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Editorial

Rapid Screening for Endophytes in Resource- Limited Settings

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Endophytes are organisms that live and grow in tissues of plants without causing the plant any discernible harm or apparent disease. The most common and widely studied endophytes are of bacterial and fungal origins. Endophytes encode a number of properties that enhance the performance of their host. These include the production of bioactive compounds such as anti-bacterial; anti-fungal, anti-cancer, anti-protozoan, insecticidal compounds and the capacity for Nitrogen fixation and Phosphate solubilisation to name a few. Given the diversity of indigenous trees and medicinal plants within the tropics, endophytes present an enormous opportunity for drug discovery and indeed other useful bioactive compounds. Furthermore, this approach to drug discovery and search for bioactive compounds presents an opportunity for the sustainable harvesting of these genetic resources.

To this end therefore, a rapid screen can be set up where following surface sterilization, tissue segments are placed on a Potato dextrose agar (PDA) plate and allowed to grow. This medium will support both bacterial and fungal growth. As bacteria colonies appear they are then placed on Luria Bertani agar plates and streak –purified to single colonies. Similarly, as fungi grow they can also be moved to fresh PDA plates until established as pure cultures. The resulting numbers of bacteria and fungi can be overwhelming especially if they are being isolated from multiple sources all at once. To analyze such numbers, a rapid and effective method of DNA isolation is required.

The DNA can be extracted using a standard buffer such as one containing 100 mM Tris HCl (pH 8.0), 60 mM EDTA, 1% Sodium dodecyl sulphate (SDS) and 150 mM Sodium chloride. If the microorganisms are pigmented, then Polyvinyl pyrrolidone (PVP) can be added to a final concentration of 2%. A subsequent incubation at room temperature and addition of a 1/10 volume of 3 M Sodium acetate (pH 5.2) and addition of an equal volume of isopropanol and centrifuging at 10 k rpm will precipitate the DNA. The DNA pellet is then washed with 70% ethanol, centrifuged once more at 10 K rpm for 3 minutes and the supernatant discarded. The DNA is then dried in a Speedvac and resuspended in 50 ul of 10 mM Tris HCl 1 mM EDTA (pH 8.0). Ten micro liters of DNA is then run on a 0,8 % agarose gel stained with Ethidium bromide(10mg/ml) and visualized under UV light then photographed. One microlitre of the DNA can be run in a standard Polymerase chain reaction (PCR) volume of 25 ul to amplify either the 16S- or 18 rRNA. These same amplicons can be sequenced, and a phylogenetic tree drawn to show the degree of relatedness with other microorganisms whose sequence is already in the database. This approach provides a rapid way of identifying the organism without undertaking a series of biochemical tests in order to identify the microorganisms.

At this point the same purified strains can be grown in liquid broth and after a number of days their supernatants can be extracted using a range of solvents in preparation for antimicrobial assays. Antimicrobial assays can be done on crude extracts or on purified spots from thin layer chromatography (TLC). Similarly, carbon source utilization assays using various substrates can be used to detect a number of extracellular enzymes the organism might be secreting. These include such substrates as carboxymethyl cellulose, for cellulase; starch for α-amylase, gelatin for protease, Tween 80 for lipase or xylan for xylanase to mention just a few. Other analyses that can be done on bacteria could be Gram staining to determine whether the microorganism is Gram positive or Gram negative, or indeed test for indole production or test for its nitrogen fixing capacity. Clearly microorganisms showing potential for useful compounds can be analysed in a much more detailed fashion.

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