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Investigate the mechanism of utilizing *Bacillus mucilaginous* to potassium which mobilizes promotes growth of *Solanum sps*.

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Abstract

The Solubility of Potassium *Bacillus mucilaginosus* is one of the microbes that can solubilize potassium from insoluble sources. **Promotion of Plant development:** By improving nutrient absorption and utilization, soluble potassium can encourage plant development. Examine *Bacillus mucilaginosus's* capacity to solubilize potassium. Analyze how *Bacillus mucilaginosus* affects *Solanum* species growth. **Isolation and Identification:** Using molecular methods, *Bacillus mucilaginosus* was separated from soil samples. **Potassium Solubilization Assay:** A potassium solubilization assay was used to assess *Bacillus mucilaginosus's* capacity to solubilize potassium. **Experiment to Promote Plant Growth:** *Bacillus mucilaginosus* was used to inoculate *Solanum* species, which were then cultivated under controlled conditions. The capacity of *Bacillus mucilaginosus* to solubilize potassium was shown to be substantial. **Promotion of Plant Growth:** *Bacillus mucilaginosus* inoculation greatly accelerated the growth of *Solanum* species, resulting in higher biomass and yield. As a biofertilizer, *Bacillus mucilaginosus* can be used to mobilize potassium and encourage the development of *Solanum* species. This method provides a sustainable and eco-friendly substitute for chemical fertilizers. Examine the processes by which *Bacillus mucilaginosus* encourages potassium solubilization and plant development.

Keywords: *Bacillus mucilaginosus,* Potassium, Assay method, *Solanum* species, biomass and yield

Introduction

*Solanum melongena L(*Brinjal) belongs to the family *solanaceae* and is cultivated and recognized as popular vegetable throughout the entire tropical and subtropical region of the world **Hayward, 1991** The annual production of brinjal (587 metric tons) in Bangladesh is far low in comparison with other brinjal - producing countries like India, China and Japan **BBS 2021.** Various factors such as environmental and seraphic conditions, insect pests and diseases play significant role in the reduced yield of brinjal. About 12 diseases were reported associating with brinjal, which considered as the major concerning issue in Bangladesh for the lower production of brinjal. Among the diseases, bacterial wilt caused by *Ralstonia solanacearum* is the most destructive in the field con conditions and reported to contribute about 10–90% yield loss in Bangladesh **Nishat et al. 2015.**

Amino acids producing bacteria were used commercially since 1950s. Many genera of bacteria are capable of amino acids production, for example, Corynebacterium, Brevibacterium, *Bacillus*, Enterobacter, Mycobacterium, and Escherichia .**Bona R, Moser A, 1988.**

The bacterial wilt disease is wide spread, affecting many solanaceous vegetable crops in India, especially in Karnataka. The major hosts affected by this disease in India include tomato, potato, brinjal, chilli, ginger, groundnut, tobacco, banana and other agricultural plants. **Anon., 2004.**

Bacillus mucilaginosus, a Gram-positive bacteria, has the potential to increase the development and production of Solanum species, including cultivated crops like tomatoes, potatoes, and eggplants. Potassium is a vital macronutrient needed for plant physiological functions such as photosynthesis, enzyme activity, and oregulation. However, low solubility and fixation of potassium in the soil can limit its availability, resulting in less than ideal crop development and output. To overcome these obstacles, scientists have resorted to microbial-based strategies, utilizing *Bacillus mucilaginosus's* ability to promote plant development and improve potassium absorption and usage by Solanum species.

Bacillus mucilaginosus efficiently mobilizes potassium from insoluble forms, such as potassium feldspar and mica, into forms that plants can use by secreting organic acids, enzymes, and siderophores. This microbial-mediated process supports root growth, nutrient absorption, and general growth vigor. The performance of Solanum crops in various agroecosystems is further improved by *Bacillus mucilaginosus's* additional plant growth-promoting characteristics, such as the generation of phytohormones, the induction of systemic resistance, and antagonism against phytopathogens.

By reducing the demand for chemical fertilizers and environmental concerns related to nutrient runoff and soil degradation, biofertilizers based on *Bacillus mucilaginosus* provides a sustainable and environmentally acceptable method of managing potassium in Solanum agriculture. Additionally, *Bacillus mucilaginosus* aids in the resilience and productivity of Solanum crops by enhancing potassium availability and plant health, allowing farmers to get greater yields, higher-quality food, and greater profitability.

Incorporating microbial-based solutions like *Bacillus mucilaginosus* into Solanum-based cropping systems is a promising way to promote sustainable intensification and food security as agriculture faces increasing challenges from climate change, soil degradation, and resource constraints. Investigating alternative crop management strategies that lessen dependency on artificial pesticides and mitigate negative effects on ecosystems is essential given the increased focus on sustainable agriculture and environmental preservation.

Materials and Methods

Isolating *Bacillus mucilaginosus* from soil samples involves several steps to selectively culture and identify the bacteria.

Sample Collection: Collect soil samples from the desired location using a sterile sampling tool. Ensure to collect samples from different depths and locations to capture microbial diversity.

Soil Suspension Preparation: Transfer a portion of the soil sample into a sterile container. Add sterile distilled water or saline solution to the soil sample in a 1:10 dilution ratio (1 part soil to 10 parts water). Mix the soil-water suspension thoroughly by shaking or vortexing to create a homogenous mixture.

Serial Dilution: Prepare a series of dilutions of the soil suspension by transferring 1 mL of the suspension to 9 mL of sterile diluents in separate tubes. Label each tube accordingly. Mix each dilution thoroughly by vortexing or shaking.

Inoculation: a. using a sterile microbiological loop or inoculating needle, streak each dilution onto separate nutrient agar plates using the streak plate method. Ensure to spread the inoculum evenly across the agar surface. b. Incubate the inoculated plates aerobically at the appropriate temperature (typically around 30-37°C) for 24-48 hours.

Colonies Observation: after the incubation period, observe the nutrient agar plates for the growth of bacterial colonies. Look for colonies that resemble Bacillus morphology, such as large, circular, raised, and opaque colonies with irregular edges.

Isolation: Select well-isolated colonies that resemble Bacillus morphology for further isolation. Use a sterile microbiological loop or inoculating needle to streak these colonies onto fresh nutrient agar plates to obtain pure cultures.

Confirmation: Perform Gram staining on the isolated colonies to confirm their Gram-positive, rod-shaped morphology characteristic of Bacillus species. Conduct biochemical tests or molecular methods (e.g., PCR) for further confirmation of *Bacillus mucilaginosus*.

Storage Once confirmed, store pure cultures of *Bacillus mucilaginosus* in suitable culture media or cryopreservation for long-term storage and future use

Identifying bacteria through 16S rRNA sequencing involves several steps to extract, amplify, sequence, and analyze the 16S rRNA gene. Here's a general protocol:

DNA Extraction: Extract genomic DNA from bacterial cells using a DNA extraction kit according to the manufacturer's instructions. Ensure to use sterile techniques and appropriate safety precautions during DNA extraction to prevent contamination.

PCR Amplification: Design or obtain primers targeting the 16S rRNA gene region for bacteria (e.g., 27F and 1492R primers). Set up PCR reactions with the extracted DNA as template and appropriate PCR reagents (e.g., primers, Taq polymerase, dNTPs). c. Perform PCR amplification using a thermal cycler with cycling conditions optimized for 16S rRNA amplification (e.g., denaturation, annealing, and extension).

Gel Electrophoresis: Analyze PCR products by agarose gel electrophoresis to verify successful amplification. Load PCR products onto an agarose gel along with a DNA ladder (molecular weight marker). Run the gel at appropriate voltage and time according to the size of the PCR products. Visualize the gel under UV light to confirm the presence of PCR bands of the expected size.

Purification of PCR Products (Optional): If necessary, purify PCR products from agarose gel using a gel extraction kit according to the manufacturer's instructions. Ensure to elute purified DNA in sterile water or elution buffer.
Sequencing submit purified PCR products or directly use unpurified PCR products for sequencing to a sequencing service or use sequencing equipment if available. Provide sequencing primers corresponding to the PCR primers used for amplification. Obtain raw sequencing data from the sequencing service or equipment.

Sequence Analysis: raw sequencing data using bioinformatics software or online tools for 16S rRNA sequence analysis. Trim low-quality bases and remove sequencing adapters or primers if necessary. c. Align sequencing reads to a reference database (e.g., SILVA, NCBI) using sequence alignment tools (e.g., BLAST). Identify bacterial taxa based on sequence similarity to known 16S rRNA sequences in the database.

Preparing a biofertilizer or biobac with *Bacillus mucilaginosus* involves culturing the bacterium in a suitable medium and formulating it into a bioinoculant.

Preparation of Culture Medium: Prepare nutrient broth or other suitable growth medium according to the manufacturer's instructions. Sterilize the medium by autoclaving or using a suitable sterilization method.

Inoculate a sterile container or flask containing the prepared culture medium with *Bacillus mucilaginosus* culture. Use a sterile inoculating loop or needle to transfer a small amount of *Bacillus mucilaginosus* culture into the medium. Ensure that the inoculum is evenly distributed in the medium.

Incubate the inoculated culture flask or container at the appropriate temperature (typically around 30-37°C) for 24-48 hours. Monitor the growth of *Bacillus mucilaginosus* culture by observing changes in turbidity or optical density.

pH Adjustment (optional): Measure the pH of the culture medium using a pH meter. Adjust the pH of the medium, if necessary, to optimize the growth of Bacillus mucilaginosus.

Harvesting After incubation, harvest the *Bacillus mucilaginosus* culture by transferring the culture to sterile tubes or containers. Centrifuge the culture at low speed (e.g., 3000 rpm) to separate bacterial cells from the culture medium. Discard the supernatant, leaving behind the bacterial pellet.

Formulate the harvested *Bacillus mucilaginosus* culture into a bioinoculant by mixing it with a carrier material (e.g., peat, vermiculite, compost). Ensure thorough mixing of the bacterial culture with the carrier material to achieve uniform distribution.

Packaging and Storage: Transfer the formulated bioinoculant into sterile tubes or containers for storage. Store the bioinoculant at appropriate conditions (e.g., refrigerated or room temperature) to maintain viability.

***Bacillus mucilaginosus* for potassium (K) supplementation in tissue culture media** for mustard plants, you can follow this protocol:

Preparation of *Bacillus mucilaginosus* Inoculum: Start by inoculating *Bacillus mucilaginosus* culture into a suitable liquid medium (e.g., nutrient broth) and incubate it at the appropriate temperature (around 30-37°C) for 24-48 hours until reaching the desired growth phase.

Preparation of Tissue Culture Media: Prepare mustard plant tissue culture media according to standard protocols (e.g., Murashige and Skoog medium). Ensure the media are free from potassium supplements or have low levels of potassium.

Inoculation with *Bacillus mucilaginosus:* Add the desired volume of *Bacillus mucilaginosus* inoculum to the tissue culture media. Mix the inoculum thoroughly into the media to ensure even distribution of the bacteria.

Adjustment of pH (if necessary): Measure the pH of the media using a pH meter. b. Adjust the pH to the desired level using sterile solutions of acids or bases as needed.

Sterilization: Autoclave the media containing *Bacillus mucilaginosus* at appropriate conditions to sterilize the media while maintaining the viability of the bacteria. Allow the media to cool down to room temperature before use.

Plant Tissue Inoculation: Prepare mustard plant tissues for tissue culture, such as explants or seeds. Inoculate the prepared plant tissues into the tissue culture media supplemented with *Bacillus mucilaginosus* using sterile techniques.

Incubation: Place the inoculated tissue culture containers or flasks in an incubator set to the appropriate temperature for mustard plant tissue culture (typically around 25-28°C). Incubate the cultures under suitable light conditions and photoperiod according to the requirements of mustard plant tissue culture.

Monitoring and Maintenance: Regularly monitor the growth and development of mustard plant tissues in the culture media supplemented with *Bacillus mucilaginosus*. Check for any signs of contamination or adverse effects on plant growth.

Harvesting: Harvest mustard plant tissues from the culture media when they have reached the desired growth stage or when sufficient biomass has accumulated.

Analysis of Potassium Content: Analyze the potassium content in the harvested plant tissues using suitable analytical methods (e.g., atomic absorption spectroscopy, flame photometry). Compare the potassium levels in tissues grown in media supplemented with *Bacillus mucilaginosus* with those grown in control media to assess the effectiveness of the bacterial supplementation in enhancing potassium uptake. By following this protocol, you can apply *Bacillus mucilaginosus* for potassium supplementation in tissue culture media for mustard plants and evaluate its effects on potassium uptake and plant growth.

Analyzing the growth parameters of plantlets supplemented with a bio-bac (biofertilizer or biobac) involves assessing various morphological and physiological characteristics to evaluate the impact of the bio-bac on plant growth. Here's a general protocol for conducting such analysis:

Preparation of Experimental Setup: Ensure that plantlets treated with bio-bac and control plantlets are grown under identical environmental conditions, including light intensity, photoperiod, temperature, and humidity. Plant the treated and control plantlets in separate containers or pots filled with the same growth medium or soil.

Monitoring of Growth Parameters: Record initial measurements of growth parameters for both treated and control plantlets before the start of the experiment. These parameters may include:

Plant height: Measure the distance from the base of the plant to the tip of the tallest leaf.

Leaf number: Count the total number of leaves on each plant.

Stem diameter: Measure the diameter of the main stem at a specified height from the base.

Root length: Measure the length of the primary root or the total root system.

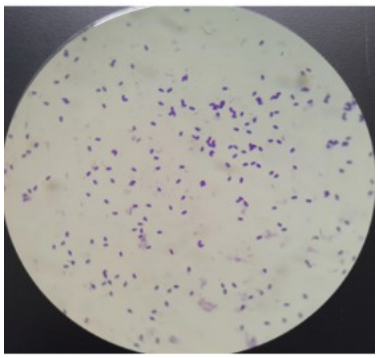
Biomass: Weigh the fresh weight of the entire plant or specific plant parts (leaves, stems, roots). Repeat measurements at regular intervals (e.g., weekly) throughout the experimental period to track changes in growth parameters over time. Assessment of Physiological Parameters: Measure physiological parameters that Chlorophyll content: Assess chlorophyll levels using a chlorophyll meter or spectrophotometric methods. Photosynthetic rate: Measure photosynthesis using a photosynthesis measurement system or gas exchange analyzer. Transpiration rate: Determine the rate of water loss from leaves using a porometer or gravimetric method. Stomatal conductance: Assess stomatal conductance using a leaf porometer or gas exchange analyzer. Conduct physiological measurements at appropriate time points to capture changes induced by bio-bac supplementation.

Soil and Nutrient Analysis (if applicable): Collect soil samples from the containers or pots containing treated and control plantlets. Analyze soil properties, including pH, nutrient levels (e.g., nitrogen, phosphorus, potassium), and microbial activity, to assess any changes induced by bio-bac supplementation.

Data Analysis: Compile and organize the collected data on growth and physiological parameters for treated and control plantlets. Analyze the data statistically using appropriate methods (e.g., t-test, ANOVA) to determine significant differences between treatments. Interpret the results to assess the impact of bio-bac supplementation on plant growth and performance.

Results and Discussion

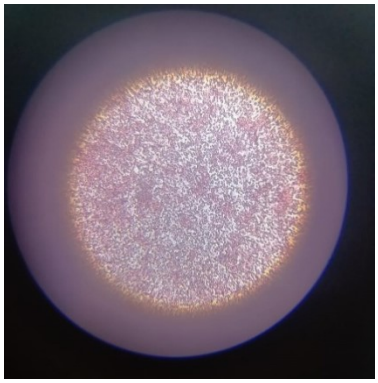
Bacillus mucilaginosus

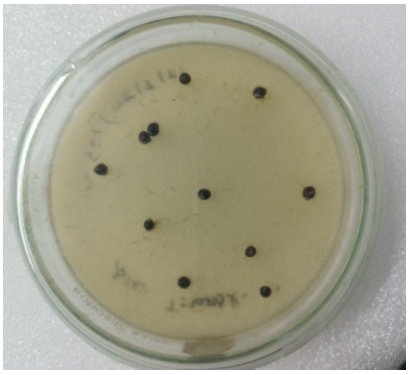


Preparation of Bio-Bac material with *Bacillus muciloginosus*

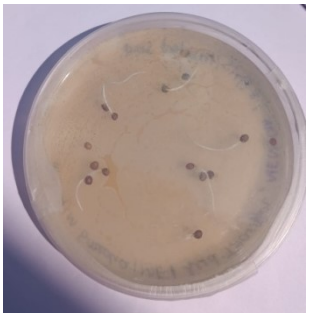
Bacteria broth was blended with calcium carbonate, Talk powder and carboxy methyl cellulose materials. During inoculation the bacterial cfu was 10^9 after preparation as powder it was 10^6.The culture were re-stained in grams test and identified as *B.muciloginosus.*

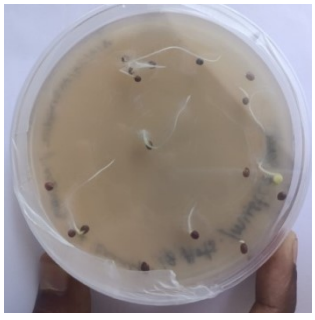
Treatment of Bio-bac with mustard seeds and inoculation on MS-TC media



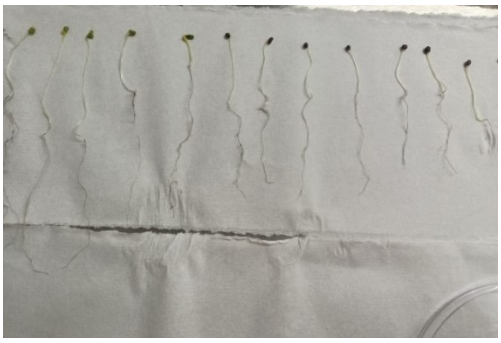


The seeds not only on TC also planted on earthen pots for vigour index and K solubilisation test





Effects of Bio-bac on mustard seedlings



The *B. muciliginosus* treated with different concentration on TC media showed variable and higher growth and germination rate on TC media. In that 1mg/ml showed higher root and shoot length on seedlings. The seedling produced on pots also checked for the growth a parameters and the results were same like TC plantlets.

Estimation of K on seedling treated Vs non treated

Instead of using K meter, I used potassium testing kit for the K solublisation effect on mustard seedlings. In treated the K was 2.2 to 4.0% K, whereas in non-treated it was 1.2 to 3.0% K On estimation with K detection kit on plants part showed higher K rate was in treated plantlets to non-treated.

Conclusion

Bacillus mucilaginosus is a powerful bacterium that generates organic acids that solubilize potassium from insoluble sources, such as citric acid, gluconic acid, and ketogluconic acid. These acids chelate potassium ions, allowing plants to absorb them. This process improves nutrient absorption, cell division, and cell expansion, promoting plant development. *Bacillus mucilaginosus* is an eco-friendly and sustainable alternative to chemical fertilizers, reducing the need for synthetic fertilizers. It also enhances soil health by making potassium and other minerals more accessible. The molecular processes of *Bacillus mucilaginosus* promote plant development and solubilize potassium, and it can be used as a biofertilizer to ensure efficient colonization and transport to the plant rhizosphere.

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