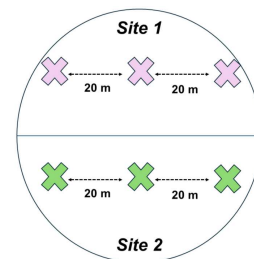


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Soil Collection Protocol

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Protocol status: Working

We use this protocol and it's working

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Abstract

Soil harbors diverse microorganisms, including fungi that play crucial environmental roles but can also harm plants, animals, and humans through infections or spore exposure. In 2022, the WHO released several pathogenic fungi, including *Fusarium* group, Mucorales and *Eumycetoma* agents, which are prevalent in Thailand's agricultural soils and have caused significant human infections. Despite this, the genetic mechanisms behind their infections and antifungal resistance remain poorly understood, particularly in Southeast Asia. Antifungal resistance to major drug classes, such as azoles, echinocandins, and polyenes, is increasing, driven by specific resistance genes. To address these challenges, we propose establishing a Southeast Asian (SEA) network of mycologists to study fungal pathogens, focusing on collecting soil samples, extracting fungal genomic DNA, and creating a comprehensive genome database. This initiative will improve detection and management of drug-resistant fungi, enhance clinical treatments, and strengthen agricultural biosecurity. By leveraging genomic technologies and fostering international collaboration, the project aims to develop targeted strategies against fungal infections and resistance, benefiting public health, food security, and sustainable agriculture across SEA. Establishing a fungal biobank and expert network will support long-term efforts to combat antifungal resistance, providing critical resources for swift and accurate responses to fungal threats.

Materials

1. Dry ice
2. Ethanol 70%
3. Soil tester (pH, temperature and moisture)
4. Sterile centrifuge tube (50 mL)
5. Shovel or Cylinder crop
6. Sterile plastic ziplock bag
7. Tape measure
8. Cooler box



Soil sampling collection

- 1 Choose Sampling Location: Select a location close to an agricultural area for soil sampling. Ensure the area is representative of the study conditions.
- 2 Measure Soil Condition: Use a soil tester to measure and record parameters such as pH, moisture content, and temperature.
- 3 Collect Soil Sample: Dig ± 10 cm below the soil surface using a shovel or a cylinder crop. Transfer the soil sample into a 40 mL centrifuge tube. Ensure the sample is compacted enough to avoid air gaps but not overpacked.
- 4 Replicate Sampling: Move to a new position ± 20 m from the initial sampling site to collect the second replicate and third replication.
- 5 Store Samples: Place all soil samples into an ice box.
- 6 Ensure that at least the e-DNA extraction and isolation processes are performed within the same week or, at the latest, within a month.



Protocol references

Brooks, J. (2016). Soil sampling for microbial analyses. *Manual of Environmental Microbiology*, 2.6. 3-1-2.6. 3-11.