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Plant Beneficial Microbes: An Overview

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Abstract: Use of harmful chemical fertilizers and pesticides that have large negative impacts on environmental and human health have generated increasing interest in the use of beneficial microorganisms for the development of sustainable agri-food systems. A successful microbial inoculant has to colonize the root system, establish a positive interaction and persist in the environment in competition with native microorganisms living in the soil through rhizo competence traits. Recently, several approaches based on culture-dependent, microscopic and molecular methods have been developed to follow bio inoculants in the soil and plant surface over time. Culture-dependent methods are commonly used to estimate the persistence of bio inoculants; it is difficult to differentiate inoculated organisms from native populations based on morphological characteristics. Therefore, these methods should be used complementary to culture-independent approaches. Microscopy-based techniques (bright-field, electron and fluorescence microscopy) allow to obtain a picture of microbial colonization outside and inside plant tissues also at high resolution, but it is not possible to always distinguish living cells from dead cells by direct observation as well as distinguish bio inoculants from indigenous microbial populations living in soils. The present paper deals with the study of beneficial microbes benefit to plants growth and development.

Key Words: - ***Beneficial Microbes, Harmful, Chemical Fertilizers, Culture Based Methods, Microscopy Based Methods.***

I. INTRODUCTION

Beneficial microorganisms are naturally occurring bacteria, fungi, and other microbes which play a crucial role in plant productivity and health. Two types of beneficial microorganisms, mycorrhizal fungi and nitrogen-fixing bacteria, are considered beneficial to plant health. These are able to control plant diseases can colonize or compete with the pathogens for nutrients and sites of interaction, or exert antagonism through antimicrobial compounds, develop hyperparasitism or directly attach to the pathogen cells, interfere with pathogen signals, or induce resistance into the plant host. These are used as in the form of Bio-inocualts in place of chemical fertilizers.

The increasing demand to reduce the use of chemical fertilizers and pesticides for the development of an agri-food system sustainable for environmental and human health, as well as the current shifting in the agricultural legislation of several countries, have led to an expanded use of bio-inoculants. Although the manipulation of soil micro biomes to optimize crop productivity is an ancient practice regarding mechanistic studies of plant–microbe interactions and microbial persistence in heterogeneous communities in diverse locations, soils, and hosts. Numerous bacterial or fungal strains used as bio inoculants. Plant growth-promoting microbes (PGPM) are the most commonly applied. They may affect plant performance through multiple mechanisms of action, operating directly by the production of specific substances that are able to promote plant growth and increase the availability and uptake of nutrients in soil (i.e., phosphate solubilization, siderophore and indole-3-acetic acid production, nitrogen fixation) or indirectly through the suppression of plant pathogens (Ribeiro and Cardoso, 2012). Several plant growth-promoting rhizobacteria (PGPR) have also been demonstrated to exert a beneficial effect on plant growth under nutritional and abiotic stress.

A successful microbial inoculant has to colonize the external and internal part of plant tissues and establish a compatible interaction with the host as well as to persist in the soil against autochthonous microorganisms living in environment through its rhizo competence traits. In general, rhizosphere colonization occurs through several different mechanisms, such as bacterial movement, survival in the rhizosphere by competition against other microbes, adherence to and colonization of root surfaces, for instance by biofilm formation, and the creation of synergistic interactions with the host plant. If PGP inoculants colonize the plant initially, their persistence over time is not guaranteed. Measuring the

persistence of microbial inoculants in soil poses technical difficulties, as the inoculant needs to be identified from within a complex community. The tracking and monitoring of the persistence of PGPM released in the environment have been widely studied to understand their behaviour in soil and which factors influence their survival under various conditions. Several sets of techniques are currently used to detect root colonization and persistence in the soils: microbial enumerations by culture-based methods, microscopy-based techniques, and DNA-based methods. The results may depend on the choice of technique since each has advantages and limitations, and each technique may have bias in favour of specific microbial taxa.

This review examines and presents an overview of the current methodological approaches used to assess and detect plant colonization and soil persistence of microbial bioinoculants in the rhizosphere environment.

II. Rhizosphere Sampling And Soil Preparation

In soils, several variables or factors can influence the results due to the highly heterogeneous distribution of microbial cells in the environment. To ensure good results in microbiological analysis, the first fundamental prerequisite is the correct soil sampling, both in laboratory and in greenhouse trials and in field experiments, to obtain representative samples for each treatment to be analyzed. Soil and rhizosphere samples can be collected by different sampling approaches, as extensively detailed by Wollum (1994):

- simple random, which ensures that each sample has the same opportunity to be selected, usually by using a grid;
- stratified random, similar to simple random, except the area to be sampled is broken into smaller subareas;
- systematic, which ensures that the entire area is sampled and represented by individual samples that are obtained by establishing predetermined points.

The number of soil samples to take depends on the microbial population distribution and can be calculated using the formula suggested by Wollum (1994 the sample variance and the sample mean. However, it is recommended to brush away stone, rubbish, trash or grass from the soil surface before taking samples. Then, using a sanitized shovel, it is possible to take the samples from topsoil to an adequate depth (for instance, 0-20 cm) or to collect plant roots by excavating or uprooting plants to study microbial diversity in bulk soil and/or rhizosphere. For rhizosphere studies, after plant sampling, roots should be shaken vigorously by hand to remove bulk soil and to collect soil adhering to roots. During the sampling, it is necessary to avoid root damage. Manual excavation using spades and hand tools and working progressively in layers or sectors could minimize the corruption of soil architecture and ensure the safety of the roots. It is also fundamental to take a sufficient number of replications for data analysis. Following this, the samples must be recovered in sterile polyethylene bags or vessels and stored at 4°C to avoid desiccation during transport to the laboratory.

To evaluate external and internal root colonization, which generally occur in the rhizoplane and endosphere, respectively, several steps for sample preparation are necessary (Fig.1.). Plant roots should be washed by agitation in sterile water or buffer without tearing or cutting plant tissues to facilitate the separation between soil/root particles and microorganisms (Kloepper and Beauchamp, 1992).

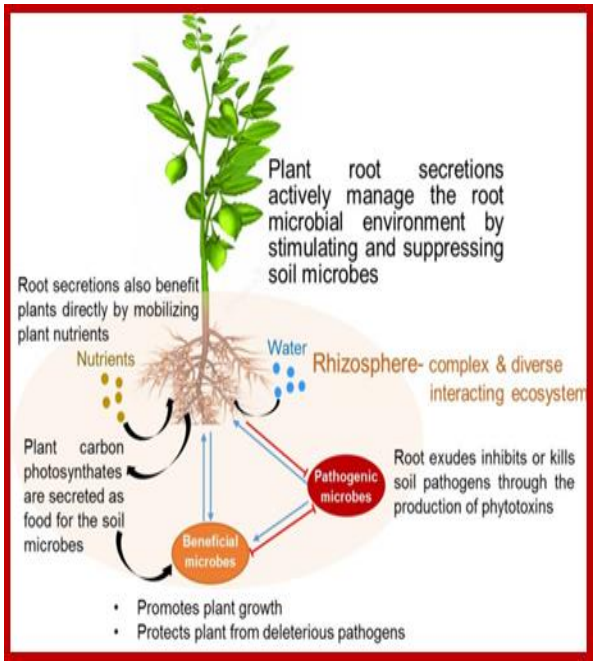

Fig.1. Schematic description of sampling collection, separation of different soil fractions, and methods for the detection of microbial inoculants

III. Microbial Enumerations By Culture-Dependent Methods

This approach has been successful, and it allowed the detection of a higher diversity of cultivable populations compared with other methods. Although culture-dependent methods have been used to detect bio inoculants in different experimental conditions which are useful when the experiment is carried out in sterile conditions and interference by soil autochthonous microbial populations can be avoided.
Growth chambers are usually performed using sterile synthetic substrates or hydroponic conditions for plant growth, allowing the control of all environmental parameters, such as temperature, relative humidity, light/dark cycle, and light intensity. Therefore, this approach is particularly suitable for the detection of inoculated strains in plant tissues by enumeration on culture media.
Greenhouse experimental conditions could be considered a variation of farming in a controlled environment, which provides favorable growing conditions and protects crops from unfavorable weather and various pests. Therefore, this approach could be suitable for evaluating the viability of inoculated microorganisms by culture-dependent methods.

IV. Microscopy-Based Techniques

Today, a wide range of microscopy-based techniques are available and have been used to detect microorganisms inoculated on plant tissues and to evaluate the colonization patterns of bacterial endophytes through molecular interactions and dynamics within living cells in specific vegetative tissues. Root colonization by bacteria and AMF has been studied by several types of microscopy, which can be divided into three major groups: light microscopy, electron microscopy and fluorescence microscopy.
Microscopy techniques that use different dyes are also usually used to assess mycorrhizal relationships with host plants. A wide number of staining procedures, which each have advantages and disadvantages, have been developed for studying AMF colonization. Among these is a very simple, nontoxic, reliable and inexpensive staining technique for AMF colonization in root tissues; this technique is based on the use of an ink-vinegar solution after adequate clearing with KOH. This solution stains all fungal structures, rendering them clearly visible by bright-field light microscopy.
Electron microscopy was further developed into scanning electron microscopy (SEM), which can be used to examine plant surfaces and microorganisms at high resolution, highlighting the adhesion of microbial cells to plant tissues. SEM was used to observe chickpea root colonization by *A. chroococcum* and *Trichoderma viride*. The plants were cultivated in sterile media composed of sand and vermiculite (1:1), and samples were taken at 40 days' post inoculation. SEM microphotographs revealed the proliferation of *Azotobacter* cells, both individually and attached to the fungal mycelia. SEM observations have also highlighted the production of exopolysaccharides by *A. chroococcum.*
Environmental scanning electron microscopy (ESEM) is another powerful method to evaluate the survival of a bacterial inoculant and its ability to colonize plant tissues. It provides new possibilities compared to conventional SEM and enables the investigation of nonconductive and hydrated samples without complex histological preparation steps (i.e., air drying, chemical fixation, dehydration, and coating), which are critical in conventional SEM.
Fluorescence microscopy has become an essential technique in biology for the study of living tissues or cells. Although this method requires more complex and expensive instrumentation than conventional transmitted-light microscopy, it is widely used for the detection of bacteria inside plant tissues. This is possible because fluorescence microscopy reveals the position of fluorescent substances that were previously introduced into living cells. Several fluorescent dyes and protein tags and other methods to fluorescently label cells can be employed, providing a range of tools to track a microbial inoculant.

V. Conclusion

Assessing the root colonization of inoculants with beneficial effects on plant growth as well as their persistence over time in a soil is a critical issue in sustainable agriculture. Currently, several approaches that use culture-dependent, microscopic and molecular methods have been developed to follow bioinoculants in the soil and on the plant surface. However, to ensure good results in microbiological analysis, the first fundamental prerequisite is the correct soil sampling and sample preparation for the different methodological approaches that will be assayed.

Although plant colonization of bacterial endophytes can be assessed by microscopy-based techniques through molecular interactions and dynamics within living cells in a specific vegetable tissue, the measurement of the persistence of inoculants in soil poses technical difficulties, as the inoculant needs to be identified from a complex community. Culture-dependent methods are commonly used to estimate the persistence of inoculated bacteria in soil and/or rhizosphere, mainly for their ease of use, but this analysis is limited since it is difficult to represent the high diversity of bacteria on culture media and, at the same time, it is difficult to differentiate inoculated organisms from native populations based on morphological characteristics. Therefore, culture-dependent methods are especially useful when the experiment is carried out in sterile conditions to avoid interference by native microbial populations living in the soil.

Moreover, a microscopy-based approach allows us to obtain a picture of bacterial colonization outside and inside plant tissues, but it is not possible to always distinguish living cells from dead cells by direct observation. The auto fluorescence of the plant cells and interference by soil particles make it difficult to visualize microbial cells inside different plant tissues. Tagged microbial cells should be used only in limited and controlled experimental conditions (growth chamber and greenhouse), and the evaluation of the survival and colonization ability of an inoculant in a natural real ecosystem cannot be performed because the strains could be released into the environment.

All the described methods have advantages and disadvantages and provide only partial results, and most of them are time-consuming, expensive and unable to detect specific inoculated microbial strains. Therefore, to better explain the behaviour of bioinoculants in the natural soil ecosystems, culture-dependent and culture-independent (molecular and microscopic approaches) methods should be used in combination to examine the variations in microbial communities after inoculation treatment and to track the inoculated microbial strains in different systems.

The main challenge for the application of PGPM as bioinoculants in unsterilized greenhouse or field conditions is the establishment of effective methods for the assessment of plant colonization and soil persistence. Moreover, modern soil microbiology lacks efficient methods for the detection and estimation of the effective PGP activities that inoculated strains have on the soil. This is another main bottleneck in the use of microbial inocula for rhizosphere engineering. Therefore, the development of specific and easy methodologies for the evaluation of PGP activities could help to understand what actually occurs in a natural soil system during plant–soil–microbe interactions.

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