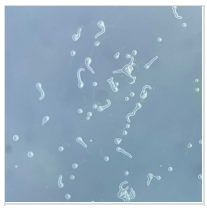

Version 2

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In vitro germination of *Austropuccinia psidii* urediniospores V.2

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ABSTRACT

Optimisation of *Austropuccinia psidii* urediniospore germination for use in RNA extraction and cytogenetics.

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Spore collection, desiccation, and storage 2d

1 Harvest fresh spores from heavily infected leaves by shaking into a paper bag.

2 Move fresh spores to a glass petri dish with no lid. Transfer petri dish to a desiccator with silica gel beads for 24 - 48 hours to dry the spores.

3 Sieve spores to remove any plant matter or dirt.

4 Aliquot spores into Nunc CryoTubes or similar for short or long term storage.

> Spores can be frozen at -80ºC or in liquid nitrogen for long-term storage but may reduce germination rates. Use of spores immediately after desiccation is recommended.

Inocula preparation and plating 2h

5 Create 2% water agar plates.

6 In a 15 mL centrifuge tube, make up inocula to a concentration of approximately 1 mg/mL in sterile distilled water (SDW) with 5ul/mL Tween 20 (0.05%). Mix thoroughly by gently inverting the tube several times.

> A haemocytometer can be used to count and confirm spore concentration.

7 Rehydrate spores in inocula for 30 minutes prior to use.

Pipette 2 uL aliquots of inocula onto agar plate, covering the entire plate with distinct 2 uL

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8 droplets and seal the plates with parafilm.

Incubation 8h

9 Germinate in incubator for a minimum of 8 hours in the dark at 18°C and 75% relative humidity.

Determine Germination Percentage 1h

10 For each aliquot, count the number of germinated spores under a dissecting microscope. A germinated spore is considered to be a spore with a germination tube at least double the length of the urediniospore.

Germination rates of approximately 70 - 80% are expected when using fresh spores.

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