut off, and the potting soil (containing the spores) is collected. Various physical techniques are used to physically separate the spores from the potting soil, and concentrate them in dry form.

How are VA Mycorrhizal Fungi Spores Counted and Used for Products?

Once separated and concentrated from potting soil, the spores can be directly counted using sample aliquots of known weight. Small aliquots are removed and examined with a dissecting microscope. Spores are visually removed and counted using a small artist's brush. The number of spores counted per unit weight (or volume) is averaged over several small samples. There are various techniques published for counting spores. But the resulting number is always expressed as the number of *spores*. This number cannot be expressed as CFU's.

Ectomycorrhizal Fungi

Ectomycorrhizal Fungi typically produce puffballs or mushrooms. Since puffballs produce the most spores, commercial ectomycorrhizal fungi are usually puffball-producing species, rather than mushrooms. These will grow at the base of host trees. To produce spores, tree roots are inoculated with a particular species of ectomycorrhizal fungus in the nursery. These trees are then planted to field sites. Puffballs or mushrooms develop under these trees, and typically return annually for many years. Alternatively, puffballs can also be collected from natural forest sites. Once collected, puffballs are dried, ground, and the spores are physically separated from the rest of the tissue.

How are the Ectomycorrhizal Mycorrhizal Fungi Spores Counted and Used for Products?

Published research has revealed the most probably number of ectomycorrhizal fungi spores in a concentrated gram. For example, a gram of concentrated spores of *Pisolithus tinctorius* is said to contain about 1.1 billion spores. For practical reasons in commerce, this number is rounded to an even 1 billion. Again, the resulting number is always expressed as the number of spores. This number cannot be expressed as CFU's, since there is no "colony" involved in this figure.

References

Smith, S. E. and D. J. Read. 1997. Mycorrhizal Symbiosis. 2nd Ed. Academic Press, Ltd. London. 605 p.

Brundrett, M. et al. 1994. Practical Methods in mycorrhizal research. Mycologue Publications.