Cornmeal Agar (CMA)

Per Liter:	20.0	g	cornmeal
	20.0	g	peptone
	20.0	g	glucose
	15.0	g	agar

Cook cornmeal in 500 ml water for 1 h at 60˚C. Add agar, peptone and dextrose in 500 ml water. Filter cornmeal mix through cheesecloth. Combine liquids, adjust volume to 1000 ml. Autoclave. ***NOTE:*** *ARSEF routines uses a commercial preparation of this medium rather than making it from scratch.*

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Entomophthora Complete Medium (ECM)

I. Ben-Ze'ev. 1980. Systematics of entomopathogenic fungi of the '*sphaerosperma* group' (Zygomycetes; Entomophthoraceae) and their prospects for use in biological pest control. PhD thesis, The Hebrew University of Jerusalem; pp 11-15 and 35-39).

Per Liter:	10.0	g	casein hydrolysate (as salt-free as possible) ***or*** casamino acids
	10.0	g	yeast extract
	100.0	mg	tryptophane (if using casein hydrolysate; ***omit*** tryptophane if using casamino acids)
	10.0	g	dextrose ***or*** maltose ***or*** soluble starch
	15-20	g	agar

Dissolve in warmed water, stirring continuously until a perfectly transparent, honey-colored solution is obtained. Continue heating to boiling if necessary. If using starch, dissolve it first. Agar should be added in small quantities and allowed to dissolve before adding more. Autoclave for 15 min at 121˚C, let cool to 55-60˚C and then add:

10 or 20	ml	Vogel's modified solution (*see below*)
10	ml	Vitamin solution (*see below*)
10	ml	Antibiotic solution (*see below*)

These solutions should be kept sterile (membrane-filtered) and added to the medium aseptically, but it is advised to mix all three solutions inside a 50 ml disposable syringe and to inject them into the medium through a disposable sterile 0.22 µm filter. Mix well and dispense into petri dishes or slants.

VOGEL'S MODIFIED SOLUTION:

Modified by Ben-Ze'ev from: Vogel VH. 1956. A convenient growth medium for *Neurospora* (Medium N). *Microbiol. Genet. Bull.* 13: 42-43.

1000	ml	distilled water
123	g	Na_3 citrate•$2H_2O$
250	g	NH_2PO_4 (anhydrous)
165	g	$(NH_4)_2HPO_4$ ***or*** 118.4 g tri-ammonium orthophosphate ***but not both!***
10	g	$MgSO_4$•$2H_2O$ ***or*** 15.8 g $MgSO_4$•$7H_2O$ ***but not both!***
5	g	$CaCl_2$•$2H_2O$
5	ml	Trace Elements Solution (*see below*)
2	ml	chloroform

Use cold water and DO NOT heat! Stir vehemently and continuously with a magnetic stirrer, and add chemicals successively in the order listed. Do not add the next ingredient before the former is completely dissolved. Calcium chloride should be added in very small amounts, each after the former is completely dissolved. Before adding the chloroform, filter sterilze the solution with 0.22 µm cellulose nitrate membranes; cellulose acetate tends to clog). Disperse the 1000 ml sterilized solution into 10 presterilized 100 ml bottles and add 0.2 ml chloroform to each. Stopper well and keep at room temperature. Heating during preparation will result in irreversible precipitation. The same will happen if too large amounts of salts are added at a time. If not sterilized fungal contamination will occur. If stored at low temperatures, the saturated solution will crystallize, and because it cannot be heated, it may take days at room temperature to redissolve.

TRACE ELEMENTS SOLUTION:

95	ml	distilled water
5	g	citric acid•H_2O
5	g	$ZnSO_4$•$7H_2O$
1	g	$Fe(NH_4)_2(SO_4)_2$•$6H_2O$
250	mg	$CuSO_4$•$5H_2O$
50	mg	$MnSO_4$•H_2O
50	mg	H_3BO_3 (anhydrous)
50	mg	Na_2MoO_4•$2H_2O$
1	ml	chloroform

Dissolve salts successively in cold water on a magnetic stirrer. Before adding chloroform, sterilize by membrane filtration. Keep at room temperature in a well-stoppered, presterilized bottle.

VITAMINS SOLUTION

Vogel 1956, after Beadle and Tatum, 1945. *Neurospora*. II. Methods of producing and detecting mutations concerned with nutritional requirements. *Amer J Bot* 32: 678-688.

1000 ml	distilled water
100 mg	thiamine
50 mg	riboflavine
50 mg	pyridoxine
200 mg	Ca pantothenate
50 mg	para-amino benzoic acid
200 mg	nicotinic acid
200 mg	choline chloride
400 mg	inositol
50 mg	folic acid

Sterilize by 0.22 µm membrane filtration. Disperse in 10 ml aliquots in presterilized stoppered tubes. Keep frozen at -20˚C; thaw just before use. Add only to presterilized media.

ANTIBIOTICS SOLUTION

From I. Ben-Ze'ev's thesis:

100	ml	distilled water
6	g	streptomycin sulfate
2.5-3	g	sodium penicillin G (or other highly soluble penicillins)

Sterilize by 0.45 µm filtration. Disperse in 10 ml aliquots in presterilized stoppered tubes. Keep frozen at -20˚C; thaw just before use. Equivalent to 417,500 – 500,000 units penicillin + 450,000 units streptomycin per liter of medium.

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Peptone-Yeast-Glucose (PYG, PYG + oil)

Fuller MS, Jaworski A. 1978. *Lower Fungi in the Laboratory*. Univ. of Georgia Press: Athens, GA. Taken here from: Kerwin JL, Petersen EE. 1997. Fungi: Oomycetes and Chytridiomycetes. In *Manual of Techniques in Insect Pathology*, L Lacey ed., pp 251-268. Academic Press: London.

Per Liter:

1.25	g	peptone
1.25	g	yeast extract
3.0	g	glucose
15.0	g	agar

This medium is a variant of one developed by Ralph Emerson. Some researchers add 1.36 g/L KH_2PO_4 and 0.71 g/L Na_2HPO_4, especially when using this medium for lilquid culture. In order to promote sporulation, $CaCl_2 \cdot 2H_2O$ and $MgCl_2 \cdot 6H_20$ are often added at concentrations of 0.5-5.0 mM. Sterol deprivation will result in the gradual loss of the ability of cultures to sporulate; therefore, PYG is commonly supplemented with vegetable oils or purified sterols with a suitable solubilizing agent. Commonly used vegetable oils include soybean, safflower and corn oil. Wheat germ, linseed and cod liver oil have also been used, unusually in conjunction with corn oil. Oils are usually added at a concentration of 0.5-2 mL/L (Kerwin, Simmones & Washino, 1986, *J Invertebr Pathol* 47: 258-270). Purified sterols such as cholesterol, ergosterol and sitosterol (10-100 mg/L) can be added with oil or solubilized using Tween 20 or crude preparations of lecithin (phosphatidylcholine).Lecithin (50-100 µm/L) is solubilized in 20-15 mL distilled water using a stir bar and gentle heating, and added to culture media after complete dissolution. If this is not done, the lecithin will form large clumps during autoclaving, and the sterol, which forms complexes with this lipid, will not be solubilized in a form that can be utilized by L. giganteum. A variation of this medium (PYG4S; Frances, Sweeney & Humber, 1989, J Invertebr Pathol 54: 103-111) used a mixture of sterols — 0.0625% each of cholesterol, lanosterol, ergosterol and cholestan-β-ol — plus 5% lecithin added to basal PYG medium. Difco Laboratories (Detroit, Michigan) is a common source for peptone and yeast extract. We have used a variety of commercial sources for yeast extract. Peptones, however, vary greatly in source, preparation and composition, and unless preliminary evaluations prove otherwise, Difco peptone should be used.

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Oatmeal Agar (OA)

Per Liter:

30.0 g	rolled oats
15.0 g	agar

Cook oats in 1 L water at 60˚C for 30 min. Strain through cheesecloth, add and dissolve agar. Adjust volume to 1 L, and autoclave.

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SOLID MEDIA

Synthetischer nährstoffärmer Agar (SNA)

Source: Gerlach W, Nirenberg H. 1982. The genus *Fusarium*: a pictorial atlas. Mitteil. biol. Bundesanstalt für Land- und Forstwirtschaft Berlin-Dahlem 209: 1-406. Parul Parey: Berlin.

Per Liter:	1.0	g	KH_2PO_4
	1.0	g	KNO_3
	0.5	g	$MgSO_4 \cdot 7H_2O$
	0.5	g	KCl
	0.2	g	glucose
	0.2	g	sucrose
	15.0	g	agar

Combine and autoclave above ingredients, dispense in petri dishes. Place a piece of sterile filter paper (ca. 1 cm square) on the surface of the agar in each dish after the medium solidifies.

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Sabouraud Dextrose Agar + Yeast Extract (SDAY, SDAY/4)

Per Liter (SDAY):		Per Liter (SDAY/4):	
40.0 g	dextrose	10.0 g	dextrose
10.0 g	neopeptone	2.5 g	neopeptone
10.0 g	yeast extract	2.5 g	yeast extract
15.0 g	agar	15.0 g	agar

Adjust to pH 6.5 before autoclaving.

Note that the standard formula of Sabouraud dextrose agar (SDA) includes no yeast extract. The ARSEF culture collection always uses yeast extract in its media. A quarter-strength dilution of this medium (SDAY/4)–which supports good growth and retention of ability to sporulate–is now the most used general medium at the ARSEF culture collection. Half- or tenth-strength dilutions of SDAY (still containing 1.5% agar) may also be useful for specialized purposes.

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Sabouraud Maltose Agar + Yeast Extract (SMAY, SMAY/4)

Per Liter (SDAY):		Per Liter (SDAY/4):	
40.0 g	maltose	10.0 g	maltose
10.0 g	neopeptone	2.5 g	neopeptone
10.0 g	yeast extract	2.5 g	yeast extract
15.0 g	agar	15.0 g	agar

Adjust to pH 6.5 before autoclaving.

SMAY (full-strength) has traditionally been thought to be preferable to SDAY for supporting sporulation by Nomuraea rileyi. Variants of Sabouraud media substituting maltose for dextrose (or using reduced quantities of both sugars) are rarely used at ARSEF.

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Sabouraud-Egg-Milk Agar (SEMA)

Part A:	1.0	g	neopeptone
	1.5	g	agar
	8.5	ml	whole milk (≥4% butterfat)
	40.0	g	water
Part B:	4.0	g	dextrose
	40.0	g	water

Autoclave parts A and B separately, allow to cool slightly, and then one egg yolk (aseptically obtained, and preferably with yolk membrane already broken before adding to a medium) to either part. Mix A and B, and shake vigorously to break yolk and to make a uniformly mixed medium before pouring plates.

This is one of the the series of media including egg yolk that are used to cultures some fungi of the Entomophthorales. This is easier to prepare than many and tends to dry out (and to crack) more slowly than other media containing much higher concentrations of egg yolk and less (or no) agar.

Breaking the egg yolk is made easier by including a magnetic stirring bar in the container to which egg yolks aree added so that shaking the medium, yolk, and bar quickly breaks and disperses the yolk. To obtain egg yolks aseptically separated from the white may require some practice. *All egg yolk-based media should be retained at room temperature (or above) for at least 24 hours after preparation and then checked for visible growth of bacterial contaminants.*

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(Emerson's) Yeast Extract-Potassium Phosphate-Soluble Starch Agar (YPSS)

Per Liter:	4.0	g	yeast extract
	1.0	g	KH_2PO_4
	0.5	g	$MgSO_4 \cdot 7H_2O$
	15.0	g	soluble starch
	15.0	g	agar

Separately dissolve agar and starch in ca 400 ml water. Heat to boiling. Dissolve the inorganic salts and yeast extract spearate in ca 500 ml water. Mix both solutions thoroughly and adjust volume to 1000 ml before autoclaving.

This medium is available as a commercial pre-mix from Difco, and has proven to be an excellent one for supporting good growth and sporulation by *Nomuraea rileyi* (and may be at least as good or better than SMAY for this fungus).

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GLEN

Beauvais A, Latgé J-P. 1988. A simple medium for growing entomophthoralean protoplasts. *J Invertebr Pathol* 51: 175-178.

Per Liter:	4.0	g	glucose
	5.0	g	yeast extract
	6.5	g	lactalbumin hydrolysate
	7.7	g	NaCl
	50-100	ml	fetal bovine serum

Addition routinely used in J Eilenberg Lab (Univ. of Copenhagen):

1.952	g/L	MES (2-[N-morpholino]ethane sulfonic acid] buffer (Sigma M-8250)

Useful pH range: 5.5-6.7. Adjust base medium (above ingredients without FBS) to pH 7.0 with 1N NaOH before autoclaving. ***Important note***: ***Always*** add fetal bovine serum after all other ingredients are autoclaved or filter sterlized.

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Grace's Medium ± Fetal Bovine Serum (GF, GF10, GF20)

This widely used liquid insect tissue culture medium is available commercially from several source and is usually supplemented with 5% (v/v) fetal bovine serum; adding 10% FBS (GF10) is favored in some laboratories, but this expensive ingredient may be used at a rate as high as 20% (GF20) in some highly specialized media.

Two major forms of Grace's medium–the original formula devised by Grace to culture lepidopteran tissues, and a later modification by Yunker et al. that contains lactalbumin hydrolysates among other modifications) are commercially available.

The ARSEF culture collection uses Grace's medium primarily for entomophthoralean fungi whose vegetative stages are wall-less protoplasts that cannot be grown on solid media.

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Modified Medium 199 ± Fetal Bovine Serum (M199)

Medium 199 is a commercially available and widely used liquid tissue culture medium devised for mammalian tissue cultures. **ARSEF always modifies Medium 199 with the addition of 26.68 g/L sucrose** to help support the osmolarity of this medium for entomopathogenic fungi (which generally cannot metabolize this sugar). This concentration of sucrose taken from the formula for Grace's medium since M199 includes no sucrose. This modified medium is usually supplemented with fetal bovine serum, mostly at 5% v/v, and has been used most often for entomophthoralean fungi.

To grow fungal entomopathogens in this medium, use the version ***with Hanks' salts*** (which equilibrate for pH under standard air) rather than Earle's salts (that are intended for use under enhanced carbon dioxide atmospheres).

This medium contains phenol red as a pH indicator. The pink color becomes yellow as the pH falls with age; cultures usually need to be transferred to fresh medium soon after this color change becomes apparent.