Batch Culture Fermentation of Endophytic Fungi and Extraction of Their Metabolites

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[Abstract] Antibiosis is one of the possible modes of action shown by endophytic fungi having antifungal activity. To test if antifungal activity in endophytic fungi is due to antibiosis, assay of the metabolites of endophytic fungi was needed. To obtain metabolites for bioassay batch culture fermentation and extraction of metabolites was done. Fungus was multiplied on wickerham media at incubation temperature of 25 ± 2 °C for 4 weeks and then extracted with solvents of different polarity. All the solvent extracts were dried under vacuum rotary evaporator to get dried crude fungal extract, which was subjected to further fractionation and bioassay.

Materials and Reagents

1. Ethyl acetate (Rankem)
2. Butanol (Qualigens)
3. Methanol (Qualigens)
4. Hexane (Qualigens)
5. Fungus culture
6. Measuring cylinder
7. Whatman filter paper
8. Detergent
9. Glass jars of 5 L capacity
10. Malt extract (HiMedia Laboratories)
11. Yeast extract (HiMedia Laboratories)
12. Peptone (HiMedia Laboratories)
13. Glucose (Qualigens)
14. Needle
15. Spirit lamp
16. Commercial disinfectant(70% ethanol)
17. Vacuum pump
18. Malt extract

19. Yeast extract
20. Wickerham medium (see Recipes)

Equipment

1. Inoculation flasks
2. Conical flasks of 1 L capacity
3. Vacuum rotary evaporator (Heidolph Instruments GmbH)
4. pH meter (Eutech Instruments pH tutor)
5. Autoclave (Nat Steel)
6. BOD incubator (Toshiba)
7. Laminar air flow hood (Toshiba)
8. Filteration assembly
9. Hand blender (inalsaappliances.com)
10. Fume hood
11. Microbalances (Sartorious, model: RC210P)

Procedure

1. 5 discs of 5 mm diameter of endophytic fungus from petridish were inoculated in Wickerham medium.
2. Flasks with inoculated media were incubated at 24 °C for 24 days under static culture condition without light.
3. One flask of medium without any inoculam served as a control.
4. After 24 days of incubation, 250 ml of ethyl acetate was added to each flask, mixed, and left overnight.
5. Ethyl acetate immersed fungus culture was blended with a hand blender for 15 min and filtered by Whatman filter paper under vacuum (Wicklow *et al*., 1998).
6. The filtrate was collected and residual aqueous phase was partitioned thrice times with equal volumes of ethyl acetate in a separator funnel (Figure 1).

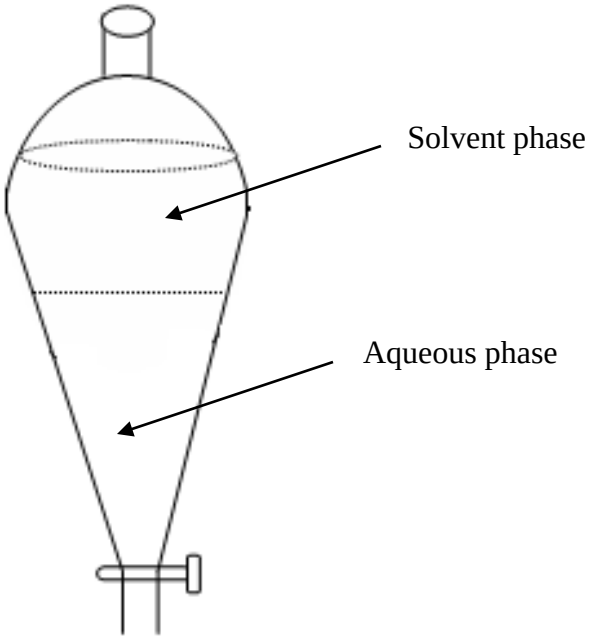

Figure 1. Partitioning by separator funnel

7. Aqueous phase obtained after ethyl acetate extraction was further partitioned three times with equal volumes of saturated butanol.
8. Aqueous phase obtained after butanol extraction was discarded after immersing in detergent.
9. The ethyl acetate and butanol extracts were dried with vacuum rotary evaporator.
10. Dried ethyl acetate extract resuspended in 90% methanol and extracted with *n*-hexane.
11. After drying the hexane, butanol, and methanol extracts with vacuum rotary evaporator they were subjected to further experimentation. Schematic diagram for the extraction of the metabolite has been given in Figure 2.

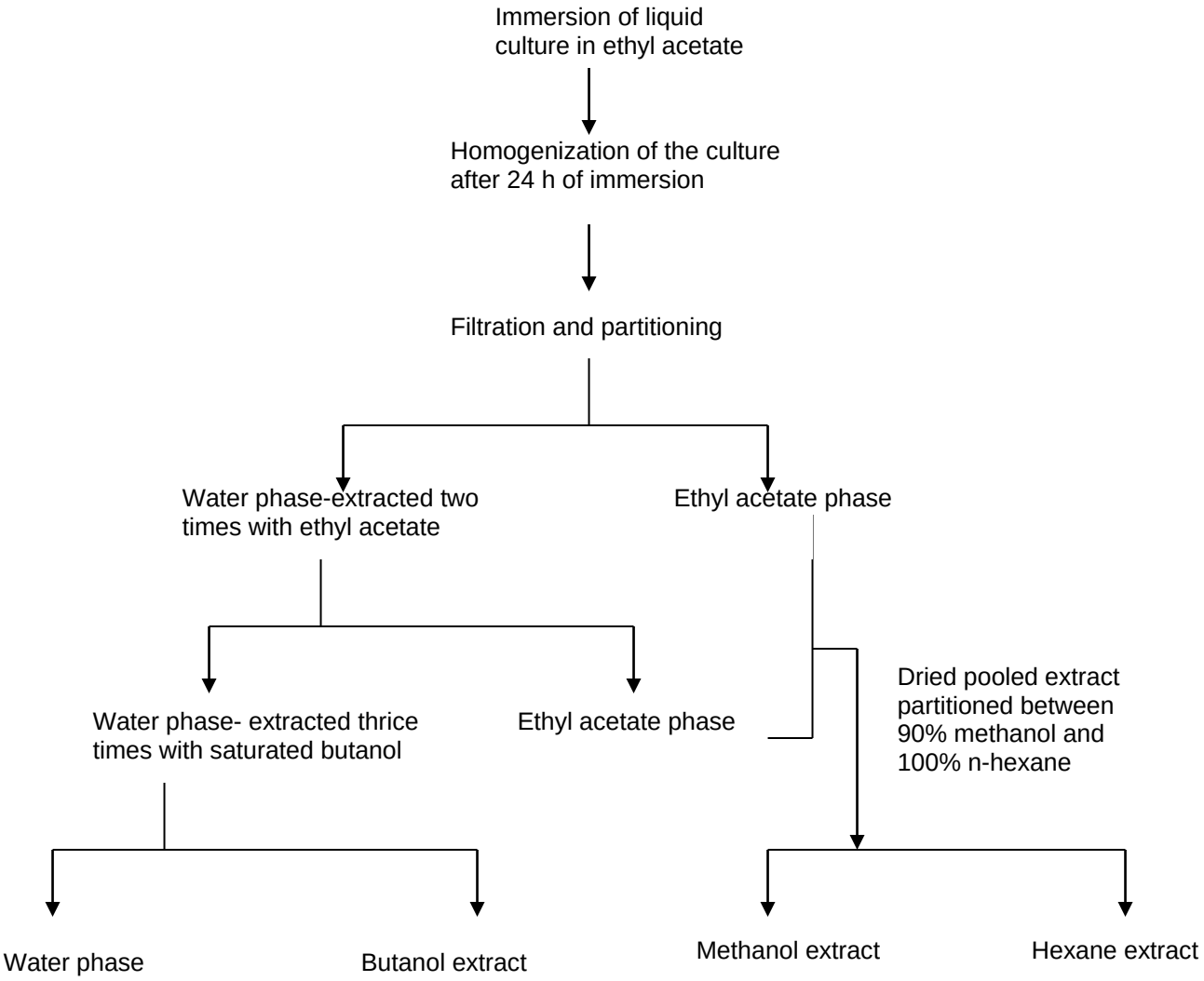

Figure 2. Schematic diagram of extraction procedure for obtaining crude fungal extracts

Recipes

1. Wickerham medium

   Malt extract 3 g/L

   Yeast extract 3 g/L

   Peptone 5 g/L

   Glucose 10 g/L

   All the media chemicals were weighed and dissolved in distilled water and pH was measured. After adjusting the pH in range of 7.2-7.4, media was distributed in conical flasks (300 ml in 1 L conical flask). These flasks were subjected to autoclaving at 121 °C

temperature and 15 psi pressure for 20 min.

Acknowledgments

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References

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