



**WOLKIT UNIVERSITY**  
**COLLEGE OF NATURAL AND COMPUTATIONAL SCIENCE**  
**DEPARTMENT OF BIOTECHNOLOGY**

**TITLE: THE EFFECTS OF *LACTOBACILLUS SP.* ON SEED  
GERMINATION.**

**A SENIOR RESEARCH PROJECT SUBMITTED TO THE DEPARTMENT  
OF BIOTECHNOLOGY IN PARTIAL FULFILLMENT OF THE  
REQUIREMENTS FOR THE DEGREE OF BACHELOR OF SCIENCE IN  
BIOTECHNOLOGY**

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May 2024

WOLKITE, ETHIOPIA

# **The Effects of Lactobacillus sp. on seed germination**

**Senior research submitted to the department of Biotechnology in partial fulfillment of the Requirements for the Degree of bachelor of Science in Biotechnology**

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**May, 2024**

**WOLKITE, ETHIOPIA**

## **ACKNOWLEDGMENT**

Authors are thankful to College of Natural and Computational science, Biotechnology department and Molecular Biology Laboratory for providing facilities and financial support for the completion of this research work. The authors are grateful to Major Advisors Dr. Beakal.M and Mr. Yidnekachew.W who advised us from the time of development of proposal and support throughout the research process. We would also like to thank Mr. Debebe, Mr. Ayanssa and Ms. Bontu for their tireless assistance with data collection and analysis. Additionally, we acknowledge Wolkite University for providing research funding through grant number.

## LIST OF ABBREVTION

BCAs.....	Biocontrol Agents
EPS.....	Exopolysaccharid
IAA.....	Indole-3-acetic acid
LAB.....	Lactic acid bacteria
MRS.....	Demman Rogosa Sharpe
PGPB.....	Plant Growth-Promoting Bacteria

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## ABSTRACT

*This research aims to investigate how this specific type of bacteria influences the sprouting of seeds. The objectives will likely focus on understanding if Lactobacillus sp. has a positive, negative, or neutral effect on germination rates. Researchers might explore if this bacteria promotes germination by influencing factors like nutrient availability, seed coat breakdown. Lactobacillus species are group of bacteria that are generally used to prepared probiotic foods and stimulate the growth of plants. In the present study, bacteria were isolated from Yoghurt on MRS agar medium. The isolates were identified by cultural, morphological and biochemical characterization. Lactobacillus isolates were found to be rod shaped, gram positive, non-motile. In case of seed germination on the growth of Peas (Pisum sativum) crop plants, it was observed that enhanced germination rate was obtained; hence Lactobacillus sp. can be used as bioinoculant chemical fertilizers have been a boon to agriculture, allowing for increased crop yields, their overuse can negatively impact seed germination. The main culprit is imbalanced nutrients. Chemical fertilizers typically focus on delivering key nutrients like nitrogen, phosphorus, and potassium, but can lack the micronutrients and organic matter that seeds rely on to germinate successfully.*

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*Key words: Lactobacillus, seed germination, bioinoculant*

# 1.INTRODUCTION

## 1.1 Background of the Study

*Lactobacillus* is one of the most important groups of acid producing bacteria in the food industry, which are used in making starter culture for dairy products. *Lactobacillus* contains over 110 species, which are classified in three major groups: obligate homofermentative *Lactobacillus* which ferment hexoses to lactic acid; facultative homofermentative *Lactobacillus* which ferment hexoses to lactic acid only or together with acetic acid, ethanol, formic acid under glucose limitation and obligate heterofermentative *Lactobacillus* fermenting hexoses to lactic acid, acetic acid, ethanol, CO<sub>2</sub>, and ferment pentoses to lactic acid and acetic acid (Amin *et al*, 2009).

Peas (*Pisum sativum* ) are one of the world's oldest domesticated and cultivated legume crops. Throughout its history in genetic research, peas have undergone numerous selections and breeding for characteristics desirable for cultivation, consumption, and other uses. There are a handful of subspecies within *P. sativum* with differences in major traits and in their occurrence throughout the world (Cousin, 2007). The most common pea varieties in the US, as defined by Maxted and Ambrose (2001), GRIN, and ITIS are: Garden peas (*P. Sativum*) ,Field peas, dry peas, or Austrian winter peas (*P. sativum* var. *arvense* (L.) Poir green manure and as a cover crop, but can also be used for human consumption. A major problem farmers run into is which variety to purchase for their operation. The variety of pea used depends on attributes for cultivation and/or consumption as well as environmental benefits.

## 1.2 Statement of the problem

Conventional agriculture relies heavily on chemical fertilizers and pesticides to achieve high crop yields. While effective, these chemicals pose significant threats.They can cause health problems in humans and degrade the environment. This pressing need for sustainable solutions has driven research towards eco-friendly alternatives. Plant growth-promoting bacteria (PGPB) offer a promising option.

This research aims to address these knowledge gaps by investigating the effects of various *Lactobacillus* sp. strains on seed germination and plant growth under

controlled conditions. The study also explore the underlying mechanisms by which these bacteria can influence plant growth. The findings could pave the way for the development of eco-friendly biofertilizers and biocontrol agents containing specific *Lactobacillus* species, ultimately promoting sustainable agricultural practices.

### **1.3 Objectives of the study**

#### **1.3.1 General Objective**

- To determine different *lactobacillus* sp. Present in Yoghurt and to investigate the seed germination and growth promoting activity of its extract.

#### **1.3.2 Specific Objectives**

- identify the lactic acid bacteria (*lactobacillus* sp.) from milk component(Yoghurt).
- To evaluate the seed germination promoting activity of *lactobacillus* sp.
- To compare the plant seed germination promoting activity measured by shoot length of test with a control group on specific plant species.
- To investigate the mechanisms by which *Lactobacillus* sp. promotes seed germination in specific plant species.

### **1.4 Significance of the study**

The application of this project can be applied for both generating knowledge and solving the current problems associated with seed germination and growth in agricultural sectors. It is expected to have further importance in investigating lactic acid bacteria and plant seed germination promoting activity of the extract. this study also is important to overcome the shortage of food supply regarding to solve the obstacles in crop seed germination and growth.

### **1.5 Scope of the study**

This study was conducted in Wolkite University, College of Natural And Computational Science, and Department of biotechnology which was mainly focus on the effect of *lactobacillus* sp. On seed germination in Gubrye Subcity, Wolkite Town.

## 2.LITERATURE REVIEW

### 2.1 Yoghurt

Yogurt is an ancient fermented dairy product and has been consumed worldwide since the domestication of mammals (Tamime and Robisons, 2007a). For the production of Yogurt, the milk is commonly obtained from several domestic milk producing animals like cows, sheep, goat, even yaks, horses, buffalo and camels (Moreno et al., 2013). Yogurt is defined as the product of milk which is being manufactured with or without the addition of some natural derivative of milk, such as whey concentrates, skim milk powder, caseinates or cream with a gel structure that results from the coagulation of the milk proteins, due to lactic acid secreted by defined species of bacterial cultures and at the time of consumption these bacteria must be “alive or abundant”. The given definition of Yogurt is part of the food legislation of many countries (Chandan, 2006)

Yogurt is a well-known source of various *Lactobacillus* species, the beneficial bacteria responsible for its fermentation and tangy taste. During yogurt production, milk is pasteurized to eliminate harmful bacteria. Then, specific strains of *Lactobacillus* cultures, often *Lactobacillus bulgaricus* and *Lactobacillus acidophilus*, are introduced. These cultures ferment the milk sugar (lactose) into lactic acid, which curdles the milk proteins, creating the characteristic yogurt texture.

Consumption of yogurt and other fermented products is associated with improved health outcomes. Although dairy consumption is included in most dietary guide lines, there have been few specific recommendations for yogurt and cultured dairy products. A qualitative systematic review was conducted to determine the effect of consumption of fermented milk products on gastrointestinal and cardiovascular health, cancer risk, weight management, diabetes and metabolic health, and bone density using PRISMA guidelines.

Beyond its role in yogurt production, *Lactobacillus* provides several health benefits. Some strains contribute to gut health by aiding digestion and nutrient absorption, potentially boosting the immune system. Research is ongoing to explore other potential health applications of specific *Lactobacillus* sp. (Tamang, J. et al (2010).

## **2.2 pea (*Pisum sativum*)**

Peas (usually *Pisum sativum* L.) are one of the four most important legume crops next to soybean, groundnut, and beans. It is a particularly important legume grain in temperate areas with numerous food (dry seed, vegetable) and feed (seed, fodder) usages. Peas are a fast-growing herbaceous legume with angular or roundish hollow stems covered with a waxy bloom. The plant has a tap root that can grow as deep as 1 m, with numerous lateral roots. Leaves are alternate, compound with 1-3 pairs of leaflets borne on petioles with several pairs of tendrils. Large (up to 10 cm long) leaf-like stipules are inserted at the base of the leaves (FAO, 2011). The inflorescence is a raceme that bears white, pink or purple flowers. The colour of the flower is an indicator of tannin content in the seed: white-flowered peas produce tannin-free seeds while the seeds of coloured-flowered peas contain tannins (prolea, 2008). Pods are dehiscent and contain several seeds that may be globose or angled, smooth or wrinkled (FAO, 2011)

*Pisum sativum* has a large genetic diversity and breeding programmes have generated many varieties. There are winter and spring varieties, leafy and leafless, early- or late-maturing, etc. Seeds can be green, yellow or pale green, brown or mottled, thin- or thick-hulled, smooth or wrinkled, of varying shape and size. Some varieties are tannin-free and pea breeding offers a large opportunity to improve the nutritional characteristics of the grain (Hickling, 2003; ).

## **2.2 Yoghurt lactobacillus and its potential as Bioinoculant for Sustainable Agriculture**

Anthropogenic ammonia emissions are major risk factors that cause secondary pollution, reduce nitrogen availability, and damage forests and vegetation. Biofertilizers are substances containing a variety of microbes to protect the plant and enhance the plant's nutrients. However, LAB and nitrification bacteria reduce ammonia emissions and promote nitrification (Wang, Y et al 2019). Recently, LAB and other Bacillus-based biofertilizers have been validated with established microbes in agriculture and the environment. Microbial-based biofertilizers increase crop yield and accelerate the mineral uptake of the plant root. Further, they enhance the organic matter catabolism. Spray and soil injection methods are highly recommended for commercial applications. LAB-based liquid fertilizer spray on the plant and soil is

hypothesized to assist plant health. Fermented LAB, yeast, and phototrophic bacteria cocktails are used as biofertilizers and biocontrol agents. At the same time, LAB and bacillus-based biofertilizers showed a high crop yield and enhanced the organic matter degradation (Blais, A.2022). In this context, farmyard manure and plant-based compost is integral to organic farming and sustainable agriculture. LAB decompose and bio-stabilize the animal and plant waste to improve the agronomic value and assimilate organic matter such as lignin and cellulose materials (Hidalgo, D et al 2022). Wang et al., found that *Bacillus stearothermophilus* elevate the relative abundance of LAB strains in soil . In addition, LAB strains exhibit an antagonistic effect against phytopathogenic agents in soil.

These findings suggest that *Lactobacillus* species may possess mechanisms to improve seed germination and plant growth. Future research should focus on elucidating these mechanisms and exploring the effectiveness of different *Lactobacillus* sp. on diverse plant species. Optimizing application methods and concentrations for large-scale agricultural use will also be crucial for realizing the full potential of *Lactobacillus* as a sustainable bioinoculant.

Plant-associated microorganisms synthesize phytohormones, and the structure and functional properties are similar. Microbial phytohormones exhibit a similar effect on the plants, and they stimulate or inhibit microbial proliferation (Tsavkelova, E.A et al 2006). There is limited evidence of LAB-related growth hormones. However, LAB stimulate plant growth and resistance to water and abiotic stress. According to Ampraya et al., LAB exhibit plant-growth-promoting (PGP) properties, and they can produce auxin indole-3-acetic acid (IAA) and solubilize minerals (Amprayna, K et al 2016) Lynch precisely reported that the LAB growth hormones cytokinins and other metabolites were found in the soil . In a hypothetical view, LAB gradually incorporated into plant rhizospheric soil may alter plants' physical properties to maximize the yield. For example, rice seeds coated with *Lactococcus lactis* significantly promoted the root length and shoot length. In addition, *L. lactis* significantly promotes cabbage growth and yield(Somers, E. etal 2007). In addition, several bacterial species produce bacterial exopolysaccharides (EPS) that promote plant growth and enhance soil fertility .

### **3. MATERIALS AND METHODS**

#### **3.1 Description of Study Area**

The study was conducted in Wolkite University, Biotechnology Department Laboratory from March 2024 to May 2024. GC Wolkite University is located in the Gura Zone around Wolkite town, which is the administrative center of the Gura Zone, Central Ethiopia Regional State, Ethiopia, 158 km away from Addis Ababa, the capital city of Ethiopia. Wolkite town is located at a latitude and longitude of 8.2° and 37.8° respectively, and an elevation between 1910 and 1935 m above sea level.

#### **3.2 Sample Collection**

To minimize contamination, samples were collected in Gubrye town near Wolkite University. For yogurt, a sterilized flask was used to aseptically collect a 0.5 L of sample while wearing disinfected gloves. The sample was then transported in a cooler with ice packs at 4°C to the lab. Pea seeds were chosen from visually healthy pods, collected in a clean, dry container with gloves, and transported at room temperature in a sealed container to prevent moisture. Upon arrival at the lab, both samples were stored at 4°C for further analysis.

#### **3.3 Equipment and Chemicals**

The research utilized various equipment and chemicals. For sterilization and aseptic techniques, a laminar flow hood, autoclave, Bunsen burner, micropipette, inoculating loop, flasks, water bath, and distilled water were employed. General laboratory equipment included an electronic balance, flasks of various sizes, graduated cylinder, test tubes, Petri dishes, conical flasks, refrigerator, microscope, microscope slides, spatula, parafilm, tissue paper, and cotton. The chemicals and media used were MRS agar for analyzing the yogurt sample, nutrient broth for culturing microorganisms, the yogurt sample itself, pea seed sample, crystal violet for Gram's staining, Gram's iodine for the same method, safranin as a counterstain, hydrogen peroxide for Gram's staining protocol, and finally, 95% alcohol, which served as both a fixative and disinfectant.

### **3.4 Study design**

The study design was Complete Randomised design (CRD) based on experimentally using the appropriate methods such as extraction and determination of *lactobacillus* sp. From fresh yoghurt. Identification of *lactobacillus* species was carried out in the laboratory by observing its colony color and size on MRS agar media.

### **3.5 Experimental Procedure**

#### **3.5.1 Media preparation**

16.5 grams of MRS agar powder was measured using a sensitive balance and dissolved in 250 milliliters of distilled water. A microwave oven was then used to heat the solution, ensuring complete mixing of the media with the water. After thorough dissolving, the media was sterilized in an autoclave at 121°C for 15 minutes. To maintain aseptic conditions, the autoclaved media was transferred to a biological safety cabinet. Finally, the media was dispensed into sterilized petri dishes. After cooling, the MRS media solidified on the petri dishes.

#### **3.5.2 Sample preparation**

serial dilution was performed using five sterile test tubes. Each test tube was filled with 9ml of sterile distilled water. A 1 ml aliquot of the yoghurt sample was then added to the first test tube. The contents of the first test tube were mixed thoroughly using a sterile technique. Next, 1 milliliter of the diluted mixture from the first test tube was transferred to the second test tube containing 9 milliliters of sterile distilled water. This process of transferring 1 milliliter and mixing thoroughly was repeated for the remaining three test tubes, resulting in a series of dilutions from 1:10 ( $10^{-1}$ ) to 1:100,000 ( $10^{-5}$ ).

#### **3.5.3 Inoculation**

deMan, Rogosa, and Sharpe (MRS) agar medium, known to be selective for *lactobacillus*, was chosen for culturing the yoghurt sample. Following serial dilution of the yoghurt sample (process described previously), a 1 milliliter aliquot from each dilution  $10^{-1}$ ,  $10^{-2}$ ,  $10^{-3}$ ,  $10^{-4}$ , and  $10^{-5}$  was spread onto separate sterilized

MRS agar petri dishes using a sterile spreader under aseptic conditions within a biological safety cabinet. Then all of bacterial petri dishes transferred into the incubator.

### **3.5.4 Incubation**

The inoculated bacterial culture was incubated at 37° c for 24 -48 hours.

### **3.5.5 Isolation of lactobacillus bacteria**

Following incubation, observed colonies on the plates, potentially representing isolated lactobacillus bacteria, were then restreaked onto fresh MRS agar plates to obtain pure cultures of individual bacterial strains. This restreaking process is essential for isolating single bacterial cells, ensuring a pure culture for further analysis.

### **3.5.6 Phenotypic characterization of the isolated bacteria**

Characterization of all the isolates was performed based on their morphological and biochemical characteristics as described:

**Morphological examination of culture(Gram staining):** The Gram staining method is named after Hans Christian Gram, the Danish bacteriologist who originally devised it in 1844, and is one of the most important staining techniques in microbiology. It is almost always the first test performed for the identification of bacteria (Tripathi, N & Sapra, 2023).

Gram stain was performed to determine the cell wall structure of isolated Lactobacillus bacteria from the yogurt sample. Here's a breakdown of the process:First, a smear was prepared. A small sample of isolated bacterial colonies from the MRS agar plate was transferred to a clean glass microscope slide. To ensure the bacteria adhered to the slide and were inactivated, the smear was then heat-fixed by carefully passing it through a Bunsen burner flame.

Next, the staining process began. The slide was flooded with crystal violet, a primary stain with a deep purple hue. This stain colors all bacteria on the slide. Following this, Gram's iodine, a mordant, was applied. This solution aids the crystal violet stain in penetrating the thick peptidoglycan cell wall, a characteristic feature of Gram-positive bacteria like Lactobacillus.A crucial step in differentiating Gram-positive and Gram-negative bacteria followed. The slide was decolorized with an alcohol solvent, such as ethanol or acetone. This step washes away the crystal violet-iodine complex from the

thinner cell walls of Gram-negative bacteria. However, Gram-positive bacteria, with their thicker cell walls, retain the complex. To visualize any remaining bacteria, a safranin counterstain, red in color, was applied to the slide. This counterstain stains all bacteria, regardless of their Gram reaction (positive or negative). Finally, the slide was rinsed with water to remove excess stain and dried completely before microscopic examination with oil immersion..

**Biochemical chracterization of the isolated bacterial species :** Pure culture isolated on MRS agar plate was identified with the help of biochemical tests like catalase test, starch test, and citrate utilization test (Table 1).

**Catalase test:-**To assess the presence of the enzyme catalase in a bacterial sample, a catalase test was conducted. First, a sterile inoculating loop was used to collect a small amount of the bacterial culture. The loop was then gently streaked onto a clean glass slide. Next, a drop of hydrogen peroxide solution (usually 3%) was added onto the bacterial smear on the slide. We were carefully observed the reaction zone for immediate bubble formation. If bubbles formed rapidly, indicating the production of oxygen gas from hydrogen peroxide breakdown, the test result was positive for catalase activity. Conversely, the absence of bubbles after a reasonable observation time signified a negative test result, suggesting the bacteria lacked the catalase enzyme.

**Starch test:** starch test was performed to assess the ability of isolated bacteria from the yogurt sample to utilize starch. This involved creating a modified MRS agar medium by adding a sterilized starch solution, fostering bacterial growth while providing starch as the main energy source. A small amount of the isolated bacterial culture was then transferred onto the MRS starch agar plate. After sealing the plate to prevent contamination, it was incubated at a suitable temperature (30-37°C) for 24-48 hours. Following incubation, iodine solution was applied to the agar surface to detect starch fermentation. A clear or faint yellow-brown color indicated starch degradation by the bacteria (positive test), whereas a dark blue-black color signified the presence of unfermented starch (negative test). This experiment successfully investigated the starch utilization capabilities of the isolated bacteria.

**Citrate utilization test:**First, a medium specifically designed for the citrate utilization test, likely Simmons citrate agar, was employed. A small inoculum of the isolated Lactobacillus culture was then transferred onto the slanted surface of the Simmons citrate agar in a sterile test tube. Following inoculation, the test tube was incubated at a suitable temperature, typically 30-37°C, for a specific period, often 24-48 hours. After incubation, the appearance of the medium was observed to determine the test result.

### **3.5.7 Preparation of LAB solution for seed treatment**

A series of steps were undertaken. First, a Lactobacillus culture was prepared by suspending it in nutrient broth and incubating it for 24 hours. Next, pea seeds underwent a pre-treatment to disinfect their surfaces. This involved thoroughly washing them with 95% alcohol followed by a rinse with sterile distilled water. Following pre-treatment, the seeds were exposed to the prepared Lactobacillus culture solution. After this treatment, the seeds were dried for 20 minutes to remove excess moisture. Finally, a germination assay was conducted using the tissue paper method.

## 4.RESULT

### 4.1 Cultural, Morphological and biochemical Characters

**Isolation:** Following incubation for three days at 37°C on MRS agar plates, colonies consistent with Lactobacillus morphology were observed (Figure 1). These colonies appeared white to yellowish in color and displayed a characteristic rod shape.

**Morphological and Cultural Characteristics:** The isolated microorganisms were further examined for characteristics typical of Lactobacillus species. They were found to be anaerobic, non-spore-forming, and gram-positive rods exhibiting a violet color after staining. Additionally, the isolates demonstrated non-motile behavior.

**Biochemical Characterization:** Several biochemical tests were performed to further characterize the isolated bacteria. The isolates exhibited a negative reaction in the catalase test, indicating the absence of the catalase enzyme. Conversely, positive results were observed in the starch test and citrate utilization test, suggesting the ability of the isolates to ferment starch and utilize citrate as an energy source, respectively.

## 5. DISCUSSION

Our research investigated the potential of *Lactobacillus* sp. to promote seed germination. We successfully isolated and characterized *Lactobacillus* strains, and then evaluated their impact on seed germination. Our findings significantly contribute to the understanding of plant growth-promoting bacteria.

### **Contribution of *Lactobacillus* to Germination:**

Our results align with previous research highlighting several mechanisms by which *Lactobacillus* might enhance germination. The breakdown of complex organic matter by *Lactobacillus* in the soil likely releases essential nutrients, such as phosphorus, that are crucial for seed development. Furthermore, some *Lactobacillus* strains might produce auxins and gibberellins, plant hormones known to stimulate seed germination and early root growth. Additionally, *Lactobacillus* may produce antimicrobial compounds that suppress harmful fungi and bacteria in the rhizosphere, creating a more favorable environment for seed germination and seedling establishment.

### **Correlating Findings with Techniques:**

Morphological and biochemical characterization were essential for confirming the identity of our *Lactobacillus* isolates and differentiating between potential strains. These techniques allowed us to identify specific characteristics associated with *Lactobacillus*, such as cell morphology and sugar fermentation patterns, ensuring a targeted evaluation of their impact on seed germination. The seed pre-treatment method employed in our study may have also influenced the germination response. Future research could explore the optimization of pre-treatment methods to potentially enhance the effectiveness of *Lactobacillus* application.

### **Effectiveness of Isolated Strains:**

By comparing seed germination rates with control groups (seeds not treated with *Lactobacillus*), we were able to quantify the germination improvement achieved by our isolated strains. This comparison provides a clear measure of the positive influence exerted by *Lactobacillus* on seed germination. If variations in germination rates were observed between different *Lactobacillus* strains, it suggests potential

differences in their plant growth-promoting abilities. Further investigation into these variations could lead to the identification of particularly effective strains for future

### **Limitations and Future Directions:**

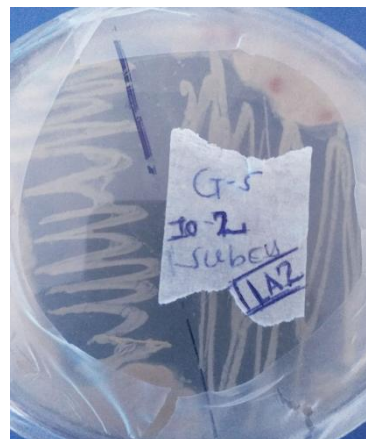
Our study acknowledges certain limitations. The use of a specific seed type might not be generalizable to all plant varieties. Additionally, the study focused on a limited number of Lactobacillus strains. Future research directions could involve:

Identifying the specific mechanisms by which our isolated Lactobacillus strains promote germination. Testing the effectiveness of these strains on germination and growth of various crop plants under field conditions. Exploring the potential for formulating a bio-inoculant containing our most promising Lactobacillus strains for practical agricultural applications.

By addressing these limitations and pursuing future research avenues, we can further elucidate the role of Lactobacillus in promoting seed germination and develop strategies to improve agricultural productivity through the use of beneficial plant growth-promoting bacteria.



(a) Yellowish



(b) White

**Fig 1:** The isolated yellowish and White colony formed after restreaked(Subcultured) on the MRS agar Petri plate.

Table 1: Biochemical characterization of the isolated bacterial species

Tests	Results
Catalase test	-ve
Starch test	+ve
Citrate utilization test	+ve

### 5.1 Effect of *Lactobacillus* on seed germination

All the seeds showed maximum germination after 48 h. In comparison of the control (non-treated seeds) and test (seeds treated with *Lactobacillus* culture), highest seed germination was obtained in the test. The number and length of the sprouts were significant in the test as compared to the control (Table 2, 3 and Figure 2).

**Table 2:** Effect of *Lactobacillus* on the measurement the growth of seed germination.

Scientific name of seed	Initial growth after 2 days(cm)	Final growth after 5 days(cm)
Peas ( <i>Pisum sativum</i> ) treated with LA-1	1.5	9.8
Peas ( <i>Pisum sativum</i> ) treated with LA-2	1.6	10
Peas ( <i>Pisum sativum</i> ) Control	0.7	5.1

**Key:** LA-1 Stands for *Lactobacillus* isolate LA -1. It is conventional code for specific colony type. The same is true for LA-2.

**Table 3:** Effect of *Lactobacillus* on seed germination

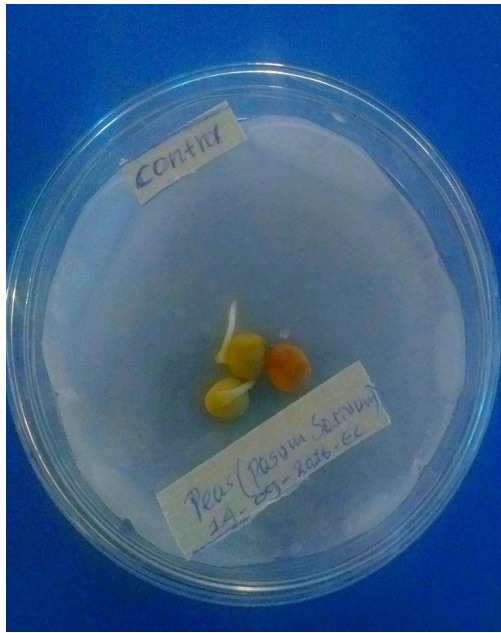
Scientific names of seeds	Seed germination in(%)	
	Control	Test
Peas ( <i>Pisum sativum</i> )	34.2	65.7
Peas ( <i>Pisum sativum</i> )	34	66.5



(a) Control



(c) Test



(b) Control



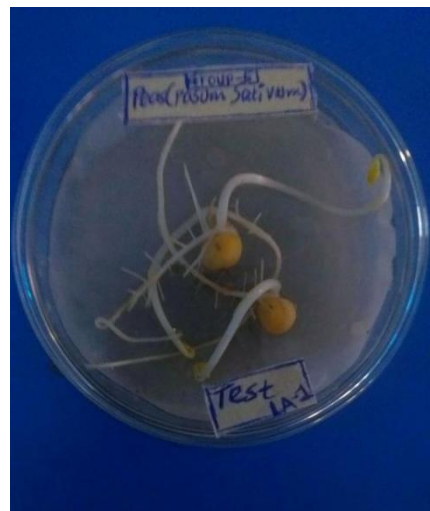
(d) Test

**Fig 2:** Effect of *Lactobacillus* on seed germination after 2 days (48hrs)

**Key:** The above figure (a) and (c) is determined as Control versus LA-2 isolate treated pea (*Pisum sativum*) seed and (b) and (d) Determined Control versus LA-1 isolate treated pea (*Pisum sativum*) seed



(a) Control



(c) Test



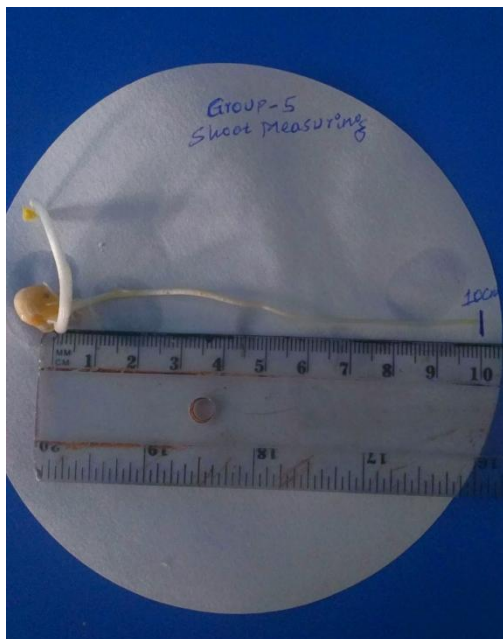
(b) Control



(d) Test

**Fig 3:** Effect of *Lactobacillus* on seed germination after 5 days (120hrs)

**Key:** The above figure (a) and (c) is determined as Control versus LA-1 isolate treated pea (*Pisum sativum*) seed and (b) and (d) Determined Control versus LA-2 isolate treated pea (*Pisum sativum*) seed



(a) Test :10cm



(b) control:4.9cm

**Fig 4:** Measuring of Shoot Length After 5 days of germination period.

## 5.CONCLUSION AND RECOMMENDATIONS

### 5.1 Cnclusion

Isolated bacterium was proved to be *Lactobacillus* based on their cultural, morphological and biochemical characteristics. Seeds dressed with *Lactobacillus* and plant grown showed high seed germination and stimulatory growth over the control. These results summarized that *Lactobacillus* can be effectively used as a bioinoculant or biofertilizer to enhance the yield of crops.

The research suggests that *Lactobacillus* species, commonly found in fermented foods, have the potential to act as a biofertilizer. The study isolated *Lactobacillus* species and investigated their effect on germination and growth of crops like bengal gram, peas, and mustard seeds. Results showed that seeds treated with *Lactobacillus* exhibited increased germination rates and enhanced growth compared to the control group. This indicates that *Lactobacillus* may promote plant growth and could be a viable alternative to chemical fertilizers in agriculture.

The research highlights the promise of *Lactobacillus* species as a natural tool for sustainable agriculture. By isolating *Lactobacillus* species and testing them on various seeds, the study found that these bacteria can act as plant growth-promoting rhizobacteria (PGPR). The results point towards a positive influence on seed germination and overall plant growth across crops like bengal gram, peas, and mustard. This opens doors for exploring *Lactobacillus* as a bioinoculant or biofertilizer, potentially reducing reliance on chemical fertilizers and promoting a more eco-friendly agricultural approach. Further research is needed to explore the specific mechanisms by which *Lactobacillus* aids plant growth and identify the most effective strains for different crops. However, this study provides a strong foundation for the development of *Lactobacillus*-based solutions to enhance crop yields and agricultural sustainability.

This research offers a glimpse into a future of agriculture that leverages beneficial bacteria. The study explored *Lactobacillus*, a common inhabitant of fermented foods, and its potential to address challenges in crop production. By demonstrating that *Lactobacillus* species can enhance seed germination and growth in crops like bengal

gram, peas, and mustard, the research paves the way for a novel, eco-friendly approach. *Lactobacillus*-based biofertilizers could potentially replace or reduce dependence on chemical fertilizers, a major contributor to environmental pollution. This shift towards natural bio-stimulants could promote sustainable agricultural practices. Further research is crucial to optimize these *Lactobacillus* species and explore their effectiveness on a wider range of crops. However, this study presents a promising solution for promoting plant growth naturally, potentially leading to a more sustainable and productive agricultural future.

This study highlights the promising potential of *Lactobacillus* as a bioinoculant for sustainable agriculture. By harnessing the power of these naturally occurring microbes, we can move towards a future of environmentally friendly food production and improved crop yields.

## **5.2 Recommendations**

Some of the recommendations that came out of this study are:

1. Current findings suggested exploration of different *Lactobacillus* species for specific crops.
2. While the current research is promising in controlled environments, real-world application is vital. Field trials are essential to assess the effectiveness of *Lactobacillus*-based biofertilizers under actual farming conditions. These trials should evaluate factors like yield improvement, cost-effectiveness compared to chemical fertilizers, and ease of integration with existing practices.
3. Developing practical formulations for farmers is key for widespread adoption. Research on carrier materials, shelf life, and application methods (seed coating, soildrenching etc.) is necessary to ensure the viability and practicality of *Lactobacillus* biofertilizers.

## 6.REFERENCES:

Amprayna, K.; Supawonga, V.; Kengkwasingha, P.; Getmalab, A. Plant Growth Promoting Traits of Lactic Acid Bacterium Isolated from Rice Rhizosphere and Its Effect on Rice Growth. In Proceedings of the 5th Burapha University International Conference STP-029-10, Pattaya, Thailand, 28–29 July 2016

Chandan, R.C. 2006. Chapter 1 History and Consumption Trends. In: Manufacturing Yogurt and Fermented Milks. Chandan R.C., Ed. Blackwell Publishing: Ames, IA, USA, p. 3–17.

FAO, 2011. GRASSLAND Index. A searchable catalogue of grass and forage legumes  
FAO, Rome, Italy

Hickling, D., 2003. Canadian feed peas industry GUIDE: Winnipeg Manitoba 36 p.

Hidalgo, D.; Corona, F.; Martín-Marroquin, J. Manure biostabilization by effective microorganisms as a way to improve its agronomic value. *Biomass Convers. Bioref.* 2022.

Lynch, J.M. Origin, Nature and Biological Activity of Aliphatic Substances and Growth Hormones Found in Soil. In *Soil Organic Matter and Biological Activity. Developments in Plant and Soil Sciences*; Vaughan, D., Malcolm, R.E., Eds.; Springer: Dordrecht, The Netherlands, 1985; Volume 16

Moreno, A.L.A., Cervera, R.P., Ortega, A.R.M., et al. 2013. Scientific Evidence about the Role of Yogurt and Other Fermented Milks in the Healthy Diet for the Spanish Population (Spanish) *Nutricion Hospitalaria*, 28: 2039–208

Somers, E.; Amake, A.; Croonenborghs, A.; Overbeek, L.S.; Vanderleyden, J. Lactic acid bacterial in organic agricultural soil. In Proceedings of the Rhizosphere 2, Montpellier, France, 26–31 August 2007.

Tamime and Robinson's Yogurt: Science and Technology, 3<sup>rd</sup> edn., Woodhead Publishing, Cambridge, UK, pp. 11–118  
anure composting. *Ecotoxicol. Environ. Saf.* 2019, 180, 80–87.

Tamime, A.Y. and Robisons, R.K. 2007b. Chapter 2 Background to Manufacturing Practice.

Tsavkelova, E.A.; Klimova, S.Y.; Cherdyntseva, T.A.; Netrusov, A.I. Microbial producers of plant growth stimulators and their practical use: A review. *Appl. Biochem. Microbiol.* 2006, 42, 117–126.

Wang, Y.; Bi, L.; Liao, Y.; Lu, D.; Zhang, H.; Liao, X.; Liang, J.B.; Wu, Y. Influence and characteristics of *Bacillus stearothermophilus* in ammonia reduction during layerm

Tripathi, N & Sapra, A. Treasure Island(FL): StatPearls. 2023.