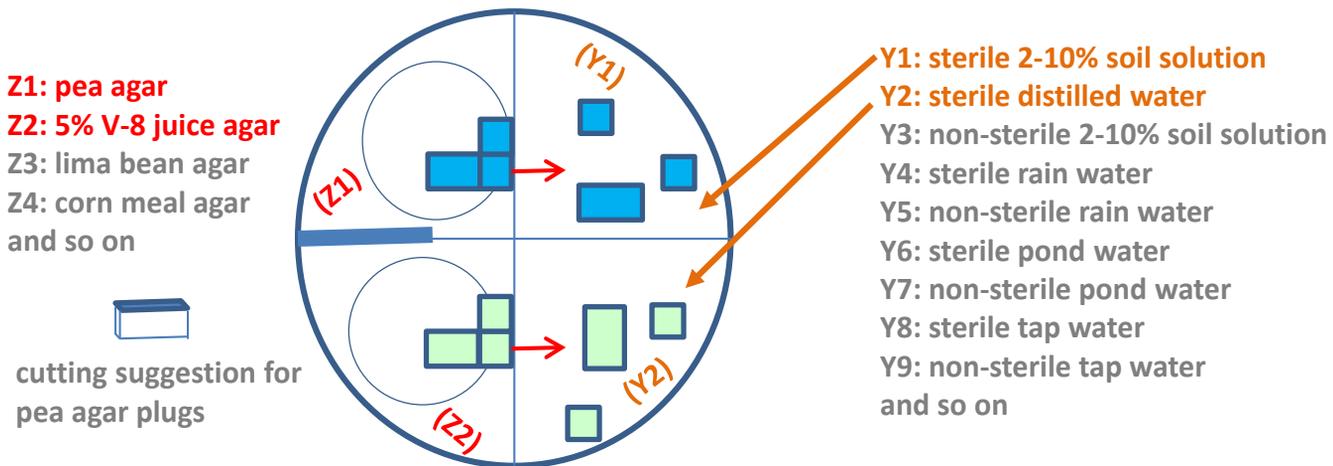


SOP-PID-07.01: Quick reference guide for *Phytophthora* spp. original isolation and methods for the production of asexual and sexual phases

Original isolations of *Phytophthora* species are made in corn meal agar (CMA) plus antibiotics: pimaricin, ampicillin, rifampicin and pentachloronitrobenzene (CMA-PARP). Grow isolates in the dark for 3-7 days. Transfer isolates into CMA and grow for an additional 3-7 days.

Asexual phase: sporangia, sporangiophores, hyphal swellings, and chlamydospores

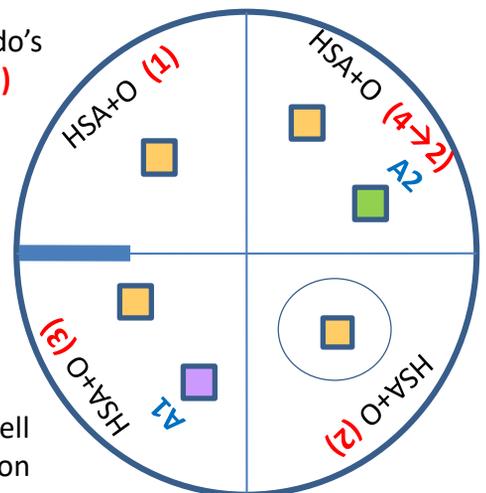
1. Transfer isolates into X-plate with agar (Z; such Z1, Z2, Z3, or Z4); grow for 3-7 days (18-24°C).
2. Transfer a few plugs from the border of the colony into the neighboring cells (Y). Add liquid (such as Y1, Y2...) only to the level of the plugs; do not overflow. Keep plates under continuous fluorescent light (24-48 hrs) for sporangia formation (oospores of homothallic species might be produced using this method).
3. After sporangia are produced, to induce zoospore release (1) incubate the plates at 5°C for 10-20min, then bring back to room temperature; or (2) keep a bottle of DD H₂O at 5°C, and place a couple drops of the cold water on the slides you use to observe sporangia.



Alternative: Grow on pea agar, V-8 agar, or carrot potato agar, transfer mycelia into pea broth for 4-7 days; rinse the mat of mycelia with DD H₂O, and grow in DD H₂O for 24-48 hrs under light.

Sexual phase: oogonia, antheridia and oospores

1. Transfer isolates into one X-plate with hemp seed agar + Udo's Oil (HSA+O) or 5% V-8 juice agar: (1)(2) sample  alone, (3) sample + tester A1, (4) sample + tester A2. Incubate in the dark for 7 days (18-24°C).
2. If oospores are produced in (1) and (2), the species is **homothallic**. If not produced, it may be **heterothallic**, then observe (3) and (4). If oospores are produced in (3) it is **A2**, if produced in (4) it is **A1**. Use cell (2) for asexual phase (if needed).
3. If oospores are not produced in 7 days, you can try using polycarbonate membrane covering the sample mycelia in cell (2), and then put A1 and A2 tester (1 day old) upsidedown on top. Some species of *Phytophthora* may require weeks or months to produce oospores.
4. If still not produced, the species may be **sterile (A0)**.



pea medium (400ml broth or agar, Coffey)

120 g frozen petite peas in ~240 ml DD water, boil for 12-15 min, pass through coarse sieve and discard peas; add 3.2 g sucrose, 0.8 g L-Asparagine, 0.4 g L-methionine, 0.04 g β -Sitosterol. Add DD water to bring volume to 400 ml. For agar medium add 6 g Difco Bacto agar, autoclave for 40 min.

lima bean agar (LBA)

20 g of baby lima bean in 200 ml of DD H₂O, sterilize for 5 min (121°C), filter with 3 layers of cheesecloth, add vol. to 400 ml and 7.2 gr of agar, autoclave (20 min at 121°C).

corn meal agar (CMA)

7.2 gr of Difco agar in 400 ml of DD H₂O, autoclave (20 min at 121°C).

5% V-8 Juice agar (5% V8A)

Clarified V8- juice: 1.g of CaCO₃ for 100 ml of V-8 juice, stir for 15 min, centrifuge at 7000 rpm for 10 min. Store in 50ml vials at -20°C. For 400 ml medium, use 20 ml of clarified V8-juice, 6 g agar, and 380 ml DD H₂O.

hemp seed agar (HSA) + Udo's oil

Soak 20 g of hemp seed in 400 ml of DD H₂O overnight. Filter through 3 layers of cheesecloth. Add vol. to 400 ml and 7.2 g of agar and autoclave (20 min at 121°C). Before dispensing HSA into petri dishes, drip 0.2 or 0.8 ml sterile Udo's oil, or 0.2 ml of Macadamia oil into the bottle of 400 ml HSA (v:v=1:2000 or 1:500).

Citation

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10% soil solution

Stir 100 g of sandy soil in 1 L of DD H₂O for 1 min, let sit 5 min to settle. Filter upper part through 3 layers of cheesecloth, dispense into bottles of 100 ml, and autoclave (20 min/121 °C).

Note: some species sporulate better in UNSTERILIZED soil solution.

Phytophthora testers (any of)

- 1) *P. meadii* A1 and A2
- 2) *P. cryptogea* A1 and A2
- 3) *P. cinnamomi* A1 and A2
- 4) *P. nicotianae* A1 and A2
- 5) *P. capsici* A1 and A2
- 6) *P. cambivora* A1 and A2

alternate: polycarbonate membrane

Polycarbonate membrane (47 mm, 0.2 μ m; Sterlitech) is placed on top of culture, and a plug of sample in HSA (15 x 15 mm) is placed on top with mycelia in contact with the membrane and incubated in the dark.

to observe sporangia caducity

1. Carefully shake the plate with the block of agar in it (sporangia and sporangiophores on the agar block).
2. Take the block of agar (with sporangia and sporangiophores on it) and knock against a drop of H₂O on microscopic slide.

Document revision history

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