The British Mycological Society

Chemical Reagents

by Peter Smith

GENERAL MOUNTING MEDIA

All sections can of course be mounted without any staining, and this can be in distilled water, however, potassium hydroxide (KoH), or ammonium hydroxide is generally preferred. as they produce a clearer mount. potassium hydroxide does not keep as well as ammonium hydroxide as the former will crystallise out in time. Potassium hydroxide or ammonium hydroxide should be used on all dried material to facilitate swelling to near the original size. Note. some hyphal pigments are soluble in these alkaline mounts, and alkali mounts often alter the colour of tissue and spores. In this guide I will refer to ammonium hydroxide however, this can be read as ammonium hydroxide or potassium hydroxide. Some mycologists use a general mounting medium called L4, which is suitable for dry or fresh fungi and is based on potassium hydroxide but also contains sodium chloride and invadin IFC. Coloured stains are then added to the L4 as required.

METHODS

The methods described here are for tissue cut directly from the fungus and not for tissue that has been wax embedded and microtomed. In many cases reasonable results can be obtained by mounting the fungal tissue to be examined directly in the stain, however for the best results the tissue should be left in the stain usually for a few minuets and then the surplus stain is washed away. Two methods can be employed; method a) place the tissue in a drop of the stain with fine forceps, leave for a few minuets, then transfer and wash in a drop of ammonium hydroxide, place on a clean slide apply fresh ammonium hydroxide and cover. method b) Place tissue on the slide and apply the stain, cover and leave for a few minuets, then apply ammonium hydroxide to one edge of the cover slip and at the same time apply absorbent material to the opposite edge of the cover slip to draw the ammonium hydroxide through the tissue clearing away surplus stain. In Van Waveren (1985) cystidia are stained by first applying a drop of ammonium hydroxide, removing this with tissue, applying congo red stain, heating till dry, then washing all dry stain away with potassium hydroxide. If this technique is superior maybe it can be used for the cystidia in other genera. The use of aceto-carmine to stain siderophilous granules in basidia also requires heating, this is usually done by heating the tissue in one drop of stain until almost dry and then adding another drop and repeating several times.

WHITE SPORES

Amyloid & Dextrinoid Spores

In Melzers Iodine, amyloid spores stain blackish and dextrinoid spores stain brown, although this is usually easily to see when the test is done on a pile of spores, it is not so easy to determine under the microscope as colours appear much lighter. In Russula and Lactarius and some other genera the spore ornimentation is amyloid and melzers iodine is invaluable for examining this ornamentation. Lugols Iodine is OK for the Amyloid reaction but not the Dextrinoid one

Cyanophilous spores (i.e. Lepista)

In a concentrate solution of cotton blue spore walls and appear dark blue when stained with lacto-phenol cotton blue.

Metachromic spores (i.e. Macrolepiota, Leucoagaricus, Leucocoprinus, and Hassiella)

These stain a reddish purple when placed in cresyl blue.

Epispore (i.e. some species of the Coprinus group)

Erythrosin can be used to make the spores stand out, this is particularly useful for spores which have an epispore which is a transparent bag like outer layer (i.e. some Coprinus).

Ornamentation that is hard to see (i.e. Laccaria)

For hard to see ornamentation and for Tricholoma spores, stain with Phloxine B

Oil Drops

To stain the oil drops in spores use Sudan III or Sudan IV.

Callistosporium

When dried the spores and hyphae show a reddish internal pigment in Ammonia or KOH

Hypiszgus

Acetocarmine for spore walls and basidia

BROWN SPORES

General mountant

Brown spores are best observed in ammonium hydroxide.

Plage (i.e. Galerina spores)

The plage on brown spores should also be observed in ammonium hydroxide and best results or obtained by first treating with 50 % chloral hydrate.

Coloured Spore Wall (i.e Crepidotus)

Use KOH or water for coloured wall of spores

HYPHAE

Hyphae walls and contents

Stain with Congo Red

Hyphae walls and septa only

Stain with Chlorozole Black

Hyphae contents only

Stain with Phloxine B, or Cotton Blue but cotton blue will stain debris and bacteria as well

Congo red and phloxine can be mixed together before staining, and for best results use one drop of combined stain and one drop of ammonium hydroxide, and wash away surplus stain with ammonium hydroxide.

Hyphal protoplast

Stain with Erythrosin.

Amyloid hyphae (i.e. Chroogomphus)
Dextrinoid hyphae (i.e. Crinipellis and some Mycena)

Stain with melzers iodine. A positive reaction for amyloid hyphae is blue-black and brown for dextrinoid hyphae.

Amyloid Septa (i.e. Some Boletus)

As for amyloid hyphae above.

Metachromatic hyphae (i.e. some Mycena)

Stained with cresyl blue, the positive reaction is magenta red to blue-violet and the colour will vary with species and also the manufacture of the dye. True metachromic reaction is magenta-red to violet When the tissue stains dark blue it is said to be orthochromic but there may be tissues of some species which stain a colour in-between metachromic and orthochromic.

Lactiferous hyphae (i.e. Lactarius)

These hyphae can be identified by staining with sulphobenzaldehyde,

Gelatinous hyphae (i.e Hygrocybe, Hygrophorus, Hebeloma, Hebelominia, Melanotus, Myomphalia, Oudemansiella, Psilocybe, Gymnopilus, Limacella, Gyrodon, Flammulina, Gamundia, Gomphidius, Stropharia, Resupinatus , Rhodotus Tylopilus and Hohenbuelhelia)

Stain with cresyl blue, or toluidine blue.

Hyphal pigmentation

First treat with a strong salt solution to separate the cytoplasm from the cell walls and observe in ammonium hydroxide, if unsuccessful due to alkaline soluble pigments mount in distilled water.

Cystoderma

The hyphae from cap surface stain reddish brown with potassium hydroxide

Xeromphalina

For cap cuticle use KOH = yellow or orange-red dependent on species

Cortinarius – End cells

To distinguish hyphae and end cells use conc. hydrochloric acid (+ reaction is green or red).

GILL TRAMA

Stain as for hyphae but in Agrocybe use cresyl blue, and for Xeromphalina and Collybia use ammonium hydroxide.

Agrocybe: The trama of many species is Metachromatic (turns reddish violet in Cresy Blue)

Collybia: For trama of some species use Ammonium Hydroxide (green reaction)

Gyroporus: Use cresyl blue for the hyphae of the trama

Resupinatus: : Cresyl blue for gelatinised trama of gills

Xeromphalina: For the trama use Ammonium Hydroxide.

Boletus: Use cresyl blue for the trama

CYSTIDIA

Use ammonium hydroxide for the following:

a) chrysocystidia (i.e. Hypholoma, Stropharia & Pholiota), or use Patent Blue

b) Pseudocystidia,

c) Mucilage caps (i.e. Psathyrella) which stain green,

d) Mucoid deposits on cheilocystidia

e) the cystidia of Xeromphalina which stain reddish-brown.

The gloeocystidia (i.e. Corticeacea), and the cystidia in Melanoleuca use cresyl blue.

The encrusted cystidia in some Inocybe stain in Guiac.

For fuchinophile hyphae & dermatocystidia in Russula use strong Carbol Fushin for 15 minutes and wash with 10% hydrochloric acid for one minute, or use Sulphovanillin

The contents of macrocystidia stain with sulphobenzaldehyde.

Oxalate crystals on cystidia or in tissue stain with anthracene green.

Chaetocalanthus: : Melzers Iodine for cystidia & hairs on cap

Crinipellis: Melzers Iodine for the cap hairs. They also stain grey with Potassium Hydroxide. The walls of the hyphae stain well in cotton blue.

Faebaria: Melzers Iodine for the cystidia

Hypholoma: For marginal cystidia use ammonium hydroxide, or potassium hydroxide (internal contents stain yellow, or try Patent Blue).

Inocybe: For the incrusted cystidia in some species use Guiac

Pholiota: For the marginal cystidia use Ammonium Hydroxide

Psathyrella: For cystidia stain in congo red (gently heat till dry) and then wash away surplus stain with ammonium hydroxide. For Mucilage caps on pseudocystidia use Ammonium Hydroxide (green reaction).

Stropharia: : For marginal cystidia use ammonium hydroxide (inclusions stain yellow

Suillus: Iodine for cystidia = Red-brown or yellow globules in encrusted material.

Xeromphalina: For cystidia use Ammonium Hydroxide (red-brown reaction

BASIDIA

For siderophilous basidia (i.e. Tephrocybe) heat tissue while in aceto carmine. The basidia of Tricholoma stain well in cresyl blue.

Tricholoma: The basidia are Metachromatic (turn reddish violet in Cresy Blue

Asterophora: Basidia contents stain in Acetocarmine (Siderophilous)

Calocybe, Lyophyllum: Contents of Basidia stain in Acetocarmine (Siderophilous granules),

VEIL CELLS AND CRYSTALINE DEBRIS

Coprinus group: Veil cells, diverticulate hyphae and crystalline debris stain in hydrochloric acid.

FLESH (some of the common reactions)

Agaricus: KOH, & the Schaeffer test = Intersection of lines made from: Glacial Acetic Acid and Aniline.

Allopsalliota: Flesh turns green with ammonia and grey to brown with KOH, Schaeffer-reaction purple to dark pink

Cortinarius: KOH + reaction = dark brown (most Telemonia taxa), but can also be red, olivaceous-green, or yellow

Guiac a + reaction = bluish-green. Lugol's iodine a + reaction = lilac/purple. Silver nitrate a + reaction = yellow (in section Leprocybe).

Gymnopilus: Cap surface turns black with KOH or ammonium hydroxide

Gyrodon: FeS = flesh olivaceous, KOH = fulvous, Ammonia = no reaction.

Leccinum: Ammonia, KOH, ferrous salts and formalin used for various testing reactions the flesh.

Russula: Ferrous Sulphate, Guiacum, KOH, Ammonia, Sulphovanillian, O-toludine

Tylopilus: FeS = flesh grey-olivaceous

Tricholom stiparophyllum = green with Sulfoformol

FORMULAE FOR CHEMICAL STAINS AND REAGENTS

The formula for many of the reagents used in mycology varies from author to author, as does the exact methods used. I have quoted the most frequently used formula for each reagent and also commented on some of the variations in use.

Ammonium hydroxide
For microscopical use a solution of between 5% – 10% seems to be the normal range, although 2% solutions are used by some mycologists. For macroscopical application stronger solutions are often used, these range from 10% to 75%, but 40% seems the most frequent used. Keeps for several years.

Acetocarmine
Various methods of preparation the simplest is as follows: Boil 45% acetic acid (45cc acetic acid in 55cc water) for ½ hr., filter and dilute with 45% ethanol (45cc ethanol in 55cc water), add 1 or 2 drops of ferric hydroxide per 50ml of solution.

Keeps for several years.

Anthracene Green
0.5 g dissolved in 100 ml of 2.5% ammonium hydroxide.

Chlorazol Black
An aqueous solution (carcinogenic !)

Congo Red
A saturated solution in ammonium hydroxide. (or a 1 % aqueous solution)

Cresyl Blue (Brilliant Cresyl Blue)
Dissolve 0.5 – 1.0 g cresyl blue in 100 ml water. Allow to stand for 5 – 10 minutes then filter out the excess dye. Aqueous solutions only keep for a few weeks. The colour of the stained tissue may vary with different manufacturers of the dye.

Alternative formula that is said to keep well:

0.2 – 0.5 g of cresyl blue, 0.5 ml invadin IFC, 17 ml pure glycerine, 27 ml of 96% ethonol, 55.5 ml of distilled water. Filter after one day.

Erythrosin
0.5 % solution in 10% ammonium hydroxide. Keeps for several years.

Ferrous Sulphate
10 % aqueous solution. Keeps for a few months.

Formalin
Standard solution. Keeps for a few years.

Fuchsin
Strong solution of carbol fuchsin is used with 10 % hydrochloric acid. (stain with fuchsin and wash with hydrochloric acid and observe in water),

Guaiac
A saturated solution of gum guaiacum in 70 % ethanol. Keeps for about a year.

Lacto-Phenol Cotton Blue
Dissolve 50 ml of 1 % solution of cotton blue (1 g of cotton blue in 99 ml water) in a mixture of: 100 g lactic acid, 100 g phenol, 150 ml glycerine, 50 ml water. Keeps for several years.

L4 General Mounting Fluid
84 ml distilled water, 16 ml glycerine, 0.5 ml invadin IFC, 0.72 g potassium hydroxide, 0.76 g sodium chloride.

Melzers Iodine
Add 1.5 g Iodine, 5.0 g potassium iodide, and 100 g chloral hydrate to 100 ml water , warm but do not boil. Keeps for several years (I have been using the same batch for over 12 years).

Phenol
2% aqueous solution of crystalline phenol.

Phloxine
Dissolve 1.5 g phloxine in 100 ml water.

Potassium hydroxide
As for Ammonium hydroxide but only keeps for about a year as there is a tendency for crystallisation.

Sulphobenzaldehyde
Dissolve 6 ml of benzaldehyde in 3 ml of distilled water and add 10 ml of concentrated sulphuric acid.

Sulphovanillin
Add 4 ml concentrated sulphuric acid to 2 ml distilled water and when required dissolve 5 mg pure vanillin in the dilute acid. Only keeps for a few days.