

Common Media Recipes

(All recipes make 1L unless otherwise specified)

Rye V8 (for growth of *Phytophthora infestans*)

To make rye broth:

- Soak 100g rye grain in 2200mL dH₂O overnight. Use a 4L flask
- Autoclave using the 1000mL liquid cycle (45 min). Let cool.
- Strain broth through 4 layers of cheesecloth
- Use for making media or store in freezer bags in the freezer for later use.

Recipe:

Rye broth	950mL
V8 juice	50mL
Granulated agar	20g
Calcium carbonate	0.2g

Add a stir bar and autoclave using the 1000mL liquid cycle (30 min).

To make antibiotic Rye V8, add 10.4mL PCNB (5mg/mL) and 2mL rifampicin (10mg/ml) to Rye V8 that is cooled but still molten. Stir gently for approximately 30 seconds before pouring plates.

Clarified V8 (for mating type testing)

To make clarified V8:

Centrifuge 150ml of V8 juice at maximum speed for 5 min. Pour supernatant into clean Falcon tubes and use immediately or freeze for future use.

Recipe:

V8 Supernatant	100 ml
CaCO ₃	1.5g
B-sitosterol	0.05 g
H ₂ O	900 ml
Agar	17 g

Autoclave using the 1000mL liquid cycle (30 min).

Lima bean agar (for general *Phytophthora* growth)

To make lima bean broth:

- Autoclave 200g lima beans in 1L dH₂O using the 1000mL liquid cycle (30 min). Let cool.
- Strain broth through 4 layers of cheesecloth.
- Use for making media or store in freezer bags in the freezer for later use.

Recipe:

Lima bean broth	1000mL
Granulated agar	18g
Dextrose	1g

Add a stir bar and autoclave using the 1000mL liquid cycle (30 min).

Pea broth – for storage revival/harvesting mycelium

- Autoclave 120g peas in 1L dH₂O using the 1000mL liquid cycle (30 min).
- Strain through 4 layers of cheesecloth.
- Pour into 500mL bottles and cap loosely.
- Autoclave again using the 500mL liquid cycle (30 min).

1.5% water agar – for detached leaves

- Mix 15g granulated agar with 1L dH₂O
- Autoclave using the 1000mL liquid cycle (30 min).

PARP (for non-*P. infestans* culture cleanup)

- Prepare corn meal agar according to package. *ADD A STIR BAR*
- Autoclave using the 1000mL liquid cycle (30 min). Let cool until agar can be comfortably touched with gloved hands.
- While waiting for agar to sterilize/cool, prepare the following antibiotic cocktail in a sterile 50mL Falcon tube:

Pimaricin (10mg/mL)	1mL
Ampicillin (25mg/mL)	10mL
Rifampicin (10mg/mL)	1mL
PCNB (5mg/mL)	20mL

Store in fridge until ready to use.

- When agar is cool but still molten, aseptically add the antibiotic cocktail to the agar. Stir gently for approximately 30 seconds before pouring.

Rye A Agar – For long-term maintenance of *P. infestans*

1. Soak 60 g of rye grain in distilled water for 24 hours at room temperature. This is done in a small tray so that water just covers grain. Cover tray tightly with aluminum foil.
2. Next day, pour supernatant off germinated grain and put aside.
3. Place grain in a beaker, add distilled water (about 1 inch above grain) and blenderize on high for 2 minutes. Cook in water bath for 1 hour at 68°C. Don't modify extraction time or temperature.
4. Filter through 4 thicknesses of cheese cloth squeezing gently to remove residual liquid. Discard cheese cloth and grain sediment.
5. Combine original supernatant (liq. poured off grain at the beginning) with filtrate. (At this point the preparation can be frozen for use later).
6. Add 20g sucrose, 15g Bacto Agar then adjust volume to 1 liter.
7. Autoclave at 15 psi for 20 minutes.

Reference: Caten, C. E. and J. L. Jinks. 1968. Spontaneous variability of single isolates of *Phytophthora infestans*. I. Cultural variation. Can. J. Bot. 46: 329-348.