Bioassay of Extracts of the Endophytic Fungi

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[Abstract] Many of the microbes including fungi produce metabolites possessing antifungal activity and in some cases fungal metabolites are main cause of antifungal activity resulted by fungus. To infer that antifungal activity is due to fungal metabolites, there is a need to develop a repeatable procedure to assay these metabolites. Here we are presenting the poisoned food technique for bioassay of extract from endophytic fungi, where culture media is supplemented with extract to testpathogen viability as proxy for antifungal activity.

Materials and Reagents

1. Culture of plant pathogenic fungi (*Sclerotinia sclerotiorum*)
2. Methanol (Qualigens)
3. Potato dextrose agar (PDA) media (HiMedia Laboratories)
4. Commercial disinfectant(70%ethanol)
5. Distilled water
6. PDA media (see Recipes)

Equipment

1. Measuring cylinder
2. Inoculating needle
3. Spirit lamp
4. Cork borer
5. Conical flask
6. Measuring scale
7. 10 cm Petri dishes
8. pH meter (Eutech Instruments pH tutor)
9. Autoclave (Nat steel)
10. BOD incubator (Toshiba)

11. Laminar air flow hood (Toshiba)
12. Micropipette (Eppendorf)
13. Microbalances (Sartorious, model: RC210P)

Procedure

1. Solvent extracts from endophytic fungi were tested against *Sclerotinia sclerotiorum* a well known plant pathogenic fungus.
2. 30 mg of dried extract was dissolved in 800 µl of methanol.
3. 200 and 400 µl of the dissolved extract were added to 30 ml of molten PDA media, mixed and then poured equally into three 10 cm Petri plates to obtain 250 and 500 µg/ml extract concentrations, respectively.
4. To obtain 1,000 µg/ml concentration, 30 mg of dried extracts were dissolved in 400 µl of methanol and 400 µl were added to 30 ml of molten PDA.
5. Control growth plates contained 400 µl of methanol.
6. *S. sclerotiorum* was inoculated at the centre of the plate and radial growth was measured at 48 h intervals till the control plate attained the full growth.
7. Per cent growth inhibitions (%GI) of the extracts were calculated relative to the growth on the control plate.

   %GI = {(A-B)/A} x 100

   Where A = Growth of plant pathogenic fungus in control plate

   B = Growth of plant pathogenic fungus plates supplemented with extract from endophytic fungus

Recipes

1. PDA media

   39.1 g of PDA media was dissolved in 1,000 ml of distilled water and pH was adjusted to 7.0

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References

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