

## CULTURE MEDIA FOR FUNGI

### Introduction

A wide range of media are used for growing fungi. Most mycologists develop preferences for certain types of media based on experience and peculiarities of the type of fungi that are routinely grown. Media will affect colony morphology and color, whether particular structures are formed or not, and may affect whether the fungus will even grow in culture. For example, some fungi lack the necessary enzymes to utilize different carbon sources. All fungi require several specific elements for growth and reproduction. The requirements for growth are generally less stringent than for sporulation, so it is often necessary to try several types of media when attempting to identify a fungus in culture. Most fungi thrive on Potato Dextrose Agar (PDA), but this can be too rich for many fungi, so that excessive mycelial growth is obtained at the expense of sporulation. I have found that most of the fungi isolated from soil, or from substrates in the soil, i.e., plant debris, grow well on Corn Meal Agar (CMA), a relatively weak medium compared to PDA. Similarly, wood-inhabiting fungi and dematiaceous (dark pigmented) fungi often sporulate better on CMA or Oat Agar, both of which have less easily digestible carbohydrate than PDA. Cellulose-destroying fungi and spoilage fungi retain their ability to produce cellulase when grown on a weak medium such as Water Agar (WA) or Potato Carrot Agar (PCA) with a piece of sterile filter paper, wheat straw or lupin stem placed on the agar surface. The introduction of pieces of tissue, such as filter paper, wheat straw, rice, grains, leaves or dung, often produces good sporulation dependent on the organism grown.

### Constituents of Media

Media generally contain a source of carbon, nitrogen and vitamins. Glucose (dextrose) is the most widely utilizable carbon source, and hence is the most commonly used in growth media. Fructose and mannose are the next most commonly utilized sugars by fungi and are found in media from natural sources. Sucrose (table sugar) may be used in some media. Nitrogen sources include peptone, yeast extract, malt extract, amino acids, ammonium and nitrate compounds. Casamino Acids, a Difco product, is acid-hydrolyzed casein, a mixture of amino acids. It is a good general source of nitrogen but is vitamin-free. Bacto-Peptone, another Difco product, contains nitrogen and a high peptone and amino acid content. Salts, including Fe, Zn and Mn, are often added to 'defined' media, but are usually not added to the common media used for routine culture. Fungi have natural deficiencies for vitamins that are satisfied at  $\mu\text{M}$  to  $\text{nM}$  concentrations. The most common naturally occurring vitamin deficiencies are thiamin and biotin. Deficiency of both is quite common among the Ascomycota. Other organic nutrients such as glucose are often contaminated with vitamins sufficient to supply the growth requirements of fungi.

### **Isolation Media--Water Agar, Antibiotic Agar and Acidified Cornmeal Agar**

Most fungi are difficult to isolate from infected tissues, infested soil or decaying organic material due to the rapid and often antagonistic development of associated fungi, bacteria and actinomycetes with faster growth rates. Successful isolation of many of these fungi can be achieved by the use of selective media that either slow down the growth or inhibit the growth of these antagonistic organisms. One approach is to surface sterilize the material to eliminate surface contaminants, then place the material on Water Agar. Most fungi and bacteria will grow on WA, but at such a slow rate that it is relatively easy to isolate the target fungus. Selective exclusion can be accomplished by several approaches. One is by selective inhibition, i.e., the use of antimicrobial chemicals, primarily antibiotics such as streptomycin sulfate and penicillin. We have Antibiotic Agar (AA) in the lab for this purpose. Antibiotic Agar is a good agar to use when isolating from material that cannot be surface sterilized and hence will have a heavy load of bacteria. Another approach is to create an unfavorable environment in the isolation medium such as high pH in which the fungus will grow but the bacteria will be inhibited. Acidified cornmeal agar (ACMA) works well for the isolation of fungi from various types of plant material. It is best to use surface-sterilized material in conjunction with ACMA.

### **Growth Media**

We will use mostly 'natural' media based on materials such as cornmeal, carrots, hay, potatoes, oatmeal, soil, etc. 'Semi-synthetic' media, containing both natural ingredients and defined components include Malt Extract Agar, Malt Agar. We probably will not be using 'Synthetic' or 'defined' media which contain precise amounts of a carbon source, vitamins and minerals.

As previously noted, Cornmeal Agar is a good medium for the culture of dematiaceous fungi and fungi isolated from soil, soil substrates and wood. Malt Extract Agar (MEA) is also frequently used for culturing fungi from soil and wood.

Water molds, such as those obtained from baiting with hemp seed and snake skin, can be grown on Cornmeal Agar, Water Agar or Emerson's YpSs agar. See 'Zoosporic Fungi in Teaching and Research' pages 125-127 for a full description of methods.

Ascomycota isolated from fruiting bodies forming on dung, wood or soil can be grown on Malt Agar, Potato Carrot Agar and Potato Dextrose Agar. The first step is to attempt to get the ascospores to germinate by streaking them out or getting forcible discharge onto a selective or isolation medium. Look for spore germination and transfer a small piece of agar with the germinating spore(s) to one of these richer media.

Basidiomycota growing on wood can be grown on Malt Extract Agar. Cultures derived from mushrooms can be grown on Potato Dextrose Agar, Potato Sucrose Agar and Malt Extract Agar. Potato Dextrose Yeast Extract Agar (PDYA) also is a recommended

medium for growing cultures of *Agaricus*, *Pleurotus*, *Lentinus*, *Stropharia*, *Flammulina*, and some of the *Psilocybe* species.

### **Summary of media and common use**

**Water Agar (WA)**--use for isolating fungi from surface-sterilized substrates.

**Antibiotic Agar (AA)**--use for isolating fungi from substrates not readily surface-sterilized, or to clean up a culture contaminated with bacteria.

**Acidified Cornmeal Agar (ACMA)**--use for isolating fungi from substrates that are likely to be contaminated with bacteria. Not a substitute for AA, but the acidity inhibits bacteria and the medium supports the growth of a wide range of fungi.

**Cornmeal Agar (CMA)**--use for growing a wide range of fungi, particularly members of the Fungi imperfecti; provides a good balance of mycelial growth and sporulation.

**Potato Carrot Agar (PCA)**--considered a relatively weak medium somewhat comparable to CMA, good for some Fungi imperfecti.

**Malt Agar (MA)**--lacks peptone, and is useful for culturing many Ascomycota; sporulation in some species is inhibited by peptone.

**Malt Extract Agar (MEA)**--a good growth medium for soil fungi, fungi isolated from wood, basidiomycetes, etc. An all-purpose type of medium.

**Potato Dextrose Agar (PDA)**--a relatively rich medium for growing a wide range of fungi.

**Potato Dextrose-Yeast Extract Agar (PDYA)**---good for growing cultures derived from mushrooms.

## REFERENCES

Descriptions of methods for isolating and growing all groups of fungi can be found in the following reference, available in room 326:

Stevens, R. B., editor. 1981. *Mycology Guidebook*. University of Washington Press, Seattle.

Descriptions of methods for isolating and growing mushrooms can be found in the following references, also available in room 326 or from the instructor:

Stamets, P. and J. S. Chilton. 1983. *The Mushroom Cultivator. A Practical Guide to Growing Mushrooms at Home*. Agarikon Press, Olympia, WA.

Stamets, P. 1993. *Growing Gourmet and Medicinal Mushrooms*. Ten Speed Press and Mycomedea, Olympia, WA.

Watling, R. 1977. *How To Identify Mushrooms to Genus V: Cultural and Developmental Features*. Mad River Press, Eureka, CA.

Techniques for isolating and growing all types of zoosporic fungi can be found in:

Fuller, M. S. and A. Jaworski. 1987. *Zoosporic Fungi in Teaching and Research*. Southeastern Publishing Corporation, Athens, GA.

Techniques for isolating and growing members of the Protoctista and Chytridiomycota can be found in:

Margulis, L., J. O. Corliss, M. Melkonian and D. J. Chapman. 1990. *Handbook of Protoctista*. Jones and Bartlett Publishers, Boston.