Technique Sheet: Culture Media for Fungi

Many types of media with various formulations are used to culture fungi. Some are general in application, while others are used for specific purposes (standardization of descriptive data, isolation of fungi from special habitats, induction of reproductive structures, physiological or for growing fungi for molecular studies).

Media can be grouped into three broad categories based on their composition:

A. Non-reproducible natural media—occur in nature and require no preparation
B. Reproducible natural media—entire chemical composition not known
C. Synthetic media—complete chemical composition known

For more detailed information on culture techniques and formulations of culture media see:

Difco Laboratories. 2003. Difco and BBL *Manual of Microbiological Culture Media*. Becton, Dickinson and Company, Sparks Maryland. 696 pp.

Johnson, L.F., and E.A. Curl. 1972. *Methods for research on the ecology of soil-borne plant pathogens*. Burgess Publishing Co., Minneapolis. 247 pp.

Stevens, R.B., ed. 1974. *Mycology guidebook*. University of Washington Press, Seattle. 703 pp.

Some of the media used in preparation of class cultures are outlined below. For each, raw ingredients are given. When medium is commercially available in pre-mixed ready-to-use, dehydrated form, its name is followed by an asterisk.

A. Non-reproducible natural media:

1. Dung
2. Wood
3. Fruits and Vegetables

B. Reproducible Natural Media:

1. Corn Meal Agar (CM)*

   Corn meal .....20 g
   Agar.....18 g
   Distilled Water..... 1 liter

2. Lactose-Yeast Extract Agar (LY)

   Lactose*.....1 g
   Yeast Extract* .....0.5 g
   Agar*.....18 g
   Distilled Water..... 1 liter

3. Malt Extract Agar* (ME)

   Malt Extract* 10lg
   Peptone* 4.0lg
   Agar*.....18lg
   Distilled Water..... 1 liter

4. Oat Agar, whole (OA)

    Quaker Oats* ..... 10 flakes/20 ml
    Agar* ..... 15 g
    Distilled water ..... 1 liter

5. Potato Dextrose Agar (PDA)*

    Potato extract (see below) ..... 200 ml
    Glucose* ..... 10 g
    Agar* ..... 15 g
    Distilled Water ..... 1 1 liter

    To prepare potato extract: place 200 g diced potatoes into 500 ml dist. water, cook 1 hour in steamer or 40 min. in autoclave. Strain potato infusion through cloth, melt agar in 500 ml dist. water, then add 200 ml potato extract to melted agar, add glucose, adjust volume to 1000 ml, and autoclave. This is commercially available from Difco.

6. Acidified Potato Dextrose Agar (PDA)

    Lactic acid (25% [vol/vol])* 2.5ml

    Add 2.5ml of lactic acid per liter of agar, then autoclave and plate.
    If not using commercially available PDA Agar, amend formulation above to have 20 g of agar, then add acid, autoclave, and pour plates.

7. Soil Extract Agar (SEA)

    Soil ..... 50 g
    Yeast Extract ..... 1 g
    Agar* ..... 15 g
    Distilled Water ..... 500 ml

    Boil soil for 15 minutes in 500 ml water, filter, then add yeast extract, then agar.
    Increase volume to 1 liter. Melt, autoclave, and pour plates.

8. Yeast powder-soluble starch Agar (YPSS)*

    K_2HPO_4 ..... 1 g
    $MgSO_4 \times 7H_2O$ ..... 0.5 g
    Soluble Starch ..... 15.0 g
    Yeast Extract ..... 4 g
    Agar ..... 20 g
    Distilled Water ..... 650 ml
    Filtered Pond Water ..... 300 ml
    Double Distilled Water ..... 50 ml

    Dissolve salts in 65 0ml distilled water, add 20 g agar, and melt.
    Dissolve 15 g of soluble starch in 300 ml of filtered pond water by bringing to a boil while stirring. The starch should dissolve and become transparent.
    Dissolve 4 g yeast extract in 50 ml double distilled water.
    Add yeast extract solution and the dissolved starch to the melted agar solution, stir well, autoclave, and pour plates.

9. Rose Bengal Agar*

   Soytone ..... 5 g
   Dextrose ..... 10 g
   KH_2PO_4 ..... 1 g
   Magnesium Sulfate ..... 0.5 g
   Rose Bengal ..... 0.05 g
   Agar ..... 15 g

   Rose Bengal Agar can be supplemented with Rose Bengal Antimicrobic Supplement C, which utilizes chloramphenicol as a selective supplement to inhibit bacterial growth.

C. Synthetic media:

1. Czapek's Agar (CZ)*

   Sucrose (commercial grade) ..... 30 g
   $NaNO_3$ ..... 3 g
   K_2HPO_4 ..... 1 g
   $MgSO_4$ x 7 H_2O ..... 0.50 g
   KCl ..... 0.50 g
   $FeSO_4$ x 7 H_20 ..... 0.01 g
   Agar ..... 20.0 g
   Distilled Water ..... 1 liter

2. Czapek's Rose Bengal Agar (CZ-RB)
   This modification of Czapek's agar is made by adding the following to the above formulation just prior to sterilization:

   Rose Bengal* ..... 100mg/L (=0.1g/L)

3. Spezieller Nahrstoffarmer agar (SNA) [Nirenberg, H. 1976. Untersuchungen tiber die morpholoigische und biologische Differenzierung in der Fusariuru-Sektion Liseola. Mitt. Biol. Bund. Land- und Forstw. Berlin-Dahleru 169: 1-117}

   Spezieller Nährstoffarmer agar contains (per liter)

   Sucrose ..... 0.2 g
   Glucose ..... 0.2 g
   KNO_3, ... 1.0 g
   KH_2PO_4 ..... 1.0 g
   $MgSO_4$ x $7H_2O$ ..... 0.5 g
   NaCl ..... 0.5 g
   Agar ..... 12 g
   10 (4 x 4 mm) pieces of filter paper

Information on Agar (Agar-Agar)

Agar is obtained from certain seaweeds. The red algae (Class Rhodophyceae), *Gelidium cartilagineum* and *Gracelaria confervoides*, are mainly used. Species other than those of *Gelidium* and *Gracelaria*, which are used for agar production on a small scale, are *Pterocladia lucida*, *P. capillacea* (New Zealand), *Subria vittata* (South Africa), *Phyllophora rubens*, and *Ahnfeltia plicata* (Russia). In the past, the main agar-producing countries have been Japan, the United States, Russia, Australia, New Zealand, South Africa, and India, but as with most modern trends, China has now burst into the scene.

Agar is a complex polysaccharide that forms a colloidal solution in hot water and has a remarkable gel-forming capacity, some eight to ten times greater than that of gelatin. *Gelidium* agar dissolves in water at about 90ºC whereas the gel melts at 80ºC. Gelation of the dissolved agar occurs when the temperature drops to about 40ºC or less. The strength of agar gels depend on the moisture content and source of the agar. Dehydrated agar absorbs water, and melted agar precipitates in ethyl alcohol or acetone as a white fibrous mass. Agar also dissolves in glycerine, ethylene glycol, and other polyhydroxy alcohols; *Gelidium* and *Gracelaria* agars do not gel in these liquids unless water is present.

Gelidium agar is a sulfuric acid ester of linear galactan that exists in nature as a calcium salt. The galactan, a very complex carbohydrate, is composed mainly of D-galactose units, but also contains L-Galactose.

Since agar readily dissolves in boiling water, the agar is easily extracted from boiled seaweeds. The agar is then purified by freezing the gel and allowing it to thaw. Agar is marketed in granulated form, as flakes.

Walter Hesse (1891) is often credited with the introduction of the use of agar as a solidifying agent for culture media which was an important step in the advancement of microbiology. However, agar was discovered in 1658 by Minora Tarazaemon in Japan. According to legend, this Japanese innkeeper put surplus seaweed soup into the winter night and noticed it latter transformed into a gel by the night's freezing and day's warmth. Walter Hesse, a country doctor from Saxony, introduced Koch to this powerful gelling agent and in 1882 Koch was the first to use agar in microbiology. The main advantage of agar over gelatin, is the stability at temperatures above room temperature and the inability of most organisms to hydrolyze the galactan polymers.

References:

Difco Laboratories. 2003. Difco and BBL *Manual of Microbiological Culture Media*. Becton, Dickinson and Company, Sparks Maryland. 696 pp.

Hitchens, A.P. and M.C. Leikind. 1939. *The introduction of agar-agar into microbiology*. Journal of Bacteriology 37: 485-493

Humm, H. J. 1947. *Agar—a pre-war Japanese Monopoly*. Economic Botany 1: 317-329.

Moore, L. R. 1944. *New Zealand seaweed for agar manufacture*. New Zealand Journal of Science and Technology 25; 183-209.

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