Isolation of endophytic fungi

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Abstract

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Protocol

Step 1.
Plant materials were thoroughly washed with running tap water, cut under sterile conditions into small pieces (2-3 cm).

Step 2.
Surface sterilization was carried out by dipping in 75 % ethanol for 1 min.

Step 3.
The samples further sterilized in bleach solution containing 1.3 % sodium hypochlorite for 3 min.

Step 4.
Followed by surface sterilized in 75 % ethanol for 30 s.

Step 5.
Traces of sodium hypochlorite were removed by rinsing with sterile Millipore water three times (3 min each).

Step 6.
The tissues were dried the sterile laminar air flow and briefly passing through the flame, the outer tissues of the stem cuttings were removed.

Step 7.
The tissues were cut into small segments (5mm X 5mm) using a sterile scalpel.

Step 8.
Single isolate were transferred to fresh PDA plates using the hyphal tip method.10. The effectiveness of the surface sterilization was confirmed by making imprints of disinfected plant fragments on PDA plates from which no fungal growth was observed.

Step 9.
Small segments and were incubated on PDA plates supplemented with streptomycin to suppress bacterial growth at 28 °C in darkness for 2-4 weeks and were checked daily for hyphal growth.

The effectiveness of the surface sterilization was confirmed by making imprints of disinfected plant fragments on PDA plates from which no fungal growth was observed.

Step 10.

Step 11.