



Utilization of expiry food and microorganisms in development of biofertilizer

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ABSTRACT

The growing accumulation of expired packaged food, wet biodegradable waste, and non-degradable plastics poses major environmental and waste-management challenges. Plant growth-promoting microorganisms improve soil fertility through nitrogen fixation, phosphate solubilization, organic matter decomposition, and pathogen control. *Rhizobium* and *Azotobacter* enhance soil nitrogen, while *Pseudomonas* improves phosphorus availability and disease resistance. *Bacillus subtilis* supports nutrient mineralization and soil structure, promoting eco-friendly agriculture and reduced chemical fertilizer use. A systematic isolation and confirmation strategy was followed for biofertilizer production. Microorganisms from rhizosphere soil, legume root nodules, and organic waste were identified using common tests such as Gram staining, catalase test, and selected biochemical observations, excluding specific tests like Hofer's alkaline test, starch hydrolysis, and Ashby's mannitol growth. The confirmed cultures of *Rhizobium*, *Azotobacter*, *Pseudomonas*, and *Bacillus subtilis* were purified and applied for microbial decomposition during biofertilizer preparation. This study focuses on converting organic waste into value-added biofertilizer through efficient microbial decomposition while ensuring proper segregation and recycling of plastic materials. The integrated approach promotes sustainable waste management and supports circular economy principles. The effectiveness of integrating microbial biofertilizer production with sustainable waste management. Conversion of expired food and wet biodegradable waste into biofertilizer reduces waste accumulation and enhances soil fertility and microbial activity. The resulting biofertilizer provides a cost-effective, eco-friendly alternative to chemical fertilizers, supporting sustainable crop productivity.

Keyword: Biofertilizer, Microbial treatment, Expired food waste, Plastic recycling, Soil fertility, Sustainable agriculture, Circular economy.

INTRODUCTION

Soil microorganisms are required for both effective nutrient control and soil biodiversity. They are necessary for plant development and

growth. In recent years, chemical fertilizers have been employed in agriculture to increase food production and independence for the country, but at the expense of the environment and the health of all living beings. Overusing these fertilizers in farming is costly and has many negative consequences for soil fertility. Beneficial microorganisms offer alternatives to traditional farming approaches for addressing our agricultural needs. Biofertilizers are safer compared to chemical fertilizers, they are more concentrated have fewer negative environmental effects, and function better in smaller quantities. Furthermore, microbial inoculants degrade faster and are less likely to cause resistance to diseases and pests. The plant needs mineral nutrients, which can only be obtained through the direct or indirect application of chemical fertilizers in conjunction with organic manure and biofertilizers to boost the content of organic soil and ensure sustainability in fields and horticultural crops. Organisms like bacteria, fungi, and other microbes are microbial inoculants that are introduced into an environment to achieve a specific aim, such as biocontrol or plant development. The word "bio-fertilizer" therefore contains a wide range of products all of which contain active or dormant microorganisms, including bacteria, fungi, actinomycetes and algae. Some bacteria secrete compounds that improve plant growth. These bacteria help fix atmospheric nitrogen and solubilize or mobilize soil nutrients on application. In addition to numerous other products, biofertilizers, and biopesticides are now available as alternatives to standard synthetic pesticides, inorganic fertilizers, and inorganic fertilizers, respectively. From 2022 to 2027, the biofertilizer market is predicted to rise at a compound yearly 12.1% growth rate, from 1.57 billion in 2018. The market is tremendously fragmented because of the vast number of small and large businesses operating in various locations. Because it is now mostly unregulated, the biofertilizer market is dominated by numerous small businesses; however, if rules are enforced, as has occurred in the global market for biopesticides, the market may become more concentrated. Furthermore, because they occur naturally in the rhizosphere, are non-pathogenic, environmentally friendly, and directly increase plant yield, PGPRs are a distinct class of microbes that influence plant defense mechanisms and provide host resistance to further pathogen attack via an incredibly diverse mechanism. As a result, they are regarded as more effective biocontrol agents than traditional

chemical fertilizers (Mishra et al., 2020; Salman et al., 2024).

Biofertilizers encompass various types of microorganisms, each contributing uniquely to soil fertility and plant growth. Nitrogen-fixing bacteria are among the most important biofertilizers, as they convert atmospheric nitrogen into a form that plants can use. For instance, *Rhizobium* forms symbiotic relationships with leguminous plants, inhabiting root nodules where it fixes nitrogen, directly benefiting the host plant by supplying essential nitrogen for growth. Similarly, *Azospirillum* has proven effective in cereals and grasses, where it promotes plant growth by fixing nitrogen in the rhizosphere, thus enhancing nitrogen availability. *Azotobacter*, on the other hand, is a free-living nitrogen fixer that benefits non-leguminous crops by enhancing nitrogen levels in the soil, supporting overall plant health and growth. Phosphate-solubilizing microorganisms play a crucial role in making phosphorus an essential nutrient for plant development available to plants. Species like *Bacillus* and *Pseudomonas* release organic acids that solubilize insoluble phosphate compounds in the soil, thereby increasing phosphorus uptake by plants. This process is particularly beneficial in soils where phosphorus is present in forms that are not easily accessible to plants. In addition, potassium-solubilizing microorganisms enhance plant nutrition by releasing potassium from soil minerals, ensuring that this essential macronutrient is available to support plant physiological processes such as photosynthesis and water regulation. Lastly, plant growth-promoting rhizobacteria (PGPR) offer multifaceted benefits to plants. PGPR, such as species of *Bacillus*, promote plant growth through several mechanisms, including the production of growth hormones like indole-3-acetic acid (IAA), cytokinin's, and gibberellins, which stimulate root and shoot development. Additionally, PGPR improve nutrient availability by producing siderophores, compounds that bind to and solubilize iron, making it more accessible to plants. These microorganisms also play a role in enhancing plant resistance to environmental stresses and pathogens, making them valuable allies in sustainable agriculture. Mycorrhizal fungi, particularly Arbuscular Mycorrhiza (AM), establish symbiotic relationships with plant roots, forming an extensive hyphal network that significantly increases the surface area for nutrient absorption. These fungi are particularly important for phosphorus uptake, a nutrient often present in limited amounts in the soil.

By associating with mycorrhizal fungi, plants benefit from enhanced access to phosphorus and other micronutrients, improving overall growth and yield. (Durre et al., 2023; Ahamad et al., 2023).

Food waste has emerged as a large international environmental issue with tons of food waste being produced every year from households, food processing and retail sectors, causing pressure on land use, greenhouse gas emissions and pollution when disposed of through landfills or incineration (ScienceDirect Bioresource Technology). To overcome this challenge, sustainable ways of converting food waste into value-added products have attracted a lot of attention. One such promising solution is the upcycling of expired food into biofertilizer, which is nutrient-rich organic amendment that can help to enhance soil health and plant productivity and reduce the dependence on chemical fertilizers (Journal of Umm Al-Quar University for Applied Sciences). Research has proven to be full of macro- and micro-nutrients in food waste and through various processes such as microbial digestion and composting, the nutrients can be stabilized and released efficiently when they are used in soils (RSC Publishing). Additionally, various research has shown that using biochar, plant ash or specific microbe combinations during food waste valorization increases nutrient content and agronomical performance of the biofertilizer produced, thus helping to improve environmental sustainability and even economics, by reducing the cost of disposal and addressing circular bioeconomy targets (Chemosphere). Therefore, the conversion of expired food into biofertilizer is a holistic solution to food waste management that can improve both soil fertility and also promote sustainable agriculture while reducing environmental damage (Lei et al., 2025; Anastasiia et al., XXXX; Pramodkumar et al., 2XXX).

In recent years, the emphasis of agricultural research has moved towards eco-friendly inputs that not only produce greater crop yields, but can also ensure soil health in the long-term. Excessive use of chemical fertilizers has been noted to lead to decline in soil structure, microbial diversity, and environmental pollution of water bodies by leaching of nutrients from the soil. In this scenario, biofertilizers derived from organic waste such as expired food have become a sustainable alternative. Several studies demonstrate that food waste-derived biofertilizers help support

beneficial soil microorganisms, improve nutrient availability and enhance plant growth parameters when compared to traditional fertilizers. Microbial inoculation in the conversion of food waste is important in the mineralization of nutrients, fixing of nitrogen, and solubilization of phosphorous which enhance the efficiency of fertilizers. Furthermore, the use of expired food as a source of biofertilizer production is also in the spirit of waste valorization and circular economy by turning waste into a valuable agricultural resource. Such approaches not only alleviate the environmental burden that is caused by the disposal of food, but they also help in sustainable agriculture practices, more so in developing countries where soil amendments such as the low cost and organic amendments are highly beneficial. (Mamun et al., 2024; Zhen et al., XXXX; Mustafa et al., XXXX).

The experimental procedures used in this study were intended to isolate, cultivate and identify selective bacterial populations related to biofertilizer development from expired food substrates. The methodology involved sequential steps including the processing of the samples, bacteria isolates in selective culture media, germination and growth characters observation, and taxonomic identification of bacteria isolates. All laboratory work was performed with controlled aseptic conditions to get reproducibility and avoid microbial contamination. The identification of the bacterial strains was done based on morphological, cultural, staining and biochemical analyses following the standard microbiological protocols. These methods were chosen to get reliable and consistent results and test the suitability of the isolated bacterial strains for application in the production of biofertilizer. For the efficient development of a biofertilizer that could have multifunctional benefits, the process of selecting appropriate microbial strains is a very important part. In the present research, four agriculturally important bacteria genera namely *Rhizobium*, *Pseudomonas*, *Bacillus* and *Azotobacter* have been selected for their proven role in nutrient cycling, improvement of soil and plant growth promotion. *Rhizobium* species are symbiotic nitrogen-fixing bacteria, being responsible for fixing atmospheric nitrogen in easily available forms for plants, contributing to the improvement of soil nitrogen status. *Azotobacter* species are free-living nitrogen fixers that contribute to soil fertility and also produce biologically active compounds (vitamins, amino acids, and phytohormones) that stimulate plant growth. *Pseudomonas* species are responsible for

phosphate solubilization, production of siderophore and biological control of soil-borne pathogens, which contribute to enhanced nutrient availability and plant health. *Bacillus* species, as spore-forming, metabolically versatile organisms are valued in their capacity to survive in different environmental conditions and their involvement in the decomposition of organic matter and their production of enzymes and antimicrobial compounds. Collectively, these genera of bacteria were chosen in order to bring complementary functions to improve soil quality and increase effectiveness of the biofertilizer. Following the selection of these 4 bacterial genera, selection, cultivation and identification of the strains from appropriate samples have been carried out in this study. Standard microbiological procedures were applied to obtain the successful isolation of the pure cultures and controlled conditions ensured the germination and growth of the bacterial populations. Subsequent identification of the isolates was carried out by means of morphological, cultural, staining and biochemical tests, thus enabling any bacterial genus to be precisely characterized.

The details of the specific procedures used to realize the isolation, germination and identification of the isolates of *Rhizobium*, *Pseudomonas*, *Bacillus* and *Azotobacter* that were employed in this study are described in the following sections. Efforts to achieve sustainable management of food waste have been increasingly focused on recycling food waste after consumer use and agriculture back into value-added organic amendments used to improve soil health and crop productivity. Food waste valorization, aside from the problems of waste disposal, contributes also to the recycling of nutrients through the transformation of organic residuals to biofertilizers and soil amendments, through biological, physical or chemical treatments. Recent studies have shown that food waste-based fertilizers can play a key role in improving soil chemistry, microbial activity and plant growth, and in reducing the use of synthetic nitrogen products, which will promote sustainable crop production systems and nutrient cycling within agroecosystems. (Nasir et al., 2025, Bernal and Graham, 2001; Pawar et al., XXXX).

In this present paper the experimental studies demonstrate that amendments produced from food waste assist in the modification of soil properties such as pH and nutrient availability, promote microbial

enzyme activities linked to carbon, nitrogen and phosphorus cycling and ultimately contribute to the improved nutrient use efficiency in plants. In addition, food waste conversions methods such as anaerobic digestion and microbial bioconversion techniques have been found to be effective options in the production of nutrient-rich materials that can be used either separately or combined with mineral fertilizers in sustainable soil management practices. These findings highlight the potential of food waste valorization as a waste management approach, but also as a way to achieve circular economy principles and sustainable agriculture. The methodology for transforming expired food into biofertilizer and evaluating the performance of biofertilizer in soil systems.

MATERIAL AND METHODS

Collection of plant sample:

Healthy, mature and not infected with any disease root nodules were collected from the leguminous plants like pea, bean or soybean. Nodules have been selected according to their firm texture and pinkish interior, which is an indication of active nitrogen-fixing bacteria. Such bacteria were isolated from above selected plants.

Collection of soil sample:

Soil samples will be collected from different areas near Chh.Sambhajinagar, following a zig zag pattern to access soil fertility levels and analyze nitrogen fixing bacteria. Soil sample containing root nodules were collected from various areas such as Adgaon Bk, Balapur, Gadiwat, Daulatabad, Patoda near chh. Sambhajinagar city.

Isolation, Cultivation and Detection of bacterial Strain:

The isolation of bacterial from the leguminous plant in laboratory was done using standard aseptic microbiological procedures so that pure and viable cultures of bacteria can be obtained.

Surface Sterilization of Nodules: In order to remove epiphytic or surface microorganisms, nodules were washed in running tap water for the removal of adhering soil particles. They were then surface-sterilized by immersion of them in a 0.1% solution of mercuric chloride for 30-60 seconds or in 70% ethanol for 1 minute. Following sterilization, nodules were

rinsed 5-6 times with sterile distilled water to get rid of any possible sterilant left over. Proper sterilization is important in order to isolate only endophytic *Rhizobium* and not contaminating bacteria.

Nodule Crushing / Homogenization: Sterilized nodules were put inside sterile mortar or sterile micro-tube containing 1-2 mL of sterilized saline solution (0.85% NaCl) in aseptic condition. Nodules were slightly crushed by the use of sterile pestle or homogenizers to introduce the bacterial cells of infected tissues. Care was taken that the bacterial cells were not damaged by over-crushing.

Preparation of Dilutions: Nodule suspension was plated in serial dilution to attain various low levels of bacteria to allow discrete colony formation on agar plate. Usually 10^{-1} to 10^{-5} dilutions were made in sterile saline with aseptic pipettes.

Streaking on Selective Media: Aliquots (50-100 μ L) of suitable dilutions were streaked on plates (Yeast Extract Mannitol Agar; YEMA, supplemented with Congo Red dye 0.0025%). Congo Red acts as a differential, the *Rhizobium* colonies do not take up much or any Congo Red. The plates were streaked by quadrant streak method for isolation of the colonies.

Incubation: Inoculated plates were incubated at temperature 28°C to 30°C for 3-7 days at aerobic condition. Colony development was monitored on a daily basis. Characteristic *Rhizobium* colonies are small, circular, convex, translucent, whitish and often mucoid as a result of the production of exopolysaccharides.

Purification of Isolates: Well isolated colonies were picked with the help of sterile inoculating loops and re-streaked on fresh YEMA plates for obtaining pure cultures. This procedure was repeated 2-3 times to ensure that contaminants were removed. Pure cultures were kept in YEMA slant at the 4°C for short-term storage and sub-cultured periodically to maintain viability.

Cultivation Media for *Rhizobium*:

YEMA is popularly used for the isolation and cultivation of *Rhizobium* due to its advantages of supporting nitrogen-fixing bacteria while preventing the growth of a large number of non-target microorganisms (Urban-Chmiel et al., 2022). For the

cultivation and enrichment of *Rhizobium*, pure colonies obtained from YEMA plates were inoculated into sterile Yeast Extract Mannitol Broth (YEMB) under aseptic conditions. The broth, prepared with 10 g/L mannitol, 0.5 g/L yeast extract, 0.5 g/L K_2HPO_4 , 0.2 g/L $MgSO_4 \cdot 7H_2O$, and 0.1 g/L NaCl with pH adjusted to 6.8, was sterilized by autoclaving at 121°C for 15 minutes. A small loopful of the pure *Rhizobium* culture was transferred into the broth and incubated at 28–30°C on a rotary shaker at 120–150 rpm to ensure uniform aeration and growth. Growth was monitored by observing turbidity and, if required, by measuring optical density. After 3–5 days of incubation, the enriched culture, containing a high population of actively growing *Rhizobium* cells, was used for further experiments such as biofertilizer preparation or inoculation studies. For long-term storage, the cultures were maintained on YEMA slants at 4°C and periodically sub-cultured to preserve viability and functional activity.

Cultivation Media for *Azotobacter*:

Soil samples are collected from the rhizosphere region and air-dried to lower the number of non-spore-forming bacteria. A gram of the soil sample is carefully transferred into 9 mL of sterile distilled water and diluted up to 10^{-6} . From the appropriate dilutions, usually 10^{-5} , 0.1 mL of the suspension is spread evenly on Ashby's Mannitol Agar medium. This medium is nitrogen-free and selects for *Azotobacter*. The plates are incubated at 28 to 30 °C for 3 to 5 days. Colonies that appear large, opaque, slimy, or mucoid are chosen as possible *Azotobacter* colonies. To cultivate the selected *Azotobacter* colonies, they are picked with a sterile inoculating loop and streaked on fresh Ashby's Mannitol Agar plates to obtain pure cultures. For mass cultivation, a loopful of pure culture is added to Ashby's Mannitol Broth and incubated at 28 to 30 °C for 3 to 4 days while shaking. Turbidity in the broth shows that the organism is actively growing. The pure cultures are kept on agar slants at 4 °C for future studies (Ambily and Jisha, 2012).

Cultivation Media for *Bacillus subtilis*:

Soil samples come from the rhizosphere region of plants and are air-dried briefly to lower the number of non-spore-forming bacteria. One gram of the soil sample is transferred into 9 mL of sterile distilled water and mixed well to create a soil suspension. This suspension is diluted serially up to a 10^{-6} dilution. From suitable dilutions, usually between 10^{-4} and

10^{-6} , 0.1 mL of the suspension is spread evenly on nutrient agar medium, which encourages the growth of *Bacillus* species. The inoculated plates are kept at 30–37°C for 24–48 hours. Colonies that appear large, irregular, dry, rough, and opaque are chosen as presumptive *Bacillus subtilis* colonies. To cultivate *Bacillus subtilis*, selected colonies are picked with a sterile inoculating loop and streaked on fresh nutrient agar plates to get pure cultures. For mass cultivation, a loopful of pure culture is added to nutrient broth and incubated at 30–37°C for 24–48 hours while shaking. Turbidity in the broth shows that the organism is actively growing. The pure cultures are stored on agar slants at 4°C for further studies (Gouda et al., 2018).

Cultivation Media for *Pseudomonas*:

Scoop rhizosphere soil with a sterile spatula or forceps and put the material into sterile tubes or containers; get it back to the lab quickly — don't let the sample dry out or form a crust. Weigh 1 g of soil into 9 mL of sterile distilled water, then vortex or shake hard until you have a homogenous suspension — imagine a thin, muddy broth, a bit like weak chocolate milk. Make serial tenfold dilutions down to 10^{-6} . From the suitable dilutions (usually 10^{-4} to 10^{-6}), pipette 0.1 mL and spread it evenly over cetrimide agar, which selects for *Pseudomonas*. Incubate the plates at 28–30°C for 24–48 hours. After incubation, pick colonies that are smooth and circular sometimes slightly irregular and show greenish to bluish pigmentation. Those hues often come from pigments such as pyoverdine or pyocyanin, so treat these as presumptive *Pseudomonas*. To purify, touch a single colony with a sterile loop and streak it onto fresh cetrimide agar. For larger cultures, transfer a loopful of the pure isolate into nutrient or cetrimide broth and incubate with shaking at 28–30°C for 24–48 hours. How can you

tell it's growing? Increased turbidity the broth will cloud. Store confirmed pure cultures on agar slants at 4°C for future use (Sarwar, et al., 2020).

Common confirmational test for the detection of all bacteria:

1. **Indole Test (I):** This test detects the ability of bacteria to produce indole from the amino acid tryptophan using the enzyme tryptophanase.
2. **Methyl Red Test (M):** This test detects mixed acid fermentation of glucose resulting in stable acid production.
3. **Voges-Proskauer Test (V):** This test detects acetoin production during glucose fermentation via the butanediol pathway.
4. **Citrate Utilization Test (C) Principle:** This test determines the ability of bacteria to use citrate as the sole carbon source.
5. **Hoffers alkaline test:** It is a biochemical test used to differentiate *Rhizobium* from *Agrobacterium* based on their ability to grow in an alkaline medium.
6. **Catalase test:** It is a biochemical test used to detect the presence of the catalase enzyme, which breaks down hydrogen peroxide into water and oxygen.
7. **Gram staining:** It is a differential staining technique used to classify bacteria based on their cell wall composition.
8. **Fluorescent Test:** It is used to detect the production of fluorescent pigments (like pyoverdine) by bacteria under UV light.
9. **Growth on Ashby's Mannitol Agar:** It is a selective medium used to isolate nitrogen-fixing bacteria that can grow without combined nitrogen.
10. **Gelatin hydrolysis test:** It is a biochemical test used to detect the ability of bacteria to produce gelatinase enzyme that liquefies gelatin.

Table 1: Bacterial culture identification using IMVIC TEST

Bacterial Isolate	Indole Test (I)	Methyl Red Test (M)	Voges-Proskauer Test (V)	Citrate Utilization Test (C)
<i>Rhizobium</i> spp.	Negative (–)	Negative (–)	Negative (–)	Positive (+)
<i>Azotobacter</i> spp.	Negative (–)	Negative (–)	Negative (–)	Positive (+)
<i>Bacillus</i> spp.	Negative (–)	Positive (+)	Positive (+)	Positive (+)
<i>Pseudomonas</i> spp.	Negative (–)	Negative (–)	Negative (–)	Positive (+)

RESULTS

Firstly, the soil sample and the Healthy, mature and not infected with any disease root nodules were collected from the leguminous plants. The isolation of bacterial from the sample in laboratory was done using standard aseptic microbiological procedures so that pure and viable cultures of bacteria can be obtained. The process for the isolation of bacterial culture from plant sample includes: Surface Sterilization of Nodules, Nodule Crushing / Homogenization, Preparation of Dilutions, Streaking on Selective Media, Incubation, Purification of Isolates. Once the bacterial culture was isolated from the soil or plant sample then the bacterial strains were confirmed using the confirmational test such as Hoffers alkaline

test, Catalase test, Gram staining, Growth on Ashby's mannitol agar, Gelatin hydrolysis test.

Once the bacterial strain were confirmed using the confirmational test then the bacterial culture were grown on the enrichment media for the large quantity production of bacteria. And after enrichment the culture is used in the fertilizer. Plants grown in soil blended with the homemade biofertilizer performed noticeably better than those in untreated pots. Germination in the treated mix started a little earlier, and seedlings marched on at a steady, measured pace during the whole observation no sudden surges, just reliable, healthy growth. By contrast, plants in plain soil lagged: slower to establish, shorter and generally more fragile. The takeaway was obvious a clear, positive effect from the biofertilizer.

Table no. 2: Confirmational test for all bacterial strains.

	Bacteria	Confirmational test	Observation	Result
1	<i>Rhizobium</i>	(a) Hoffers alkaline test	Good / Luxuriant Growth (Broth becomes turbid; colonies appear on agar medium)	Negative Hofer's test, confirming the isolate of <i>Rhizobium</i> .
		(b) Catalase test	Immediate bubble formation is observed	Formation of immediate bubbles confirms the <i>Rhizobium</i> isolate.
		(c) Gram staining	<i>Rhizobium</i> cells appear pink to red, rod-shaped under oil immersion microscopy	Gram negative <i>rhizobium</i> bacteria were confirmed.
2	<i>Azotobacter</i>	(a) Growth on Ashby's mannitol agar	Large, smooth, opaque and slimy colonies observed on Ashby's Mannitol Agar	Positive for <i>Azotobacter</i>
		(b) Catalase test	Rapid bubble formation is seen	Formation of immediate bubbles confirms the <i>azotobacter</i> isolate
		(c) Gram staining	<i>Azotobacter</i> cells appear pink to red, rod-shaped under oil immersion microscopy	Gram negative <i>azotobacter</i> bacteria were confirmed.
3	<i>Bacillus subtilis</i>	(a) Gelatin hydrolysis test	As the gelatin medium remain liquid after refrigeration, it confirms the presence of the <i>bacillus subtilis</i> .	<i>Bacillus subtilis</i> shows positive gelatin hydrolysis
		(b) Catalase test	Rapid bubble formation is seen	Formation of immediate bubbles confirms the <i>bacillus</i> isolate
		(c) Gram staining	<i>Bacillus subtilis</i> cells appear purple	Gram positive <i>bacillus subtilis</i> were confirmed.
4	<i>Pseudomonas</i>	(a) Catalase test	Rapid bubble formation is seen	Formation of immediate bubbles confirms the <i>Pseudomonas</i> isolate
		(b) Gram staining	<i>Pseudomonas</i> cells appear red or pink	Gram negative <i>pseudomonas</i> was confirmed.
		(c) Fluorescent test	When observed under UV light, the colonies emitted a distinct yellowish-green fluorescence	Positive fluorescent test indicates the presence of presence of <i>pseudomonas</i> .

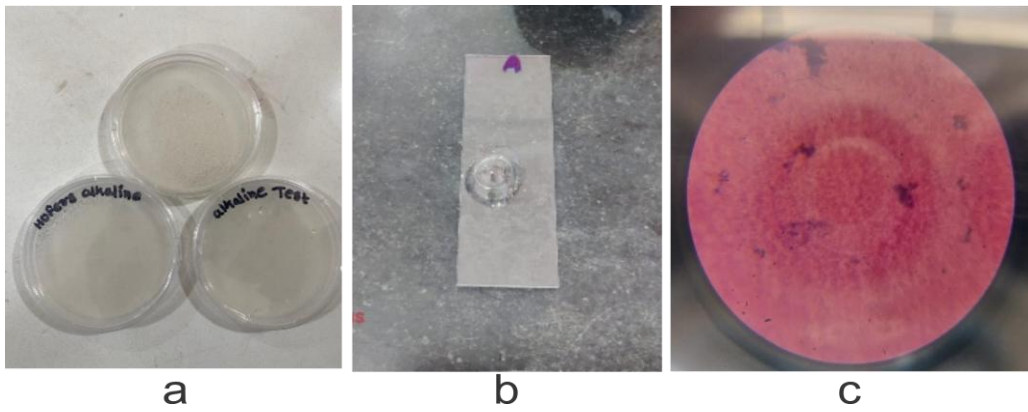


Fig 3: Confirmational test for rhizobium

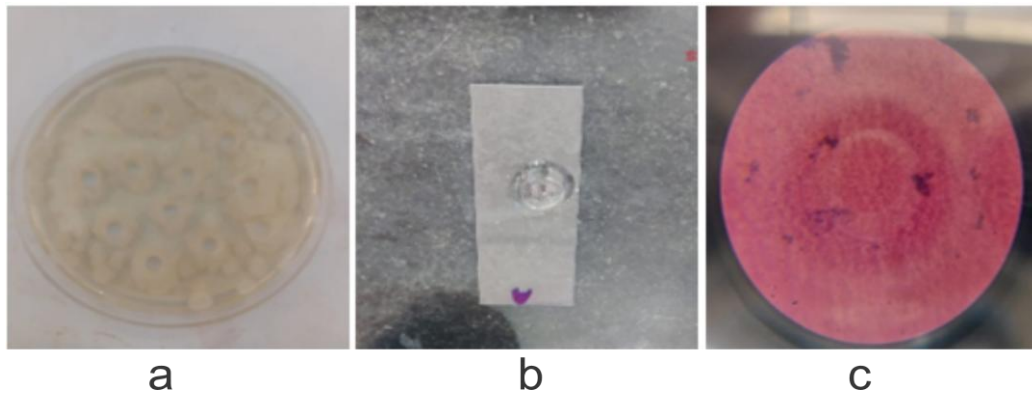


Fig 4: Confirmational test for azotobacter: (a) growth on Ashby's mannitol agar (b) catalase test (c) gram staining

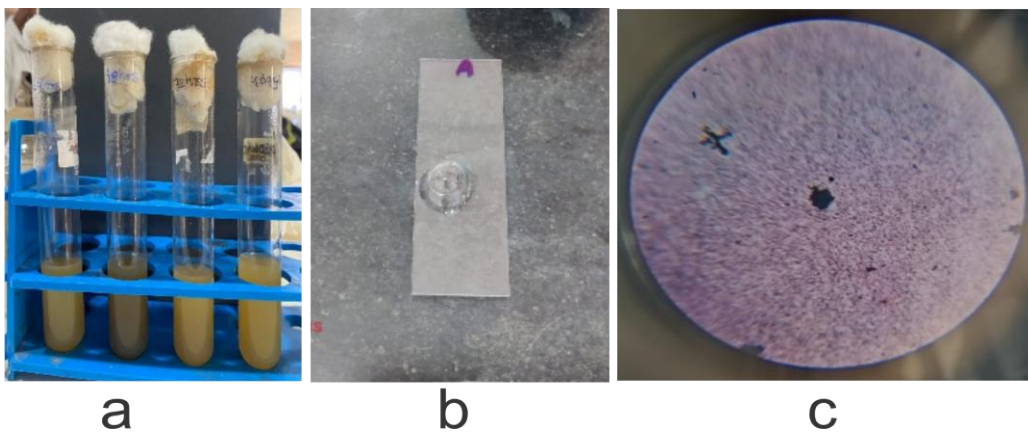


Fig 5: Confirmational test for bacillus subtilis, (a) Gelatin hydrolysis test, (b) catalase test, (c) gram staining

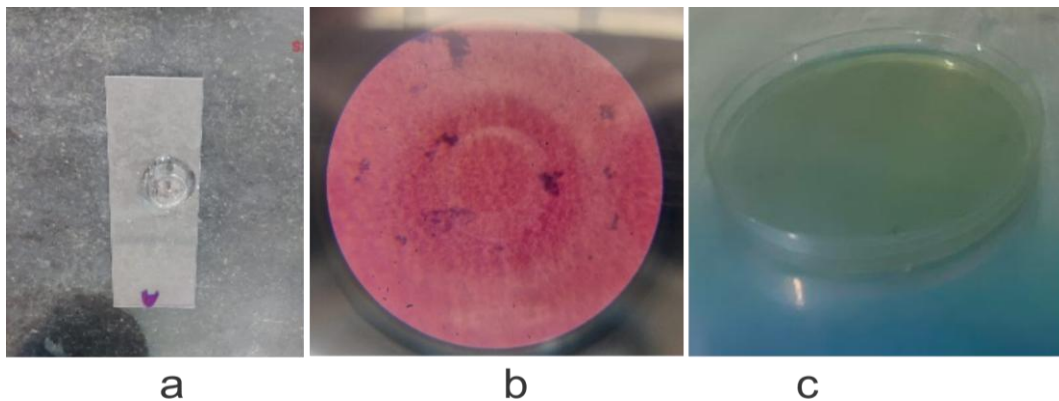


Fig 6: Confirmational test for pseudomonas, (a) catalase test, (b) gram staining, (c) fluorescent test

We ran a small trial using household biodegradable waste mostly overripe fruit and vegetable peels and after inoculating it with a mix of plant-growth-promoting microbes, decomposition crawled at first. Plastics were hand-sorted out and tossed into a separate bin before any processing began; nobody wanted contamination. When we plated samples, colonies erupted in wildly different morphologies: pinprick dots beside sprawling sheets. Textures ranged from a glossy, mucous-like sheen to brittle, dry crusts, and colors shifted across the palette findings that matched what you'd expect for *Rhizobium*, *Azotobacter*, *Pseudomonas* and *Bacillus subtilis*. Gram stains showed both Gram-positive and Gram-negative cells, and routine biochemical assays backed those tentative IDs. After we purified and blended the cultures into a biofertilizer, decomposition accelerated; the pile turned dark, uniform and earthy-smelling clear proof the microbes were doing their job.

DISCUSSION:

Plants grown in soil treated with biofertilizer did noticeably better; the microbes basically turned the root zone into a friendlier, more supportive neighborhood. They worked around the clock, weaving between soil and roots and releasing nutrients little by little instead of dumping one big dose. That quiet, steady feeding produced more even growth fewer stunted shoots, more consistent foliage across the bed. Treated specimens pushed out wider root systems, a sign of improved soil structure and greater moisture retention where microbial life was active. By contrast, plants on untreated ground, without that biological backup, lagged and appeared in patches, like a lawn with thin spots. Mix the right beneficial microbes and they build a microhabitat that helps seedlings establish and mature not magic, just better conditions.

CONCLUSION

It turns out ordinary microbes can turn organic waste into a practical biofertilizer. In paired field and lab trials, teams introduced selected microbes the same bacteria and fungi you'd spot in a steaming compost heap and decomposition sped up; soil crumb loosened, and nutrients became more available. Plants grown in that soil visibly improved: steadier growth, fuller, greener leaves, and far fewer pale, nitrogen-starved

tips than plants in conventional plots. That's a clear payoff from seeding soil with helpful microbes. The approach also keeps kitchen scraps and crop residues—banana peels, carrot tops, corn stalks out of landfills, offering a lower-impact substitute for synthetic fertilizers. Taken together, the findings suggest real promise for waste-derived biofertilizers in sustainable farming and smarter waste handling.

Conflict of interest

The authors declare no competing interests.

Data Availability Statement: Not applicable.

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