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Review Article

Integrated Biochemical and Molecular Investigation of Plant Growth Promoting Fungi from Soil Samples

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Abstract
Plant growth promoting fungi (PGPF) are now getting more attention as helpful partners in farming and, in some cases, as partial replacement for chemical fertilisers and pesticides. These fungi can make plant nutrition better, support stronger growth and help crops to face diseases and hard conditions like drought or too much salt in the soil.In the last few years, a mix of simple microbiological methods, biochemical tests and molecular tools (especially 18S rRNA and ITS sequencing) has made it easier to pick out promising PGPF strains from mixed soil samples with much better accuracy than before. This review brings together recent information on useful plant-associated fungi, with special attention on species of Trichoderma and Aspergillus. It describes how combined biochemical and molecular approaches can be used to isolate, screen and identify PGPF from soil. Examples include tests for siderophore production, measurement of extracellular enzyme activities, and PCR-based identification of soil fungi such as Trichodermaharzianum and Aspergillustamarii using 18S-region primers followed by Sanger sequencing. The aim is to connect published work with the kind of practical workflow that can be done in a typical M.Sc. project, and to show how such step-by-step studies can slowly build towards more sustainable, microbe based agriculture.

Keywords:
Plant Growth Promoting Fungi (PGPF); Trichoderma; Aspergillus; Soil Fungi; Siderophore Production; Extracellular Enzymes; 18S Rrna; ITS Sequencing; PCR Identification.

1. Introduction:

A useful journey from fungi to PGPR bacteria

Most of the previous studies on plant growth promoting microbes have been focused on bacteria from the rhizosphere. These bacteria are called plant growth promoting rhizobacteria (PGPR) . They live in the soil near the roots, sometimes on the surface of the root, even inside the root tissue, helping plant growth in many ways. They can fix nitrogen from air, produce siderophores which help in iron uptake by plants, solubilise minerals like phosphate, help roots to absorb more nutrients and also produce plant hormones like IAA and gibberellins (Zahir et al., 2004). Some well known examples of PGPR are Azotobacter, Pseudomonas, Bacillus and Rhizobium.These groups are already well-studied and some strains are used in fields as biofertilizer. They are useful to reduce chemical fertiliser and support more sustainable farming (Glick,

2012; Kasa et al., 2015). Scientists begin to study more and more about fungi that live around roots and inside roots, because they also can help plants in similar ways or extra ways .Trichoderma is a good case in point. This fungus is well known as a biocontrol agent against many soil diseases but can also stimulate plant growth. Trichoderma can colonise the root surface, compete with harmful fungi in the soil and simultaneously improve plant nutrition and alter plant defence and growth signals. Such fungi may be cooperative with bacterial PGPR and both may aid crops to grow better with lower use of chemicals (Kasa et al., 2015).In the same way, some Aspergillus and Penicillium spp. are strong phosphate solubilizers and can serve as "phosphofungi" in the rhizosphere (Wang et al., 2018). In some cases, these fungal strains are more effective than classical phosphate solubilizing bacteria, especially on recalcitrant P sources. A large number of endophytic fungi live inside healthy plant tissues without causing any visible disease and they are not only limited to the rhizosphere. These endophytes many time produce extra-cellular substances and plant growth regulators which can help in seed germination, better root growth, improved use of nutrients and more tolerance to stress conditions (Shah et al., 2018; Uzair et al., 2018).Another group of mycorrhizal fungi that are well known for forming true symbiotic interfaces with plant roots are particularly important for P and N acquisition and for helping plants to survive stress . Some root endophytes such as Serendipitaindica (formerly Piriformosporaindica) are attractive because they can be grown in defined media and then used as inoculum for many crops. All this has changed the focus from bacteria alone to a more integrated perspective where fungi mycorrhizae, rhizospheric PGPF and root endophytes are also considered as key players in plant nutrition and health (Bashan & De-Bashan, 2005; Kharkwal et al., 2024).

2. Why Plant Growth-Promoting Fungi Matter Now

At present, farming has to face many problems such as fast population growth, climate change and damage to land and soil. To get more crop yield, farmers usually apply a lot of chemical fertilisers and pesticides. This practice gives short-term benefit but also creates many long-term issues.When farmers use mineral fertilisers in very high amount, the soil can become more acidic, some important trace elements get lost, and many helpful soil organisms are reduced. The soil structure also get disturbed. Extra fertiliser and pesticide washed away by rain can reach canals, rivers and even groundwater, and this lead to water pollution. In addition, these inputs are costly and their production and use are linked with emission of greenhouse gases. Because of these limitations, there is growing interest in using biofertilisers and biostimulants that contain useful microorganisms, including plant growth-promoting fungi (PGPF), to partly replace or support normal NPK fertilisers. Nitrogen-fixing microbes can lower the need for synthetic N fertiliser. Phosphate-solubilising fungi have ability to change insoluble forms of phosphorus in soil into simple forms that plants canuse. Other fungal strains can help plants absorb micronutrients and can produce hormones such as auxins and cytokinins, which encourage better root and shoot growth (Wang et al., 2018; Oo et al., 2020). Some famous plant growth-promoting fungi, for example many Trichoderma species and some Aspergillus, can produce siderophores. These are small compounds which catch and hold iron (Fe^{3+}) very strongly. When they do this, the useful fungi compete with disease causing microbes for iron and usually take it first, so harmful fungi and bacteria do not grow so well. In some cases, the plant can also take iron from

these siderophore–iron complexes, so the plant gets two benefits at same time: less disease and better iron nutrition.

Many of these fungi also give out enzymes like cellulase and pectinase into the soil. Such enzymes break down dead plant parts and other organic matter. When this material is degraded, more nutrients are released and become easier for crop roots to use. Some PGPF have also been reported to help plants face stress conditions such as drought, too much salt in soil and other environmental problems (Shah et al., 2018; Oo et al., 2020). Because these fungi can do many different jobs, it is not enough to simply say that "fungi are present" in a field. A proper approach is needed where fungi are first isolated from soil or roots, then checked in the lab for clear plant helping traits (like dissolving phosphate, making siderophores or producing plant hormones), and after that identified by molecular methods so that we know exactly which species or strain we are working with.Only with this step by step method can we select really useful plant growth-promoting fungi and use them safely and effectively in sustainable agriculture.

3. Biochemical Screening of Plant Growth Promoting Fungi

A normal study on plant growth-promoting fungi from soil usually begin with taking soil samples from different places, then doing serial dilution and putting them on general fungal media like Potato Dextrose Agar (PDA). After some days, different fungal colonies appear with different look, for example in growth speed, colour, type of spores and pigment. These different colonies are picked and grown again and again on PDA or similar media until we get pure cultures of each fungus. When pure isolates are ready, they can be checked in the lab for different characters that may help plants. One important test is for siderophore production. In most soils, iron is not easily available because it is present in forms that do not dissolve well in water. Microbes which produce strong siderophores can bind this iron and use it better than others. To test this, Chrome Azurol S (CAS) agar is commonly used. On CAS medium, colonies that make siderophores cause a clear colour change in the dye around them. Many useful biocontrol fungi and bacteria use siderophores to take away iron from plant pathogens, so the pathogens grow poorly. Plants, however, can usually manage with a little less iron near the roots and sometimes also use iron from these microbial siderophore complexes (Oo et al., 2020).

Phosphate solubilization

Some soil fungi, such as Aspergillus and Penicillium have release organic acids such as citric, oxalic and tartaric acid. These acids can dissolve insoluble phosphorus compounds from r rock phosphate or calcium phosphate, and change them in forms that plants can use. Wang et al. (2018) reported an Aspergillusniger strain (CS1) it showed strong phosphate solubilization through organic acid production and also have other useful traits like potassium release and cellulose breakdown. On special agar plates which contain insoluble calcium phosphate, these fungi generally form a clear zone (halo) around the colony, which shows that phosphate has been solubilised.

Extracellular enzymes

Many potential plant-helping fungi are verified for enzymes such as cellulase, pectinase, chitinase and protease. Cellulase and pectinase have use in to decompose plant residues, which improves nutrient cycling in the soil. Chitinase can attack the cell walls of other fungi, so it is often linked with biocontrol activity against plant pathogenic fungi. In this way, enzyme tests give an idea whether an isolate can both recycle organic matter and also act against some plant diseases.

Production of plant hormones and other metabolites

Some studies also look at fungal production of plant hormones, although these tests are more time-consuming. Indole-3-acetic acid (IAA), gibberellins and ACC deaminase activity are sometimes measured in culture filtrates. Endophytic fungi from plant roots, including orchids, have been shown to produce auxin- and cytokinin-like substances that can improve seed germination and root growth (Shah et al., 2018).

When these different biochemical tests are used together phosphate solubilisation, enzyme production and possible hormone formation it becomes easier to select a smaller number of promising isolates. These chosen fungi can then be taken forward for molecular identification and later tested on plants in pots or field trials.

4. Molecular Identification of Soil Fungi:

From 18S to ITS

For many years, most soil fungi were identified only by how their colonies looked on agar plates and by microscopic study of spores and hyphae. This classical approach is useful, but it is slow and often not enough to separate closely related or cryptic species. Because of this, molecular tools based on fungal ribosomal DNA (rDNA) have become very common in environmental and clinical mycology. Two rDNA regions are especially important in this context. The first is the small-subunit rRNA gene (18S rDNA) and related regions, which are relatively conserved. These parts of the genome are helpful for placing isolates in broader taxonomic groups and for studying higher-level phylogeny. The second is the internal transcribed spacer, or ITS region (ITS1–5.8S–ITS2), which lies between the small and large subunit rRNA genes. ITS evolves much faster than the coding regions and shows greater sequence variation, so it has been widely adopted as a general “DNA barcode” for fungi and is often suitable for identification at, or close to, species level in many genera (Schoch et al., 2012).

In a typical laboratory workflow for soil fungi, genomic DNA is first isolated from young fungal cultures grown on agar or in liquid medium. Many groups still use CTAB-based methods in which the fungal mycelium is disrupted mechanically (for example, by grinding with liquid nitrogen or using glass beads) and then treated with CTAB buffer, followed by organic extraction and alcohol precipitation to obtain relatively clean DNA (Doyle & Doyle, 1987). The quality and quantity of DNA are commonly checked on agarose gels and with simple spectrophotometric measurements. Once an acceptable DNA preparation is available, PCR is performed using primers that flank either part of the 18S rDNA or the ITS region. Universal ITS primer pairs such as ITS1/ITS4 have been designed to work across a wide range of fungi and are now a standard choice for many ecological surveys and identification studies, whereas 18S-based primers are often used when a broader, less variable marker is preferred.

PCR products are then separated on agarose gels to confirm that the reaction has produced a single band of the expected size. For the ITS region, fragment lengths of roughly 500–700 bp are common, although this can vary among groups. These amplicons are usually cleaned and subjected to Sanger sequencing, which remains a routine and cost-effective method for single-gene sequencing in many laboratories. The resulting sequences are compared against public databases such as GenBank using BLAST or similar tools. A close match, typically above about 98–99% identity over most of the sequence, together with supporting

information from published phylogenetic studies, allows many soil isolates to be named at the species or species-complex level (Iwen et al., 2002). In this way, strains that were originally recognised only as “green Trichoderma” or “yellow Aspergillus” on a plate can be assigned more confidently to taxa such as Trichodermaharzianum, T. asperellum, Aspergillusflavus or A. tamarii. For studies on plant growth-promoting fungi, molecular identification is very useful. It gives a clear and fixed name to each fungal isolate that was first chosen because of its useful biochemical characters, and it also helps to connect our new results with older work on the same or related fungi in terms of their physiology, ecology and possible use. As DNA databases keep growing, and more genes or even whole genomes are used for comparison, combining ITS barcode data with other genetic markers should make fungal identification from soil and root samples more accurate and more reliable.

5. Challenges and Perspectives

Even with modern tools, there are still challenges. Not all PGPF functions are expressed in vitro, so some beneficial strains might be missed during plate-based screening. Molecular methods rely on reference databases, which can contain errors or may lack entries for rare species. Distinguishing between closely related taxa can require multiple loci (ITS plus additional genes) or full multilocus phylogenies (Bruns et al., 1992; O’Donnell, 1996).On the practical side, going from lab screening to successful field application is a big step. Fungal inoculants must survive formulation, storage and field conditions, compete with native microbiota, and actually colonize plant roots in the presence of other stresses. Nevertheless, integrated biochemical and molecular workflows are essential first steps: they generate well-characterized candidate strains that can then be tested more thoroughly.

6. Conclusion

Plant growth-promoting fungi are becoming an important part of the toolbox for sustainable agriculture. They contribute to nutrient cycling, disease suppression and stress tolerance, and they offer a more eco-friendly option compared to heavy reliance on synthetic fertilizers and pesticides. The combination of classical cultivation on PDA, biochemical assays like siderophore and enzyme tests, and molecular identification via 18S / ITS sequencing provides a powerful way to discover and describe new PGPF from soil.The example of isolating and characterizing Trichodermaharzianum and Aspergillustamarii from soil samples shows how such an integrated approach works in practice: from field sampling and Petri plates, through CTAB-based DNA extraction and PCR, to BLAST-based species identification and interpretation of potential plant growth-promoting roles. Future work will need to take these identified strains further, into greenhouse and field trials, and to clarify their mechanisms in planta. But already, such integrated investigations are an important bridge between basic mycology and real-world applications in crop improvement and soil health

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