Pilot project: Plant hormones and soil bacterial populations in plant growth enhancement

Ada Sardaña Sambola and Dr. Chad Welsh
Department of Natural Sciences, Lindenwood University, St. Charles, MO

Introduction

Numerous microscopic organisms, including bacteria, fungi, actinomycetes, protozoa, and algae thrive in soil environments. Among these, bacteria are the most prevalent, making up 95% of the soil's microbial population. It has been long known that there are millions of bacteria in every gram of soil, usually around 10^8 to 10^9 cells (Schoenborn et al. 2004). However, they are not uniformly distributed, as a greater concentration of bacteria is found near the roots of plants. This is due to the nutrients secreted by plants like sugars and amino acids. Bacteria can be either beneficial, harmful, or neutral to plants. Those that provide plants with nutrients that enhance growth and help them thrive are called plant growth-promoting bacteria (PGPB) (Glick 1995).

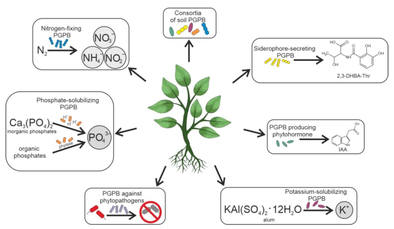

Figure 1. Different PGPBs contribute to plant growth and development in various ways.

Purpose

The way microorganisms affect plant health has been thoroughly studied, but the reverse of this phenomenon has not been studied as deeply. This initial investigation aims to delve into the less-explored aspect of how the introduction of external plant hormones impacts the population of soil bacteria and its relationship with plant growth. By unraveling this intricate relationship, we seek to uncover potential implications for agricultural practices and ecosystem management. Our study will not only shed insight into the reciprocal connections between plants and soil bacteria, but it will also open the way for novel techniques to improve plant health and productivity sustainably.

Acknowledgments

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Plant Growth Promoting Bacteria (PGPB)

Plant growth-promoting bacteria encompass diverse groups such as nitrogen fixation bacteria, phosphorus solubilizing bacteria, and indole-3-acetic acid (IAA) producing bacteria. Beneficial bacteria can enhance plant growth directly by aiding in resource uptake or adjusting plant hormone levels. (Glick 1995). These can be found in the rhizosphere, residing on or in close association with the root surface (Dimkpa et al. 2009).

- Nitrogen-fixing bacteria: N_2 fixers, also called diazotrophs reduce atmospheric nitrogen into ammonia, and sequentially, nitrifying bacteria turn ammonia into nitrite or nitrate in a process called nitrification (Ji SH et al. 2014).

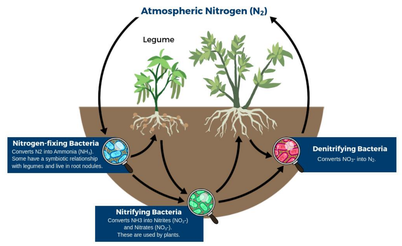

Figure 2. Biological nitrogen fixation cycle (BNF).

- Phosphorus solubilizing bacteria (PSB): Transform insoluble phosphorus into a soluble form. This conversion enhances the accessibility and utilization of phosphorus by plants in the soil (Yu et al. 2022, Glick 1995).
  - Inorganic: Lower the pH of the surrounding environment with phosphorus for binding sites on soil particles bound to cations like calcium, forming soluble complexes, and facilitating the release of phosphorus.
  - Organic: Carried out by enzymes like non-specific acid phosphatases (NSAPs), phytases, phosphonatases, and C-P lyases (Sharma et al. 2013).
- Indole-3-acetic acid-producing bacteria: Hormone naturally produced in plants, belonging to the auxin family. Some bacteria are capable of producing it, influencing seed germination, xylem, and root growth, and nutrient uptake (Calvo et al. 2010, Glick 1995).

Data Analysis

The soil samples have already been procured from the BSC 32000 Plant Biology laboratory Course-Based Undergraduate Research Experience (CURE), where plants are subjected to gibberellin hormone treatments. We aim to examine the influence of this hormone application on the soil bacterial population. These samples were obtained both before and after the hormone treatment to analyze any alterations in microbial community composition and abundance.

The bacterial colonies will be plated in a Biolog Ecoplate, which is a specialized instrument for investigating microbial populations and metabolic processes. The Biolog Ecoplate contains 31 distinct carbon sources immobilized in each well, allowing for the simultaneous investigation of microorganism metabolic pathways. This plate is often used in environmental microbiology and soil science to evaluate the functional diversity and metabolic capability of microbial communities found in materials such as soil, water, and sediment. Using various carbon sources by bacterial colonies on the Ecoplate reveals important information about their metabolic capacity and ecological responsibilities within a given environment.

The bacterial colonies will undergo incubation within the Biolog Ecoplates, followed by monitoring their growth using a microplate reader at intervals of 24 and 48 hours post-plating.

Biolog EcoPlate	1	2	3	4
A	Water	β-methyl-D-glucoside	D-galactonic acid γ-lactone	L-arginine
B	Pyruvic acid méthyl ester	D-xylose	D-galacturonic acid	L-asparagine
C	Tween 40	i-erythritol	2-hydroxy benzoic acid	l-phenylalanine
D	Tween 80	D-mannitol	4-hydroxy benzoic acid	L-serine
E	α-cyclodextrin	N-acetyl-D-glucosamine	γ-hydroxy butyric acid	L-threonine
F	Glycogen	D-glucosamic acid	Itaconic acid	Glycyl-L-glutamic acid
G	D-cellobiose	Glucose-1-phosphate	α-ketobutyric acid	Phenylethyl-amine
H	α-D-lactose	DJ-α-glycerol phosphate	D-malic Acid	Putrescine

Amines
Carbohydrates
Complex carbon sources
Carboxylic acids
Amino acids
Phosphate-carbon

Figure 3. Carbon sources present in Biolog Ecoplate wells.

Future Research

After collecting the data from the Biolog Ecoplates, it will be organized into graphs. Figure 4 shows the bacterial growth over a period of 24 and 48 hours from soil samples collected at the LARC and Young Hall.

By contrasting the graphs of soil bacteria growth, it will be possible to determine how the introduction of plant hormones affects soil bacteria. To further characterize the colonies present in the samples, DNA will be extracted from soil microbes using the Soil DNA extraction kit from MidSci. DNA concentrations for each will be determined and samples will be stored at -80ºC until they are sent for sequencing. With this thorough analysis, it will be feasible to determine if there is a correlation between plant treatments and changes in soil bacterial diversity.

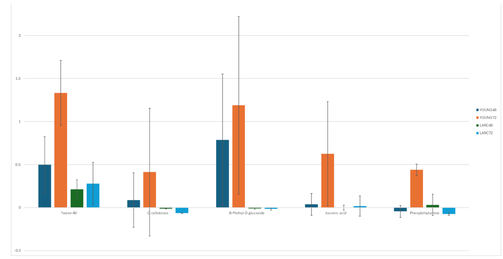

Figure 4. Data from the 5 most significant wells with soil collected from the LARC and Young Hall.

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https://theory.labster.com/bacteria-nitrogen-cycle/ (Figure 2)