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Biofertilizer production from indigenous microorganisms for sustainable agriculture in Nigeria: A case study of Bauchi State

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Abstract
The increasing cost of inorganic fertilizers and their associated environmental impacts have necessitated the search for sustainable alternatives for crop production. This study investigated the potential of indigenous soil microorganisms for biofertilizer production and their application in improving soil fertility and maize (*Zea mays* L.) growth in Bauchi State, Nigeria. Rhizospheric soil samples were collected from soybean fields and subjected to microbiological isolation, biochemical characterization, and molecular identification using 16S rRNA gene sequencing. The isolates were screened for phosphate-solubilizing and nitrogen-fixing abilities before being utilized for biofertilizer production. Molecular analysis revealed that the predominant isolate showed 99.93% similarity with members of the genus *Comamonas*, particularly *Comamonas testosterone.* The formulated biofertilizer was evaluated in a pot experiment alongside NPK fertilizer and an untreated control. Results indicated that biofertilizer application enhanced soil nutrient status and improved maize growth performance compared with the control treatment. Biofertilizer-treated plants consistently produced higher leaf numbers and greater plant height than plants receiving NPK fertilizer or no fertilizer treatment. The findings demonstrate that indigenous microorganisms possess considerable potential for sustainable biofertilizer production and could contribute significantly to reducing dependence on synthetic fertilizers in Nigeria.

Keywords: Biofertilizer, indigenous microorganisms, *Comamonas testosteroni*, sustainable agriculture, maize, soil fertility, Nigeria

Introduction

Background to the Study

Agriculture remains a critical sector in the Nigerian economy, contributing significantly to food security, employment, and national development. However, agricultural productivity in Nigeria is increasingly constrained by declining soil fertility, land degradation, and the rising cost of agricultural inputs, particularly chemical fertilizers. Continuous and excessive use of inorganic fertilizers has led to adverse environmental consequences such as soil acidification, nutrient imbalance, groundwater contamination, and loss of beneficial soil microorganisms (Babalola, 2010; Fasusi *et al*, 2021) [5]. These challenges underscore the urgent need for sustainable and environmentally friendly alternatives to maintain soil health and improve crop productivity.

Biofertilizers have emerged as a viable and eco-friendly solution to these challenges. Biofertilizers are formulations containing living microorganisms which, when applied to seeds, plant surfaces, or soil, colonize the rhizosphere and promote plant growth by increasing the availability and uptake of essential nutrients (Ahemad & Kibret, 2014) [2]. These microorganisms enhance soil fertility through various mechanisms, including biological nitrogen fixation, phosphate solubilization, production of plant growth regulators such as indole-3-acetic acid (IAA), and suppression of plant pathogens (Glick, 2012; Olanrewaju *et al*, 2017) [10].

Among the various types of biofertilizers, plant growth-promoting rhizobacteria (PGPR) such as *Bacillus*, *Pseudomonas*, *Azotobacter*, and *Rhizobium* species are widely recognized for their significant role in sustainable agriculture. These microorganisms not only improve nutrient availability but also enhance plant tolerance to environmental stresses such as drought and salinity (Adesemoye *et al*, 2009; Bashan *et al*, 2014) [1, 6]. Additionally, mycorrhizal fungi contribute to improved nutrient and water uptake, particularly phosphorus, thereby further supporting plant growth and soil structure (Smith & Read, 2008) [13]

Despite the proven benefits of biofertilizers, their adoption in Nigeria remains relatively low. One major limitation is the reliance on imported or non-native microbial strains, which may not perform optimally under local environmental conditions. Indigenous microorganisms, on the other hand, are naturally adapted to the local soil and climatic conditions and are therefore more efficient and resilient (Babalola, 2020). Harnessing these native microbial resources offers a promising pathway for developing cost-effective and locally relevant biofertilizer technologies

Recent advances in molecular biology and bioinformatics have significantly improved the identification and characterization of beneficial microorganisms Techniques such as 16S rRNA gene sequencing, phylogenetic analysis, and functional gene annotation now allow for precise identification of microbial species and their plant growth-promoting capabilities (Tamura *et al*, 2021; Turenne *et al*, 2001) [14]. These tools provide a deeper understanding of microbial diversity and enable the selection of highly efficient strains for biofertilizer production

In Nigeria, several studies have reported the presence of beneficial microorganisms in agricultural soils; however, there is still a gap in translating these findings into practical, scalable biofertilizer products. Furthermore, limited integration of advanced molecular techniques in local research has hindered the optimization and standardization

of biofertilizer formulations (Olorunfemi *et al*, 2020; Ukaegbu-Obi & Achi, 2019).

Research Objectives
This study seeks to bridge these gaps by:
Isolating and characterizing indigenous microorganisms using conventional and advanced molecular approaches.
Utilizing selected strains in the production of high-quality biofertilizers.
Evaluating their effectiveness on the growth and yield of crops like maize (*Zea mays*) and okra

Materials and Methods
Study Area
The production of biofertilizer and comparative evaluation experiments were conducted at the laboratories of the Department of Science Laboratory Technology (SLT), Abubakar Tatari Ali Polytechnic, Bauchi State, Nigeria. The inorganic fertilizer used for comparison was NPK (20:10:10), obtained from the Bauchi State Agricultural Development Programme (BSADP), Bauchi

Collection and Isolation of Soil Samples
Rhizospheric soil samples were collected from soybean (*Glycine max*) fields in Durum, Bauchi State. The samples were aseptically collected into sterile polythene bags and transported to the laboratory in an ice-packed container.
Ten grams (10 g) of soil were suspended in 90 mL of sterile distilled water (10^{-1} dilution), and serial dilutions were prepared up to 10^{-9}. Aliquots were inoculated onto Nutrient Agar (NA) and Yeast Mannitol Agar (YMA) plates using the spread plate technique. Purified distinct colonies were maintained for further analysis.

Characterization and Biochemical Identification
Bacterial isolates were characterized based on colonial morphology and biochemical traits:
Gram staining and microscopic examination
Temperature tolerance assessment up to 65°C
Standard tests: motility, catalase, urease, oxidase, citrate utilization, H_2S production, and carbohydrate fermentation.

Screening for Phosphate-Solubilizing Bacteria (PSB)
Isolates were streaked onto Pikovskaya (PKV) agar plates and incubated at 37°C for 24 hours. Clear halo zones surrounding colonies indicated positive phosphate solubilization

Screening for Nitrogen-Fixing Bacteria (NFB)
Isolates were inoculated into Jensen's nitrogen-free broth medium with phenol red and incubated at 37°C for 5 days. A color change from pink to red confirmed nitrogen-fixing potential.

Molecular Characterization (PCR and 16S rRNA)
Molecular identification was carried out using Polymerase Chain Reaction (PCR) with universal bacterial primers:
27F: 5′-AGAGTTTGATCMTGGCTCAG-3′
1525R: 5′-AAGGAGGTGWTCCARCCGCA-3′
The 25 µL PCR reaction mixture contained: 2.5 µL 10× PCR buffer, 1 µL $MgCl_2$ (25 mM), 1 µL each of forward/reverse primers, 1 µL DMSO, 2 µL dNTPs (2.5 mM), 0.1 µL Taq DNA polymerase (5 U/µL), and 3 µL template DNA (extracts.docx). Amplified products (~1,500 bp) were visualized on 1.5% agarose gels using ethidium bromide and sequenced via an ABI 3500 Genetic Analyzer. Sequences were aligned using BioEdit and analyzed via NCBI BLAST.

Biofertilizer Production Process
1. **Starter Culture:** 100 mL Nutrient Broth flasks were inoculated and placed in a shaker incubator at 37°C and 100 rpm for 7 days

2. **Mass Propagation:** 10 mL of starter culture was transferred into 1,000 mL conical flasks of Nutrient Broth and incubated for another 7 days

3. **Packaging:** Formulated composite biofertilizer was packaged in breathable polythene bags and stored under cool, dry conditions

Physicochemical Analysis of Biofertilizer and Soil
Analyses were conducted at the National Cereals Research Institute (NCRI), Badeggi, Niger State, evaluating

pH: Calibrated pH meter (buffers 4.0 and 7.0)

Nitrogen: Kjeldahl method (digestion, neutralization, titration)

Phosphorus & Potassium: Colorimeter (470 nm) and flame photometer respectively

Physical Properties: Soil color (Munsell Chart) and texture (feel method)

Pot Experimental Design
A pot experiment was established under a Completely Randomized Block Design (CRBD) with 15 experimental pots distributed into three treatment groups (5 replications each)
- **Control (T_0):** Untreated soil
- **Inorganic Fertilizer (T_1):** NPK (20:10:10) application
- **Biofertilizer (T_2):** Produced indigenous bacterial biofertilizer application

Results and Discussion
Biochemical Screening
Out of the 14 isolates screened, the majority demonstrated excellent metabolic profile viability necessary for biofertilizer performance

Characteristic	Positive Isolates	Percentage (%)
Gram-negative	13	92.9%
Gram-positive	1	7.1%
Catalase Production	13	92.9%
Citrate Utilization	12	85.7%
Urease Production	13	92.9%
Starch Hydrolysis	8	57.1%
Motility	7	50.0%
H_2S Production	11	78.6%
Gas Production	4	28.6%
Growth at 37°C	14	100.0%

Sequencing and Molecular Identification
Gel electrophoresis revealed distinct bands at the expected 1500 bp region for the 16S rRNA

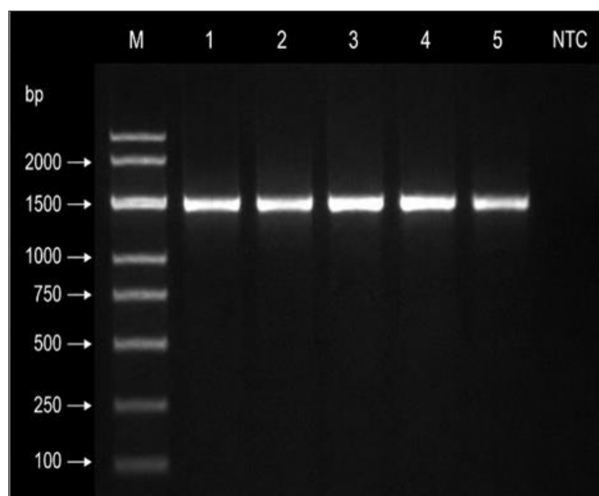

Fig 1: Agarose gel electrophoresis profile showing amplified DNA fragments of bacterial isolates. M: 100 bp DNA ladder; Lanes 1–5: Bacterial isolates; NTC: Non-template control

NCBI BLAST alignment showed that the predominant isolate had 99.93% identity to the genus *Comamonas*, matching closely with *Comamonas testosteroni*.

Table 1: NCBI BLAST Sequence Alignment Identification

Target Crop Isolate Species	Max Score	Total Score	Query Cover	% Identity	Accession No.
Comamonas testosteroni	2562	20499	100%	99.93%	LN879547.1
Comamonas testosteroni	2562	2562	100%	99.93%	CP090445.1
Comamonas thiooxydans	2562	23028	100%	99.93%	OQ255866.1
Comamonas sp. C11	2562	23061	100%	99.93%	KM462142.1

Soil Properties Analysis (Before vs. After Planting)

Baseline analysis before planting showed grey, moderately coarse sandy soil with acidic conditions, 5% organic matter, low Nitrogen (0.52%), and low Potassium (0.69 cmol kg^{-1})

Table 2: Variations in Key Soil Nutrient Values After Cultivation

Parameter	Baseline (Pre-planting)	Control Soil (Post)	NPK Treated (Post)	Biofertilizer Treated (Post)
Soil Color	Grey	Grey	Grey	Darker / Granular
Nitrogen (%)	0.52%	Low (~3.5%)	Medium (~5.0%)	Highest (~9.0%)
Available P	Baseline	Moderate	High	Highest (Max)
Organic Matter	5.0%	Low (~5.0%)	High (~13.0%)	Highest (~15.0%)
pH Status	Acidic	Acidic	Near-Neutral	Near-Neutral

Note: Statistical verification using one-way ANOVA and Bonferroni post hoc tests indicated that while pH changes were statistically gradual, Nitrogen, Phosphorus, and Organic Matter improvements were significantly higher ($P < 0.05$) in biofertilizer treatments.

Agronomic Performance of *Zea mays*

Leaf Production (Weekly Changes)

Biofertilizer-treated plants (MBF) consistently produced a higher number of leaves throughout the 7-week cultivation period compared to NPK and Control treatments. Week 1-3: Gradual uniform setup.

Week 4-7: MBF diverged sharply, reaching a peak leaf count average significantly above the control and NPK.

Plant Height (cm)

Weeks 1 & 2: No structural height variation among groups ($P > 0.05$) (extracts.docx).

Week 3: Height of biofertilizer-treated plants increased markedly (Highly significant, $P < 0.001$)

Weeks 4 to 7: Biofertilizer treatment consistently recorded the highest average plant height, demonstrating superior growth performance.

Conclusion

This study successfully demonstrates that indigenous soil bacteria isolated from soybean fields in Bauchi State specifically *Comamonas testosterone* possess potent nitrogen-fixing and phosphate-solubilizing capabilities the formulated biofertilizer outperformed conventional NPK synthetic inputs across major soil health indexes and plant vegetative growth parameters. This offers a scientifically validated, economically viable alternative for sustainable agricultural production within Nigeria.

Recommendations for Future Studies

1. Optimize shelf-life parameters and carrier material formulations
2. Establish structured commercialization frameworks for local farmers

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