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STUDIES ON FINDING MICROBIAL GROWTH AND YIELD ENHANCER FOR PLANTS

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Abstract

The purpose of this work was to find microbial growth and yield enhancer for plants. This paper presents the results obtained in the research conducted for the case study included in the Bachelor's Thesis. The soil sample was taken from the family vegetable garden located in Crucea de Piatra, Calugareni commune, Giurgiu county from a depth of 5 - 20 cm, after removing the vegetal layer from the surface. The soil sample was transported in a refrigerated box to the laboratory of Ecology and Environmental Microbiology within the Faculty of Land Reclamation and Environmental Engineering within the U.S.A.M.V. Bucharest. In order to achieve the proposed goal, the soil sample taken from the family vegetable garden was analyzed in the laboratory of Ecology and Environmental Microbiology from a microbiological point of view. Thus, the in vitro studies carried out highlighted the great biological diversity of the analyzed soil sample and of some relationships that exist between the different groups of microorganisms in this microcosm. Thus, the use of consortia of microorganisms present in this soil sample allowed us to obtain new strains of microorganisms with properties for growing and stimulating plant development, which we can continue to use in other studies that we will do in the laboratory and in vivo in the vegetable garden.

Key words*: new microbial growth and yield enhancer strains for plants, soil microbial microcosm.*

INTRODUCTION

The soil supporting today's agriculture activities is lacking sufficient microbial activity to promote healthy plant growth. This is due primarily to an over reliance on pesticides to control disease and insect infestation. These synthetic chemicals also destroy non-target organisms, namely the essential bacteria and microbes found in healthy soil. Beneficial microorganisms are necessary to promote healthy, vigorous plant growth. When microbial population is depleted the plant growth system becomes stressed giving rise to a myriad of problems. Our soil microbial consortium is an all-natural product with unique microbial systems as a response to this problem (https://www.malatechwater.com/).

This soil microbial consortium should be a microbial system with unique properties like helping soil to establish beneficial microbes by providing certain unique nitrogen fixing, phosphorus solubilizing, and plant growth factor producing natural microbes. It is not only how many bacteria but also the type and functionality of the bacteria that is very important for overall plant growth. These microbes should be able to extract nutrients from the mineral part of the soil and eventually pass the nutrients on to plants.

A good plant growth and yield enhancer should have a number of features, such as these:

- Promotes conversion of soil elements including phosphorus, into plant available forms.
- Increases resistance to plant diseases.
- Revitalizes the soil. Helps decompose soil organics.
- Biodegradable and not harmful to the soil.
- Increases soil buffering properties by increasing humus level.
- Chelates metal ions in alkaline conditions, increasing plant availability.
- Stimulates plant growth by naturally accelerating cell division.
- Increases seed germination and viability.
- Stimulates root growth, thus increasing root density.
- Increases root respiration.
- Stimulates plant enzymes.

- Helps reduce fertilizer load.
- Helps reduce dependence on chemical applications.
- Better quality and yield.
- Helps faster flowering and faster fruiting.
- Completely organic.

Soil bacteria are very important in biogeochemical cycles and have been used for crop production for decades. Plant–bacterial interactions in the rhizosphere are the determinants of plant health and soil fertility. Free-living soil bacteria beneficial to plant growth, usually referred to as plant growth promoting rhizobacteria (PGPR), are capable of promoting plant growth by colonizing the plant root. PGPR are also termed plant health promoting rhizobacteria (PHPR) or nodule promoting rhizobacteria (NPR). These are associated with the rhizosphere, which is an important soil ecological environment for plant–microbe interactions (Hayat et al., 2010).

Plants have co-evolved with soil microbes over hundreds of millions of years. As bacteria colonized the Earth and transformed the atmosphere over three billion years ago, they created conditions which made it possible for the evolution of soil fungi (approximately 900 millions of years ago). Together, bacteria and fungi shaped Earth's soil structure and created habitable conditions for the evolution of plants around 700 millions of years ago.

Soil microbes are ubiquitous, meaning they are abundant in most terrestrial environments. For example, more microbes can be found in one gram of soil than there are people on the Earth! This is important because these tiny soil microbes play a huge role in supporting plant growth.

Bacterial and fungal species work together in clusters (i.e. consortia) to support plant growth along the rhizosphere (i.e. the soil root zone) primarily by delivering nutrients and preventing disease. For example, soil bacteria and fungi continually increase soil nutrient availability by transforming unavailable nutrients into bioavailable forms for plant uptake. Microbes also act as a biofertilizer by releasing critical nutrients when they die. Without microbes, plants wouldn't have the constant supply of nutrients they need to grow.

Beyond nutrient cycling, microbes produce hormones and other chemicals to stimulate plant growth. Soil microbes can also prevent pathogen infection by inducing plant systemic disease resistance and by coating root surfaces to physically shield the plant from getting infected by pathogens (Bell, C, 2021).

Plant growth-promoting rhizobacteria (PGPR) are free-living bacteria of beneficial agricultural importance. The PGPR present encourage beneficial effects on plant health and growth, suppress disease-causing microbes and accelerate nutrient availability and assimilation. Thus, in the quest to improve soil fertility and crop yield and to reduce the negative impacts of chemical fertilizers on the environment, there is a need to exploit PGPR for continued beneficial agricultural purposes. PGPR exist in the rhizosphere and this is defined as the region around the root (Babalola, 2010).

Co-inoculation of multiple PGPRs, inoculation with mixed different strains could be an alternative to inoculation with individual strains, likely reflecting the different mechanisms used by each strain in the consortium. Combined inoculation with N2-fixing bacteria and phosphate solubilizing bacteria were more effective than a single microorganism for providing a more balanced nutrition for plants. There are numerous examples in wheat whereby synergistic effects of multiple PGPRs are observed (Çakmakçı et. al., 2017).

A *Bacillus subtilis* strain showed a variety of colony growth patterns on agar plates. The bacterium grew to fractal colony through the diffusion-limited aggregation process, a round colony reminiscent of the Eden model, a colony with a straight and densely branched structure similar to the dense branching morphology, a colony spreading without any openings, and a colony with centric rings, on plates with various agar and nutrient concentrations. The microstructures of these colonies were also characteristic and dynamic. The patterns of these bacterial colonies were thought to grow in relation to the diffusion of nutrient in agar plate (Fujikawa, 1994).

The Gram-positive, rod-shaped bacterium *Bacillus subtilis* is usually found in soil. *B. subtilis* is considered to be non-pathogenic to humans and was shown to be beneficial to plants when in association with plant roots. The species is widely used in microbiology research and is considered to be a facile model organism for the

study of biofilms, particularly due to its ability to form distinctly segmented three-dimensional colony biofilms. Under conditions of stress, *B. subtilis* forms endospores that can withstand extreme environmental conditions for prolonged periods of time, thus enabling the survival of the organism under conditions such as nutrient depletion or under other various unfavorable environments (Gingichashvili et al., 2017).

This paper presents the results obtained in the research conducted for the case study included in the Bachelor's Thesis. The purpose of this work was to find microbial growth and yield enhancer for plants. In order to achieve the proposed goal, the soil sample taken from the family vegetable garden was analyzed in the laboratory of Ecology and Environmental Microbiology from a microbiological point of view. Thus, the in vitro studies carried out highlighted the great biological diversity of the analyzed soil sample and of some relationships that exist between the different groups of microorganisms in this microcosm. Thus, the use of consortia of microorganisms present in this soil sample allowed us to obtain new strains of microorganisms with properties for growing and stimulating plant development, which we can continue to use in other studies that we will do in laboratory and *in vivo* in the vegetable garden.

MATERIALS AND METHODS

The soil sample was taken from the family vegetable garden located in Crucea de Piatră, Călugăreni commune, Giurgiu county from a depth of 5 - 20 cm, after removing the vegetal layer from the surface. The soil sample was transported in a refrigerated box to the laboratory of Ecology and Environmental Microbiology within the Faculty of Land Reclamation and Environmental Engineering within the U.S.A.M.V. Bucharest.

The laboratory work steps were as follows:

1. Preparation of culture media - in these experiments we used two types of culture media - the culture medium with agar potato extract PDA (20 g dextrose, 200 g potatoes infusion, 15 g agar) and the solid culture medium LB (Luria-Bertani) which after preparation we sterilized for 20 minutes at 120 degrees Celsius in an autoclave;

   Preparation of Potato Dextrose Agar Medium for 1000 mL
   - boil 200 g of sliced peeled potatoes in 1000 mL water for 30 minutes;
   - filter through cheesecloth, saving effluent, which is potato extract, infusion;
   - add dextrose and agar and shake the ingredients in bottle with a magnetic stirrer to dissolve the reagents and add distilled water to a final volume to 1000 mL;
   - sterilize by autoclaving at 1.2 atm pressure (120°C) for 20 minutes;
   - pH 5.6 ±0.2

   Preparation of LB (Luria-Bertani) Agar Medium for 1 000 mL
   - weigh out 10 g tryptone, 10 g sodium chloride (NaCl) and 5 g yeast extract and add to a 1 L Duran bottle;
   - measure out approximately 900 mL of distilled water and add to the Duran bottle;
   - shake the ingredients in bottle with a magnetic stirrer to dissolve the reagents and add distilled water to a final volume to 1000 mL;
   - sterilize by autoclaving at 1.2 atm pressure (120°C) for 20 minutes;
   - pH 7 ± 0.2
2. Obtaining the soil extract - realization of the soil extract for obtaining consortia of microorganisms with potential in stimulating plant growth. We weighed 5 grams of soil with a Precisa balance, which we suspended in 10 ml of sterile distilled water;
3. Observation of the soil extract suspension under an optical microscope. To highlight some morphological characteristics of microorganisms (the size, shape, and arrangement of cells, types of microorganisms – bacteria or fungi) we prepared living, unstained preparations as a wet mount;
4. Cultivation of the soil extract on solid PDA and LB (Luria-Bertani) culture media. The soil extract containing the consortia of microorganisms was inoculated in accordance with the sanitary rules in the microbiological hood, 1 ml

each on petri dishes with solid medium LB (Luria-Bertani) and PDA, then incubated at room temperature for 18-24 hours (LB plate), and respectively 4-5 days (PDA plate);
5. Isolation in pure culture of soil bacteria. We used the serial dilution technique from the soil extract to dilution 1/104. After that we cultured on solid culture medium LB (Luria-Bertani) 1 ml of the last dilution, then we incubated the petri dishes inoculated at room temperature for 18 hours;
6. Obtaining new bacterial colonies with potential properties in germination, plant growth and protection against specific phytopathogenic fungi that come from the soil. We used the loop impoverishment technique to obtain isolated colonies in pure culture of soil bacteria, in order to characterize them microbiologically (macroscopic appearance of the colony, type of colony, color, appearance of colony edges, microscopic appearance of cells, morphology, form and grouping mode).

RESULTS AND DISCUSSIONS

The purpose of this work was to find new microbial growth and yield enhancer strains for plants.
This paper presents the results obtained in the research conducted for the case study included in the Bachelor's Thesis.
The culture media prepared in the laboratory allowed us to observe and identify new strains of microorganisms with properties for growing and stimulating plant growth and protection against infection with phytopathogenic fungi from the soil.
Obtaining the soil extract according to the method presented in the section Materials and methods and observing it in wet mount preparations under the optical microscope allowed us to highlight the microorganisms present in the soil consortia, which we then isolated in pure culture to use in subsequent experiments.
The cultivation of the soil extract on PDA and LB specific media highlighted the development of microorganisms from the consortium of the soil microcosm. Then from these colonies of new microorganisms we isolated in pure culture a new strain of bacteria.
The new bacterial strain from the soil sample taken from the vegetable garden of the Zurini family in the locality Crucea de Piatră, Călugăreni commune, Giurgiu county was isolated in pure culture on solid LB medium.
When cultured on ordinary nutrient agar, the morphology circular colony of this bacteria was rough, opaque, fuzzy white or slightly yellow with jagged edges.
The appearance of cells under a microscope is characteristic of bacillus-type cells, rod-shaped and Gramm staining revealed that they are gram-positive bacilli.

CONCLUSIONS

The purpose of this work was to find microbial growth and yield enhancer for plants. This paper presents the results obtained in the research conducted for the case study included in the Bachelor's Thesis. The soil sample was taken from the family vegetable garden located in Crucea de Piatra, Calugareni commune, Giurgiu county from a depth of 5 - 20 cm, after removing the vegetal layer from the surface. The soil sample was transported in a refrigerated box to the laboratory of Ecology and Environmental Microbiology within the Faculty of Land Reclamation and Environmental Engineering within the U.S.A.M.V. Bucharest. In order to achieve the proposed goal, the soil sample taken from the family vegetable garden was analyzed in the laboratory of Ecology and Environmental Microbiology from a microbiological point of view. Thus, the in vitro studies carried out highlighted the great biological diversity of the analyzed soil sample and of some relationships that exist between the different groups of microorganisms in this microcosm. Thus, the use of consortia of microorganisms present in this soil sample allowed us to obtain new strains of microorganisms with properties for growing and stimulating plant development, which we can continue to use in other studies that we will do in the laboratory and in vivo in the vegetable garden.
Studies conducted in the laboratory of Environmental Ecology and Microbiology have allowed us to isolate in pure culture a new

bacterial strain, which we will use in subsequent experiments to test the ability to stimulate germination and plant growth and also for the ability to inhibit phytopathogenic fungi.

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SECTION 02
SUSTAINABLE DEVELOPMENT OF RURAL AREA