Double staining with Aniline blue and Calcofluor white

Materials

- *Sodium phosphate buffer (0.07M, pH9)*
  1. 12.46 g $Na_2HPO_4 \cdot 2H_2O$
  2. 1l dH_2O
  3. Adjust the pH to 9 using 0.07M $NaH_2PO_4 \cdot 2H_2O$ (0.966g of $NaH_2PO_4 \cdot 2H_2O$ in 100ml dH_2O).

- *Aniline blue solution*
  1. Aniline blue stock 0.05 %in dH_2O
  2. Dilute to 0.005 % in Sodium phosphate buffer (0.07 M, pH9)

- *Calcofluor white solution*
  1. Calcofluor White (calcofluor white M2R.S.new) solution 0.1% (w/v) in Tris-HCl (0.1M pH=8.5).
  2. Cover the bottle with aluminium foil, as calcofluor white is light sensitive.

Method

1. De-stain overnight in 96% ethanol in a Petri dish.
2. Incubate in sodium phosphate buffer (0.07 M, pH9) for 30 minutes.
3. Remove the buffer and add Aniline blue to cover the sample (write down the volume).
4. Let the tissue impregnate for 60 minutes, discard the aniline blue solution.
5. Add 1/10 of the aniline blue volume of 0.1 % calcofluor white into the dish.
6. Observe the samples immediately under a microscope with fluorescence illumination.
7. Aniline blue: excitation 370nm, emission 509nm.
8. Calcofluor white: excitation 370nm, emission 440nm.

Notes

Aniline blue is used to reveal callose structures in plant tissue, which appear after infection (papillae, apposition) or during pollen tube formation.

The calcofluor stains chitin present in fungal cell membranes and also binds to cellulose at locations where the cuticle is damaged.

This staining method can be used in combination with Trypan blue staining.