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Isolation and Enrichment of Plant Growth Promoting Actinobacteria in Cow Dung Manure for Sustainable Biofertilizer Application

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Abstract:

The isolation and enrichment of plant growth-promoting Actinobacteria in cow dung manure were examined to improve its application as a biofertilizer. The sample is collected from the animal slaughterhouse (abattoir) in Geidam, Yobe State, Nigeria, dried in an oven at 45 °C for 24 hours, and homogenized for analysis of microbes. Dilution series were initiated from 10^{-1} to 10^{-6}, and species of Actinobacteria were cultivated on Starch Casein Agar (SCA) as selective media supplemented with ampicillin and nystatin. After primary cultivation, actinobacterial colonies were further enhanced by inoculating them in tryptic soy broth (TSB) and subsequently enriching them in dried cow dung manure at a moisture content of 50-60%, based on cell density (10^{-4} CFU/g). Enriched cow dung is kept at mesophilic temperature for the growth of bacteria, additionally by a secondary mode of isolation as well as colony counting. The outcomes reveal a notable growth of Actinobacterial colonies, with CFU counts increasing from 3.9×10^{-4} CFU/g prior to enrichment to 8.5×10^{-4} CFU/g after completing the enrichment. Colony quantification verified the success of the enhancement, with fine, applicable and distinct actinobacterial strains viewed as well as the absence of contamination from the other bacteria. This investigation recommended that Actinobacteria enrichment can enhance the bio-fertilizer capacity of cow dung manure through boosting valuable strains of Actinobacterial species, resulting in soil well-being and eco-friendly agriculture production. Advanced investigation should place emphasis on land applications to confirm the prolonged effectiveness of enhanced cow dung manure as a biofertilizer or organic fertilizer.

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INTRODUCTION

The agricultural production widely is experiencing a model change toward sustainability because of the various consequences of inorganic fertilizers on soil health and environmental safety (Pahalvi *et al.*, 2021). The consistent applications of chemical-based agrochemicals have not just contributed to nutrient depletion; rather, they have resulted in a decrease of valuable soil microbes that are important for stabilizing agricultural ecosystem practicability (Chen, 2006). As an attention to these problems, microbial bio-based fertilizers have attracted serious recognition for their potentiality to improve soil productivity as well as plant growth via sustainable methods (Ramasamy *et al.*, 2020). Actinobacteriaum, a group of Gram-positive bacteria with high G-C content, serve an important function in soil interactions between living and non-living components (Boubekri *et al.*, 2022). These beneficial microorganisms are well-known for their metabolic adaptability and are capable of generating several bioactive substances, along with biocides, siderophores, and plant hormones like indole-3-acetic acid (IAA) (Barka *et al.*, 2016). Many strains, especially the genus *Streptomyces* and relevant taxa, displayed nitrogen fixation potentiality (Aasfar *et al.*, 2024) dissolving of phosphate, and detestation against plant pathogens, rendering them the best microorganisms for bio-based fertilizer utilisation (Gopalakrishnan *et al.*, 2011).

Cow dung manure, a degradable substrate, is enriched with nutrients and native microorganisms, showing an untapped reserve for cultivating plant growth-enhancing actinobacteriaum. Conventionally applied in organic agricultural productions, cow dung manure creates a favourable condition for microbial proliferation and exertion (Yadav *et al.*, 2015). In spite of its capability, scientific attempts to cultivate and enhance plant growth by promoting actinobacteriaum from cow dung manure are still very rare. Eexamining this sample might result in the finding of novel genera with improved agricultural applications.

The species of Actinobacteria are among the major groups of microbes that exist naturally in virtually all environments and are not mainly found in soil, alpine, abyssal, or harsh environmental conditions; also, they are found on the surface and in animals' bodies and in the root nodules and the roots of plants. Actinobacterium are an abundant group that are divergent and are additionally essential sources of bioactive compounds. Moreover, they have exhibited significant application benefits in several disciplines like health, agriculture, and food (Nazari *et al.*, 2022). The valuable actions of actinobacterium in boosting plant development and controlling crop pathogens have been completely ascertained (Du *et al.*, 2022). Cultivated Streptomyces albidoflavus St-220 from Salvia miltiorrhiza rhizosphere soil with powerful phosphorus solubilizing, indole acetic-acid generation and iron transporter-promoting capabilities, and additionally with powerful biocontrol characteristics (Omar *et al.*, 2022).

The incorporation of nitrogen-fixing Actinobacteria isolated from cow dung into agricultural production usually provides simultaneous advantages (Boukaew *et al.*, 2022), efficient waste usage and improved plant growth. Studies have shown that the application of cow manure can enhance the number of beneficial microorganisms in the rhizosphere, resulting in enhanced soil well-being along with the growth of plants (Ngone *et al.*, 2023). However, the application of such bio-based fertilizers can limit reliance on inorganic fertilizers, encouraging eco-friendly production. (Aasfar *et al.*, 2024).

The current investigation intended to cultivate and enhance plant growth-boosting actinobacteriaum from cow dung organic fertilizer and assess their capability for application in eco-friendly biofertilizer compositions. Through giving emphasis on indigenous microbial declines, this study will contribute to promoting environmentally sustainable as well as locally endurable products for soil productivity optimization and eco-friendly crop production.

MATERIALS AND METHODS

Sample collection and preparation

Cow dung manure was obtained from the animal slaughterhouse and dried in an oven at 45 °C for 24 to 48 hours; the dried sample was homogenized using mortar and pestle. A dried and homogenized cow dung sample is dissolved in 50 mL of distilled or saline water, and it's mixed thoroughly to create a uniform suspension and create a dilution series from 10^{-1} to $10^{-7.}$

Preparation of Media

Exactly 6.3.0 grams of Starch Casein Agar (SCA) were suspended in 100 mL of distilled water. Sterilize by autoclaving at 121 °C for 15 minutes. Allow the medium to cool approximately at 40 °C and pour into sterilize petri dishes/plates.

Isolation Experiment

1 gram of dried and homogenised cow dung sample was suspended in 50 mL of distilled water or saline water and serially diluted up to 10^{-7}. To create a uniform suspension, each tube for serial dilution is vortexed. 0.5 ml of each dilution is plated out and is overlaid with approximately 20–25 mL of starch casein agar (SCA) and incubated at 28–30 °C for 7 days. After the incubation period the CFU was observed and estimated.

Preparation of inoculant (Biofertilizer) using Actinobacterial isolates

3 grams of tryptic soy broth (TSB) was poured into a conical flask and dissolved in 100 mL of distilled water. The flasks were sealed with the cotton plugs, wrapped in aluminium foil, and heated at 121 °C for at least 45 minutes. After it cools, a fine and single colony of actinobacterial isolate was picked up with a sterile inoculation loop from the agar plate and partially dipped into the TSB to transfer the viable colony. Incubate at 28–30 °C with 200 rpm for 72 hours.

Enhancement experiment (Post-Enhancement)

Based on the cell density (10^{-4} CFU/g), 1000 mL of actinobacterial inoculant (Biofertilizer) was inoculated into 10 grams of cow dung manure at a ratio of 1:10. Enriched cow dung was dried in an oven at 45 °C for 24 hours. Dried and enriched cow dung was serially diluted and cultivated on SCA media for verification of enhancement efficiency.

Colony Forming Unit (CFU) Count

Plates with distinct and countable colonies ranging from 30 to 300 for Actinobacteria before enrichment and after enrichment were selected, and the colonies were counted using a digital colony counter. The number of colonies per gram in the sample is calculated using the formula CFU/g = Number of Colonies x Total dilution factor/volume of culture plated, then compare the CFU count between pre-enhancement (first replicates) and post-enhancement (second replicates).

RESULTS AND DISCUSSION

Actinobacterial colony morphology identification

Colonies were isolated on SCA agar petri dishes as selective media for pure culture. Actinobacterial colonies were subjected to cultivation using a concentration of 0.5 mL dilution of the cow dung manure sample before enrichment and the control experiment, as shown in figures (1 and 2), as well as after enrichment in (Figure 3). The existence of distinct and fine actinobacterial colonies was observed on SCA plates, showing the successful cultivation process without any contamination or growth of other non-target bacteria. The presence of convex colonies was observed, which verified the morphological features of Actinobacteria. While the control experiment showed contamination and growth of other non-target bacteria.

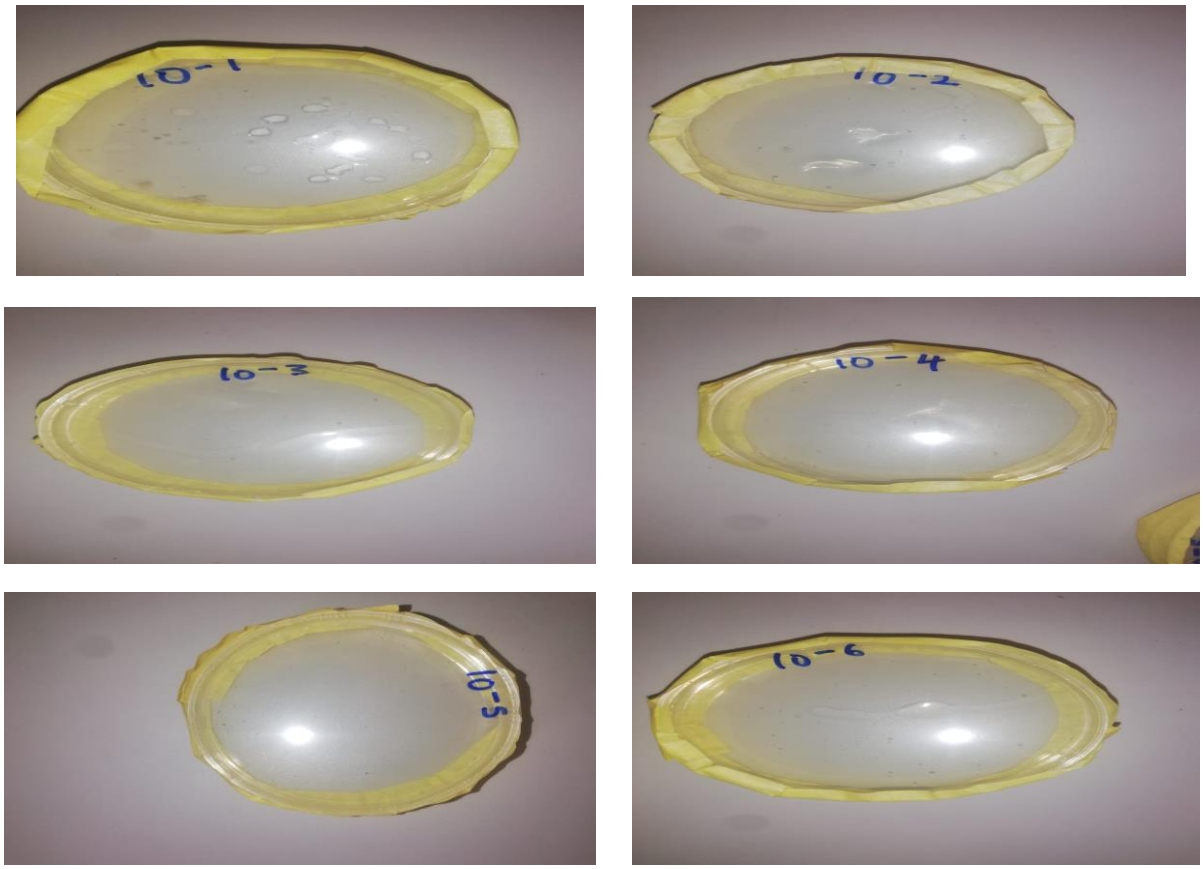

Fig. 1. Plate "**1-6**" showing cells containing actinobacterial colonies from the agar plate from dilution series 10^{-1} to 10^{-6} for cow dung manure before enhancement.

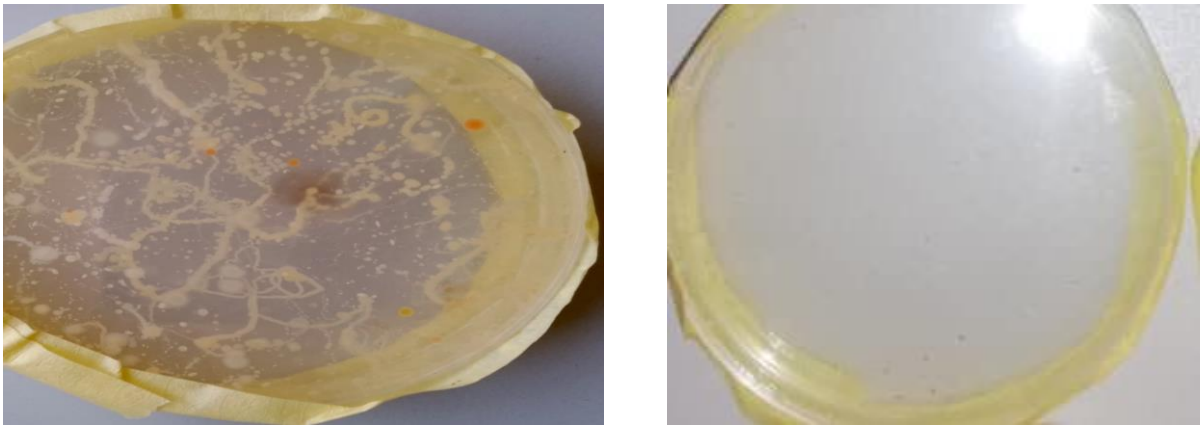

Fig. 2. Plate "**A**" Positive control, showing a contaminated Actinobacterial isolate on SCA without antibiotics, Plate "**B**" Blank control, SCA only without a single colony of Actinobacterial isolate.

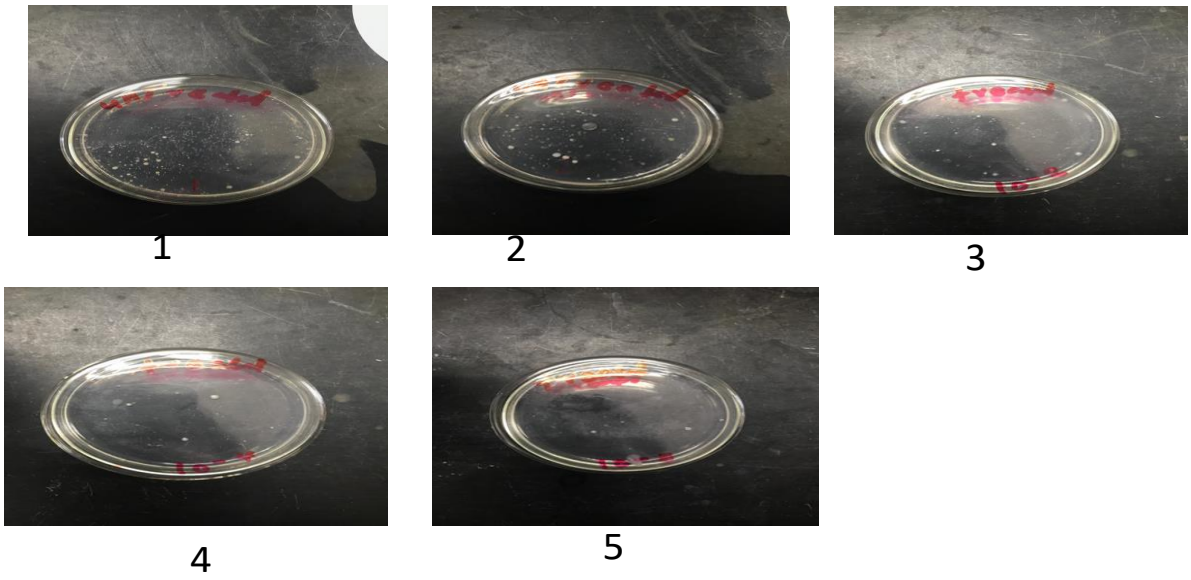

Fig. 3. Plate "**1-5**" showing cells containing actinobacterial colonies from the agar plate from dilution series 10^{-1} to 10^{-6} for cow dung manure after enhancement.

CFU Quantification Before and After Enhancement

The fine, distinct, and viable colonies from the best total dilution factor that are within the range of 30-300 for cow dung manure samples before and after enhancement are calculated and recorded in table (1). This shows the presence of an actinobacterial population in cow dung manure before enhancement was in average quantity.

Table 1. Changes in CFU count of Actinobacteria and percentage increase.

Sample	CFU	Fold Change	% Increase
Cow dung manure Before Enhancement	3.9 X 10^{-4} CFU/g	1.00	0
Cow dung manure After Enhancement	8.5 X 10^{-4} CFU/g	2.18 x	118%

Fold change: (After enrichment divided by before enrichment)

$\frac{8.5}{3.9}$ = **2.18**

% Increase: $\frac{8.5 - 3.9}{3.9}$ x 100 = 117.95% **≈118%**

Cow dung manure after enhancement has a higher number of CFU than the one before enhancement, with 3.9 x 10^{-4} CFU/g, while cow dung manure after enhancement has 8.5 x 10^{-4} CFU/g, which represents a 2.18-fold increase (118%) as displayed in figure (4). The population of Actinobacteria in cow dung manure before enhancement is not much, and it has been improved by the enhancement technique up to the desired level for agricultural applications. This has confirmed the hypothesis that cow dung manure is safe to be used as biofertilizer, and plant growth-promoting Actinobacteria was at an optimum level for better crop production.

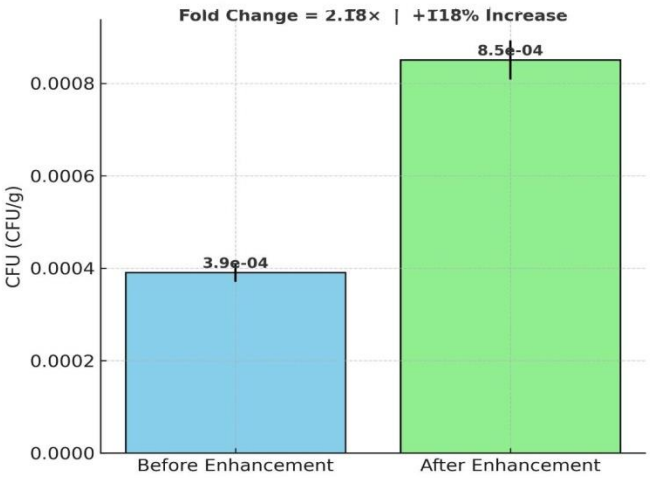

Fig. 4. Illustrating CFU count before and after enhancement, with an error bar along with the computed 2.18× fold change (+118% increase).

The increased notice in Actinobacterial CFU counts prior to enhancement indicated that the enrichment technique gives more room for the required nutrients as well as favourable conditions for actinobacterial multiplication and division. This enhancement has correlated with the previous investigations by previous studies (Aguilar-Paredes *et al.*, 2023; Alori *et al.*, 2017; Prisa *et al.*, 2023), who outline a rise in streptomyces actions after subsequent organic waste enrichment in manure-typed biofertilizer development.

CONCLUSION

This investigation revealed that the enrichment of plant growth-promoting actinobacterial populations in cow dung manure was attained via strategic isolation and enrichment techniques. An increase in colony-forming units (CFU) post-enhancement showed a successful increase in populations of valuable microorganisms. The research emphasizes the potential of using cow dung manure as a biofertilizer enhanced with the plant growth-promoting Actinobacteria.

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CONFLICT OF INTEREST

There is no conflict of interest.

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